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Lecture and Poster Abstracts

$L = lecture \quad P = poster$
Host Cell Proteins (HCP) need to be adequately monitored and cleared during downstream purification of biopharmaceuticals due to their potential impact on product quality. Protein A affinity chromatography was demonstrated to be an effective and robust process step for HCP clearance. However, there is still a small amount of HCP remaining in the purified product, often attributed to non-specific interaction with the monoclonal antibody (mAb) molecule or Protein A resin. We developed an approach to profile the separate interactions of HCP with mAb and resin during Protein A capture. To study HCP interaction with the resin, we utilized null cell supernatant as a source of a representative preparation of HCPs that were allowed to interact with different Protein A resins. We observed that, while no HCPs were detected in the elution pool of an agarose-based Protein A resin, both glass- and silica-based resins had significant amounts of non-specific HCP interactions. Mass spectrometry analysis shows that the glass-based and silica-based resins interacted with similar HCP populations, which suggests shared characteristics in adsorption mechanisms. To profile mAb-HCP interactions, a purified mAb was immobilized by cross-linking to Protein A resin. HCPs from the null cell supernatant were incubated with the immobilized mAbs. HCPs which bound to the mAb molecule were subsequently eluted and analyzed by mass spectrometry. This approach allowed HCP identification without interference from the mAb. We utilized this approach for identification of HCP species which interacted with several mAb molecules. Each mAb interacted with a distinct HCP subpopulation. However, since these mAbs have high similarity in the amino acid sequence, particularly in the region of the heavy chain, we found that many common HCPs interacted with all the mAbs. This approach can potentially be used to improve understanding of mechanisms by which HCPs interact with mAbs, and to facilitate development of new strategies to mitigate HCP-mAb interaction during both upstream and downstream process development. Because individual HCPs that interact with the mAbs can be identified and assessed for potential effects on patient safety and mAb stability, it may be possible to use this approach to develop targeted HCP control strategies.

Process Characterization of a Design Space for an Extraction Process. Karthik Mani, Genentech Inc., South San Francisco, CA, USA
The characterization of an extraction process for a E. coli derived recombinant protein is presented. This step functions to remove insoluble cell debris and host cell impurities, while maximizing product yield. First, a risk ranking and filtering (RRF) tool is used to identify parameters for process characterization. Second, characterization studies are designed and executed based on the output of the RRF tool. Third, the results of the characterization studies are analyzed to evaluate the impact of each process parameter on pre-determined Critical Quality Attributes (CQAs). A dimensionless indicator of parameter criticality, called an Impact Ratio, is used as a quantitative and consistent measure of parameter impact on each CQA over the parameter's characterized range. The planning and execution to characterize the unit operation design space, within a Quality by Design (QbD) framework, is discussed.

A process optimization initiative, utilizing quality by design (QbD) approach, to increase production throughput will be presented. A purification step using Q sepharose FF step of an early phase non-mAb protein was redeveloped. The load capacity of this column limited potential scale-up and created concerns regarding buffer consumption and processing time. To enhance scalability and facility fit, resin scouting was initiated. Batch mode resin scouting was performed to identify promising candidates with higher binding capacity and equal or better selectivity. Subsequent linear and step gradient separations were performed to identify the optimal operating conditions. A preliminary full factorial experimental design with the three most important factors was conducted on the top resin candidate to optimize the column operating conditions. Side-by-side confirmation runs through the entire purification train were then performed using Q FF and the new resin to compare process performance and product quality. The new chromatography step was characterized using a sequence of fractional factorial and central composite designs including multiple operational variables. These designs allowed the impact of input variables to be quantified on process performance (e.g. yield) and product quality, thus identifying the design space. Using the identified models, Monte Carlo simulations were then performed to establish in-process ranges for operating and performance parameters. The new resin was subsequently implemented for a late stage purification process operated at a larger scale.
L-104 Application of Small-scale Models for the Development, Optimization, and Life-cycle Management of Industrial Scale Protein Chromatography. Samuel Hernandez¹, Tara Jones², ¹Amgen, Juncos, Puerto Rico, USA; ²Amgen, Thousand Oaks, CA, USA

The design and use of downstream protein purification processes has the ultimate goal of delivering a drug substance that meets product quality expectations. One major aspect of a downstream process is to reduce host cell protein impurities that are ubiquitous in all industrial scale mammalian and bacterial cell culture processes. This case study examines the use of a laboratory chromatography model (small-scale model or SSM) to investigate unexpected process variability for the reduction of host cell impurities observed at industrial scale. The cause for the unexpected variability and correction at industrial scale is discussed. Additionally, this case study examines optimization of a reversed phase chromatography step to further enhance the clearance of host cell proteins. Specifically, the unexpected process variability was leveraged to understand and correct the performance of this chromatography step at the commercial scale. Potential root causes were evaluated using a statistical model that took into consideration process variables known to influence the clearance of host cell impurities. Furthermore, process development and characterization work utilizing the SSM is followed from laboratory to industrial implementation to ensure expected performance and robustness. Finally, the importance for on-going life-cycle management of a laboratory chromatography model to predict industrial scale performance for investigational purposes and process improvements is highlighted.

L-105 Structure and Protein Adsorption Mechanisms in Clean and Fouled Anion Exchange Resin. Timothy Iskra¹, Rachel Corbett², Jeffery Salm¹, Christopher Gallo¹, Ranga Godavarti¹, Giorgio Carta², ¹Pfizer, Andover, MA, USA; ²University of Virginia, Charlottesville, VA, USA

Pfizer’s two column mAb platform has a single anion exchange chromatography polishing step operated in the weak partitioning mode. Consistent and robust clearance of impurities has been demonstrated for multiple mAb processes. A recent study determined an unusual linkage between process steps potentially resulting in lower capacity on the AEX resin. The cause for reduced capacity was hypothesized to be a result of resin fouling. An investigation into the mechanism of fouling was undertaken. For this study several techniques were used to visualize the fouling (such as SEM, TEM, and confocal microscopy). This visualization has revealed the formation of a skin layer on the outside of the resin beads that result in a drop in the capacity for impurities. The effects of fouling was and can be measured through the use of adsorption kinetics of larger molecules such as thyroglobulin whereas smaller proteins show little effect.

L-106 Understanding and Enhancing the Selectivity of Multimodal Protein Chromatography. Steven M. Cramer, S. Parimal, J. Woo, S. Garde, K. Srinivasan, Rensselaer Polytechnic, Troy, NY, USA

In this work a detailed investigation into the engineering of multiple weak interactions to create selective multimodal protein separation systems was carried out. This research seeks to determine what conditions are required to achieve selective separations of similar protein variants and to provide fundamental insight into the mechanisms underlying these separations. The retention of protein libraries on several multimodal cation-exchange systems, including Capto MMC and Nuvia cPrime was first under a range of fluid phase modifier conditions. While these ligands are constructed from similar functional groups (a phenyl ring and carboxylic acid), the retention of many proteins proved to be sensitive to subtle changes in the ligand chemistry and geometrical presentation that affected the exposure of the phenyl ring to the surrounding solvent. Further, the effects of fluid phase modifiers were found to be quite different for the adsorption of various proteins in the two MM systems. All-atom explicit Molecular Dynamics (MD) simulations were then carried out to shed light on the multiple weak interactions that resulted in the unique selectivities achieved in these multimodal chromatographic systems. The knowledge from these simulations was also used to deconvolute synergistic MM interactions into its key contributors. This was coupled with protein surface characterization techniques to evaluate the strength and importance of electrostatic and hydrophobic interactions in these systems. Simulations were also performed to evaluate the interactions of fluid phase modifiers with proteins and to study how they enhance/reduce protein-ligand binding. Single molecule force spectroscopy was then employed to study the energetics of face specific binding of proteins to self-assembled monolayers (SAMs) of MM ligands. Force measurements were performed on the exposed protein face using a force probe functionalized with SAMs of MM ligands and the data was employed to obtain equilibrium values of free energy and to obtain further insight into protein binding energetics at the molecular level. Finally, kinetics of protein and MM ligand interactions were investigated using quartz crystal microbalance (QCM). This work provides fundamental understanding of the nature of these interactions at the molecular level and insight into the design of MM ligands, the roles of synergy and the modulation of selectivity using fluid phase modifiers with important implications for addressing challenging problems in downstream bioprocessing.
Predicting Protein Adsorption Equilibria on Ion-exchange Resins. Rushd Khalaf\textsuperscript{1}, Matteo Costioli\textsuperscript{2}, Massimo Morbidelli\textsuperscript{3}, \textsuperscript{1}ETH Zurich, Zurich, SWITZERLAND; \textsuperscript{2}Merck Serono S.A., Fenil-sur-Corsier, SWITZERLAND

Due to the recent advances in upstream processing, the chief portion of the cost involved in the development of production processes for pharmaceutical drugs is now associated with downstream processing. More specifically, the vast majority of purification costs are directly linked to chromatography. The design of these chromatographic steps is often done with respect to a classical 3-step platform (i.e. a Protein A capture followed by two additional ion exchange chromatography steps), which is slightly modified to suit the needs of each new drug. While the use of a platform significantly reduces the cost and time involved in the design of the process, real optimal conditions in terms of yield and cost are almost never achieved. As such, a predictive tool allowing fast and robust chromatographic optimization is of primordial importance, with the only time and cost constraint being computing power. In this talk, a predictive model for ion exchange chromatography will be presented. The model, adapted from Guélat et. al. [1], is based on Derjaguin–Landau–Verwey–Overbeek (DLVO) theory and statistical thermodynamics. The proper calibration steps and the involved parameters will be shown to be solely dependent on the protein or peptide sequence. Several examples of predicted results will be presented and discussed. Through these examples, the model will be shown to be suitable for the design and development of ion exchange chromatographic steps, for proteins and peptides of varying size and charge. In all cases, the model's physical basis will be shown to be consistent with known facts. [1] Bertrand Guélat, Guido Ströhlein, Marco Lattuada, Lydia Delegrange, Pascal Valax, Massimo Morbidelli, Simulation model for overloaded monoclonal antibody variants separations in ion-exchange chromatography, Journal of Chromatography A, Volume 1253, 31 August 2012, Pages 32-43

High-Throughput Measurements of Protein Transport in Ion-exchange Media. Steven Traylor, Abraham Lenhoff, University of Delaware, Newark, DE, USA

Protein transport mechanisms in chromatographic media remain an area of significant interest, in both traditional and especially polymer-functionalized media. Uptake rates have been reported to change as a function of both ionic strength and protein concentration and these effects have generally been attributed to contributions from the mobility of bound protein. Significant changes in protein displacement rates compared with uptake rates of the same protein have been reported and attributed to kinetic and steric limitations. Similar limitations have also been blamed for sluggish protein elution rates from polymer-functionalized materials. This talk will address two main research objectives: first, developing the methodology for measuring high-throughput batch uptake, displacement and elution kinetics even under conditions of very fast transport; and second, applying these methods along with relevant theory to elucidate transport behavior in specific systems of interest. The method development focus is on integration of high-throughput kinetic measurements with more traditional batch kinetic and isotherm measurement methods. Special emphasis is on time-dependent batch uptake measurements in a standard liquid handling system, specifically our success in adapting the system to obtain time-resolved measurements accurate to well under one minute, with minimal investment in auxiliary equipment. We will also discuss displacement and elution measurements using the same format. The principal application is the effect of protein concentration and ionic strength on protein uptake and displacement rates in traditional and polymer-modified ion exchange media. We will compare microscopic measurements of protein mobility on chromatographic media by fluorescence photobleaching microscopy (FRAP) with macroscopic high-throughput measurements of protein transport. We will additionally discuss theoretical insights into binding kinetics and transport by testing appropriate models and attempting to describe and predict observed uptake and displacement behavior.

Thermodynamic Study of Effects of Mixed Aqueous Electrolytes on Adsorption of PEGylated Lysozyme, Native Lysozyme and Pure PEG on a Mildly Hydrophobic Resin. Albert Werner, Hans Hasse, University of Kaiserslautern, Laboratory of Engineering Thermodynamics, Kaiserslautern, Rhineland-Palatinate, GERMANY

PEGylation of pharmaceutical proteins, i.e. the covalent binding of poly(ethylene glycol) (PEG) to the protein, can lead to improved residence time of the drug in the body. Usually, the protein is already purified before PEGylation. Therefore, the purification task after PEGylation is reduced to a separation of the PEGylated isoforms or a fraction with a certain PEGylation degree from the unPEGylated protein. As the PEGylation changes the hydrophobicity of the proteins, the hydrophobic interaction chromatography (HIC) is a promising technique for the separation of PEGylated protein forms. The state of the art is using pure electrolytes for reducing the protein solubility. Using mixed electrolytes for that task has great potential, due to different interactions of the different anions and cations with the different parts of the proteins. This can lead to synergistic effects in protein adsorption. In the present work, the adsorption of different forms of PEGylated lysozyme as well as native lysozyme and pure PEGs on a mildly hydrophobic resin (Toyopearl PPG-600M) was studied by measuring both the adsorption equilibrium isotherms and the specific enthalpies of adsorption along the
isotherms. This gives a complete thermodynamic picture of the adsorption. The measurements of the adsorption equilibrium isotherms were carried out using a fully automated robotic liquid handling station. For the calorimetric measurements, a high precision isothermal titration micro-calorimeter was used. The studied salts are sodium chloride, ammonium sulfate, sodium sulfate, ammonium chloride and their mixtures. A sodium phosphate buffer was used to adjust the pH value. It was found, that adsorption mechanism changes completely upon variation of the salt. A molecular picture is discussed which describes the complex adsorption mechanisms based on the interpretation of the interactions of the different ions with the proteins. It is shown that the specific interactions of the individual ions with particular regions of the proteins are responsible for the varying adsorption behavior. A quantitative structure property relationship (QSPR) is discussed, which describes the influence of the different salt ions and their cross interactions on the adsorption.

L-110  Evaluating the Performance of Different Multicolumn Setups for Chromatographic Separation of Proteins on HIC Media. Roman Bochenek, Wojciech Marek, Wojciech Piatkowski, Dorota Antos, Rzeszow University of Technology, Rzeszow, POLAND

The effectiveness of isolating a target protein from a multi-component protein mixture using different column arrangements has been analyzed. Different continuous systems have been considered capable of performing the solvent gradient separation: open loop simulated moving bed (OL-SMB), multicolumn countercurrent solvent gradient purification (MCSGP) and its modifications and carousel multicolumn setup (CMS). The first system is based on the standard SMB configuration but the regeneration zone of the liquid phase is eliminated, the second one exploits the internal countercurrent recycling of the solid phase whereas the liquid phase loops can be open after each or some of zones. CMS refers to continuous systems in which feed and desorbent streams are delivered subsequently through parallel columns to mimic their countercurrent movement with respect to the fluid flow. As a case study separation of a ternary mixture of proteins on HIC media has been selected. The target protein of the separation was either the most strongly adsorbed component of the mixture or exhibited intermediate adsorption strength. The performance of the continuous processes has been examined and compared to a single-column batch system. Two conflicting performance indicators have been considered in the multi-objective optimization routine, such as yield and productivity. A mathematical model has been used to simulate the process dynamics and to optimize operating conditions for the feed loading and separation. Different systems have been ranked with respect to both objective functions. Moreover, the eluent consumption has been considered.

L-111  How to Purify a Monoclonal Antibody in One Shot: Continuous Chromatography Applied to the Entire Purification Process. Benoit Mothes, Sanofi, Vitry-sur-Seine, FRANCE

The current state of the art in Monoclonal Antibodies purification consists in a sequence of distinct unit operations: 2 to 3 chromatography steps + filtration steps. Up to now, these units operations could not be operated in a continuous mode as adjustment of pH, conductivity and protein concentration are necessary between each chromatography or filtration step. A new purification process has been developed by Sanofi, enabling to run 3 chromatography steps in continuous mode with no holding time nor open phase. This means that a cell culture bulk containing the Monoclonal Antibody can be now fully processed through this continuous process to obtain a pure Monoclonal Antibody batch without human intervention, decreasing also resin and buffer costs.

L-112  Purification of Monoclonal Antibodies by Hydrophobic Interaction Chromatography under No-salt Conditions. Sanchayita Ghose, Yining Tao, Lynn Conley, Biogen Idec, Research Triangle Park, NC, USA

Hydrophobic interaction chromatography (HIC) is commonly used as a polishing step in monoclonal antibody purification processes. HIC offers an orthogonal selectivity to ion exchange chromatography and can be an effective step for aggregate clearance and host cell protein reduction. HIC, however, suffers from the limitation of use of high concentrations of kosmotropic salts to achieve the desired separation. These salts often pose a disposal concern in manufacturing facilities, require dilution of the product pool and at times can even cause precipitation of the product. Here, we report an unconventional way of operating HIC in the flowthrough (FT) mode with no salt in the mobile phase. This new strategy was tested with a wide panel of antibodies with varying pI's and surface hydrophobicity. Comparable performance was seen to the existing HIC step under high salt conditions. The concept described here can be extremely useful for designing HIC processes not only for antibodies, but any neutral or basic protein.
L-113  A New Disposable Process for Post-protein A Monoclonal Antibody Polishing. David Gruber1, Mikhail Kozlov2, Romas Skudas3, MedImmune Ltd, Cambridge, Cambridgeshire, UK; 2EMD Millipore, Bedford, MA, USA; 3EMD Millipore, Darmstadt, Hessen, GERMANY
We describe a novel MAb purification process replacing a typical post-Protein A step of bind/elute CEX chromatography with new disposable flow-through technologies. Two new adsorbers were designed by EMD Millipore for removal of process- and product-related impurities and tested with early MedImmune MAb drug candidates. The first adsorber is based on a highly porous media preferentially removing protein species smaller than a MAb, including most HCP's, leached Protein A, while the second adsorber is optimized for removal of product aggregates. The two new adsorbers and an AEX flow-through membrane device were assembled into a polishing train and tested for performance in a number of device combinations, loadings, and solution conditions. Experimental results demonstrate robust performance, including maintaining high product yield, removal of HCP, and aggregates. The new approach could enable a simplified and more powerful platform for MAb polishing than existing technologies.

L-114  Purification of a Common Light Chain Bispecific Antibody using Contichrom (MCSGP). Thomas Muller-Spath1, Nicole Ulmer1, Guido Strohlein1, Michael Bavand1, Linda Kaldenberg-Hendriks2, Lex Bakker3, John de Kruif1, Mark Throsby2, ChromaCon AG, Zurich, SWITZERLAND; 2Merus BV, Utrecht, THE NETHERLANDS
A human bispecific common light chain IgG antibody (AB) was purified from PER.C6 cell culture harvest with high yield and high purity using a three-step chromatographic downstream process. The purity of AB in the harvest with respect to the parental antibodies AA and BB was 40%. The downstream process comprised a Protein A capture step, a first polishing step using MCSGP cation-exchange chromatography and a second polishing step using mixed-mode chromatography. The key to successful purification of AB was the operation of the first polishing step with Contichrom in MCSGP mode that enabled a step yield of 87% and a purity of > 99% AB. In contrast the step yield of the regular batch chromatography polishing step in the benchmark process was 37%. The overall AB yield was 78% in the former case and 34% in the latter case. The MCSGP process also showed excellent aggregate, HCP and DNA clearance. The presented data shows that Contichrom in MCSGP mode enables the economic production of bispecific antibodies thereby removing the bottleneck in downstream processing for this product class.

L-115  Fractionation of Peptides by High-throughput Ion-exchange Chromatography - Unspecific Interactions of the Peptides with the Stationary Phase. Elena Leeb, Seronei Cheison, Ulrich Kulozik, Technische Universität München, Freising, GERMANY
A food-grade process to fractionate complex peptide mixtures obtained from tryptic hydrolysis of β-lactoglobulin was developed using Ion-exchange Membrane Adsorption Chromatography (MAC). The application of a two-step purification process, whereby a strong anion and cation MAC module are coupled (Sartobind Q and Sartobind S), enables the fractionation of peptides with isoelectric points (pI) in the range of 4 to 10. Process conditions for maximum separation efficiency were determined, using 0.03 mol/l phosphate-buffer at pH 7 for the anion exchange process. This gave seven individual fractions in the first fractionation process step. In the second step the flow-through of the anion exchange process was further separated using a cation exchanger and 0.03 mol/l phosphate buffer (pH 3). With a step-wise NaCl-gradient for both fractionation processes the production of 12 individual fractions in total was achieved. However, following analysis of the peptide composition of the fractions by mass spectrometry pointed out that not only electrostatic interactions between the peptides and the ligands were responsible for the fractionation. The elution of the peptides was partly independent of their pI. Therefore hydrophobic interactions between the peptides and the membrane as well as between the peptides and to the membrane attached spacers were assumed to play a role during the elution process. To investigate the influence of the column material and the spacers on the elution profile of peptides the process was transferred to monolithic columns. Thereby also a strong anion and cation exchanger were used in series and comparable fractionation conditions were provided. The elution profile of the hydrolysate using membrane and monolithic columns was compared and thus the role of unspecific interactions of the peptides with the stationary phase evaluated.

L-116  Novel Ion-exchange Doped Reversed Phase Material for Peptide Purification. Nicola Forrer1, Rushd Khalaf2, Massimo Morbidelli2, Zeochem AG, Uetikon am See, Zurich, SWITZERLAND; 2ETH Zurich, Zurich, SWITZERLAND
Pharmaceutical grade peptides have recently gained much importance because of their great efficacy in treating diseases and illnesses. As with most pharmaceutical drugs, the high purity requirements of the active ingredient are obtained by liquid chromatography. In the case of peptides, reversed phase chromatography is most often used. As such, the development of more efficient stationary phases, which would lead to increases in yield and or productivity, has become an important part in the improvement of the purification process. In this talk, a novel,
ion exchange doped reverse phase material will be presented. The performance of this new material will be compared to traditional reversed phase materials. It will be shown that the doped material displays an increase in all the conventional performance markers for chromatography (yield, selectivity between closely eluting products, productivity, concentration of the purified product fraction, etc.). Examples depicting the increase in purification performance, from crude peptide solutions to PEGylated proteins, will be provided. The choice of cation or anion exchange ligands as the doping agent will be shown to be closely related to peptide charge. Finally, the effect of organic modifier, counter-ion concentration, and concentration of doping groups on the retention time of peptides is described. The influence of these parameters on retention time is then characterized using a model based on the DLVO theory and statistical thermodynamics (for the ion exchange interactions) and on empirical correlations (for the reversed phase interactions).

L-117 Versatile Use of Mixed-mode Sorbents for Intermediate Purification of a Fab Fragment. Karl Rogler, Rene Gantier, Pall Life Sciences, Northborough, MA, USA
Mixed-mode chromatography has been demonstrated to be a very useful alternative technique to ion-exchange (IXE) and hydrophobic interaction chromatography (HIC) for intermediate and polishing steps in downstream processing of monoclonal antibody. The versatility of mixed-mode chromatography sorbents, coming from the combination of both ionic and hydrophobic interactions, makes them usable for extended type of purification applications including the emerging antibody fragments. In this study we used different mixed-mode sorbents for intermediate purification of a Fab fragment from E. coli periplasmic extract after a first capture step using cation-exchange (CEX) chromatography. High-throughput screening (HTS) on 96-well filter plates was employed to identify optimum conditions for the Fab purification in both bind/elute and flow through modes. Column based performance was then evaluated for Dynamic Binding Capacity (DBC), purity, and yield of recovery. While some sorbents achieved a high binding capacity, Fab purity and yield of recovery in bind and elute mode, other sorbents provided similar purification performance when loading up to 150 mg of Fab per ml of sorbent in flow through mode. Overall, this study gives insight on the multipurpose applicability of mixed-mode sorbents and their ability to be used efficiently as a practical Fab purification step.

L-118 Protein Adsorption on Chromalite PCG Resins: Effect of Porosity and Surface Area on Purity and Yield. Ksenia Sochilina1, Alessandra Basso2, Luca Froment2, 1Purolite, Moscow, RUSSIA; 2Purolite, Llantrisant, South Wales, UK
The adsorptive and chromatographic behavior of different proteins has been studied for PCG1200, PCG900 and PCG600 adsorbents. PCG adsorbents are a macroporous family of hydrophobic polydivinylbenzene materials of varying porosity and surface area. They can be used for preparative purification of biomolecules including proteins, and peptides varying in dimension and charge. PCG adsorbents functionalized with sulphonic or amino groups are ideal ion-exchange resins for interaction with charged proteins thanks to the optimal surface area. PCG1200 15 um and 50 um in their sulphonated form can find application in MAbs polishing step, whereas the PCG1200 aminated form is ideal for negatively charged proteins and peptides. The PCG1200 15 micron, in its neutral form, is a valid alternative to C18 silica for insulin purification. The smaller porosity of PCG600 is optimal for smaller molecules and exceptional resolution has been shown for purification of bacterial peptidoglycan fragment, used as immune enhancing drug obtained from bacterial cells and purified at the final stage from its alfa- and beta- anomers. The particular polymerization method confers to PCG extremely high mechanical robustness as shown by back pressures profiles.

L-119 Investigation of the Adsorption Phenomena on Cinchona Alkaloid-based Zwitterionic Chiral Stationary Phase. Attila Felinger1, Csaba Szmolnik1, Wolfgang Lindner2, 1University of Pecs, Pecs, HUNGARY; 2University of Vienna, Vienna, AUSTRIA
Cinchona alkaloid-based zwitterionic chiral stationary phases (ZWIX) show excellent selectivity for a number of chiral separations. In our study the zwitterionic chiral stationary phases was employed for the enantiomer separation of rac-erythro-mefloquine. The overloaded peak profiles have confirmed that there is sigificant difference between the adsorption and desorption phenomena of the two enantiomers. Whereas one enantiomer shows a biLangmuir behavior, which is commonly observed in chiral separations, the less retained enantiomer has only a moderate retention. The weak retention is caused a combination of repulsive and attractive interactions between the analyte and the chiral stationary phase. We describe the adsorption phenomena of both enantiomers through the experimental determination and modeling of their equilibrium adsorption isotherms. The overloading studies show that the band of the less retained enantiomer exhibits a rather steep front and rear, which is ideal for overloading and preparative purification. We will present the modeling and the optimization of the chiral separation.
New Retention Models and Interaction Mechanisms of Monovalent Solutes with Amylose Tris[(S)-alpha-methylbenzylcarbamate] Sorbent. Hung-Wei Tsui, Elias I. Franse, Nien-Hwa Linda Wang, Purdue University, West Lafayette, IN, USA

Amylose tris[(S)-alpha-methylbenzylcarbamate], or AS, is a sorbent often used for chromatographic chiral separations. A typical mobile phase is n-hexane containing isopropanol (IPA) as a polar modifier. The interaction mechanism between the solute, the modifier molecule, and the sorbent has attracted a lot of attention in the literature. Various stoichiometric displacement models in the literature have been widely used for understanding these competitive adsorption mechanisms of solutes and polar modifiers. The models were used to explain the often-observed linear log-log plots of the solute retention factor versus the concentration of the modifier in the mobile phase. The slope of the plot was sometimes inferred to be equal to the number of displaced modifier molecules upon adsorption of a solute molecule, or upon adsorption of the solute-modifier complex, or both, and was generally found to be greater than 1. In this study, five monovalent solutes, acetone, cyclo hexanone, benzaldehyde, phenylacetaldheyde, and hydrocinamaldehyde are chosen for a controlled study. Each solute has one C=O functional group, which can form one H-bond with the sorbent or the IPA. The observed slopes were found to range from 0.25 to 0.45. Slopes less than 1 cannot be explained by the literature displacement models. New results of Infrared Spectroscopy, combined with Density Functional Theory simulations, provide an indication that the small slopes are due not only to acetone-IPA complexation but also to IPA aggregation with an average aggregation number of n=3. A new retention model has been developed to take into account the observed IPA aggregation and the solute-IPA complexation. The model shows that the aggregation leads to a significant reduction of the IPA monomer concentration, which affects the IPA-sorbent adsorption and the IPA-solute complexation. A general analysis using dimensionless concentrations and equilibrium constants shows that aggregation of IPA can indeed lead to slopes below 1. The limiting slope at high IPA concentrations approaches the values of 2/n. The IPA aggregation number and the equilibrium constants for the various processes are estimated from the HPLC data. Moreover, they are further validated with dynamic chromatography simulations. The differences in the slopes between the five solutes are due to different equilibrium constants for the solute-IPA complexation. Hence, the solute-modifier complexation and the alcohol modifier aggregation in the mobile phase must be accounted in the retention models used for the interpretation of the retention factors.

Overcoming the Yield Limitation of Thermodynamically Constrained Bioreactions by Integration of Biocatalysis and SMB. Nina Wagner, Andreas Bosshart, Matthias Bechtold, Sven Panke, Bioprocess Laboratory ETH Zurich, Basel, SWITZERLAND

Biocatalysis has matured into an established technology in the field of fine chemistry due to its high selectivity, absence of side reactions and the ability to operate under ambient temperature and pH conditions. However, a considerable set of highly attractive enzyme reactions (e.g. C-C bond formations by aldolases or isomerizations) shows an unfavorable position of the thermodynamic equilibrium impairing their implementation on industrial scale [1]. The yield limitation can be overcome by a continuous in-situ product removal process using simulated moving bed (SMB) technology for the separation of substrate and product [2]. In the scope of this work we present an integrated process setup for the production of the rare sugar D-psicose which can be obtained directly by epimerization from readily available D-fructose employing the enzyme D-tagatose epimerase (DTE). Specifically, we demonstrate a number of milestones toward a fully optimized integrated process: (i) the proof of principle (ii) model-based characterization of all involved units (iii) optimization of the stand-alone SMB unit and (iv) model-based simulation of the integrated process. The principal feasibility of the process concept was demonstrated by a fully integrated process on lab-scale consisting of an 8-column SMB based on calcium-substituted ion exchange material, an enzyme membrane reactor (EMR) retaining DTE and a nanofiltration (NF) device for concentration of the fructose-rich SMB raffinate before recycling to the EMR. It was operated for several days and provided 97% yield [3]. Next, a detailed characterization of all involved unit operations was conducted by model-based experimental analysis. In case of the chromatographic process the inverse method was used to populate parameterizations of different chromatography models. Model discrimination was conducted by lack of fit analysis and by comparison with experimental SMB results. After identification of a suitable chromatography model a steady state TMB model was applied for numerical optimization of the SMB process. Again, the results were validated by SMB experiments. Finally, an integrated process model was developed that enables the calculation of process yield, purities and process costs under different scenarios allowing comparison with SMB stand-alone operation. [1] Bechtold, M., Panke, S., 2009, CHIMIA, 63(6): 345-348. [2] Bechtold, M., Makart, S., Panke S., 2006, J. Biotechnol, 124: 146-162. [3] Wagner, N., Fuereeder, M., Bechtold, M., 2012, OPBD, 16(2): 323-330.
In vaccine production downstream processing often constitutes a bottleneck in terms of productivity and economy. Established separation techniques either lack in selectivity (and resolution) like density gradient centrifugation or are limited in throughput and scalability as for batch chromatography (BC). One way to design more efficient purification trains could be the implementation of continuous chromatographic methods. In this study, continuous size exclusion chromatography (SEC) in cell culture-based influenza vaccine production was investigated using a simulated moving bed (SMB) approach. Experiments were performed in an open loop configuration at different operation conditions using one or two columns per zone. For some experiments, the product fraction was subsequently passed through an anion exchange column (Capto Q®, GE Healthcare) to reduce the level of remaining host cell DNA contaminations. The separation performance was evaluated for virus yield (hemagglutination assay) as well as for total protein (Bradford) and for host cell DNA (PicoGreen®) contamination. Finally, yield, purity and productivity of the SMB process were compared to BC operated under corresponding conditions. Similar yields of influenza virus were obtained for SMB and BC in the product fractions whereas purity in terms of the level of contaminating protein (related to the amount of virus) of the SMB process did not match the results obtained for BC. However, the calculated amount of total protein per vaccine dose met the criteria required for human influenza vaccines prepared in cell culture for both methods (< 100 µg per strain and dose). Further improvements would, however, increase process robustness with respect to batch to batch variations in upstream processing. Nevertheless, it could be shown that SMB surpasses BC in terms of productivity. The host cell DNA could not be removed via SEC since it co-eluted with the virus. The combination of SMB-SEC and Capto Q® chromatography, however, resulted in an overall DNA depletion of more than 99 %. However, DNA levels in the product fractions still exceeded the level required for production of influenza vaccines for human use (< 10 ng per dose). Nevertheless, a further depletion of DNA could be achieved by treatment with Benzonase®, a unit operation which is typically implemented in the process train for DNA fragment reduction and to reduce the risk of viral cross contaminations. Altogether, the results indicate that SMB-SEC in combination with subsequent DNA capturing by anion exchange chromatography is a promising method for the purification of cell culture-derived human influenza vaccines.

**Adsorption of Pegylated Proteins on Macroporous and Polymer Grafted Anion Exchangers.** Mimi Zhu, Giorgio Carta, University of Virginia, Charlottesville, VA, USA

This work compares protein adsorption and transport in UNOsphere Diol Q, which is a macroporous anion exchanger, and Nuvia Q, which has the same backbone matrix but with grafted polymeric surface extenders. Native BSA and PEGylated BSA were used as model proteins. Inverse size exclusion chromatography revealed an apparent pore diameter of about 100 nm for UNOsphere Diol Q and about 12 nm for Nuvia Q. The equilibrium protein binding capacities for UNOsphere Diol Q at pH 7 were only about 50 mg/ml resin for both native and PEGylated BSA, but were much larger (283 ± 7 mg/ml resin and 170 ± 5mg/ml resin for native and PEGylated BSA, respectively) on Nuvia Q, indicating that the grafted polymeric surface extenders provide more favorable binding. Despite the small apparent pore size, the rates of adsorption on Nuvia Q were also much larger than those on UNOsphere Diol Q. In fact, the effective pore diffusion, determined by fitting the pore diffusion model to batch uptake curves, were about 10 times larger for Nuvia Q than for UNOsphere Diol Q and less dependent on whether the protein was PEGylated or not. Confocal laser scanning microscopy (CLSM) conducted with individual fluorescently labeled BSA and PEGylated BSA showed sharp adsorption fronts within the UNOsphere Diol Q particles for both proteins, but smooth profiles in the Nuvia Q particles. Two component simultaneous adsorption CLSM experiments also showed sharp fronts in UNOsphere Diol Q with clear evidence of displacement of the more weakly bound PEGylated protein by the more strongly bound native BSA. Displacement was also evident for Nuvia Q, but only at the particle surface, with both proteins co-diffusing within the particles at similar rates. Comparing the rates of sequential adsorption, in experiments where the particles were first saturated with PEGylated BSA alone and then exposed to BSA showed rates and intraparticle concentration patterns consistent with a pore diffusion mechanism for UNOsphere Diol Q, but only very small rates for Nuvia Q indicating that counterdiffusion of the two protein forms is severely hindered in this resin. A rate model taking into account a single file diffusion mechanism is presented to describe the Nuvia Q behavior. Considerations on the relative merits of macroporous resins and resins containing grafted polymeric surface extenders are made with regards to chromatographic processes that can be used to resolve mixtures of native and PEGylated proteins with these resins.
L-124 A Novel Silica Surface Hybrid Material with Chemical Stability Over a Wide pH Range for Reversed Phase Preparative Chromatography. Fredrik Limé, Robert Fredriksson, Joakim Högblom, Kristina Hallman, AkzoNobel/Kromasil, Bohus, SWEDEN

Silica is widely used as a stationary phase matrix in liquid chromatography with superior mechanical stability and ensuring high efficiencies. However, the chemical stability for reversed phase silica materials usually is in the range of pH 2 to 8, limiting the number of applications. At low pH, the siloxane bond between the silane and silica matrix will break producing free silane and exposed silanols on the silica matrix, while high pH leads to dissolution of the silica matrix. In order to increase the chemical stability of the silica matrix, a bonding with an organosilane that penetrates the silica surface to create an interfacial gradient was performed maintaining the superior mechanical stability and pore structure. This hybrid silica material were further derivatized with n-octadecylsilane (C18) and end-capped to completely shield the silica matrix from silanol interactions. The new hybrid material has been predominantly developed for preparative scale chromatography, with selectivity and efficiency similar to classic C18 bonded phases. Loadability was high for small basic compounds at elevated pH, as well as for purification of peptides. The chemical stability of the material was evaluated using accelerated stability test at elevated temperatures, with different buffers known to hydrolyze silica stationary phases at pH above 10. The resistance to high pH under extreme alkaline conditions were proved by regenerations of the columns by cleaning-in-place procedures using 0.1 – 1.0 M NaOH followed by testing column performance with anti-depressants as analytes. Anti-depressants are highly affected by silanol interactions due to the loss of bonded phase. This will immediately show up in terms of increased retention times and peak asymmetry.

L-201 Open Access Prep HPLC Consolidation and Centralization at Novartis Cambridge. Joseph Twomey, David Dunstan, Novartis, Cambridge, MA, USA

Open Access Prep HPLC Consolidation and Centralization at Novartis Cambridge Historically Prep and Semi Prep HPLC at Novartis was under the care and control of chemistry and supported by the users themselves. This led to a rapid expansion in the number of systems purchased, issues with service and performance , resource drain from chemistry projects and platforms to support systems, increased costs of consumables, and inefficiencies in utilization. Over the last year the Separations team here in Cambridge has taken over all aspects of the Prep HPLC environment from chemistry. This includes system maintenance, user support, method development, troubleshooting, and consumables. By centralizing the systems we were able to reduce the number of systems by over 50% while increasing the throughput and productivity of the end users. This has also led to cost savings by standardizing all consumables used during the process as well as reducing the amount of solvents used and out in the laboratory environment. Two of the major cost savings were in Vendor service and column purchases, by bring both of these aspects into the control of the Separations team and supported by technicians both costs have been greatly reduced. An examination of how this was achieved will discussed during the presentation. The selection of the system vendor and configuration of the systems, the application of the general methods for everyday use, how the general methods are selected and scaled up will also be presented. The use of the systems is also based on the scale of the chemistry, we have several platforms in place that all run in Open Access mode and can support samples from as low as several mg to batches of over 25g all run in the OA environment. Finally the results and a discussion of what information the actual run data has shown will be presented in a real application cost per injection discussion.

L-202 Outsourcing Services in Drug Discovery: How to Build a Successful Partnership. Christina Kraml, Princeton University, Princeton, NJ, USA

Many, if not all, major pharmaceutical companies have outsourced, or are evaluating the benefits of outsourcing to a third-party service provider. Overall cost savings, access to a pool of competent trained personnel, continued access to state of the art technologies and timeliness are all reasons for the trend. The positive effects of outsourcing are enhanced if the provider compliments the existing internal competencies of the company. The cooperation model of ‘leased competence’ offers additional access to specialists that can support projects flexibly and quickly. This cooperation helps build up a high internal competence level and, if successful, creates and maintains a mutually beneficial relationship between the parties. The collaboration allows the sponsor to benefit from the provider’s flex capacity. The sponsor can chose a CRO to provide expertise for specific needs, which in turn, can add momentum to its drug discovery and R&D processes. The most successful sponsor-CRO partnerships are built on trust and effective collaboration while using good communication as the underlying tool. Projects which have intense needs and constrained timelines require a close coordination of efforts. This talk will illustrate how to get the most from such a partnership in one area of drug discovery: small molecule purifications.
Aggregated Singletons for Automated Purification (ASAP) Workflow. Bhagyashree Khunte, Pfizer Inc., Groton, CT, USA
Aggregated Singletons for Automated Purification (ASAP) is a purification workflow that provides singleton sample purification, registration, and delivery to Materials Management as 30mM DMSO solutions for biological screening. The singleton samples submitted are aggregated in a mini array of 10-12 samples and then analyzed using an automated purification process consisting of various steps. These steps include pre-QC, preparative chromatography, solvent evaporation, reformat dilution and duplication, final QC, and final registration. The turn-around time from samples received to delivery to Materials Management is 2-3 business days. The final QC data is uploaded in the Global Analytical Database and is available to the chemists via Chemistry e-Notebook. The final purity, weight recovery, and registration information is available in the Research Database and an email notification of completion is sent to the Chemist. ASAP supports projects across all therapeutic areas. Centralizing this activity in the purification group allows greater time for higher value tasks to be completed by practicing chemists; the purification scientists can provide expert level service and technology, and also more opportunity for harmonization with screening (consistent and high quality samples delivered for biological assays). ASAP enables high purification success rate and increases likelihood to run mini-arrays (generate 5-10 analogs in a final step rather than 1-2) with the same 2-3 day turn-around time. Even though the samples are purified in a high throughput purification mode, the versatility in technology allows purification of diverse molecules in regards with the compound solubility, polarity, and molecular weight.

Leveraging Partnerships to Build a Centralized Purification Service. James Paulson, Bristol-Myers Squibb, Wallingford, CT, USA
A centralized service for purifying medicinal chemistry compounds has been built over the last five years at Bristol-Myers Squibb [1]. The story of how the service grew from a pilot study with a handful of customers to a full-scale, multi-site business serving hundreds of customers is a story of successful partnerships between the purification team and several internal and external organizations, including customers, technology groups, and an external analytical service provider. This presentation will describe how we leveraged the benefits of these partnerships to build a customer-focused, cost-effective process for purifying small molecules. It will also highlight some of the technical challenges we encountered as the service was built and how we overcame them. [1] H. N. Weller, D. S. Nirschl, J. L. Paulson, S. L. Hoffman, and W. H. Bullock. “Addressing the Medicinal Chemistry Bottleneck: A Lean Approach to Centralized Purification.” ACS Comb. Sci. 14 (2012): 520–526.

Using SFC to Drive Productivity and Increase Workflow Efficiency for Small-scale Purification Laboratories. Christine Aurigemma, Pfizer Inc., San Diego, CA, USA
With many pharmaceutical companies under pressure to cut R&D costs, efforts to outsource purification support and reduce headcount have created a need for increased efficiency and utilization of chromatography for small scale purification. Given the inherent advantages of speed and solvent usage, it’s only natural that SFC supplant HPLC as an impact technology for internal chemistry support. Demonstration of the ways SFC reliance in our day-to-day analytical and purification activities has driven productivity gains and increased workflow efficiency in support of medicinal chemistry workgroups will be presented. Other examples of applications that have contributed to the mainstream use of SFC, such as plate-based purification, achiral/chiral purification, and the implementation of a walk-up analytical SFC/MS screening system with automated method selection software will be discussed.

The Amazing Ability of Continuous Chromatography to Adapt to a Moving Environment. Roger-Marc Nicoud, Consultant, founder of Novasep, Lay-Saint-Christophe, FRANCE
During the 20th century, chemical engineering transformed batch separation processes into continuous processes. Liquid-liquid extraction, gas-liquid adsorption, distillation, reactions are relevant examples. Chromatography, due to intrinsic complexities, resisted longer but industry needs pushed UOP to invent the very clever concept of SMB. Not only chromatography became continuous but it also took benefit from a counter-current contact between liquid and solid phases. That is a first adaptation to the environment. In the late 80’s I contributed to adapt the SMB concept for offering efficient purification processes for pharmaceutical intermediates or actives. When I look at the picture of the first machine exhibited at Achema in 1992, I feel like contemplating a Dinosaur and don’t feel like living in the same geological era! The adaptation to pharmaceuticals required systems to be less specific (who knows what is going to be produced tomorrow?) so that more versatile pilot plants had to be designed. The visible part of the iceberg is the change from rotary valves to dual valves, the move from 24 columns to 12 then about 6 columns. The expensive nature of some specific adsorbent as well as their availability at small particle size pushed towards columns with very small length over diameter ratio. This challenging design required significant development in column technology but also systems design improvement for compensating the effect of dead volumes. This is certainly the third generation. Many contributions aimed at modifying the SMB scheme in order to maximize the productivity
obtained from a limited set of columns have then been proposed. Strange animals named Powerfeed, ISMB, SSMB, Modicon, VariCol and many others came to earth. The fourth generation is thus abundant, even if some animals are unlikely to have progeny. In the recent years, two specific needs emerged: • Separations of very complex mixtures containing very similar products (cyclosporine, paclitaxel, omega3) leading to systems with multiple entries and outlets or to associate several SMB in series in an optimum manner. • Separations of two very different categories of species associated with a large selectivity (ion versus non ion separation, affinity chromatography) leading to systems with open loops, able to deal with different solvent compositions, possibly in a gradient mode. Continuous chromatography development imposed nonlinear modeling of chromatography as a critical development tool. In return, simulation tools allow to continuously reaching the limits of the technology so to improve it.

L-207 MCC in-the-field: Return of Experience. Veronique Pinilla, UCB Pharma, Braine l’Alleud, BELGIUM
Multicolumn continuous chromatography is now a widely spread out technology in pharma and biopharma industry. Beside sugar and petroleum derivatives separations, quite a few MCC units are running all year long, to produce API for patient. This presentation will summarize UCB experience in MCC through 15 years of use. From the lab unit up to production scale, goals are clearly different and will be detailed to understand why this technology can fit in a research environment as well as in a manufacturing process. Constraints, regulations, objectives might change, equipment can be design for purpose to achieve those goals.

L-208 Case Studies of the Use and Utility of Small Molecule Preparative Chromatography in Early Phase Clinical Development. Olav Lyngberg, Benjamin Hritzko, Rajendra Deshpande, Bristol-Myers Squibb, New Brunswick, NJ, USA
The utility of preparative chromatography in late pre-clinical and early clinical development is substantial and likely to increase in the future. The largest drivers for additional use are increased pressures on route development resources and timelines and improvements in chromatographic efficiency. Critical aspects afforded by chromatography at this stage of route development is assurance of delivery, favorable timelines and reduction in synthesis resource spend. The typical chromatographic separation for an IND toxicology or Phase I delivery ranges from tens of grams to tens of kilograms. The development time available for a separation is rarely on the critical path due to the relative speed at which the majority of the separations can be developed. However limited molecular property information at the early stage can lead to non-ideal choices for the placement of the separation in the synthesis and low or modest productivity for the overall separation. Avoiding such pitfalls and ensuring productivities above approximately 0.5 kg/kg/day is important to execution success within the required time frame. Full automation of chromatographic equipment at the 2-15 cm column ID sizes have enabled 24 h operation with reduced staffing, but solvent handling issues are often a limitation for the overall productivity when running in multipurpose facilities. For SFC these handling issues are generally less pronounced but can still exist. The most common separation is a binary separation such as those of enantiomers, diastereomers, or atropisomers on a chiral or normal phase media. The choice between batch or continuous chromatography most often comes down to total batch requirements and the associated execution time and for separations with expensive stationary phases the reduction in expense for solid media. The presentation will focus on several case studies highlighting aspects of the above mentioned drivers as well as lessons learned on strategic placement of chromatography to drive overall route development while funding ongoing clinical programs with API.

L-209 Continuous Matrix-assisted Refolding SMB of Self Cleaving Fusion Proteins with Complete Buffer Recycling. Martin Wellhoefer1, Wolfgang Sprinzl1, Rainer Hahn2, Alois Jungbauer2, 1ACIB, Vienna, AUSTRIA; 2University of Natural Resources and Life Sciences Vienna, Vienna, AUSTRIA
Continuous processing with buffer recycling can be used in downstream processing to increase productivity and reduce buffer consumption. In this work, we present a proof of concept of a continuous four-stage process where recombinant proteins expressed as Npro fusion protein from inclusion bodies (IBs) are refolded by matrix-assisted refolding (MAR) and subsequently purified while the buffers used are continuously recycled. In the first stage of this process, NaOH is used to dissolve IBs continuously. The use of NaOH for dissolution instead of chaotropes such as urea or guanidine hydrochloride allows isocratic operation and recycling of refolding buffer by tangential flow filtration (TFF). This led to a significant reduction of the buffer consumption. In the second stage, matrix-assisted refolding SMB size exclusion chromatography (MAR-SMB-SEC) was carried out to refold the dissolved proteins. A significantly increased refolding yield was achieved by MAR-SMB-SEC compared to classical batch refolding which was related to the separation of aggregated proteins generated during refolding from partly folded and native proteins. In the third stage, refolded and partly folded proteins were led to an off-column refolding tank where the refolding reaction was finished. In the fourth and last stage of the process, a second SMB-SEC unit was used to separate the target protein in the refolding solution from high molecular impurities, e.g. large proteins. We determined for each SMB operation individually important process parameters.
such as refolding and cleavage yield and kinetics, purity, recovery, productivity and buffer consumption. Furthermore, a comparison between classical batch refolding and MAR-SMB-SEC using an Npro fusion protein as model protein was drawn. It was demonstrated that the refolding and cleavage yield of this model protein could be increased from 60% with classical batch refolding to 76% by MAR-SMB-SEC. Moreover, the model protein was entirely recovered from the system at high purity. In summary, it can be stated that the higher complexity of the system is rewarded by a significant buffer reduction exceeding 90% and higher refolding yield.

L-210 Fundamentals of QbD Applied to Downstream Processing. Guillermo Miro-Quesada, David Robbins, Gisela Ferreira, MedImmune, Gaithersburg, MD, USA
Quality by Design is a development framework that results in well understood and controlled processes, consistently delivering product of suitable quality. Risk assessment tools are used to systematically prioritize experimentation in order to generate the most useful knowledge for each unit operation. The work presented describes how scientific knowledge can be merged with different strategies on the experimental plan suggested by risk assessments for downstream unit operations, without compromising information and minimizing resources. The case study presented here includes approaches for multiple unit operations used for one antibody purification process.

Quality packing methods for large scale chromatography columns are a critical component of successful manufacturing. This case study pertains to the development of a packing procedure for a large scale column that utilizes a compressible resin over multiple cycles. The columns were packed using axial compression. A design of experiments approach was followed using smaller diameter columns to investigate the impact of several key packing parameters on performance, including different levels of compression, buffers of varying ionic strength and linear velocities. The results were subsequently evaluated using a larger diameter column. Comparisons are presented to elucidate the impact of input factors on the efficiency, asymmetry, and permeability of the packed beds. The results were correlated using the Van Deemter and Darcy models for efficiency and permeability, respectively.

L-212 QbD Strategies for Managing Chromatography Resin Lot-to-lot Variability based on Design of Experiments and Monte Carlo Simulation. Karol Lacki, Mattias Ahnfelt, Tryggve Bergander, Gunnar Malmquist, GE Healthcare Life Sciences, Uppsala, SWEDEN
Quality by Design highlights the need for better process understanding, both regarding Critical Process Parameters and Critical Raw Material Attributes (CMA’s). Chromatographic media contain several potential CMA’s, and GE Healthcare is considering a multifaceted raw material framework that focuses on identification of CMA’s through dedicated studies and intensified supplier – end user collaboration efforts. The framework will be illustrated by examples describing process characterization studies performed using high-throughput process development tools and chromatography medium batches with varying attributes within respective specification ranges. The underlying principle behind this approach should be contrasted to typical resin screening where different resins are compared, as in the proposed framework all attributes reflect the normal manufacturing variation within one resin product. Special emphasis will be placed on ligand density, one of the top CMA candidates as it can affect chromatographic selectivity. The framework employs HTPD data, Design of Experiments and Monte Carlo simulation to establish the effect of chromatography medium attributes and selected process parameters on CQA’s. The evaluation includes process parameter adaptation by looking for parameter interactions that allow an adaptive control strategy where the effect of variations in chromatography medium attributes can be counteracted by changing process parameters. The proposed approach outperforms conventional testing of a few resin lots from normal production by explicitly investigating a potential CMA. When established, the adaptive process control strategy maintains the process within the defined operating window, yet minimizes effects related to chromatography medium lot to lot variability and/or eventually leading to a non-critical assessment of the medium from the raw material classification perspective. The proposed adaptive control strategy will result in a more robust purification step and increased security of supply, and in turn better process economy.
L-213  Design Space for a Countercurrent Chromatography Protein Purification Unit. Fabian Steinebach¹, Martin Krättli², Thomas Müller-Späth², Massimo Morbidelli¹, ¹ETH Zurich, Zurich, SWITZERLAND; ²ChromaCon AG, Zurich, SWITZERLAND

For all chromatography processes the operating parameters and the physico-chemical properties of the compounds to be separated as well as column characteristics affect the separation in terms of yield and product quality (purity). As described in the PAT initiative and ICH guidelines, process understanding is necessary to define operating parameter ranges ensuring product quality and process robustness. Process understanding can be demonstrated by developing and validating a mathematical chromatography model that includes the relevant operating parameters. As shown in the case of countercurrent column chromatography (SMB (simulated moving bed) process), equilibrium theory can be used to determine regions for operating parameters where the separation task is accomplished. This presentation shows how this concept was transferred from SMB to twin-column MCSGP (multi-column countercurrent solvent gradient purification). MCSGP is a process that is capable of addressing the needs of biochromatography such as ternary separations and linear gradients. In order to account for the linear modifier gradients the trajectories were solved dependent on the modifier concentration. Moreover, the Henry constant was assumed to be a power function of the modifier concentration. Results for elution volumes were combined with mathematically formulated constraints under which a theoretical yield and purity of 100% with respect to the desired bioproduct are achieved. The results are summarized in a 3-dimensional design space of operating parameters. Examples for process operation within subregions of this space are presented and discussed. With the developed tool the robustness of the process can be investigated and the influence of different parameters can be estimated. Eventually, the understanding how process variations influence quality and robustness also supports the optimization and control of the twin-MCSGP process.


Simulated moving bed (SMB) chromatography is an attractive continuous separation process to separate binary or pseudo-binary mixtures with high productivity and low solvent consumption compared with a batch chromatography. First suitable operating conditions can be obtained using the short-cut design method, e.g. the Triangle method based on the equilibrium theory [1]. Process simulations with rigorous models and optimization techniques are required to obtain optimized operating conditions. An advanced technique to optimize the operating conditions of SMB chromatography is the dynamic optimization with optimal control concept. In this work, four optimization objectives (the purity and yield of the target component, and the average concentrations of the target and waste components at the outlet of the zone 4) were defined to find the optimized zone flow-ratios for each zone. To obtain the average concentrations of the target and waste components at the outlet of the zone 4, one more port named as the vent was added in between the outlet of the zone 4 and the desorbent port. From the process information of two cycles, the steady-state of SMB was predicted by a 1st order time invariant model. Then this predicted information was used to generate the feedback signal to control the SMB process. The separation of three isomeric reaction products from the nucleophilic aromatic substitution (SNAr) reaction of 2, 4-difluoronitrobenzene with morpholine was chosen as the model system [2]. Since the target component was retained longer than the others, the dynamic optimization was applied to optimize a pseudo-binary separation with conventional 4-zone SMB chromatography. [1] G. Storti, M. Mazzotti, M. Morbidelli, S. Carra, “Robust design of binary countercurrent adsorption separation processes”, AIChE J., 39 (1993) 471-492. [2] A. G. O’Brien, Z. Horvath, F. Levesque, J. W. Lee, A. Seidel-Morgenstern, P. H. Seeberger, “Continuous synthesis and purification by direct coupling of a flow reactor with simulated moving-bed chromatography”, Angew. Chem. Int. Ed., 51 (2012) 7028-7030.

L-215 Systematic Modeling and Start-up Acceleration of Simulated Moving Bed Chromatography by Model-based Optimization. Jason Bentley¹, Suzhou Li², Yoshiaki Kawajiri¹, ¹Georgia Institute of Technology, Atlanta, GA, USA; ²Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, GERMANY

A simulated moving bed (SMB) process exploits multiple chromatographic columns connected in series where the inlet/outlet port switching occurs between columns to simulate the counter-current flow of the stationary phase. SMB technology mimics a true counter-current operation without having to convey the solid phase. During the last two decades SMB has received increasing attention in the chemical industry, especially for sugar, petrochemical, and chiral separations. The products from SMBs may need to be discarded when the product concentrations are too low for solvent removal. The time to reach the CSS condition from a clean bed can be long, which lowers the overall process productivity. Furthermore, in some application where only a small volume of feed is processed by SMB, there may even not be enough time for the SMB unit to reach the CSS condition before shutdown begins. The recent work by Li et al. [1] presents systematic start-up and shutdown strategies for SMB by nonlinear programming. In that work the potential for significant start-up and shutdown
acceleration is shown by optimal transient control strategies. With the new transient operation strategies, the overall processing time can be shortened and average product concentrations can be increased by switching the SMB operating conditions over time. Nevertheless, this approach was not validated experimentally. In this work, we consider experimental validation of the approach proposed by Li et al. [1]. The optimal start-up operation is calculated and implemented in a lab-scale SMB unit. The SMB model is determined systematically using the recently developed prediction-correction (PC) method, which minimizes the model mismatch in transient dynamics [2]. Using the resulting mathematical model, the optimal start-up operation is obtained successfully and validated in our experimental SMB system for the case study of a linear isotherm. The results show that the start-up time can be reduced by about 44% by switching the SMB operating conditions only three times. [1] Li, S., Kawajiri, Y., Raisch, J., and Seidel-Morgenstern, A. (2011). Optimization of startup and shutdown operation of simulated moving bed chromatographic processes. Journal of chromatography. A, 1218(25), 3876–3889. [2] Bentley, J., Sloan, C., and Kawajiri, Y. Simultaneous modeling and optimization of nonlinear simulated moving bed chromatography by the prediction-correction method. Journal of Chromatography A, in press.

L-216 Analysis of Dynamic Phenomena in Liquid Chromatography Systems with Reactions in the Mobile Phase. Lei Ling, Nien-Hwa Linda Wang, Chemical Engineering, Purdue University, West Lafayette, IN, USA

Reactions in liquid chromatography systems have many important applications. One can estimate the reaction rate constants for irreversible reactions, or the reaction stoichiometry and equilibrium constants for reversible reactions. For large-scale production using a reversible reaction, if the products are well separated from each other in a chromatography column, one can obtain purified products and achieve a higher conversion than the equilibrium conversion in a batch reactor. Additives (ligands or complexants) can be added to the mobile phase to enhance solute separation if a sorbent does not have sufficient selectivity for the solutes. Proteins and polymers can aggregate in chromatography systems and result in either multiple peaks or merged broad peaks in elution chromatography or multiple breakthroughs and plateaus in capture chromatography. The number of peaks, peak retention times, and the relative peak areas in elution and the number of plateaus in capture may depend on sample concentration, sample size, temperature, linear velocity, and column length. In this study, the adsorption/separation dynamic phenomena of six typical reactions are analyzed using dimensionless groups and rate model simulations. The results provide overall guidelines for understanding, design, and optimization of chromatography systems with reactions. One can design experiments to find out whether reactions occur, whether the reactions are reversible or irreversible, and whether the reactions are first order or higher order. One can also determine the rate constants of an irreversible reaction from the peak areas as a function of the residence time. One can find the equilibrium constants and reaction stoichiometry from the peak ratios at different sample concentrations. For large scale production using a chromatography reactor, a reversible reaction can reach 100% conversion if the reaction rate is relatively large compared to the convection rate, and if the loading pulse size is sufficiently small to ensure the separation of the product bands. High purity products can be obtained if the diffusion rate is large and the axial dispersion rate is small relative to the convection rate. For reversibly aggregating systems, if the reactions reach equilibrium, one can separate the various aggregates by increasing the convection rate relative to the reaction rates. This separation can be achieved by increasing the sample concentration or the linear velocity of the mobile phase, or by reducing the temperature or the column length. One can merge multiple plateaus and increase the dynamic binding capacity in capture chromatography by increasing the reaction rate relative to the convection rate. For ligand-assisted separation, if the ligand concentration is sufficiently large and if the complexed solutes do not adsorb; then the overall selectivity is the ratio of the sorbent selectivity to the ligand selectivity, leading to a separation even if the sorbent has no selectivity. The sorbent selectivity should be opposite to that of the complexant selectivity to have synergistic effects on separation.

L-217 Peptide Ligands with High Binding Affinity and Biochemical Stability for Antibody Purification from Animal Serum. Stefano Menegatti, Benjamin Bobay, Amith Naik, Ruben Carbonell, North Carolina State University, Raleigh, NC, USA

Animal serum is an important source of proteins for therapeutic, diagnostic, and research applications. Polyclonal intravenous immunoglobulin G (IVIG), in particular, finds increasing use against autoimmune and inflammatory problems and is currently the major plasma product on the global blood product market. The purification of polyclonal antibodies from treated serum is commonly performed with Protein A / G affinity resins. These adsorbents, however, suffer from issues of high cost, harsh elution conditions, and release of immunogenic fragments in the product stream. Protein ligands, in fact, exhibit limited chemical stability against the chemical agents used for column cleaning and sanitization. Another major risk is represented by the presence in serum of proteolytic enzymes, mainly trypsin and a-chymotrypsin. These endoproteases degrade protein ligands, thus further increasing the immunogenic risk and lowering the adsorbent's lifetime. To prevent enzymatic degradation, costly enzyme inhibitors are usually added to the feed mixture. To address the problem
at its root, we propose the use of synthetic peptide ligands constructed with non-natural amino acids, which possess at the same time high target affinity and biochemical resistance. A virtual library of peptide variants was constructed by replacing aromatic and basic amino acids with corresponding non-natural residues on the IgG-binding peptides HWRGWV, HYFKFD, and HFRRHL discovered in the past by our groups. The library was screened by running molecular docking simulations of the peptide variants against the known interaction site on IgG. A pool of selected sequences were synthesized on chromatographic resins and the resulting adsorbents were tested for IgG binding and resistance to enzymatic degradation. The peptide ligands exhibited high target affinity and specificity along with high resistance to both trypsin and a-chymotrypsin. The adsorbents were used to efficiently recover IgG from animal plasma and IgG-rich fractions of human plasma, with yield and purity up to 84 % and 93 %. The strategy herein presented is very general and amenable to modulate the binding affinity and the biochemical stability of any synthetic as well as protein-derived peptide ligands meant for the purification of high-value therapeutics from animal sera.

Therapeutic antibodies are mainly purified by established platform processes including a Protein A affinity capture step. Although this protein ligand is accepted as the current gold standard for antibody purification it also has some severe limitations like high costs, low chemical stability and leaching. Consequently, there is a demand for alternative chromatography material for the purification of mAbs. Through a unique SPR screening platform we have identified and developed small molecule affinity ligands for cost efficient affinity purification of antibodies. For the identification of the binders, several humanized therapeutic antibodies were screened against a unique and diverse library of > 116k compounds immobilized on gold chips via linkers. A tissue culture supernatant from CHO cells served as a control to identify unspecific binders. Observed antibody specific hits were then coupled to NHS-Sepharose 4 FF for chromatographic evaluation. Generic binding to the Fc region of IgG1, IgG2 and IgG4 antibodies could be demonstrated. Best ligands were further characterized by high selectivity, high capacity and fast binding kinetics comparable to Protein A. Due to the extraordinary high chemical stability and low cost of the developed ligands they might represent an attractive Protein A replacement.

L-219 Dual Salts in Mixed-mode Chromatography and its Benefit for MAb Purification. Judtih Vajda1, Eva Bahret2, Egbert Müller1, 1Tosoh Bioscience GmbH, Stuttgart, GERMANY; 2University of Furtwangen, Villingen-Schwenningen, GERMANY
Mixed-mode applications have recently gained popularity, since modern mixed-mode resins seem to possibly satisfy the ever growing demand for purity and productivity in downstream processing and the applied chromatographic steps. In the ideal case, those resins combine high capacity and recovery, which are traditionally related to an ion exchange resin, with the potential separation power of a hydrophobic interaction chromatography resin. In this study, we investigated the impact of dual salts on various mAb applications, including mAb aggregate removal, F(ab)2 fragment purification and the purification of a scFv. Similar to HIC applications, the choice of the applied salts showed significant impact on the dynamic binding capacity for constant conductivity and pH. While a combination of citrate, phosphate and sodium chloride achieved a dynamic binding capacity of 58 mg/ml, a sodium acetate buffer containing sodium chloride led to a dynamic binding capacity of 74 mg/ml for mAb on the hydrophobic cation exchange Toyopearl MX-Trp-650M. Further, it was observed that the resolution can be modulated for different salt types relevant for production scale.

L-220 Compression Factor as a Critical Process Parameter of Design Space in Column Packing. Kimberly Brisack, Christopher Foster, Bio-Rad Laboratories, Hercules, CA, USA
A methacrylate-based chromatography resin used in downstream purification of biotherapeutics was packed using a range of compression factors and subsequently subjected to process cycling where the pH changes caused variances in bead volume of approximately 15%. Bed stability was evaluated via asymmetry testing and visual observation. Scalability of column size was addressed, as several different lots of media were packed into column sizes ranging from 1.1 cm diameter to 80 cm diameter. Chemical compression and mechanical compression were assessed to determine optimal packing conditions. Packed chromatography columns are an essential component of downstream processing. Due to differences in chromatography media attributes and column hardware, no universal procedure exists for packing process scale columns. In order to ensure packing reproducibility, the proper compression factor must be applied. The consequences of imprecise compression include bed instability and elution profile variability. Best practices for column packing at process scale will also be discussed.
L-221 Thermodynamics of Small Molecule Adsorption onto Multimodal Resin. Rebecca Desch¹, Kartik Srinivasan², Sophie Karkov², Melissa Holstein², Steven Cramer², Stephen Thiel¹. ¹School of Energy Environmental Biological and Medical Engineering University of Cincinnati, Cincinnati, OH, USA; ²Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY, USA

By combining multiple modes of separation into one unit operation, multimodal resins can improve biomolecule separations. While the efficiency enhancements of multimodal resins are known, the underlying mechanisms of biomolecule adsorption onto these resins are elusive. One popular resin, Capto MMC, contains both hydrophobic (phenyl) and polar (carboxyl, amide) functionalities on an agarose matrix. Three small molecules were chosen to probe the functionality of Capto MMC: tryptophan, sodium caprylate, and diphasphonate chloroquine. Previously, the adsorption of tryptophan has been associated with nonpolar interactions and the adsorption of sodium caprylate has been associated with electrostatic interactions with Capto MMC. Diphosphate chloroquine has been observed to selectively displace proteins from a multimodal resin. The real-time dynamic heats of adsorption of these materials onto a bed packed with Capto MMC were measured quantitatively using flow microcalorimetry. Tryptophan adsorption on Capto MMC in 0.02 M acetate buffer pH 5 was exothermic while the adsorption of sodium caprylate and chloroquine diphasphonate were endothermic. The adsorption of tryptophan and sodium caprylate were associated with sharp peaks in the thermogram, while the diphasphonate chloroquine peak was diffuse, indicating kinetic limitations. Hydrophobic interactions were isolated by attenuating electrostatics with concentrated salt. Increasing the salt concentration from 0.02 M to 0.75 M had no effect on tryptophan adsorption but attenuated the sodium caprylate endotherm. The free energy of adsorption was obtained from isotherm measurements and combined with the measured adsorption enthalpy to develop a thermodynamic summary of the adsorption with mechanistic insights. Calculated entropy values can be confirmed by varying the temperature (22°C and 35°C). By probing the thermodynamic properties of small molecule adsorption onto multimodal resins, valuable mechanistic insights for process design are uncovered.

L-222 Mechanisms of Protein Sorption and Transport in a Salt-Tolerant Cellulosic Ion-Exchanger. James Angelo, Abraham Lenhoff, University of Delaware, Newark, DE, USA

Cellulosic ion-exchange materials have recently been developed that allow high loading capacity and rapid uptake of proteins in addition to displaying a low sensitivity to changes in total ionic strength (TIS) of the solution. Cellulosic media lacking this enhanced functionalization are significantly more sensitive to even slight increases in the TIS of solution, by comparison. The structural and functional characteristics of two cellulosic anion exchange resins were investigated: Q HyperCel and a salt-tolerant derivative, STAR AX HyperCel (Pall Corporation). The salt-tolerant AEX material displayed higher adsorptive capacities than the standard resin at elevated TIS and similarly presented rapid uptake of protein measured via batch kinetics. Confocal microscopy was used to gain a physical understanding of the protein uptake profiles to relate to macroscopic batch experiments. Time-series confocal imaging of particle cross-sections revealed diffuse uptake profiles in both materials, indicative of homogeneous diffusion. However, the materials differed in their elution behavior, which warrants further investigation in order to optimize purification pool volumes. Structural characterization of both materials was performed using inverse size exclusion chromatography (ISEC) in order to determine relative pore sizes and to quantify the accessible pore volume. Analysis determined the apparent pore radii to be close to those of dextran-modified adsorbents, suggesting that the materials share some structural similarities. Additional mechanistic insight was sought using electron microscopy to allow for visualization of the microporous architecture and protein localization within the materials.

L-223 Multi-component Isotherm Modeling with the Gillespie Stochastic Algorithm. John Michael¹, Jillian Epstein¹, F Marques², A.C. Dias-Cabral², Marvin Thrash³. ¹Miami University, Oxford, OH, USA; ²University of Beira Interior, Covilhã, PORTUGAL; ³Central State University, Wilberforce, OH, USA

This work focuses on the development of a mathematical model that describes the binding equilibrium of large biological macromolecules. In prior work we have shown the Gillespie Stochastic Algorithm as a reasonable method to simulate single component isotherms provided the ratio between the forward and reverse adsorption/desorption constants are known. We have used this method to model protein adsorption on ion-exchange adsorbents and hydrophobic interactive adsorbents. In our latest efforts we have used the Gillespie Stochastic Algorithm to simulate binary and ternary isotherms from the literature involving nitrogen, methane and carbon dioxide at three different temperatures. In each case our simulated results showed good agreement with the experimental results. Moreover we have modeled competitive isotherms between two different heavy metals and we anticipate having simulation data for competitive protein adsorption. The overall goal of our work is to develop a robust modeling approach that is capable of simulating single and multicomponent isotherms for biological molecules and inorganic molecules interacting with a variety of adsorbents. References: 1. Miami University, Department of Chemical and Paper Engineering, Oxford Ohio 45056. 2. CICS-UBI – Health Sciences Research Centre and Department of Chemistry, University of Beira Interior, 6200 – 001 Covilhã, Portugal. 3. Central State University, Department of Water Resources and Environmental Engineering. Acknowledgements:
L-224 **Printed Chromatography Media.** Conan Fee, Simone Dimartino, Suhas Nawada, University of Canterbury, Christchurch, NEW ZEALAND

Conventional chromatography media suffer from a number of limitations, including packing defects caused by random or improper packing and constraints on the individual particle geometries that are able to be packed effectively. Chromatography media in the form of beads usually have a distribution in size or, if not, are extremely expensive. Other media particles such as crushed glass or resins, pack with poor flow properties. Monolithic media, likewise, suffer a number of limitations, one being difficulty in casting large-scale columns because of non-uniformities that arise during in situ polymerisation caused by the rate of removal of heat of reaction. Monoliths also suffer from random packing effects. Many of the above limitations cause axial dispersion or poor flow properties such as high pressure drops and flow channelling. Three-dimensional (3D) printing is a new approach to solid synthesis, which is capable of creating media with exquisite control of packing geometry. For example, perfect alignment of identically dimensioned spheres into close- or dense-packing or a variety of regular packing arrangements; controlled sphere dimensions with each sphere placed into a desired position within a lattice; cubes that are aligned to touch at their corners; fibres aligned axially. Indeed, by 3D printing, within the constraints of printed resolution, one could produce an exact physical replicate of any computer model, including both perfect and deliberately imperfect geometries. This would allow one to test experimentally a packing geometry modelled by computational fluid dynamics e.g. a close-packed column of identical spheres with a single "imperfection" such as a flow channel. One could also print the separation media within the containing vessel, with all flow connectors in place and create a wide variety of geometries not currently available by conventional media synthesis or packing methods. In this paper, we show, for the first time, the versatility of this approach to column design through examples of flow through packed media designed in silica, then printed and tested experimentally.

L-225 **Protein Processing Figures of Merit and Novel Ligand Chemistries Employing Capillary-channeled Polymer (C-CP) Fiber Stationary Phases.** R. Kenneth Marcus, Zhengxin Wang, Abby Schadock-Hewitt, David Jeffcoat, Clemson University, Clemson, SC, USA

Capillary-channeled polymer (C-CP) fiber stationary phases have demonstrated a number of very positive characteristics relevant to downstream processing of proteins. In physical terms, fibers packed into column structures provide very high permeability, while also promoting very efficient mass transfer to/from the fiber surface. The extruded polymer fibers have very low porosities ($r_p = \sim 2$ nm) as determined by inverse size exclusion chromatography (iSEC) measurements. In practical terms, proteins/polypeptides having molecular weights of $>10,000$ Da experience virtually no van Deemen C-term broadening. As such, analytical-scale separations can be performed at linear velocities of up to $100$ mm sec$^{-1}$ without sacrifice of chromatographic efficiency. Current studies are aimed at discerning differences between the optimum fiber packing and flow velocities for analytical and preparative applications. To this end, dynamic binding capacities and process throughput and yield characteristics will be presented for nylon 6 fibers operating in an ion exchange mode. The melt-extrusion of C-CP fibers from simple thermopolymers (polypropylene, polyester, and nylon) means that the primary materials costs are very low. A more salient feature is the fact that these base polymers present very different surface chemistries, both in terms of their native states as well as the acceptance of surface modifications. As suggested above, nylon 6 is an excellent surface for weak anion/cation exchange separations. The surface is easily modified using simple triazine chemistries under ambient conditions. Polyester provides for a more hydrophobic surface having aromatic character, which can also be used for weak cation exchange. Finally, polypropylene provides for solely hydrophobic interactions between solutes and the fiber surface. This interaction allows for very robust affixing of capture ligands through adsorption. As such, chemistries can be affected by simply passing the ligands through the assembled column. This concept, and practical figures of merit, will be demonstrated through the common approach of the adsorption of protein A to the fibers for the capture of IgG as well as for an extremely novel, yet powerful use of head-modified PEG-lipid ligands. These commercially available phospholipids can have a wide variety of head groups, including amines and carboxylic acids, succinyl/thionyl groups, polydentate metal ligands, and high-selectivity agents such as biotin. Demonstrations of the latter strategy will be supplemented with fundamental measurements of surface loading and ligand robustness using a FITC-labeled PEG-lipid. It is believed that the physical and chemical attributes of C-CP fiber columns can have substantial impact in the realm of preparative protein separations.
L-226 **New Highly Selective Mab Polishing Tools.** Bengt Westerlund, Kristina Nilsson-Välimaa, Anna Grönberg, Lena Kärff, Anders Ljunglöf, Anna Heijbel, GE Healthcare, Upplands, SWEDEN

Multimodal chromatography has proven to be a powerful tool for solving difficult separation challenges, one of them being aggregate removal while maintaining high yields. To address challenging separations, two new multimodal chromatography polishing media were developed. The performance of a multimodal anion exchanger in polishing is exemplified for two mabs run in bind/elute and flow through modes, respectively. High throughput process design methodologies were applied to set the running conditions which were verified in small columns. A multimodal cation exchanger with optimized design for Mab purification was screened in 96-well format for optimal conditions and the dataset was used to build a DoE model. The model was then used to predict suitable conditions for the sought purification performance and these conditions were validated in lab scale columns with satisfactory correspondence to the model prediction. The capability of these new multimodal chromatography media to separate Mab charged isoforms is also discussed.

L-301 **Cold Ethanol Precipitation and Flocculation and Polishing by Monoliths, a Process for Next Generation Antibody Production.** Anne Tschelesneg¹, Peter Satzer¹, Nikolaus Hammerschmidt¹, Henk Schulz², Bernhard Heik², Alois Jungbauer¹, ¹BOKU, Vienna, AUSTRIA; ²Novartis, Basel, SWITZERLAND

Antibody production based on chromatographic technologies has reached limits in terms of productivity and scalability. The intensification of antibody downstream processing by replacing chromatography capture steps with precipitation and flocculation technologies meeting the purity requirements for therapeutic antibody was aim of this work. In light of further progress in upstream processing, especially with respect to antibody titer, we have developed a completely new concept for downstream processing of antibodies to meet the challenge of improved upstream technologies. Our process does not need elution chromatography anymore and meets the requirements for parenteral pharmaceuticals. We have developed a flocculation process which is combined with cold ethanol precipitation and finally the antibodies are By adjusting the concentration of bivalent ions and pH, conditions have been designed to remove host cell proteins from 600000 ppm to 90 ppm, with a final chromatography through a monolith in flow through mode. Aggregates could be efficiently removed to below 0.01 %. Antibodies did not change the biological activity as demonstrated by biological methods and secondary structure elements where maintained as measured by ATR-FTIR and fluorescence. The method is a generic platform technology and has been tested in the purification of several human antibodies. By applying DOE the concentration of bivalent ions and ethanol is fine tuned to optimize yield and purity. A pareto curve has been established to match maximal host cell impurity with the separation power of the second step to reach the required purity. Three antibodies with different pI and hydrophobicity have been purified with this procedure. The process is also suited to produce a dried antibody powder, which is intended either for alternative formulation or storage of the product. The whole process is designed for a continuous operation. With a tubular reactor with a 185 L size up to 3 kg can be purified per day. This process is intended for rapid scale up but also for real large scale production of antibodies. On the other hand the process can be also useful for producing different antibodies in parallel.

L-302 **Development and Application of a High Throughput Protein Solubility Workflow.** Gregory Barker, Brian O'Mara, Sibylle Herzer, Siegfried Rieble, Bristol-Myers Squibb, Pennington, NJ, USA

High throughput process development (HTPD) techniques are integral to reduce process development time. At Bristol-Myers Squibb, HTPD techniques such as plate-based resin screening and robotic mini-column screening have successfully shortened development time requirements. Establishing a protein solubility map is desirable to determine the window of opportunity for downstream unit operations prior to initiating the screening experiments. BMS' comprehensive HTPD strategy includes a high throughput buffer preparation and buffer exchange workflow to assess protein solubility. To date, most sample preparation or solubility mapping techniques have significant gaps in methodology and throughput. We present a custom workflow using an AKTA(TM) Avant to enable rapid buffer preparation and protein desalting for use in high throughput solubility, purification, and formulation experiments. Protein solubility maps are established in several minutes per condition with minimal hands on time and high reproducibility. Robotic liquid handlers can be used to create buffers, but those methods are unable to trace pH and conductivity in-line and do not offer methods for high throughput buffer exchange of the protein. This approach has the unique advantage of affording both seamless preparation of buffer maps based on acid, base, and salt blending over a wide range of conditions in combination with gentle, traceable, automated introduction of the sample into the new buffer system. The ability to trace pH, conductivity, and absorbance enables in-process verification of buffer composition as well as preliminary verification of protein compatibility with each condition. This methodology allows us to prepare a matrix of proteins solutions while generating the buffers needed for high throughput experimentation. It also serves to debottleneck other HTPD workflows which are strictly dependent on the use of robotic liquid handling systems. Using the described high throughput buffer preparation and desalting method, we explored the solubility map of a monoclonal antibody to improve protein solubility and stability during and after a final tangential flow filtration (TFF) unit operation. Using
several orthogonal high throughput analytical techniques (absorbance (A280nm and A320nm), dynamic light scattering, and analytical size-exclusion chromatography), we were able to rapidly screen 144 buffer conditions for protein solubility and aggregation propensity. The results of this screen were successfully applied to a scale-up TFF experiment to improve process conditions. Through this work, we have developed a high throughput sample preparation and solubility workflow and successfully demonstrated its utility in downstream process development.

L-303 Characterization of Protein Interactions on Mixed-mode Chromatography and Augmenting Selectivity using Selective Mobile Phase Modulators. Leslie S. Wolfe, Abhinav A. Shukla, KBI Biopharma Inc., Durham, NC, USA

Mixed-mode chromatography resins are increasingly being incorporated into preparative purification processes. Mixed-mode resins have the ability to interact with target proteins through multiple types of interactions including electrostatic interactions, hydrophobic interactions and hydrogen bonding depending on the ligand. Further selectivity can be obtained through the use of modulators in buffers during the product load, wash or elution phase of the process step. Here, the mixed-mode Capto MMC resin is described in terms of its protein adsorption characteristics by using a set of four monoclonal antibodies and two non-IgG model proteins. Linear gradient elution experiments were executed to compare the elution conditions required for each antibody and non-IgG model protein at three pHs in the presence of several mobile phase modulators. For each protein, experiments performed at pHs farther from the protein isoelectric point resulted in tighter protein:resin interactions. Additionally, in the presence of modulating agents, varying binding and retention were observed that were in-line with the known abilities of these modulators to reduce different kinds of interactions (electrostatic, hydrophobic, hydrogen bonding etc.). The influence of mobile phase modulators on linear retention was characterized by the use of log k’ vs. log (salt concentration) plots in the presence of various agents. These studies enabled a classification of the key interactions that drove retention for different types of proteins. The results of these studies characterizing fundamental interactions on this mode of chromatography were employed to develop highly selective wash steps. The use of combinations of some of these mobile phase modulators in a wash step were found to augment HCP clearance by > 5 fold in comparison with a conventional wash step. The results presented in this study can significantly enhance selectivity that can be obtained during protein separations on this mode of chromatography and offer the enticing possibility of creating a pseudo-affinity separation using a non-affinity chromatographic stationary phase.

L-304 Integrated and Fully Continuous Processing of Recombinant Proteins – From Cell Culture Media to Purified Drug Substance. Rahul Godawat, Veena Warikoo, Sujit Jain, Mahsa Rohani, Konstantin Konstantinov, Genzyme - A Sanofi Company, Framingham, MA, USA

Considering the implications of increasingly diverse product candidate pipelines, rapidly fluctuating market demands and growing competition from biosimilars, biotechnology companies should be motivated to develop innovative solutions for highly flexible and cost-effective biologics manufacturing. To address these challenges, we have developed a fully continuous biologics processing platform. Our studies have focused on the integration of a perfusion bioreactor to a fully continuous downstream purification train using two four-column periodic counter-current chromatography (PCC1 and PCC2) systems. Running in an automated manner, these systems performed the protein drug capture, viral inactivation, and in-line buffer dilution along with intermediate and final polishing purification steps to generate the drug substance. To demonstrate proof of concept, we have run the process in an uninterrupted manner for 31 days without indications of time based system performance decline. The biologics product quality observed for the fully continuous process was comparable to that for a batch purification operation. Our data reveal that an integrated fully continuous process results in a dramatic increase in the process throughput (time in hours to produce a batch as compared to days), decrease in the equipment footprint, elimination of several non-value added unit operations, elimination of hold steps and reduced number of unit operations. These findings demonstrate the potential of integrated fully continuous bioprocessing as a universal platform for the manufacture of various kinds of therapeutic proteins.

L-305 Mechanism of Improved Protein Separation in PEG-modulated Cation Exchange Chromatography. Simon Kluters1, Thomas von Hirschheydt2, Sebastian Neumann3, Andreas Schaubmar3, Christian Frech3, 1Mannheim University of Applied Sciences, Mannheim, GERMANY; 2Roche Diagnostics GmbH, Penzberg, GERMANY

The addition of neutral water-soluble organic polymers such as polyethylene glycol to the eluent of ion-exchange chromatography has been reported to effect the separation of proteins. The magnitude of this effect depends on the molecular mass of the protein and the concentration of added PEG. The differentially enhanced retention of aggregates from monomeric antibodies and the improved effectiveness of their removal have been demonstrated. The effect of PEG is attributed to the preferential exclusion of PEG from the surface of proteins and chromatographic stationary phases. When proteins bind to a stationary phase in the presence of PEG, they
are able to share hydration water and in addition, the hydrated surface area of the bound protein is lower than the additive surface areas of the protein and stationary phase separately. Both phenomena are thermodynamically favorable and stabilize the association of the protein with the stationary phase. Proteins consequently elute at higher eluent concentrations than in the absence of PEG. In this work we have analyzed the effect of PEG on aggregate removal from an antibody preparation on a strong cation exchanger at different pH values and varying PEG concentration. Using the pure monomeric antibody as well as a strongly enriched aggregate fraction linear gradient elution experiments were performed. By a detailed analysis of the data according to the model framework of Mollerup (1) the influence of PEG on the number of electrostatic interactions, the equilibrium constant of the exchange reaction and the difference in reference Gibbs energy in the adsorbed and solute state for the protein and the salt was determined. The implications of the results on the mechanism of PEG-modulated separation as well as on the PEG-induced precipitation are discussed. Part of the results were recently published (2), new results on the correlation of chromatography and precipitation data will be presented in addition. 1 Mollerup et al. (2010) Adv Chromatogr. 48:57-97 2 Kluters et al. (2012) J. Sep. Sci. 2012, 35, 3130–3138

L-306 Taking PEG Solvent Modulation to the Next Level. Sebastian Neumann, Roche Pharma, Penzberg, GERMANY

Cation exchange chromatography is an integral part of many downstream processes for therapeutic antibodies. Usually the removal of antibody aggregates can be sufficiently accomplished by this chromatographic method. Nevertheless, for some antibodies the resolution of monomers and aggregates is insufficient. In these cases it is an opportunity to employ polyethylene glycol (PEG) solvent modulation in order to improve resolution, a method that has been known for many years: PEG is excluded from protein surfaces as well as the surface of chromatographic stationary phase, resulting in preferential hydration of the protein as well as the stationary phase. Under these conditions the adsorption of the protein to the stationary phase is thermodynamically favored, leading to an increased retention volume of the proteins. Since the increase in retention is dependent on protein size, the resolution of antibody monomer and aggregates increases. This concept is generally applicable for the improvement of separation of protein species that differ in size. A limitation to this method is that on one hand highest resolutions are achieved at high PEG concentrations and on the other hand high PEG concentrations induce protein precipitation. Therefore only moderate PEG concentrations are applicable. We recently identified a way to resolve this dilemma, resulting in a chromatographic method with superior resolution performance: We conducted cation exchange chromatography experiments with a mobile phase containing high PEG concentrations in the presence of certain sugars, polyols or amino acids (“solubility enhancers”) and observed significantly improved resolution performance. This method has already been tested with several antibodies and different cation exchange resins. It allows for a superior antibody monomer-aggregate separation in a generic manner and has also been successfully applied in a scale-up experiment. In one case study the separation of antibody monomers and aggregates by cation exchange chromatography under standard conditions was hardly possible, since the resolution was as low as 0.05. By employing classical PEG solvent modulation the resolution could be increased by a factor of 4 to 0.21. In this example the newly identified solubility enhancer/PEG method led to a 10-fold increase in resolution (0.52).

L-307 A Parallel Approach for the Reduction of Product Related Species in an E. coli Fermentation Producing an IL-1 Receptor Antagonist. Emily Schirmer, Kathryn Golden, Gregory Zarbis-Papastoitis, Eleven Biotherapeutics, Cambridge, MA, USA

A superior interleukin-1 receptor antagonist was engineered for the treatment of dry eye syndrome by blocking inflammation and pain pathways. The molecule, referred to as EBI-005, is expressed in the soluble fraction of an E. coli fermentation process and purified by traditional chromatography techniques. During process development, three product related species were identified as byproducts in the fermentation process. Two of the product related species differ from the product by one amino acid, and the third is an acetylated version of EBI-005. In order to reduce the levels of the product related species in the final drug substance a parallel approach was taken to develop the fermentation and purification processes. Fermentation conditions such as temperature, pH, levels of trace metals, addition of EDTA, and carbon source were evaluated to determine the effect on levels of product related species in the fermentation harvest. Temperature, pH, and trace metals had negligible effects on the levels of product related species; however, the addition of EDTA and choice of carbon source proved to have significant effects. Ion exchange and ceramic hydroxyapatite columns were used to reduce the levels of product related species and process related impurities while maintaining high product recovery. A cation exchange capture step was developed by a statistical DOE approach to resolve EBI-005 and product related species. The resulting cation exchange eluate showed complete removal of the acetylated species, and a 65% reduction of the remaining two species. Various load and elution conditions were evaluated on a hydroxyapatite column to further reduce product related species levels by 30% for one species and by 80% for the other. The fermentation and purification processes were transferred to a contract manufacturing
organization for production in a 100 L fermenter. The large scale production of EBI-005 resulted in product that was of equal quality and purity to development scale preparations and suitable for pre-clinical and clinical activities.

L-308  **Comparison of Three Protein A Affinity Resins for Purification of a Monoclonal Antibody by Multi-column Continuous Chromatography.** Anthony Grabski, Bruce Thalley, Alla Zilberman, Robert Mierendorf, Semba Biosciences Inc., Madison, WI, USA

Monoclonal antibodies (mAbs) are a predominant modality for a broad range of clinical indications including oncology and inflammatory diseases. Increasing manufacturing capacity and decreasing cost per purification campaign are critical factors for making antibody therapies more affordable. Downstream processing accounts for up to 70% of mAb production cost and Protein A column chromatography for mAb capture is the most expensive step, contributing 51% to the overall purification cost. Multi-column continuous chromatography (MCC), a form of simulated moving bed chromatography (SMBC), is a scalable technology demonstrated to provide dramatically increased productivity, purity, and efficiencies in chromatography media and buffer utilization over the standard single column batch process. This study compares MabSelect SuRe™, POROS® MabCapture™ A, and TOYOPEARL® AF-rProtein A-650F protein A affinity resins for their productivity and efficacy in an 8-column MCC process versus the traditional single-column process for mAb purification.

L-309  **Continuous Separation of Protein Loaded Nanoparticles by Simulated Moving Bed.** Peter Satzer¹, Martin Wellhöfer², Alois Jungbauer¹, ¹University of Natural Resources and Life Sciences, Vienna, AUSTRIA; ²ACIB, Vienna, AUSTRIA

Nanoparticles loaded with bioactive compounds have a high potential for drug delivery, carrier of immunogens in vaccines, or even agricultural purpose to deliver pesticides. However, the problem of separating the particle, once loaded with active compound, from free compound in solution remains to be solved in large scale. Economic processes are required. Ultracentrifugation can separate nanoparticles, but is bound to a batch-wise process with high buffer demand due to washing steps. Size exclusion chromatography (SEC) is able to separate active compound and particles and delivers the purity necessary for drug targeting by nanoparticle carriers. We used SEC to separate fully loaded nanoparticles from an excess of model proteins such as bovine serum albumin and beta-casein. To enhance the productivity, purity and buffer consumption, we used SEC in a continuous 4 zone simulated moving bed (SMB) mode. As pharmaceutical proteins are in most cases expensive, we introduced a continuous recycling, concentrating the extract by tangential flow filtration (TFF) and recycle protein as well as buffer. To further reduce buffer consumption, the raffinate can be concentrated continuously by TFF, adjusting the resulting particle concentration and recycling buffer into the SMB system.

L-310  **Development of a Purification Strategy for Enterovirus 71 Like Particles for Vaccine Production.** Matthias Meininger¹, René Djurup², Louis Villain³, Jane Cardosa⁴, Udo Reichl¹, Michael Wolff³, ¹Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, GERMANY; ²Rebidu, Gentofte, DENMARK; ³Sartorius-Stedim Biotech GmbH, Göttingen, GERMANY; ⁴Sentinext Therapeutics Sdn Bhd, Penang, MALAYSIA; ⁵Otto von Guericke University, Magdeburg, GERMANY

Enterovirus 71 (EV71) is one of the major causative agents of hand, food and mouth disease (HFMD). In some cases the infection is associated with severe neurological diseases like encephalitis, aseptic meningitis and poliomyelitis-like paralysis. Since 1997 many outbreaks have been reported in the Asia Pacific region. However, due to the previous rather mild course of the disease and a low case fatality no vaccines have been developed so far. This picture has changed recently. Only for China more than 1.000.000 cases of EV71 infections with hundreds of deaths were reported by the World Health Organization in 2010 as well as an alarming outbreak in Cambodia in 2012 where 54 of 61 diseased children died. These developments highlight the need of a vaccine against EV71. In this project virus like particles (VLP) where chosen as vaccine agent. VLPs are empty viral capsids comprising major epitopes of viral proteins but lacking viral DNA or RNA. Thus, they can induce a strong immune response comparable to whole viral vaccines without the risk of virulent revertants. For all experiments clarified and pre-concentrated EV71 VLPs produced in SF9 insect cells (Sentinext Therapeutics Sdn Bhd) were used. The initial focus was to evaluate various membrane adsorbers (MA) to purify EV71 VLPs and to develop a downstream purification scheme suitable to manufacture clinical grade vaccines. Therefore, two anion exchange (AEX) MA with different chemistries (Sartobind Q and Sartobind STIC PA, Sartorius-Stedim Biotech GmbH) were investigated in a design of experiment (DOE) approach. The factors pH, buffer concentration, ionic strength, and ligand density have been varied to find an optimum for VLP recovery and purity. All experiments were performed with a 96 deep well plate vacuum station. Results indicated that elution of bound VLPs under the tested conditions with AEX MA is rather challenging. However, in flow through mode a good recovery of VLPs and a good depletion of DNA were achieved whereas the level of contaminating proteins require further attention in the remaining purification scheme. The STIC MA bound DNA efficiently even at high salt concentrations making it an interesting candidate for DNA removal after a capture step with high salt elution.
conditions. DOE evaluation indicated a strong influence of ionic strength and only a minor effect of pH and ligand density on VLP yield and purity. Overall, the conducted experiments showed encouraging results to develop a MA-based chromatographic process for the purification of insect cell culture derived VLPs.

L-311  Purification of Virus Particles by Ceramic Hydroxyapatite Chromatography. Yae Kurosawa¹, Maiko Saito¹, Tsuneo Okuyama², ¹HOYA Corporation, Akishima-shi, Tokyo, JAPAN; ²Protein Technos Institute, Atsugi-shi, Kanagawa, JAPAN

At the present understanding, the density gradient ultracentrifugation method is believed to be the most conventional method of virus purification. However, the ultracentrifugation method reduces viral infectivity and also processing is rather time-consuming. Ceramic hydroxyapatite can be applied in the neutral region, so that we can treat the virus with its bioactivity as the protein treatment. Ceramic hydroxyapatite is a mild packing media for biological material even for the virus separation. This method is highly reproducible and applied to some virus species such as dengue virus, influenza virus, poliovirus and feline calicivirus (FCV). One of the typical preparative procedures for chromatography was as follows: dengue virus type 2 ThNH7/93 was inoculated onto C6/36 cells and culture fluid was collected at day 7 and filtered through a 0.22 μm pore size filter. Culture fluid was loaded onto the ceramic hydroxyapatite column and eluted with a linear gradient of sodium phosphate buffer (NaPB). Virus particles retained infectious activity after purification from cell culture fluid by ceramic hydroxyapatite chromatography. Elution profiles: concerning the effect of buffer pH, influenza virus A/Beijing/262/95, dengue virus type 1 Hawaii, and poliovirus Sabin type 2 were bound more strongly on ceramic hydroxyapatite at a lower pH. Culture fluid infected each virus was loaded onto the ceramic hydroxyapatite column and viruses were eluted with a linear gradient of NaPB at pH 6.4, 7.2, and 8.2. The elution at pH 6.4 required the highest concentration of NaPB among 3 pH conditions, and the elution was delayed. Virus showed the similar profiles: adsorption on hydroxyapatite increased at a lower pH, so that the virus peak was separated from large peak of protein contaminants well by reducing the pH and this may lead to easier purification of the virus. Sodium chloride (NaCl) addition to NaPB reduced the adsorption of FCV A391 on hydroxyapatite. Virus culture fluid was loaded onto the ceramic hydroxyapatite column and FCV was eluted with a linear gradient of NaPB with the different concentration of NaCl. The adsorption of FCV on hydroxyapatite was reduced and that of DNA derived from host cells was elevated at the higher concentration of NaCl. NaCl addition to NaPB elution can control the DNA elution and it may be useful to remove DNA from the target virus and improve the purity. This kind of steady and reproducible results indicates the applicable for industrial process chromatography.

L-312  Predicting the Performance of Capture Chromatography Processes. Noriko Yoshimoto, Hiroyuki Nagaoka, Hitomi Nakano, Shuichi Yamamoto, Yamaguchi University, Ube, Yamaguchi, JAPAN

Chromatography is the main purification unit operation for biopharmaceuticals. However, it is still time-consuming and laborious to find proper operating and/or chromatography conditions by tuning various variables such as sample load, flow-rate and column dimension. The mobile phase must also be carefully examined as the separation and/or the binding capacity is strongly influenced. Protein A chromatography (PAC) is an essential unit operation as the first capture step in monoclonal antibody (Mab) purifications. As both Mab and PAC reins are quite expensive, scale down experiments by using a small column and or a small-scale batch uptake setup are needed. Such experiments are also preferred for the capture by electrostatic interaction chromatography (ion-exchange chromatography, IEC). Another concern is the productivity and the bed utility since the initial resin cost is high especially for PAC. In this study, pore diffusion coefficients or overall mass transfer coefficients of proteins were determined by pulse response (isocratic elution) experiments at non-binding conditions for PAC and IEC. Dynamic binding capacity (DBC) was determined as a function of mobile phase velocity u by using small columns (0.2-1mL). Static binding capacity (SBC) was also determined by using small columns and 96-well microplate based batch adsorption experiments. A simple correlation between the DBC/SBC and a dimensionless variable udp2/DsZ (dp: particle diameter, Ds: stationary phase (pore) diffusion coefficient, Z : column bed height) was found to be valid for various different PAC and IEC media. Based on this correlation the productivity calculation method was developed, which considers the bed utility and the pressure drop limitations.

L-313  Twin Column Capture SMB: A Novel Cyclic Process to Increase the Capacity Utilization in Protein A Chromatography. Monica Angarita¹, Daniel Baur², Thomas Müller-Späth³,⁴, Roel Lievrouw⁵, Geert Lissens⁶, Guido Ströhlein⁷, Massimo Morbidelli⁸, ¹ETH Zürich, SWITZERLAND; ²Protein Technos Institute, Atsugi-shi, Kanagawa, JAPAN; ³ChromaCon AG, Zürich, SWITZERLAND; ⁴JSR Micro NV, BELGIUM

Protein A based purification of monoclonal antibodies (mAbs) is the preferred purification platform in industry for capture of mAbs from cell culture harvest, due to its high yield and specificity. The use of multi-column, cyclic processes for mAb affinity capture has gained significant interest in the last years. However, the available process solutions require at least 3-4 columns with accordingly increased hardware complexity and downtime.
probability. To overcome these drawbacks and increase the resin utilization, a novel twin-column countercurrent sequential capture process has been developed. In this work we introduce the method design as well as experimental results using AmsphereTM Protein A JWT203, showing the significant advantages over batch chromatography in terms of higher column loading, higher productivity and lower buffer consumption. Simulation results are also presented, showing a performance comparison to conventional 3- and 4-column capture processes.

L-314 Egg White Protein Fractionation with Membrane Ion Exchange Chromatography. Janina Brand, Ulrich Kulozik, Technische Universität München - Chair for Food Process Engineering and Dairy Technology, Freising, GERMANY

Egg white (EW) gains more and more attention as source for isolated, functional proteins. The most prominent target protein fraction in EW is lysozyme, which has some applications in the pharmaceutical industry. In few cases, it is also used for preservation purposes in the food industry, because of its antimicrobial properties. However, so far, established fractionation processes are not yet satisfying in terms either production costs or purity. Further to that the hygiene of established processes renders the resulting products risky, because of their limited CIP-capability, which is a critical aspect in egg processing. Therefore, lysozyme is either not available at food grade quality or prices low enough for applications in food products. Another important target EW protein is the iron binding ovotransferrin, with antimicrobial properties and the capacity to improve iron bioavailability similar to the whey protein lactoferrin. The advantage of ovotransferrin is its 140-fold higher amount in EW compared to lactoferrin in whey. In this work, we investigated membrane based ion exchange chromatography (MAC) with cation exchangers from Sartorius-Stedim Biotech (Sartobind® S). These adsorbers consist of a porous membrane as carrier system for linked sulfonic acid that is coiled around a solid core. This construction results in a tangential flow along both sides of the membrane as well as through the pores. So, the mass transfer to the ion exchange ligands is enhanced through convective flow mechanisms rather than pre-dominantly relying on diffusional mass transport of established bead columns. At lab scale, the membrane adsorbers have a column volume of 3 ml. For industrial up-scaling, adsorbers with a volume up to 5 l are available. The objective of this work was to establish an EW protein fractionation process at lab scale level to obtain lysozyme and ovotransferrin ideally in pure form by means of using MAC. Regarding the fractionation of lysozyme, the effects of ionic strength, sample and buffer pH as well as flow rate on binding and elution behavior were investigated. Afterwards, the flow through of the lysozyme fractionation was used to separate ovotransferrin. The challenge of the ovotransferrin fractionation is that the pH adjustment for the cation exchange process is in the range of the isoelectric points of some of the remaining proteins, and therefore, precipitation effects have to be considered and avoided as far as possible. Thus, the effect of pH on the extent of precipitation as well as on purity and recovery of the fraction were investigated. In summary, it was shown that MAC is a suitable method for the fractionation of lysozyme and ovotransferrin from the complex EW system.

L-315 Determination of Equilibrium Isotherms in Supercritical Fluid Chromatography. Georges Guiochon, Abhijit Tarafder, Fabrice Gritti, Fahimeh Kamarei, University of Tennessee, Knoxville, TN, USA

Compared to traditional mobile phases used in liquid chromatography, those used in supercritical fluid chromatography (SFC) are much more compressible and less viscous, which has a profound effect on the experimental conditions that are used in SFC. A low viscosity is associated with high diffusion coefficients. Thus, optimum reduced velocities in SFC are larger than in HPLC and separations have to be run at higher velocities. Accordingly, there is still a non-negligible pressure drop along SFC columns, the mobile phase density decreases continuously and the volumetric flow rate increases along the column. As a consequence of the density decrease, the retention factors of all compounds vary in a large proportion from the inlet to the outlet of the column, so the values that are measured from the chromatograms are mere “averages”. The design of SFC instruments into which the carbon dioxide and the organic modifier must be pumped separately and mixed affects markedly the experimental conditions. Slight modifications in our instrument that eliminate the significant band spreading caused by the large dwell and mixer volumes allowed the elution of fronts as sharp as or even sharper than in HPLC. The influence of the mobile phase compressibility on the various methods of measurements of equilibrium data is discussed. The methods of frontal analysis, perturbation, frontal analysis and elution by characteristic points, and RTM were studied and used. The issue of the interpretation of these “average” data for the determination of equilibrium isotherms in solid/supercritical fluid adsorption system is raised. The results obtained with different compounds, e.g., caffeine on a C18 bonded HPLC column and the enantiomers of naproxen on Whelk-O1, using methanol or ethanol as modifier are discussed. The values obtained in SFC are compared with similar data obtained in HPLC.
Supercritical Fluid Chromatography (SFC) is a useful tool for analytical and large scale separations. SFC is most often used with carbon dioxide as a mobile phase and an organic modifier such as some type of organic alcohol. It has some significant advantages over standard HPLC methods such as less pressure drop across the columns, faster column equilibration, faster method development, higher efficiency separations and significantly less generation of hazardous waste. Some of the main advantages for preparatory chromatography include solvent waste reduction, facilitated product recovery, lower solvent cost and the possibility for recycling. The major topic will include some Computer Fluid Dynamic Software simulations modeling optimization studies that were then tested against real-life methodology.

L-316 Using Modern SFC Systems for Adsorption Characterization. Torgny Fornstedt, Martin Enmark, Jörgen Samuelsson, Karlstad University, Karlstad, SWEDEN
Recently the pharmaceutical industry has started to replace preparative HPLC with preparative SFC to lower the environmental impact and to increase performance. Reliable characterization of the adsorption processes in SFC is therefore of utmost importance. The key thermodynamic phase system information is obtained by rigorous determination of adsorption isotherm data over a broad concentration range. If properly processed, this data gives not only correct information about the degree of heterogeneity but also the energy of interactions and mono layer capacities of each individual type of adsorption site in the phase system. Ultimately, this can result in identification of the types of interactions, i.e., dipole-dipole, van der Waals interactions etc. In this study we will present transfer of selected adsorption characterization methods, traditionally applied with success in LC, to SFC. We have here transferred all available knowledge from LC – from model selection to validation. We will also, using recent findings, explain the effects of pressure and temperature variations as well as how to accurately measure the volumetric flow rate on a modern analytical SFC system. We will demonstrate how the latest SFC instruments, with some critical modifications; have the potential for rapid and reliable acquisition of thermodynamic data using the ECP method. Finally we will elaborate on how the adsorption depends on density, temperature and modifier content in the mobile phase. This is a contribution from the Fundamental Separation Science Group www.FSSG.se

L-317 Scaling-up in Supercritical Fluid Chromatography. Abhijit Tarafder, Chris Hudalla, Pamela Iraneta, Waters Corporation, Milford, MA, USA
Supercritical fluid chromatography (SFC) is already recognized as the method of choice for the preparative separation of chiral molecules in pharmaceutical industries. Even for the achiral separations, especially for the separation of natural products, SFC has a huge potential to grow as the method of choice. To harness the full potential of SFC in these separations, however, we need to develop better methods for scaling-up from analytical to preparative conditions. One of the critical procedures during the method development of a preparative separation is the scale-up procedure. Screening of competing combinations of the mobile phase compositions and the stationary phases, leading to the best separation, is time consuming and expensive to carry out directly in the preparative scale. One of the better alternatives is to first identify the optimum mobile/stationary phase combination in the analytical scale and then transfer the method to a scale where the prep separation should be carried out. Although there are standard methods available in LC for scaling-up, they are not always useful in SFC. In SFC the density of the mobile phase varies significantly across the column, which results into the variation of all the mobile phase properties. The extent and the nature of variation of density in the analytical and the preparative scale can be very different, depending on (a) the dimensions of the connecting tubes and the column, (b) the particle sizes of the stationary phases, and (c) the operating temperature, pressure and flow rate. This difference in the density profiles across the column, between the analytical and the preparative system, will lead to very different chromatography unless the scaling-up procedure is guided by a systematic approach requiring additional calculations. In this presentation we will discuss a systematic approach we are developing to scale-up operations from analytical to preparative scale in SFC.

L-318 The Effect of Mobile Phase Modifiers and Additives on the SFC Separation of Two Pharmaceutical Compounds. Xiqin Yang, Linda Barton, David Thornton, Leo Hsu, GlaxoSmithKline, King of Prussia, PA, USA
Supercritical Fluid Chromatography (SFC) is an excellent and routinely used purification tool in the pharmaceutical industry. Modifiers such as MeOH, EtOH, IPA and ACN are used to increase solvent strength in SFC. On the other hand, additives such as ammonium acetate and trifluoroacetic acid are used to improve resolution. In this talk, we will present the impacts of mobile phase modifiers and additives on the SFC separation of two pharmaceutical compounds. Because of the acidic and basic moieties on these compounds, we were able to optimize throughput and recovery of the purification by adjusting modifiers and additives.

L-319 Collection Efficiency from a Theoretical CFD Perspective. John Whelan, Waters Corporation, New Castle, DE, USA
Supercritical Fluid Chromatography (SFC) is a useful tool for analytical and large scale separations. SFC is most often used with carbon dioxide as a mobile phase and an organic modifier such as some type of organic alcohol. It has some significant advantages over standard HPLC methods such as less pressure drop across the columns, faster column equilibration, faster method development, higher efficiency separations and significantly less generation of hazardous waste. Some of the main advantages for preparatory chromatography include solvent waste reduction, facilitated product recovery, lower solvent cost and the possibility for recycling. The major topic will include some Computer Fluid Dynamic Software simulations modeling optimization studies that were then tested against real-life methodology.
P-101 High-temperature Liquid Chromatography. Yu Yang, East Carolina University, Greenville, NC, USA

High-temperature liquid chromatography (HTLC) is a technique of reversed-phase liquid chromatography carried out at elevated temperatures. A major benefit of HTLC is the reduction of methanol or acetonitrile use in the mobile phase. The higher the temperature, the greater amount of organic solvents can be saved. In addition, HTLC offers ultrafast separation, temperature-programmed elution, temperature-dependent separation efficiency, selectivity, and resolution. The challenge HTLC faces is the stability of solutes and the stationary phases under high temperature conditions. The following topics will be addressed in this presentation: HTLC system development; analytes separated by high-temperature liquid chromatography; columns feasible for high-temperature liquid chromatography; and theoretical aspects of high-temperature liquid chromatography.

P-102 Purification of Infective Baculoviruses by Monoliths: Insights in Parameters Influencing Yield. Petra Gerster, Nikolaus Hammerschmidt, Alois Jungbauer, University of Natural Resources and Life Sciences, Vienna, AUSTRIA

Pure baculovirus with a size of approximately 40-50 x 200-400 nm is often used in vaccine development or for human gene therapy. A purification process directly form the culture supernatant has been developed based on monoliths with a channel size of 1500 nm to 2000 nm. Baculovirus produced in Spodoptera frugiperda cells (SF-9) were harvested by centrifugation, filtered through 0.8 μm filters and directly loaded onto radial anion exchange monolith columns (Convective Interaction Media (CIM) QA tubes) with a volumetric flow rate of one column volume per min. Infectious virus could be eluted with a step gradient at salt concentrations of 440 mM NaCl. Recovery of infectious virus was highly influenced by composition and age of supernatant and ranged from 20 to >99% active baculovirus. Precolumns such as CIM Epoxy tubes increased recovery of infective virus by removing hydrophobic compounds. We assume that lipophilic compounds foul the ion exchangers which in turn unspecifically adsorb virus. Host cell protein content and DNA could be effectively reduced and infective virus could be concentrated up to 52-fold. In contrast to state of the art purification protocols such as density gradient centrifugation, this method allows processing of laboratory batches within hours.

P-103 Kinetic Study of the Protein Refolding in Different Chromatographic and Non-chromatographic Systems. Sylwia Wos, Renata Muca, Wojciech Piatkowski, Dorota Antos, Rzeszow University of Technology, Rzeszow, POLAND

Recently, the interest in production of recombinant proteins increased rapidly, particularly in biotechnology, medicine and pharmaceutical industries. Processing proteins extracted from inclusion bodies is very common technique due to high concentration and relative high purity of the target protein in inclusion body. Proteins are extracted from inclusion bodies by use of a denaturant agent, therefore, they are devoid of biological activity. The recovery of biologically active forms requires refolding and purification. Typically, the protein refolding is performed by dilution in a stirred tank reactor, in an ultrafiltration module or in a chromatographic column. In the last case, termed as the matrix assisted refolding, the protein refolding is accompanied with chromatographic purification in situ. In the former two cases the chromatographic separation can be optionally used as a subsequent purification stage following the refolding process. In this work the efficiency of few methods of the protein refolding has been analyzed: two non-chromatographic techniques such as: refolding in a batch reactor and in a membrane module for tangential ultrafiltration, and three matrix assisted processes based on hydrophobic interaction, ion exchange and size exclusion chromatography. The analysis has been performed for a model protein. The refolding kinetics has been investigated in all cases. Moreover, the influence of the chromatographic mobile phase composition on the refolding efficiency has been quantified. Possible advantages and disadvantages of different techniques have been discussed.

P-104 Commercial Para-xylene Simulated Moving-bed (PX-SMB) with Extended Langmuir Isotherms. Young-il Lim1, Heejung Yeo2, Geun-Ha Shin3, Sang-Hun Seo3, Jinsuk Lee4, Hankyong National University, Anseong, Gyonggi-do, SOUTH KOREA; 2Keimyung University, Daegu, SOUTH KOREA; 3GNG MC Co. LTD, Osan, Gyonggi-do, SOUTH KOREA; 4SamsungTotal, Seosan, Chungnam, SOUTH KOREA

Para-xylene (PX) is the most widely used of xylenes isomers in the petrochemical industries with a 6% average annual growth rate for the next decade (Minceva and Rodrigues, 2003). Nowadays, about 60% of PX worldwide is produced by simulated moving-bed (SMB) technology, such as UOP’s Parex, Toray’s Aromax, and IFP’s Eluxyl (Minceva and Rodrigues, 2007). Conventional SMB is divided into 4 zones by the inlet ports (desorbent and feed) and the outlet ports (extract and raffinate). In practical operation, the dead volumes inflict significant influences on SMB performances. Particularly in the PX SMB configuration, each transfer line (or bed-line) connecting a rotary valve to adsorbent beds is shared for the introduction and withdrawal of process streams, which is one of the dead volumes (Lim et al., 2010, Lim and Bhatia, 2011). Eight-zone para-xylene (PX) simulated moving-bed (SMB) involves three flushing sequences to prevent purity deterioration caused by SMB bed-lines connecting the rotary valve to beds. Beside the recycle flow from the last zone to the first, the extract fluid trapped in bed-line (BL) is fed back to one bed ahead of the extract bed in accord with the line flushing (LF)
and secondary flushing (SFI), forming an internal recycle flow. Both LF and SFI form the closed loop to increase purity and recovery, while the tertiary flushing (TFo) works somewhat independently of the other flushing streams for recovery enhancement (Sutanto et al., 2012). Here, the 8-zone PX-SMB was modeled by an one-dimensional (1D) SMB model with a competitive Langmuir isotherms published in Mineva and Rodrigues (2003). In this study, a commercial 8-zone PX-SMB with an extended Langmuir isotherms is investigated. The extended Langmuir isotherm, which is also called the Fowler adsorption isotherm, represents well adsorption of xylene isolomers with p-diethylbenzene (PDEB) as desorbent on a commercial adsorbent, X-zeolite. This study aims to validate the Fowler isotherm against experimental data obtained from a commercial plant and to find an optimum operating condition maximizing recovery within a 99.7% purity. The adsorption bed was modeled by a convection-diffusion-reaction partial differential equation (PDE) with linear driving force (LDF) mass transfer. The unsteady-state 1D SMB model with dead volume was solved by a fast and accurate solution tool for chromatography and SMB (FAST-Chrom/SMB). The binary coefficients of the Fowler isotherm were adjusted to meet the experimental data. Optimal operation conditions were searched by the parametric study of the flow rates.

P-105 Chromatan and Fujifilm Diosynth’s investigation of Countercurrent Tangential Chromatography (CTC) for Protein A Capture of Monoclonal Antibodies. Oleg Shinkazh1, Gary Brookhart2, 1Chromatan, State College, PA, USA; 2Fujifilm Diosynth, RTP, NC, USA
Fujifilm Diosynth in partnership with the Chromatan corporation collaborated on testing Protein A CTC which is a new column-free technology for purification of monoclonal antibodies. Protein A CTC provides a scalable, disposable, and continuous alternative to packed-bed Protein A column capture chromatography. Experimental studies were performed using a clarified feedstock from Fujifilm Diosynth’s CHO cell culture process. We obtained high-resolution antibody purification at < 10 psi operating pressure, with MAb recovery >94%, showing great promise for this new technology.

P-106 Effect of Alcohol Modifier on the Retention Factors of Chiral Solutes with an Amylose-based Sorbent: Modeling and Implications for the Interaction Mechanism. Hung-Wei Tsui, Elias I. Franses, Nien-Hwa Linda Wang, Purdue University, West Lafayette, IN, USA
Various stoichiometric displacement models in the literature have been widely used for understanding the competitive adsorption mechanisms of solutes and the polar modifiers of the mobile phase. The models were used to explain the often-observed linear log-log plots of the solute retention factor versus the concentration CI0 of the modifier. The slopes of the plots were sometimes inferred to be equal to the number of the displaced modifier molecules upon adsorption of one solute molecule, or upon adsorption of the solute-modifier complex, and was generally found to be greater than 1. In this study, the retention factors and enantioselectivities of ethyl lactate (EL) and pantolactone (PL) enantiomer pairs were measured for the amylose tris[O-methylbenzylcarbamate] sorbent, or AS, with isopropanol (IPA) in n-hexane at 25 °C. The slopes range from less than 1 (from 0.43 to 0.76) at CI0 = 0.13 to 1.3 M, to slightly more than 1 (from 1.0 to 1.25) at higher concentrations. The literature models cannot account for such slopes. To understand such slopes, five achiral monovalent solutes were chosen and studied in detail. Infrared Spectroscopy (IR) and Density Functional Theory (DFT) simulations provided indications that there is significant IPA aggregation with an average aggregation number of n=3. A new retention model for the monovalent solutes has been developed to take into account the IPA aggregation and the solute-IPA complexation. The small slopes can only be explained if the alcohol forms aggregates in solution. The model and the HPLC data show that the limiting slopes at high IPA concentrations approach the value of 2/n. More complex multivalent models have been developed for chiral molecules, accounting for multivalent solute adsorption, multivalent solute-alcohol complexation, solute intra hydrogen-bonding, alcohol adsorption, and, most importantly, alcohol aggregation. The limiting slopes, at the highest IPA concentrations approach the theoretically expected values of z/n, where z is the number of the alcohol molecules displaced upon the adsorption of a solute-IPA complex. Here z=3 or 4. The slopes are similar for each of EL enantiomer or and PL enantiomer. Nonetheless, significant differences were observed in the intercepts of the plots, suggesting the enantioselectivities are due to different adsorption equilibrium constants of each enantiomer on the AS sorbent.

P-107 Surface Modification of Ceramic Hydroxyapatite. Shintaro Kobayashi, HOYA Corporation, Akishima, JAPAN
Hydroxyapatite (HAP) is a purification medium of biomolecules in downstream process industry. Its mixed-mode support offers unique selectivities and often separates biomolecules that appear homogeneous using other chromatographic methods. The packing material is ceramics that the matrix has integrated with rigand and media have an alkali tolerance and strong. We modified surface of HAP and produced several kinds of new unique media based on HAP. 1.Metal modified Surface modified HAP with some metal ions interacts with certain amino acid residues and demonstrates unique properties for immobilized metal affinity chromatography. We
prepared surface modified HAP with some transition metal ions: Co (II), Ni (II), Cu (II) and Zn (II), for immobilized metal affinity chromatography and compared their chromatographic properties. Zinc-modified HAP showed special characteristics, different from HAP, and it was shown to be effective for histidine peptides and histidine-tagged protein separation. 2. Polyethyleneimine-coated hydroxyapatite (PEI-HAP) Polyethyleneimine has been widely used as a coating material to produce stationary phase for ion-exchange chromatography of biomolecules. We produced PEI-HAP by using a rotary evaporator. PEI was firmly located on the HAP surface, because the PEI did not leak out from the produced PEI-HAp even if the decantation was performed several cycles. PEI-HAP could be valuable in the analysis and purification of nucleotides and proteins. The further separation of ovalbumin suggested that a phosphoprotein such as ovalbumin could be separated on the basis of the number of phosphate residues, and dephosphorylated or polymerized ovalbumin would be separated and quantified, using this material. 3. Calcium or poly-phosphate coated hydroxyapatite Soluble calcium might often be added to lower phosphate buffer for increasing binding capacity of acidic proteins. Binding capacity of acidic proteins of HAP is generally lower, however, that increases the concentration relatedly when Ca ion is added in buffer. We modified surface of HAP by calcium or poly-phosphate and produced a new media for chromatography. Those new material shifts from multi-modal original HAP to single-modal HAP by calcium or poly-phosphate modified. We modified surface of HAP and could produce a new unique media different from original HAP. Surface modified hydroxyapatite would be useful for purification of biomolecules as new unique mixed-mode chromatography with rigid ceramics matrix.

P-108 Membrane Adsorbers in a Multicolumn Chromatography Configuration as a Viable Option for Capture Chromatography. Marc Bisschops¹, Peter Schwan², Martin Lobedann¹, ¹Tarpon Biosystems, Leiden, THE NETHERLANDS; ²Bayer Technology Services, Leverkusen, GERMANY; ³Invitae, Leverkusen, GERMANY

Membrane adsorbers have been used for polishing steps in biopharmaceutical manufacturing, albeit for negative chromatography (flow-through) mainly. Main reason for this is that membrane adsorbers have a relatively low binding capacity and a high dead volume. This makes them less attractive for capture processes. When combined in a multicolumn chromatography format, these disadvantages can be mitigated and the strong points of membrane adsorbers can also be exploited for capture processes.

In this paper, we will explore the option to combine membrane adsorbers with BioSMB® technology to create a fully continuous disposable capture process based on membrane adsorber technology. One of the unique features of BioSMB® Technology is its fully disposable fluid path, including the valves that direct the fluids to and from all columns or devices in the process. As such, BioSMB® Technology enables a fully disposable continuous chromatography process. We will present experimental work on the purification of monoclonal antibodies from clarified cell supernatant, using membrane adsorbers that are functionalized with Protein A affinity ligands. The experiments have demonstrated proof of principle, showing that complete capture of the monoclonal antibody could be obtained with very high flow rates. Even at moderate titers in the feed solution, the process cycle time is relatively short, supporting a fully disposable capture process for monoclonal antibodies. We will also address some practical aspects of the use of membrane adsorbers in a multicolumn configuration. This will cover an economic evaluation as well as a projection on scalability.

P-109 Addressing Purification Bottlenecks with Radial Flow Chromatography. Mark García¹, Mark Smith², Sushil Marwaha³, Bénédicte Lebreton¹, ¹Genentech Inc., South San Francisco, CA, USA; ²Genentech Inc., Vacaville, CA, USA

Separations by conventional or axial flow chromatography (AFC) have been established as an effective means for preparative and large-scale purification processes. However, improved cell culture titers place increasing demands on downstream operations. Existing facility constraints limit the options to increase productivity, and certain chromatography steps can become rate-limiting. Routine approaches to improve the throughput of chromatographic operations include scaling AFC column dimensions and cycling a process step. Alternative approaches to increase step throughput include the use of a column with radial geometry. In this case the mobile phase perfuses the resin bed in a radial flow chromatography (RFC) column over a larger cross-sectional area, with minimal increase to the column footprint. Faster volumetric flow rates are enabled, thus reducing processing times without compromising column performance. The capability of RFC was assessed as a potential cation exchange application in a monoclonal antibody purification process. In lab scale studies, process parameters were challenged to evaluate the impact to robustness by operating in RFC mode. Pilot scale runs were also performed to demonstrate the scalability of the technology. These results demonstrate that RFC may offer desirable throughput improvements if AFC column footprint is limiting, by cycling smaller RFC columns with no compromise to product quality.
Establishment of New Downstream Processing Methods for Monoclonal Antibody Purification through Integration of Continuous and Single-use Technologies. Christopher Gillespie, Kevin Galipeau, Mikhail Kozlov, Michael Phillips, Ajish Potty, Romas Skudas, Matthew Stone, Alex Xenopoulos, EMD Millipore, Bedford, MA, USA; Merck Millipore, Darmstadt, GERMANY

As the biopharmaceutical industry evolves, the need for advanced biomanufacturing becomes critical. Recent increases in upstream expression levels have focused the need for downstream solutions that offer both increased productivity and improved economics without sacrificing process robustness. In this presentation we explore the advantages of continuous chromatography for affinity-based mAb capture where substantial productivity increases were observed. Additionally, a fully integrated flow-through downstream process post-protein A through virus filtration was evaluated using only single-use technologies, where the flow-through process was designed to robustly remove both process- and product-related impurities. Experimental data for both steps that highlight the technology’s performance will be presented. Finally, a case study will be shown where the technologies are successfully combined enabling a continuous, integrated downstream process from capture through virus filtration.

Increasing the Efficiency of a Process Development Laboratory under GMP Requirements through Operational Excellence Initiatives. David Mead, Lisandra Negron Vega, Omayra Rivera Denizard, Alejandro Toro, James Weidner, Jose Hector Rivera, Amgen, Juncos, PR, USA

In a GMP process development laboratory, procedures lay out the operational steps. Procedures are in place to specify requirements for such activities as experiment documentation, data storage, raw material inventory, handling of unexpected results, equipment lifecycle, and sample management. Compliance with these procedural requirements can affect experiment execution timelines and associated costs. To manage the impact of increased timelines on resource requirements when transitioning to GMP and/or GLP operations, laboratories must implement optimization strategies to increase productivity and reduce cost. This communication illustrates an example of how a purification process development laboratory applied operational excellence principles to small-scale purification operations in order to increase productivity. The team achieved increased productivity through (1) increasing the success rate by approximately 3% and (2) decreasing cycle time by 11%. To increase the operations productivity while working under GMP/GLP requirements, laboratory processes and procedures were modified to implement additional preventive measures. Some of these measures focused on equipment calibration, verification and preventive maintenance to further assess equipment functionality prior to experiment execution, as well as utilizing improved operation procedures related to staff qualification, data collection, and checklists. In addition, to facilitate continuous improvement, the operations incorporated multiple initiatives such as in-depth discussions for problems encountered, daily debriefing meetings, and learning/knowledge sharing forums to proactively identify and resolve new issues and opportunities before failure occurs. Lean tools were implemented to eliminate non-value added operation activities, which resulted in a 60% reduction in documentation requirements. In summary, the implementation of operational excellence initiatives into Process Development laboratories operating under GMP/GLP requirements can significantly increase laboratory efficiency and create a highly reliable organization. Additional tools such as value-stream mapping will be used in the future to further reduce cycle time.

Molecular Insights into Multimodal Interactions: Comparison of Selectivity between Different Ligands and Fluid Phase Modifiers. Siddharth Parimal, Melissa Holstein, James Woo, Shekhar Garde, Steven Cramer, Rensselaer Polytechnic Institute, Troy, NY, USA

Multimodal (MM) chromatography has proved to exhibit enhanced selectivities as compared to traditional chromatographic systems. However, current theoretical understanding of the underlying principles governing the MM interactions leaves a lot to be desired. In the work presented here, all-atom explicit Molecular Dynamics (MD) simulations have been employed to shed light on the multiple weak interactions that result in the unique selectivities achieved in MM chromatographic systems. Protein-ligand interactions between two ligands with similar chemical moieties but different structural arrangement were analyzed to understand the mechanism behind “synergism” present in these interactions. Special emphasis was provided to the role of water and how it mediates these multiple weak interactions resulting in regionally-selective non-conformational pseudo-affinity interactions. The knowledge from these simulations was used to deconvolute the synergistic MM interactions into its key contributors. This was coupled with protein surface characterization techniques to evaluate the strength and importance of electrostatic and hydrophobic interactions in these systems. Simulations were also performed to study the effect of fluid phase modifiers on protein-ligand binding. Previous work in our group on retention behavior of a library of proteins in MM chromatographic systems had suggested protein-specific effects of these cosolvents. This was probed further by performing a series of simulations with fluid phase modifiers to evaluate the interactions of these cosolvents with proteins and how they enhance/reduce protein-ligand binding. The results presented in these studies can be used to not only explain the protein retention behavior in MM
chromatographic systems, but also to design specific ligands and fluid phase conditions for challenging bioseparations.

P-113  Cadence Evaluation of Biologics Next-generation Processes using VirtECS® Plant Models. Nuria de Mas1, Dario Frisardi1, Michael Farragher1, Andrew Plummer1, Derrick Schertz2, Susan Abu-Absi1, Luis Maranga1, 1Bristol-Myers Squibb Company, Devens, MA, USA; 2Advanced Process Combinatorics, West Lafayette, IN, USA

Scale-down laboratory models and plant modeling tools are essential to analyze the value of process improvements for commercial biologics manufacturing processes at plant scale. We present a methodology to evaluate next-generation manufacturing batch processes of a commercial fusion protein by using scale-down models and two pieces of software to investigate the impact of both process and plant configuration on debottlenecking, plant cadence, and manufacturing cost. This initiative was intended to enhance process robustness and maximize plant throughput over the commercial product lifecycle. First, a scale-down model was used to generate a detailed process description and scale-independent processing times for all unit operations. Plant cycle times for each unit operation were then obtained by adding equipment, media, and buffer preparation times as well as cleaning times. With these inputs, a customized VirtECS® scheduler (Advanced Process Combinatorics, Inc.) was used to identify manufacturing bottlenecks and determine batch run time, plant cadence, number of batches in the manufacturing train, and labor demand for multiple manufacturing upstream and downstream scenarios. Finally, the process resource analyzer SuperPro Designer® (Intelligen, Inc.) was used to pinpoint the cost drivers associated with the current and improved processes. It was found that batch runtime, plant cadence, and cost of goods can be significantly reduced with relatively low risk while freeing up valuable plant space. This approach may be used to facilitate process technology transfer and commercialization of other biologics in the future.

P-114  Purification of Proteins from Human Blood Plasma by Multicolumn Solvent Gradient Purification. Daniel Baur1, Thomas Mueller-Spaeth2, Massimo Morbidelli1, ETH Zurich Institute for Chemical and Bioengineering, Zurich, SWITZERLAND; 2ChromaCon AG, Zurich, SWITZERLAND

Proteins isolated from human blood plasma are an important class of therapeutics used in management, treatment or prevention of potentially life-threatening conditions and/or diseases caused by trauma, infections or genetic deficiencies [1]. Recently, chromatography has been increasingly used in blood plasma fractionation. However, most of the applications are limited to batch purifications of a side stream of the historic Cohn process, using affinity chromatography in some cases [1-4]. When dealing with complex mixtures, where no baseline separation is possible, preparative non-affinity batch chromatography suffers from an inherent purity-yield tradeoff. In these cases, Multicolumn Solvent Gradient Purification (MCSGP) offers a way to achieve both high purity and high yield at the same time [5]. MCSGP has been successfully used to purify biomolecules from complex biological mixtures, e.g. cell culture supernatant [6]. In this work, a novel twin-column MCSGP process was applied in two cases to isolate the proteins alpha1-Antitrypsin, and Antithrombin III, respectively, from human blood plasma with high yield and purity. The design of the MCSGP process and the advantages with respect to batch chromatography processes are outlined. Furthermore, the positioning of the process in a typical Cohn fractionation cascade is discussed. [1] T. Burnouf, Transfusion Medicine Reviews 21 (2007) 101. [2] E.J. Cohn, L.E. Strong, W.L. Hughes, D.J. Mulford, J.N. Ashworth, M. Melin, H.L. Taylor, Journal of the American Chemical Society 68 (1946) 459. [3] T. Burnouf, M. Radosevich, Journal of Biochemical and Biophysical Methods 49 (2001) 575. [4] T. Burnouf, Journal of Chromatography B-Biomedical Applications 664 (1995) 3. [5] L. Aumann, M. Morbidelli, Biotechnology and Bioengineering 99 (2008) 728. [6] T. Muller-Spaeth, L. Aumann, G. Strohlein, H. Kornmann, P. Valax, L. Delegrange, E. Charbaut, G. Baer, A. Lamproye, M. Johnck, M. Schulte, M. Morbidelli, Biotechnology and Bioengineering 107 (2010) 974.

P-115  High-gradient Magnetic Fishing (HGMF): Radical or Merely Separatist? Owen R.T. Thomas1, Matthias Franzreb1, University of Birmingham Edgbaston, Birmingham, UK; 2Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, GERMANY

High-Gradient Magnetic Fishing (HGMF), a robust capture technology developed in our laboratories over a decade ago, has the potential to overcome numerous barriers associated with traditional lower throughput unit adsorptive operations. This technique combines the adsorption of the product on to cheap customized magnetic particles, with the subsequent separation of the product-loaded support from the non-magnetic components using high-gradient magnetic separation (HGMS) technology. The non-porous magnetic supports employed in HGMF have major advantages over the macro-porous adsorbents commonly used in packed and expanded bed chromatography. Apart from very rapid sorption kinetics, non-porous support particles are less prone to fouling and are easier to clean than their porous counterparts and are therefore potentially more useful in purification from fouling feed streams. In this presentation we chart the development of the technique, from its humble origins in the minerals industry to its current state of development, poised on the brink of commercialization and
Automated Method Development Strategy for Counter Current Chromatography. David Thornton¹, Gary Yanik², Xiqin Yang¹, Leo Hsu¹, GlaxoSmithKline, King of Prussia, PA, USA; ²PDR Chemical Inc, Lake Park, FL, USA

The choice of an appropriate combination of stationary phase and mobile phase is essential in any purification techniques. Unfortunately, for counter current chromatography (CCC), the major drawback is the laborious effort needed to successfully select solvent system. In this talk, we will demonstrate a custom configured method development system to execute solvent scouting process in an automated fashion. We will also present case studies to illustrate an efficiency gain of three-fold, when compared to our previous approach.

Efficient Separation of Antibody Light Chains from Bi-specific Antibody Monomer using Mixed-mode Sorbents. Audrey Uzel, Jerome Champagne, Magali Toueille, Pali Life Sciences, Cergy Saint Christophe, FRANCE

Bi-specific antibodies are currently considered as one of the most promising class of next generation therapeutic molecules and in the coming years a growing number of such products are expected on the market. Purification of bi-specific antibodies presents unique challenges compared to that of monoclonal antibodies, and chromatographic sorbents will therefore have to answer those specific requirements. In the present study, the use of mixed-mode sorbents as capture step for the purification of a bi-specific antibody was investigated, with specific focus on the separation of the abundant antibody fragments (light chains) from the monomeric form. Three mixed-mode sorbents were evaluated for their performance in antibody light chains elimination. Our data indicated that two out of the three evaluated sorbents allowed efficient removal of antibody light chains thanks to the possibility to selectively elute antibody from the column while retaining fragments on the resin. Up to 97% pure monomers were recovered after only one purification step using mixed-mode sorbents while the initial monomer purity was about 30%. Concomitantly, satisfying recovery and efficient CHOP removal (> 1 log) were obtained. Altogether, those data reveal that mixed-mode chromatography is a powerful tool to address the future challenges of purification of the growing bi-specific antibody class of biomolecules.

Maximizing the Functional Lifetime of Protein A Resins. Jennifer Zhang¹, Ryan Caple², Lynn Conley², Sanchayita Ghose², Biogen Idec, Research Triangle Park, NC, USA

Protein A chromatography is currently the industry gold-standard for monoclonal antibody and Fc-fusion protein purification. The high cost of Protein A however makes resin lifetime and resin reuse an important factor for process economics. Most industry resin lifetime studies usually examine the effect of resin re-use on binding capacity, yield, and product quality without answering the fundamental question of what is causing the decrease in performance. A two part mechanistic study was conducted in an attempt to decouple the effect of the two possible factors (resin hydrolysis and/or degradation vs. resin fouling) on column performance over lifetime of the most commonly used base-stable Protein A resin (MAbSelect SuRe). We examined the change in binding capacity (rate of hydrolysis) upon exposure to various cleaning agents at different temperatures. Additionally, binding and resin extraction studies were conducted with these cleaning agents to determine their cleaning effectiveness (resin fouling). Results from these studies showed the NaOH solutions had the most effective cleanability at the expense of higher hydrolysis rates. Cold temperature had a stabilizing effect on resin hydrolysis while providing comparable cleaning ability to ambient temperature. An effective cleaning strategy will be presented that helped to maximize resin lifetime without any decrease in column performance. This work provides a better understanding of the impact of various cleaning agents on Protein A resin lifetime and can lead to more robust and optimal cleaning studies for future manufacturing processes.

High Mass Loading of Peptides with Hybrid Particle C18 Columns and Acetic Acid Mobile Phases. Mathew Lauber, Stephan Koza, Kennet Fountain, Waters Corporation, Milford, MA, USA

Peptides have proven to be very useful as both therapeutic agents and tools for research. It is common to purify peptides for these purposes by preparative reversed phase (RP) chromatography. Typically, peptides are separated using mobile phases containing strong ion pairing agents, such as trifluoroacetic acid (TFA). Trifluoroacetate is, however, not an acceptable counter ion for pharmaceutical peptides, or peptides intended for cell based studies. So, although TFA is ubiquitous in reversed phase peptide separations, its use requires additional steps to ensure that trifluoroacetate is subsequently removed from peptide samples. Due to its lower toxicity, acetate is the preferred counter ion for many peptide pharmaceuticals. It would seem advantageous
then to avoid TFA when possible and instead use acetic acid (HOAc) mobile phases, such that the desired peptide and counter ion can be obtained with fewer steps. In this presentation, we will demonstrate the applicability of two different hybrid particle C18 column chemistries, BEH C18 and CSH C18, to preparative separations. BEH C18 is an organo-silica C18 stationary phase, based on ethylene bridged hybrid technology, and is noted for its robustness and pH stability. CSH, or charged surface hybrid, C18 is an evolution of BEH C18 in that it is modified to contain a low level positive surface charge under acidic conditions. Loading studies performed with a number of different peptides and preparative-scale 5 μm particles have shown that both BEH130 C18 and CSH130 C18 are well suited to preparative peptide separations with either TFA or HOAc containing mobile phases. CSH130 C18, with its positive surface charge, is particularly noteworthy as it tends to provide improved loadability, generally narrower target peaks, and in turn potentially less target peptide-impurity co-elution. These studies have additionally revealed that these two unique stationary phases exhibit different selectivities and optimal separation conditions that may be used to advantage for resolving challenging impurity-target peptide profiles. More remarkably, both BEH and CSH columns have been found to yield narrower target peptide peaks and greater resolution for preparative loads of a low purity synthetic peptide when using mobile phases with optimized HOAc concentrations versus 0.1% TFA. In summary, the use of hybrid particle C18 and acetic acid mobile phases may provide a significant benefit for the preparative chromatography of peptides, particularly since a streamlined purification process involving HOAc mobile phases would eliminate the need for removal of TFA.

P-120 A Novel Affinity Chromatography Resin for the Capture of G-CSF A Novel Affinity Chromatography Resin for the Capture of G-CSF. Anna Wilborg, Henrik Neu, Hanna Wlad, GE Healthcare, Uppsala, SWEDEN
The last decade’s success of monoclonal antibodies has triggered the need for new and efficient purification platforms, in both the discovery and manufacturing phase, to ensure maximum time savings and optimized process economy at small and large scale. This requirement from the industry has also raised the question regarding how new and second generation targets should be purified; by several and conventional combinations of ion exchange and hydrophobic interaction chromatography steps or by one highly efficient capture affinity step followed by one or two polishing steps? The Custom Designed Media (CDM) department within GE has developed several new affinity resins suitable for large scale purification of new and second generation molecules such as Kappa and Lambda light chains containing targets as well as different recombinant plasma proteins, e.g. Factor VII, Factor VIII and Alpha-1 Antitrypsin. GCSF (granulocyte-colony stimulating factor) is a hormone stimulating the bone marrow to produce more white blood cells. GCSFSelect is an affinity resin specifically designed for the purification of G-CSF targets from recombinant feed streams. It is based on a highly rigid agarose base matrix allowing for high flow rates and low back pressures at large scale. The base matrix has been modified with a single-chain antibody fragment as the affinity ligand via a hydrophilic spacer arm to facilitate the binding and elution of the target molecule. The ligand was developed based on the BAC B.V. technology and specifically screened to bind and elute G-CSF targets in the presence of other proteins from different expression systems. Specific features of this new chromatography resin along with results from the application work, performed on columns and on 96-well filter plates, are presented. Approaches for the capture step with high purity and yield are proposed.

P-121 Thermodynamics of Small Molecule Adsorption onto Multimodal Resin. Rebecca Desch^1, Kartik Srinivasan^2, Sophie Karkov^2, Melissa Holstein^2, Steven Cramer^2, Stephen Thiel^1. ^1University of Cincinnati, Cincinnati, OH, USA; ^2Rensselaer Polytechnic Institute, Troy, NY, USA
By combining multiple modes of separation into one unit operation, multimodal resins can improve biomolecule separations. While the efficiency enhancements of multimodal resins are known, the underlying mechanisms of biomolecule adsorption onto these resins are elusive. One popular resin, Capto MMC, contains both hydrophobic (phenyl) and polar (carboxyl, amide) functionalities on an agarose matrix. Three small molecules were chosen to probe the functionality of Capto MMC: tryptophan, sodium caprylate, and diphosphate chloroquine. Previously, the adsorption of tryptophan has been associated with nonpolar interactions and the adsorption of sodium caprylate has been associated with electrostatic interactions with Capto MMC. Diphosphate chloroquine has been observed to selectively displace proteins from a multimodal resin. The real-time dynamic heats of adsorption of these materials onto a bed packed with Capto MMC were measured quantitatively using flow microcalorimetry. Tryptophan adsorption on Capto MMC in 0.02 M acetate buffer pH 5 was exothermic while the adsorption of sodium caprylate and chloroquine diphosphate were endothermic. The adsorption of tryptophan and sodium caprylate were associated with sharp peaks in the thermogram, while the diphosphate chloroquine peak was diffuse, indicating kinetic limitations. Hydrophobic interactions were isolated by attenuating electrostatics with concentrated salt. Increasing the salt concentration from 0.02 M to 0.75 M had no effect on tryptophan adsorption but attenuated the sodium caprylate endotherm. The free energy of adsorption was obtained from isotherm measurements and combined with the measured adsorption enthalpy to develop a

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thermodynamic summary of the adsorption with mechanistic insights. Calculated entropy values can be confirmed by varying the temperature (22°C and 35°C). By probing the thermodynamic properties of small molecule adsorption onto modiomolar phase, valuable mechanistic insights for process design are uncovered.

P-122  Twin Column CaptureSMB: A Novel Cyclic Process to Increase the Capacity Utilization in Protein A Chromatography. Monica Angarita¹, Daniel Baur¹, Thomas Müller-Späth¹,², Roel Lievrouw³, Geert Lissens³, Guido Ströhlein², Massimo Morbidelli¹, ETH Zurich, Zurich, SWITZERLAND; ²CHROMACON AG, Zurich, SWITZERLAND; ³JSR Life Sciences, Leuven, BELGIUM

Protein A based purification of monoclonal antibodies (mAbs) is the preferred purification platform in industry for capture of mAbs from cell culture harvest, due to its high yield and specificity. The use of multi-column, cyclic processes for mAb affinity capture has gained significant interest in the last years. However, the available process solutions require at least 3-4 columns with accordingly increased hardware complexity and downtime probability. To overcome these drawbacks and increase the resin utilization, a novel twin-column countercurrent sequential capture process has been developed. In this work we introduce the method design as well as experimental results using AmSpHERE Protein A JWT203, showing the significant advantages over batch chromatography in terms of higher column loading, higher productivity and lower buffer consumption. Simulation results are also presented, showing a performance comparison to conventional 3- and 4-column capture processes.

P-123  Taking PEG Solvent Modulation to the Next Level. Sebastian Neumann, Roche Diagnostics GmbH, Pennington, GERMANY

Cation exchange chromatography is an integral part of many downstream processes for therapeutic antibodies. Usually, the removal of antibody aggregates can be sufficiently accomplished by this chromatographic method. Nevertheless, for some antibodies the resolution of monomers and aggregates is insufficient. In these cases it is an opportunity to employ polyethylene glycol (PEG) solvent modulation in order to improve resolution, a method that has been known for many years: PEG is excluded from protein surfaces as well as the surface of chromatographic stationary phase, resulting in preferential hydration of the protein as well as the stationary phase. Under these conditions the adsorption of the protein to the stationary phase is thermodynamically favored, leading to an increased retention volume of the proteins. Since the increase in retention is dependent on protein size, the resolution of antibody monomer and aggregates increases. This concept is generally applicable for the improvement of separation of protein species that differ in size. A limitation to this method is that on one hand highest resolutions are achieved at high PEG concentrations and on the other hand high PEG concentrations induce protein precipitation. Therefore only moderate PEG concentrations are applicable. We recently identified a way to resolve this dilemma, resulting in a chromatographic method with superior resolution performance: We conducted cation exchange chromatography experiments with a mobile phase containing high PEG concentrations in the presence of certain sugars, polyols or amino acids ("solubility enhancers") and observed significantly improved resolution performance. This method has already been tested with several antibodies and different cation exchange resins. It allows for a superior antibody monomer-aggregate separation in a generic manner and has also been successfully applied in a scale-up experiment. In one case study the separation of antibody monomers and aggregates by cation exchange chromatography under standard conditions was hardly possible, since the resolution was as low as 0.05. By employing classical PEG solvent modulation the resolution could be increased by a factor of 4 to 0.21. In this example the newly identified solubility enhancer/PEG method led to a 10-fold increase in resolution (0.52).

P-124  Development and Application of a High Throughput Protein Solubility Workflow. Gregory Barker, Brian O’Mara, Sibylle Herzer, Siegfried Rieble, Bristol-Myers Squibb, Pennington, NJ, USA

High throughput process development (HTPD) techniques are integral to reduce process development time. At Bristol-Myers Squibb, HTPD techniques such as plate-based resin screening and robotic mini-column screening have successfully shortened development time requirements. Establishing a protein solubility map is desirable to determine the window of opportunity for downstream unit operations prior to initiating the screening experiments. BMS’ comprehensive HTPD strategy includes a high throughput buffer preparation and buffer exchange workflow to assess protein solubility. To date, most sample preparation or solubility mapping techniques have significant gaps in methodology and throughputs. We present a custom workflow using an ÄKTATM Avant to enable rapid buffer preparation and protein desalting for use in high throughput solubility, purification, and formulation experiments. Protein solubility maps are established in several minutes per condition with minimal hands on time and high reproducibility. Robotic liquid handlers can be used to create buffers, but those methods are unable to trace pH and conductivity in-line and do not offer methods for high throughput buffer exchange of the protein. This approach has the unique advantage of affording both seamless preparation of buffer maps based on acid, base, and salt blending over a wide range of conditions in combination with gentle, traceable, automated introduction of the sample into the new buffer system. The ability to trace pH, conductivity, and
absorbance enables in-process verification of buffer composition as well as preliminary verification of protein compatibility with each condition. This methodology allows us to prepare a matrix of proteins solutions while generating the buffers needed for high throughput experimentation. It also serves to debottleneck other HTPD workflows which are strictly dependent on the use of robotic liquid handling systems. Using the described high throughput buffer preparation and desalting method, we explored the solubility map of a monoclonal antibody to improve protein solubility and stability during and after a final tangential flow filtration (TFF) unit operation. Using several orthogonal high throughput analytical techniques (absorbance (A280nm and A320 nm), dynamic light scattering, and analytical size-exclusion chromatography), we were able to rapidly screen 144 buffer conditions for protein solubility and aggregation propensity. The results of this screen were successfully applied to a scale-up TFF experiment to improve process conditions. Through this work, we have developed a high throughput sample preparation and solubility workflow and successfully demonstrated its utility in downstream process development.

Quality packing methods for large scale chromatography columns are a critical component of successful manufacturing. This case study pertains to the development of a packing procedure for a large scale column that utilizes a compressible resin over multiple cycles. The columns were packed using axial compression. A design of experiments approach was followed using smaller diameter columns to investigate the impact of several key packing parameters on performance, including different levels of compression, buffers of varying ionic strength and linear velocities. The results were subsequently evaluated using a larger diameter column. Comparisons are presented to elucidate the impact of input factors on the efficiency, asymmetry, and permeability of the packed beds. The results were correlated using the Van Deemter and Darcy models for efficiency and permeability, respectively.

P-126 Characterization of Protein Interactions on Mixed Mode Chromatography and Augmenting Selectivity Using Selective Mobile Phase Modulators. Leslie S. Wolfe, Abhinav A. Shukla, KBI Biopharma, Inc., Durham, NC, USA
Mixed-mode chromatography resins are increasingly being incorporated into preparative purification processes. Mixed-mode resins have the ability to interact with target proteins through multiple types of interactions including electrostatic interactions, hydrophobic interactions and hydrogen bonding depending on the ligand. Further selectivity can be obtained through the use of modulators in buffers during the product load, wash or elution phase of the process step. Here, the mixed-mode Capto MMC resin is described in terms of its protein adsorption characteristics by using a set of four monoclonal antibodies and two non-IgG model proteins. Linear gradient elution experiments were executed to compare the elution conditions required for each antibody and non-IgG model protein at three pHs in the presence of several mobile phase modulators. For each protein, experiments performed at pHs farther from the protein isoelectric point resulted in tighter protein:resin interactions. Additionally, in the presence of modulating agents, varying binding and retention were observed that were in-line with the known abilities of these modulators to reduce different kinds of interactions (electrostatic, hydrophobic, hydrogen bonding etc.). The influence of mobile phase modulators on linear retention was characterized by the use of log $k'$ vs. log (salt concentration) plots in the presence of various agents. These studies enabled a classification of the key interactions that drove retention for different types of proteins. The results of these studies characterizing fundamental interactions on this mode of chromatography were employed to develop highly selective wash steps. The use of combinations of some of these mobile phase modulators in a wash step were found to augment HCP clearance by $> 5$ fold in comparison with a conventional wash step. The results presented in this study can significantly enhance selectivity that can be obtained during protein separations on this mode of chromatography and offer the enticing possibility of creating a pseudo-affinity separation using a non-affinity chromatographic stationary phase.

P-127 Evaluating the Performance of Different Multicolumn Setups for Chromatographic Separation of Proteins on HIC Media. Roman Bochenek, Wojciech Marek, Wojciech Piatkowski, Dorota Antos, Rzeszow University of Technology
The effectiveness of isolating a target protein from a multi-component protein mixture using different column arrangements has been analyzed. Different continuous systems have been considered capable of performing the solvent gradient separation: open loop simulated moving bed (OL-SMB), multicolumn countercurrent solvent gradient purification (MCSGP) and its modifications and carousel multicolumn setup (CMS). The first system is based on the standard SMB system but the regeneration zone of the liquid phase is eliminated, the second one exploits the internal countercurrent recycling of the solid phase whereas the liquid phase loops can be open after each or some of zones. CMS refers to continues systems in which feed and desorvent streams are delivered.
subsequently through parallel columns to mimic their countercurrent movement with respect to the fluid flow. As a case study separation of a ternary mixture of proteins on HIC media has been selected. The target protein of the separation was either the most strongly adsorbed component of the mixture or exhibited intermediate adsorption strength. The performance of the continuous processes has been examined and compared to a single-column batch system. Two conflicting performance indicators have been considered in the multi-objective optimization routine such as yield and productivity. A mathematical model has been used to simulate the process dynamics and to optimize operating conditions for the feed loading and separation. Different systems have been ranked with respect to both objective functions. Moreover, the eluent consumption has been considered. Evaluating the performance of different multicolumn setups for chromatographic separation of proteins.

P-128 Purification of Cell Culture-derived Influenza Virus via Continuous Size Exclusion Chromatography. Tina Kröber, Laura M. Fischer, Michael W. Wolff, Udo Reichl, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, GERMANY

In vaccine production downstream processing often constitutes a bottleneck in terms of productivity and economy. Established separation techniques either lack in selectivity (and resolution) like density gradient centrifugation or are limited in throughput and scalability as for batch chromatography (BC). One way to design more efficient purification trains could be the implementation of continuous chromatographic methods. In this study, continuous size exclusion chromatography (SEC) in cell culture-based influenza vaccine production was investigated using a simulated moving bed (SMB) approach. Experiments were performed in an open loop configuration at different operation conditions using one or two columns per zone. For some experiments, the product fraction was subsequently passed through an anion exchange column (Capto Q®, GE Healthcare) to reduce the level of remaining host cell DNA contaminations. The separation performance was evaluated for virus yield (hemagglutination assay) as well as for total protein (Bradford) and for host cell DNA (PicoGreen®) contamination. Finally, yield, purity and productivity of the SMB process were compared to BC operated under corresponding conditions. Similar yields of influenza virus were obtained for SMB and BC in the product fractions whereas purity in terms of the level of contaminating protein (related to the amount of virus) of the SMB process did not match the results obtained for BC. However, the calculated amount of total protein per vaccine dose met the criteria required for human influenza vaccines prepared in cell culture for both methods (< 100 µg per strain and dose). Further improvements would, however, increase process robustness with respect to batch to batch variations in upstream processing. Nevertheless, it could be shown that SMB surpasses BC in terms of productivity. The host cell DNA could not be removed via SEC since it co-eluted with the virus. The combination of SMB-SEC and Capto Q® chromatography, however, resulted in an overall DNA depletion of more than 99 %. However, DNA levels in the product fractions still exceeded the level required for production of influenza vaccines for human use (< 10 ng per dose). Nevertheless, a further depletion of DNA could be achieved by treatment with Benzonase®, a unit operation which is typically implemented in the process train for DNA fragment reduction and to reduce the risk of viral cross contaminations. Altogether, the results indicate that SMB-SEC in combination with subsequent DNA capturing by anion exchange chromatography is a promising method for the purification of cell culture-derived influenza vaccines.


During the development of a therapeutic human monoclonal antibody we detected an unusual product-related impurity, a MAb containing a third light chain, which was not removed using our purification platform consisting of Protein A, anion exchange, and cation exchange (CEX) chromatographies. Through extensive resin screening studies we determined that substituting hydrophobic interaction chromatography (HIC) for CEX was effective in removing this MAb species. In an effort to optimize the separation conditions on the HIC resin, we performed early optimization experiments in a high throughput (HTP), 96-well plate, format. The results from the HTP optimization experiments were confirmed using chromatography columns indicating the scalability of this process from HTP plates to columns. Based on the data collected, we have established an antibody polishing process capable of removing 94% of the product-related impurities yielding 99% pure MAb monomer with a step recovery of greater than 80%.

P-130 Predicting Resolution in pH-gradient Protein Chromatography using Mixed-beds of Weak and Strong Anion Exchangers. Tarl Vetter1, Giorgio Carta1, Gisela Ferreira2, David Robbins2,1 University of Virginia, Charlottesville, VA, USA;2 MedImmune, Gaithersburg, MD, USA

Protein charge variants were resolved using induced pH gradients created on mixed-bed columns containing a small-pore, weak base resin and a large-pore, strong base resin. The mixed-bed design effectively decouples the pH gradient generation and protein binding into separate resins, removing the impact of protein loading on
gradient behavior and allowing the two processes to be modeled independently. Consequently, protein resolution can be improved by optimizing the weak resin/buffer combination for pH gradient generation, and the strong resin/protein interaction for selectivity, individually. A model based on a small number of experimentally-determined parameters provided predictive capabilities for both pH gradient generation and protein retention without the use of fitting parameters. The induced pH gradients, and hence protein resolution, were tuned by adjusting the concentration of weak resin in the bed, or by changing the concentration and composition of the mobile phase buffer. Experimental results obtained for the separation of mAb charge variants using mixed-beds using the weak base resin AG 4-X4 and either SOURCE 30Q or UNOsphere Diol Q as the strong base resin, show good agreement with model predictions.

P-131 Evaluation and Performance Comparison of New Core-Shell Media vs. Fully Porous Media for Preparative Purifications. Marc Jacob, Wayne Daneker, JT Presley III, Simon Lomas, Jeff Layne, Phenomenex, Torrance, CA, USA
High performance HPLC/UHPLC core-shell material is the latest technological advancement in chromatographic media. When used under analytical conditions, core-shell particles show improved efficiency and performance over fully porous particles of equivalent particle size.
With the recent commercialization of a low pressure 5 µm core-shell media, it is now possible to offer core-shell media in a preparative format (>20 mm ID) that's compatible with standard prep LC systems. In this poster, we will demonstrate that this new 5 µm core-shell particle size, available in a variety of bonded phases, can be packed efficiently in a preparative format with internal diameter greater than 2 centimeters. Additionally, we will highlight the advantage of such product and discuss applications that are pertinent for small scale drug discovery and peptide purification.

P-132 Novel Affinity Resin using an Engineered Human Fc Receptor as a Ligand for Antibody. Teruhiko Ide1, Toru Tanaka1, Yoshiharu Asaoka1, Hidetaka Kobayashi1, Koji Nakamura1, Kouta Hayataya1, Franziska Katzenwadel1, Judith Vajda5, Egbert Müller5, Tim Schröder5, Tosoh Corporation, Ayase, JAPAN; 2Tosoh Corporation, Shunan, JAPAN; 3Sagami Chemical Research Institute, Ayase, JAPAN; 4University of Stuttgart, Stuttgart, GERMANY; 5Tosoh Bioscience GmbH, Stuttgart, GERMANY; 6Atoll GmbH, Stuttgart, GERMANY
We developed a new affinity ligand engineered from human Fcγ receptor I (FcR). FcR was the only high-affinity receptor for human IgG1. FcR recognized the Fc portion of human IgG1 and human IgG3 with high affinity. The binding site of FcR was located on the hinge region of human IgGs. The static binding capacity of FcR-resin prepared from Toyopearl™ chromatography medium showed 60 mg of human IgG1/ml settled resin and 40 mg of human IgG3/ml settled resin. The dynamic binding capacity of FcR-resin varied depending on several factors, such as target antibody and flow rate. FcR-resin allowed high yields of human IgG1 at a moderate elution pH, and showed effective CHOP clearance using selected washing buffer containing some additives.

P-133 Silica Surface Hybrid Material with Chemical Stability Over a Wide pH Range for Reversed Phase Chromatography. Fredrik Limé, Robert Fredriksson, Johan Lif, Per Jageland, AkzoNobel, Bohus, SWEDEN
Silica is widely used as a stationary phase base material in liquid chromatography, with superior mechanical stability, and ensuring high efficiencies. The chemical stability of reversed phase silica materials is often limited to pH between 2 and 8. At low pH the siloxane bond between the organosilane and the silica matrix will break, thus releasing free silanes, while high pH dissolves the silica matrix. In this new hybrid material the silica matrix was reinforced with organosilane, to create an interfacial gradient that maintains the superior mechanical stability and pore structure. This hybrid silica material were further derivatized with n-octadecylsilane (C18) and end-capped, to completely shield the silica matrix from silanol interactions. The selectivity and efficiency was compared with, and tested against, classic C18 bonded phases. The chemical stability of the material was evaluated using accelerated stability tests at elevated temperatures, using trifluoroacetic acid for low pH hydrolysis, and phosphate or carbonate buffers to hydrolyze the silica stationary phase at pH above 10. The resistance to high pH under extreme alkaline conditions was proved by regeneration procedures using 0.1 – 0.5 M NaOH, followed by testing column performance with anti-depressants, since they are highly affected by silanol interactions in terms of increased retention times and peak asymmetry.

P-134 Predicting Protein Adsorption Equilibria on Ion-exchange Resins. Rushd Khalaf1, Matteo Costioli2, Massimo Morbidelli1, 1ETH Zurich, Zurich, SWITZERLAND; 2Merck Serono S.A., Fenil-sur-Corsier, SWITZERLAND
Due to the recent advances in upstream processing, the chief portion of the cost involved in the development of production processes for pharmaceutical drugs is now associated with downstream processing. More specifically, the vast majority of purification costs are directly linked to chromatography. The design of these chromatographic steps is often done with respect to a classical 3-step platform (i.e. a Protein A capture followed by two additional ion exchange chromatography steps), which is slightly modified to suit the needs of each new
P-135  A Novel Automated Enrichment Process for the Isolation of Product-related Impurities from Active Pharmaceutical Ingredients. Thomas Müller-Späth, Nicole Ulmer, Lars Aumann, Guido Ströhlein, Michael Bavand, ChromaCon AG, Zurich, SWITZERLAND

Isolation of product-related impurities in useful quantities for pre-clinical analysis is often tedious and requires sophisticated isolation strategies. A cyclical, continuous, twin-column chromatography process (N-rich™ technology) for the isolation of product-related impurities was developed and shown to be highly efficient in isolating individual impurities in useful quantities at preparative scale. This process features simultaneous enrichment of product-related impurities and concurrent depletion of the main product, therefore allowing the isolation of highly pure and concentrated product-related impurities. The process was experimentally verified for the isolation of closely eluting strongly and weakly adsorbing product-related impurities of synthesized Fibrinopeptide A using reversed phase chromatography with a twin column Contichrom® skid. The performance of the cyclical continuous process was qualitatively compared to the performance of single column batch chromatography. This novel process principle can be operated with any kind of mode or media that is used in single column batch chromatography. Its potential lies in application fields such as lead isolation in drug discovery, product-related impurity isolations in drug development (impurity profiling), and the isolation of highly pure active pharmaceutical ingredients (API), such as synthetic peptides.

P-136  Crude to Pure - Solid Compounds from a Machine. Bob Boughtflower⁴, Przemek Stasica², Tim Underwood⁴, Yosuke Iwata³, Tomoyuki Yamazaki¹, Junichi Masuda⁴, Tsutomu Okoba³, Tsuyoshi Morikawa³, Neil Loftus⁴, Masayuki Nishimura⁴, Robert E. Buco, III³, GlaxoSmithKline, Stevenage, UK; Shimadzu UK, Stevenage, UK; Shimadzu Corporation, Kyoto, JAPAN; Shimadzu Corporation, Manchester, UK; Shimadzu Corporation, Columbia, MD, USA; Shimadzu Corporation, Marlborough, MA, USA

The currently employed multiple-step process for drying down preparative HPLC fractions to a dry powder ready for use or storage can be inefficient, laborious and suffers from several bottlenecks preventing higher throughput. The relatively large fraction volumes with high aqueous content frequently require long cycle times to recover the dried powder. In addition, anything not sufficiently volatile/evaporable in the mobile phase will concentrate down with the sample leading to a loss of mass purity, especially water and common mobile phase additives such as formic acid and TFA. Accumulation of additives in the dried sample can lead to "weighing errors," which will propagate into the preparation of a known-concentration solution. In addition, HPLC additives, both chemically bound with a compound or as an unbound excess, may be unfavourable in further activity testing. In this paper we will describe the development of a new instrumentation platform designed to process preparative HPLC fractions to high purity dry powders, free from HPLC background and available in as few as three hours after the HPLC run, regardless of fraction volume or water content.

P-137  Design Space for a Countercurrent Chromatography Protein Purification Unit. Fabian Steinebach¹, Martin Krätli¹, Thomas Müller-Späth², Massimo Morbidelli¹, ETH Zurich, Zurich, SWITZERLAND; ChromaCon AG, Zurich, SWITZERLAND

For all chromatography processes the operating parameters and the physico-chemical properties of the compounds to be separated as well as column characteristics affect the separation in terms of yield and product quality (purity). As described in the PAT initiative and ICH guidelines, process understanding is necessary to define operating parameter ranges ensuring product quality and process robustness. Process understanding can be demonstrated by developing and validating a mathematical chromatography model that includes the relevant operating parameters. As shown in the case of countercurrent column chromatography (SMB (simulated moving bed) process), equilibrium theory can be used to determine regions for operating parameters where the separation task is accomplished. This presentation shows how this concept was transferred from SMB to twin-column MCSGP (multi-column countercurrent solvent gradient purification). MCSGP is a process...
that is capable of addressing the needs of biochromatography such as ternary separations and linear gradients. In order to account for the linear modifier gradients the trajectories were solved dependent on the modifier concentration. Moreover, the Henry constant was assumed to be a power function of the modifier concentration. Results for elution volumes were combined with mathematically formulated constraints under which a theoretical yield and purity of 100% with respect to the desired bioproduct are achieved. The results are summarized in a 3-dimensional design space of operating parameters. Examples for process operation within subregions of this space are presented and discussed. With the developed tool the robustness of the process can be investigated and the influence of different parameters can be estimated. Eventually, the understanding how process variations influence quality and robustness also supports the optimization and control of the twin-MCSGP process.

P-138 Direct Capture of α-amylase from CHO Cell Culture Supernatant Using Novel HyperCel™ STAR AX Salt Tolerant Anion Exchange Chromatography Sorbent. Rene Gantier1, Magali Touille2, Jérôme Champagne2, Yamuna Dasarathy3, Pall Life Sciences, Northborough, MA, USA; Pall Life Sciences, Cergy, FRANCE; Pall Life Sciences, Port Washington, NY, USA
Anion exchangers (Q or DEAE sorbents) are commonly used as the first step in purification of proteins. Using conventional anion exchangers requires cell culture supernatant (CCS) dilution to lower ionic strength or diafiltration to achieve sufficient capacity. However, these operations increase buffer consumption and processing time while limiting throughput. Using a “salt tolerant” anion exchanger such as HyperCel™ STAR AX improves significantly the process economics at production scale by allowing direct capture of recombinant proteins from undiluted feedstock of CHO (Chinese Hamster Ovary) cell culture. The ligand is based on primary amine chemistry, which provides salt tolerance to the sorbent with the ability to capture proteins in a high conductivity environment. This study describes the use of HyperCel™ STAR AX “salt tolerant” anion-exchange sorbent for the capture of an acid sensitive model protein (α-amylase) spiked in CHO CCS.

P-139 Novel Reversed-Phase Gel as an Alternative to C18. Ken Tseng1, Toshi Ono1, Tsunehisa Hirose2, Kazuhiro Kimata2, Nacalai USA Inc., San Diego, CA, USA; Nacalai Tesque, Kyoto, JAPAN
A novel reversed-phase silica gel with a cholesteryl bonded functional group (Cholester) is compared with the traditional C18. Cholesterol gel showed a similar hydrophobicity as C18 but with a better shape selectivity. This alternate selectivity allows Cholesterol to purify structural isomers or compounds with closely related structures. An example using the a- and b-carotene mixture is demonstrated successfully on the Cholester column. The two carotenes are almost structurally identical except for the position of one double bond. A C18 column does not have the right selectivity for the baseline separation. With the Cholesterol column in 4.6x150mm dimension, 5um particle size, 120A pore size, the two carotenes were completely isolated in an isocratic tetrahydrofuran/methanol mobile phase. Another example of an 8-catechin mixture is analyzed by C18 and Cholesterol HPLC columns. Both columns are 4.6x150mm in dimension and in 5um particle size. A gradient mobile phase of acetonitrile and buffer was used in 1.0 ml/min flow rate. The C18 column showed incomplete separation. The Cholesterol column was successful in baseline separating all compounds under 20 minutes. Further comparisons were done between 5um and 2.5um particle-size Cholesterol HPLC columns on the catechin mixture. With a smaller 2.5um particle size and a smaller column dimension of 3.0x75mm, the complete baseline separation was achieved and the analysis time shortens significantly to less than 5 minutes.

P-140 Chiral Recognition Mechanism of Acyloin-containing Chiral Solutes by Amylase Tris[(S)-alpha-methylbenzylcarbamate] Sorbent. Hung-Wei Tsui, Nien-Hwa Linda Wang, Elias I. Franseps, Purdue University, West Lafayette, IN, USA
Although polysaccharide sorbents have been widely used for chiral separations, the recognition mechanisms have not been fully elucidated. In this study, we focus on one important commercial sorbent, amylase tris[(S)-alpha-methylbenzylcarbamate] sorbent, or AS. Four acyloin-containing solutes were studied: ethyl lactate (EL), methyl mandelate (MM), pantolactone (PL), and benzoin (B), which consist a hydroxyl group molecular flexibility, as determined from a novel method involving the distribution of torsion
angles of this group. The distributions were quantified with molecular dynamics simulations of the solute structure. Moreover, the enantioselectivity was higher for the molecules with the lower molecular flexibility. MD simulations of a left-handed 12-mer AS polymer rod model revealed that the polymer has orderly “grooves” and cavities. Simulations of AS with 200 n-hexane molecules indicate that the n-hexane induces an energy-related “relaxation” of the side chain phenyl groups, but does not affect the intra H-bonds in AS. Finally, Monte Carlo (MC) and MD “docking” simulations were done to investigate the interactions of AS with pantolactone enantiomers. Some possible chiral cavities for PL were found. The simulations were also done for the other three solute pairs with the same cavity. The results support the hypothesis that the general mechanism involves a leading and a secondary interaction, and reveal the types of specific cavities which may allow enantioselective interactions.

P-141 New Retention Models and Interaction Mechanisms of Monovalent Solutes with Amylose tris[(S)-alpha-methylbenzylcarbamate] Sorbent. Hung-Wei Tsui, Elias I. Fransese, Nien-Hwa Linda Wang, Purdue University, West Lafayette, IN, USA

Amylose tris[(S)-alpha-methylbenzylcarbamate], or AS, is a sorbent often used for chromatographic chiral separations. A typical mobile phase is n-hexane containing isopropanol (IPA) as a polar modifier. The interaction mechanism between the solute, the modifier molecule, and the sorbent has attracted a lot of attention in the literature. Various stoichiometric displacement models in the literature have been widely used for understanding these competitive adsorption mechanisms of solutes and polar modifiers. The models were used to explain the often-observed linear log-log plots of the solute retention factor versus the concentration of the modifier in the mobile phase. The slope of the plot was sometimes inferred to be equal to the number of displaced modifier molecules upon adsorption of a solute molecule, or upon adsorption of the solute-modifier complex, or both, and was generally found to be greater than 1. In this study, five monovalent solutes, acetone, cyclo hexanone, benzaldehyde, phenylacetaldehyde, and hydrocinnamaldehyde are chosen for a controlled study. Each solute has one C=O functional group, which can form one H-bond with the sorbent or the IPA. The observed slopes were found to range from 0.25 to 0.45. Slopes less than 1 cannot be explained by the literature displacement models. New results of Infrared Spectroscopy, combined with Density Functional Theory simulations, provide an indication that the small slopes are due not only to acetone-IPA complexation but also to IPA aggregation with an average aggregation number of n=3. A new retention model has been developed to take into account the observed IPA aggregation and the solute-IPA complexation. The model shows that the aggregation leads to a significant reduction of the IPA monomer concentration, which affects the IPA-sorbent adsorption and the IPA-solute complexation. A general analysis using dimensionless concentrations and equilibrium constants shows that aggregation of IPA can indeed lead to slopes below 1. The limiting slope at high IPA concentrations approaches the values of 2/n. The IPA aggregation number and the equilibrium constants for the various processes are estimated from the HPLC data. Moreover, they are further validated with dynamic chromatography simulations. The differences in the slopes between the five solutes are due to different equilibrium constants for the solute-IPA complexation. Hence, the solute-modifier complexation and the alcohol modifier aggregation in the mobile phase must be accounted in the retention models used for the interpretation of the retention factors.

P-142 Scale-Up from Analytical to Preparative LC Transfer from a sub-2-micron Particle-size Column to a Preparative Column. Pierre Penduff1, Jeff Blacker2, Andreas Tei1, Ronald Guilliet3, Helmut Schulenberg-Schell1, 1Agilent Technologies, Waldbronn, GERMANY; 2Agilent Technologies, Stoughton, MA, USA; 3Agilent Technologies, Middelburg, NETHERLANDS

Synthesizing novel compounds or isolating natural products from biological tissues can be a laborious and time-consuming process. Most often the goal is to isolate efficiently a large amount of one or a few target compounds out from a crude mixture. After analyzing the precious sample on an analytical UHPLC system with high separation efficiency, the crucial step is to transfer the method to a preparative column. As analytical and preparative systems operate with totally different conditions such as dwell volumes, column particle sizes, pressures and flow rates for example, the most important step in a scale up process is to keep the chromatographic performance constant and minimizing the risk of losing valuable work or collecting impure compounds. In this poster, we will describe a practical way to optimize the scale-up process from an analytical UHPLC system to a preparative LC system. After analyzing a crude mixture on the UHPLC system using a generic gradient and identifying the target compound, a focused gradient will be developed. The objective of this gradient is to increase the resolution between the target and the adjacent compounds followed by a loading study to determine the maximum sample load on the analytical column before scaling up on the preparative system. Thus, an important step of an optimized scale-up process is to keep the resolution between the analytical and the preparative column constant. To achieve this goal, system characterization is required and an efficient method transfer procedure is developed using well-established scale-up formulas from literature. As a proof of performance a sample mixture will be successfully purified with high recoveries and purities in a direct
scale-up process from a sub-2-micron particle size column on an Agilent 1290 Infinity Binary UHPLC System to a preparative column on an Agilent 1260 Infinity Preparative Scale System.

P-143 Optimizing a Scale-up Process from a 4.6 mm to a 50 mm Column on a Combined Analytical/Preparative System using Focused Gradients. Pierre Penduff1, Jeff Blacker2, Ronald Guilliet3, Andreas Tei1, Helmut Schulenberg-Schell1, 1Agilent Technologies, Waldbronn, GERMANY; 2Agilent Technologies, Stoughton, MA, USA; 3Agilent Technologies, Middelburg, NETHERLANDS

Small laboratories that synthesize and purify target compounds for a variety of test purposes can benefit from a flexible analytical and preparative liquid chromatography system typically providing up to multiple grams of high purity compound without changing the plumbing of the system. This poster presents the proof of performance of a flexible and valuable system for a manual analytical to preparative scale-up process. With exchangeable pump heads and capillary sets, the flow rate range can even rise to operate 4 inch columns with excellent performance. A dual path-length UV detector cell with a 40x larger dynamic absorbance range provides optimum detection capabilities from analytical to preparative scale independently of the sample concentration used.

During this process, we developed the different steps of a manual purification process. Starting by mapping of the analytical and preparative flow paths, scale-up formulas will be applied. As a central part of this combined system, a selection valve allows choosing between analytical or preparative methods. As an evidence of the calibration, an overlay of a calibration mixture injected on both flow paths will attest of the retention of the resolution on the analytical and preparative columns. We explained how to quickly develop a method for such a system to obtain several grams of pure isolated compounds from a sample mixture. For this objective, the calibration mixture of different compounds from high to low polarity will be used to define different elution zones for the purification. From the defined elution zones, corresponding focused gradients will be generated in order to increase the resolution of the target compounds and reduced the purification runtime. Finally a dedicated focused gradient will be applied for the purification of a sample mixture. The collected fractions will be analyzed. The resulting purity and recovery data of the collected fractions will be presented as proof the quality of the proposed process. This poster demonstrated successfully a scale up process from an analytical 4.6 x 250 mm column to a self-packed 50 x 243 mm Agilent Load & Lock column on a combined analytical/preparative Agilent SD-1 purification system.

P-144 Proposing a New Adsorption Isotherm Known as Adejo-Ekwenchi Isotherm. Sylvester Adejo, Benue State University, Makurdi, NIGERIA

The derivation of an isotherm has been, largely, observed to be empirical. For any adsorption process, the rate of surface coverage, θ, depends on a number of factors, principally among which is the heat of adsorption [1]. The adsorbed molecules would usually first bind to the more attracting sites, that is, those with greatest binding power with high negative Gibb’s free energy, ΔGads [2], which can be evaluated from the adsorption equilibrium constant, K. Generally, K has been obtained through a number of adsorption isotherms like the Langmuir, Freundlich, Temkin, Frumkin, etc. which were mostly developed empirically [3]. In addition to the existing isotherm, we hereby propose another two-parameter empirical adsorption isotherm to be known as Adejo-Ekwenchi isotherm, derived using the results of our work on inhibition of mild steel using three plant extracts (leaves and roots) [4] centered on the fact that for an adsorption process, the amount of adsorbate uptake from bulk concentration is inversely proportional to the difference between the total available surface on the adsorbent surface and the fraction that is covered by the adsorbate at a given temperature, prior to the attainment of maximum value of surface coverage. That is the more the surface coverage the less the available surface and the less the uptake. Thus; \( \log \frac{1}{(1- \theta)} = K_{AE} C^b \) (1) Or \( \log 1/(1- \theta) = \log K_{AE} + b \log C \) (2) Equation (2) is the linearised form of the isotherm, where C is the concentration of the inhibitor; KAE and b are the isotherm constants. KAE is adsorption equilibrium constant known as Adejo-Ekwenchi constant, a temperature dependent parameter, giving the adsorption strength. b is a parameter we wish to call ‘variation factor’, and it relates the surface coverage by the adsorbate to the change in adsorbate bulk concentration. Progressively decrease in the value of b with rise in temperature shows that the mechanism of adsorption is chemical. Results from the isotherm are found to be in good agreement with those obtained through other known isotherms [5, 6]. References [1] P.W. Atkins: “Physical Chemistry” 2nd ed, W. H. Freeman and Coy, U. S. A. (1982) [2] L.M. Alaoui, B. Hammoui, A. Bellououchou, A. Benchachir, A. Guenbour, S. Kerti: Corrosion inhibition and adsorption properties of 3-amino-1,2,3-triazole on mild steel in H3PO4. Der Pharm Chemica (2011), 353 - 361. [3] M. Lebrini, F. Robert, P. A. Blandinieres, C. Roos: Corrosion inhibition by Isertia coccinea plant extract in hydrochloric acid solution. International Journal of Electrochemical Science (2011), 2443 – 2460. [4] S. O. Adejo, M.M. Ekwenchi: Ph. D Thesis of University of Jos-Nigeria (2012). [5] S. M. A. Hosseini, A. Azimi, A. Sh elkshoaei, M. Salari: Corrosion inhibition of 302 stainless steel with Schiff base compounds. Journal of Iranian Chemical Society (2011), 799-806. [6] L. A. Nnanna, B. N. Onwuagba, M. I. Mejeha, K. B. Okeoma:

P-145 Adsorption Isotherms of Some Amino Acids on RPLC Using 1,3 Dialkyl Substituted Imidazolium Ionic Liquids as Mobile Phase Additives. Ahmad Tarab, Azhar Alhijji, Divya Shekar, Vijaya Sree Vegesna, Prashanthi Kolanupaka, Kishore Kumar Aluguvelli, Ahlam Alalwait, Tariq Z. Ahmad, Western Illinois University, Macomb, IL, USA

Room-temperature ionic liquids (RTILs) or ionic liquids (ILs), are liquids at ambient temperature, and are usually composed of relatively large organic cations and inorganic or organic anions. ILs have negligible vapor pressure, good thermal stability, tunable viscosity, and primarily anion-dependent miscibility with water as well as various organic solvents. Because of these characteristic properties and their being recyclable, ILs have been widely investigated as “green chemistry” solvents. In liquid chromatography ILs are used as blockers for silanol activity and as mobile phase additives and as a replacement or organic modifiers. They are known to enhance the resolution and the symmetry of the peaks and they affect the retention of compounds. The objective of this work is to determine the adsorption isotherms of some amino acids on RPLC using RTILs as mobile phase additives. Tetrafluoroborate salts of 1- butyl, 3- methyl imidazolium (BMIM), 1-ethyl, 3-methyl imidazolium (EMIM) and 1- octyl, 3-methyl imidazolium (OMIM), 1- butyl, 2,3- di-methyl imidazolium (BDMIM) ionic liquids and the chloride salt of 1- butyl, 3- methyl imidazolium ionic liquid were used as mobile phase additives at different concentrations (5- 30 mM) with and without methanol in the mobile phase. Frontal analysis experiments were used to collect the adsorption data of the amino acids on two commercial C18 stationary phases (X-terra and Preval columns). The adsorption data were fitted to Langmuir and S- shaped models. The concentration and the type of the ionic liquids and the type of the analyte used were found to affect the magnitude and the direction of the curvature (concave up or down) of the adsorption isotherms on C18 stationary phase. All adsorption isotherms or phenylalanine were concave upward, while those of tryptophan were concave down. All mobile phases containing ionic liquids were found to affect the parameters of the adsorption isotherms and the amount of the adsorbed amino acid on the stationary phases used. Adsorption isotherms of tryptophan on Preval column are more affected by the ionic liquid than X-terra column. The OMIM concentration in the mobile phase was found to have the largest influence on the parameters of the adsorption isotherms for both compounds compared to the other ionic liquids.


Purification of Active Pharmaceutical Ingredients (APIs) often yields high numbers of fractions, often leading to a bottleneck of fractions to dry-down and subsequently analyze. Mass based purification offers enhanced selectivity for purifying APIs often resulting in higher purity and fewer fractions to process. Utilizing a mass signal in conjunction with conditional logic collection parameters and complimentary UV data can provide the selectivity to collect only APIs of interest. Using a Gilson GX-271 LC/MS Purification System, enhanced selectivity shows results that dramatically reduce collected fractions, collecting only the target ions of interest for an efficient purification process versus traditional UV-only based purification.

P-202 Purification of Virus Particles by Ceramic Hydroxyapatite Chromatography. Yae Kurosawa1, Maiko Saito1, Tsuneo Okuyama2, 1HOYA Corporation, Tokyo, JAPAN; 2Protein Technos Institute, Kanagawa, JAPAN

At the present understanding, the density gradient ultracentrifugation method is believed to be the most conventional method of virus purification. However, the ultracentrifugation method reduces viral infectivity and also processing is rather time-consuming. Ceramic hydroxyapatite can be applied in the neutral region, so that we can treat the virus with its bioactivity as the protein treatment. Ceramic hydroxyapatite is a mild packing media for biological material even for the virus separation. This method is highly reproducible and applied to some virus species such as dengue virus, influenza virus, poliovirus and feline calicivirus (FCV). One of the typical preparative procedures for chromatography was as follows: dengue virus type 2 ThNH7/93 was inoculated onto C6/36 cells and culture fluid was collected at day 7 and filtered through a 0.22 μm pore size filter. Culture fluid was loaded onto the ceramic hydroxyapatite column and eluted with a linear gradient of sodium phosphate buffer (NaPB). Virus particles retained infectious activity after purification from cell culture fluid by ceramic hydroxyapatite chromatography. Elution profiles: concerning the effect of buffer pH, influenza virus A/Beijing/262/95, dengue virus type 1 Hawaii, and poliovirus Sabin type 2 were bound more strongly on ceramic hydroxyapatite at a lower pH. Culture fluid infected each virus was loaded onto the ceramic hydroxyapatite column and viruses were eluted with a linear gradient of NaPB at pH 6.4, 7.2, and 8.2. The elution at pH 6.4 required the highest concentration of NaPB among 3 pH conditions, and the elution was delayed. Virus showed the similar profiles; adsorption on hydroxyapatite increased at a lower pH, so that the
virus peak was separated from large peak of protein contaminants well by reducing the pH and this may lead to easier purification of the virus. Sodium chloride (NaCl) addition to NaPB reduced the adsorption of FCV A391 on hydroxyapatite. Virus culture fluid was loaded onto the ceramic hydroxyapatite column and FCV was eluted with a linear gradient of NaPB with the different concentration of NaCl. The adsorption of FCV on hydroxyapatite was reduced and that of DNA derived from host cells was elevated at the higher concentration of NaCl. NaCl addition to NaPB elution can control the DNA elution and it may be useful to remove DNA from the target virus and improve the purity. This kind of steady and reproducible results indicates the applicable for industrial process chromatography.

P-203 **Flow Dependent Entrapment of Submicron Particles in Monolithic Media.** Petra Gerster, Astrid Dürauer, Alois Jungbauer, University of Natural Resources and Life Sciences, Vienna, AUSTRIA

Convective Interaction Media (CIM) monoliths are highly porous stationary phases for liquid chromatography. Monoliths are cast as a single block of methacrylate polymer and are traversed by channels up to 6 µm in diameter. The porous network enables convective transport through the bed, compared to packed bed transport which is driven by diffusion. Diffusivity is decreasing with increasing particle size and therefore processing of large particles in conventional bed chromatography is slow. Thus, monoliths are distinguished material for purification of large bioparticles such as viruses, pDNA or virus like particles (VLPs). Constricted pores in the monolithic media could cause a problem because particles could get entrapped by non-adsorptive mechanism. Therefore recovery of large particles in high flow rate processes would be decreased. Flow entrapment is reversible by reduction of the convective flow and introduction of conditions supporting diffusion. Hence, the entrapped particles are able to escape from the constriction by diffusion back to a large pore where they are transported through the monolith. Flow entrapment of monoliths was demonstrated for adenovirus by E. Trilisky and A. Lenhoff (2009). However experiments with baculovirus showed different behaviour and flow entrapment could not be observed. Hence for more detailed evaluation of the phenomenon nanoparticles of different sizes (100-500nm) made out of silica are used because they have defined size and are easily detectable. Injection of a of silica particles pulse under non-binding conditions to the system, to a dummy monolith (steel ring is placed between the frits instead of a monolith) or to a monolithic column results in a detectable response. In case of flow entrapment response is expected to increase with decreasing flow rate. In case of adsorption response is expected to increase but adsorption mechanism is not reversible by reducing the flow rate solely.

P-204 **Downstream and Upstream Feed Dilution for the Column Loading.** Izabela Poplewska, Roman Bochenek, Wojciech Marek, Wojciech Piatkowski, Dorota Antos, Rzeszow University of Technology, Rzeszow, POLAND

The performance of two alternative techniques of the column loading has been analyzed: the upstream (outside the column) and downstream (inside the column) feed dilution. In both cases the feed solution is preconditioned by diluting in a loading buffer. The dilution degree is adjusted with respect to the salt concentration and/or pH of the solution to diminish the influence of the feed media on the retention behavior of the target protein and to ensure binding the protein during the loading period. In the upstream dilution the feed mixture is mixed with the loading buffer prior to the purification process, whereas in the downstream dilution the feed and the loading buffer are alternately loaded into the column by multiple injections. In the latter case the volume ratio of the subsequent feed and loading buffer pulses determines the feed dilution degree. Because the protein concentration decreases rapidly after entering the column, mixing the feed solvent and the loading buffer inside the column can reduce the risk of undesirable effects which can occur in the presence of loading buffers, such as aggregation or precipitation of proteins. A case study has been performed to illustrate the influence of the dilution technique on the adsorption behavior of the protein mixture and resulting band propagation along the column. Moreover, the protein solubility pattern inside the column and the risk of precipitation have been analyzed. The loading conditions for both techniques have been optimized in terms of productivity and the buffer consumption.

P-205 **Purification of Virus Particles by Ceramic Hydroxyapatite Chromatography - Various Chromatographic Elution Parameters for Process Chromatography.** Yae Kurosawa¹, Maiko Saito², Tsuneo Okuyama², HOYA Corporation, Akishima-shi, Tokyo, JAPAN; ²Protein Technos Institute, Atsugi-shi, Kanagawa, JAPAN

The elution of target materials in the chromatography can be effected by various factors such as the column characters and the eluate properties. The elution of virus particles can be also controlled by the same factors. We report here the chromatographic parameters for virus purification on ceramic hydroxyapatite such as media size, gradient slope, and sample culture conditions. (1) The particle size of chromatography column media is an important factor for the separation and the resolution. Culture fluid of dengue virus type 2 ThNH7/93 was loaded onto the ceramic hydroxyapatite column, which was packed with CHT Type II 40 or 80 µm particles. Dengue virus was eluted with a linear gradient of sodium phosphate buffer (NaPB). Large UV peak for contaminants was
separated from the virus peak on 40 µm particles although both peaks were partially overlapped on 80 µm particles. Ceramic hydroxyapatite also gave a better separation and resolution with smaller media. (2) Effect on gradient conditions of phosphate buffer was compared with poliovirus Sabin type 2. Poliovirus was eluted under different gradient slope conditions; (a) 10-600 mM NaPB in 15 minutes, and b) 10-300 mM NaPB in 20 minutes. In the condition (a), UV peak for contaminants overlapped the virus peak, however, virus peak was separated from the UV peak when the gradient slope was decreased (condition (b)). A decrease in the gradient slope improved the separation. (3) The Comparison of the culture conditions was studied. Feline calicivirus (FCV) A391 was cultured in MEM with or without 10% fetal bovine serum (FBS), and both culture fluid were applied on ceramic hydroxyapatite columns. The elution UV peaks of FCV cultured without FBS were dramatically decreased. Dramatically decreasing protein contaminants increased the capacity of ceramic hydroxyapatite and the recovery of FCV. The virus purification on ceramic hydroxyapatite chromatography can be performed as the macro molecule treatment, under the similar considerations.

P-206 **Online Monitoring of the Simulated Moving Bed Chromatography**, Zoltán Horváth, Ju Weon Lee, Andreas Seidel-Morgenstern, Max-Planck-Institute for Dynamics of Complex Technical systems, Magdeburg, GERMANY

Since continuous production is more efficient and productive than batch production, required continuous processes and their combination become more and more important for the production of fine chemicals. However, it is then more complex to monitor product quality and to handle process disturbances than in batch to batch production. In our previous work [1] we have shown that a direct coupling of a continuous reaction performed in a tubular reactor and simulated moving bed (SMB) chromatography for product isolation can be more efficient than the consecutive realization of stand-alone processes. A reliable online monitoring of these complex processes is relevant to achieve robust operation and to handle process disturbances. In this work we will demonstrate a solution for the online monitoring of the SMB chromatography. An in-house developed online analysis unit based on flexible rapid HPLC analysis was attached and conducted the online monitoring of the SMB product streams to measure the product purity and yield, which are used for feedback control of the separation process. [1] A.G. O’Brien, Z. Horváth, F. Lévesque, J.W. Lee, A. Seidel-Morgenstern, P.H. Seeberger, Angewandte Chemie International Edition, Vol.51, (2012) 7028-7030


We investigate the adsorption behavior of several phenyl-n-alkanes (phenyl-n-octane, -decane, -undecane, -dodecane, and -tridecane) dissolved in acetonitrile on porous graphitic carbon. This system has been previously studied for a temperature of 50°C by Diack and Guiochon [1], [2], and complicated isotherm shapes were observed. We focused on measuring and modeling the adsorption isotherm courses of these phenyl-n-alkanes in a wider temperature range. The single component adsorption isotherms were measured using Frontal Analysis. Isosteric heats of adsorption were estimated from the results obtained. The occurrence and position of inflection points in the adsorption isotherm courses depend strongly on temperature. Based on the results, optimal conditions regarding preparative separation of solution mixtures, characterized by such non-Langmuirian isotherms, will be discussed.


P-208 **Anion Exchange Chromatographic Purification of Recombinant Fusion Protein in the Presence of Urea: Characterization of Association-state and Adsorption Equilibrium**, Monica Angarita¹, Thomas Mueller-Spaeth², Paolo Arosio¹, Roberto Falkenstein³, Wolfgang Kuhne³, Massimo Morbidelli⁴, ETH Zurich, Zurich, SWITZERLAND; ⁵ChromaCon AG, Zurich, SWITZERLAND; ⁶Roche Diagnostics GmbH, Penzburg, GERMANY

Apolipoprotein A-I (Apo A-I) is an important lipid-binding protein involved in the transport and metabolism of cholesterol [1]. Recombinant Apo-I was produced in the form of insoluble inclusion bodies (IB) using E. coli. Following IB solubilization, the protein is purified in an anion-exchange chromatographic step using urea as sole modifier. Although the impact of urea as denaturant agent has been previously studied using similar protein variants [2-5], so far an ion-exchange elution using an urea gradient as sole modifier has not been reported. Understanding how the conformational stability and the adsorption properties of the protein are affected by the presence of urea is of large interest for the improvement of the purification process and might also provide insights into elution mechanisms from ion-exchange resins using urea as modifier. In this work, the conformational stability and aggregation state of Apo A-I were characterized in aqueous solutions at different...

P-209 Nanoscale Hollow Spheres for Adsorptive Enantiomeric Separation using Supercritical CO2. Johannes Kern, Monika Johannsen, Hamburg University of Technology, Hamburg, GERMANY Nanoscale Hollow Spheres with modified surface properties produced by emulsion synthesis [1] offer a high potential for pharmaceutical applications either as drug carriers or for stereoselective, adsorptive separation of chiral drugs. If it is possible to bind the target enantiomer to the particles, separation and drug deposition can be accomplished in just one process step. Supercritical CO2 is especially interesting for these processes, regarding sustainability and high demands on the removal of residual solvents in pharmaceutical products. To develop such separation processes it is essential to have a tool to quantify the adsorption of the enantiomers of interest to the particles with varying pressure and temperature. In this work, a chromatographic method is developed to obtain the adsorption data needed. For this, an already present chromatographic apparatus for measuring adsorption equilibria [2] with supercritical CO2 has been modified to allow Frontal Analysis Chromatography with or without organic modifiers. Furthermore, the use of smallest particles for SFC applications is investigated. As development of stationary phases for Chromatography is focused on HPLC, particle size is limited by the pressure drop in a HPLC-System. The low viscosity of supercritical CO2 however, results in much lower pressure drops and so allows for smaller particles. SFC has proven to be useful for many enantioseparations. Particles smaller than what is currently the lower limit for HPLC stationary phases (~1.6 µm) could give rise to even better performance. Because of the expected high pressure drop of a packed bed of nanoparticles, first experiments have been carried out using commercial, non-hollow Al2O3-particles (dp≈13 nm) packed with very short bed length. Obtained pressure drops have been high but not extensive, so a slurry packing method has been used to pack the same particles in a precolumn with 1 cm bed length and inner diameter of 4.6 mm. Injection of the model substance naphthalene dissolved in n-hexane results in well shaped peaks but without suitable separation of the solvent from the solute. However, after longer use of the column peak symmetry decreased, indicating instability of the packed bed. Frontal Analysis Chromatography measurements have been carried out with the commercial Al2O3-nanoparticles and compared to measurements with a commercial Al2O3-HPLC column. Furthermore, Nanoscale Hollow Spheres produced by a project partner are used for Frontal Analysis measurements. Reference: [1] Buchold, D. H. M., Feldmann, C.: Nano letters, 7 (11) (2007), 3489-3492. [2] Pérez Molina, B.C., Johannsen, M.: J. Supercrit. Fluids 54 (2) (2010), 237-242.

P-210 Systematic Optimization and Experimental Validation of Simulated Moving Bed Chromatography Systems for Ternary Separation. Gaurav Agrawal, Yoshiaki Kawajiri, Georgia Institute of Technology, Atlanta, GA, USA Simulated Moving Bed (SMB) chromatography is an adsorptive separation process where the components are separated due to their varying affinity towards the stationary phase. Compared to the conventional chromatography, SMB enables high throughput and low desorber consumption and thus has been successfully applied in various areas such as sugar, petrochemical and pharmaceutical separations. Over the past decade, many modifications have been proposed in SMB chromatography in order to effectively separate a binary mixture. However, the separation of multi-component mixtures using SMB is still one of the major challenges. Since SMB systems are efficient means of performing large-scale chromatographic separations, there has been
a continuous effort to find modified SMB operating strategies that allow higher throughput yet meeting the product specifications at the same time. Several ternary SMB operating strategies are proposed in past including the JO process [1, 2]. Recently, a computational study was performed which compared various existing ternary separation operating strategies in terms of the maximum throughput attained and minimum desorbent to feed ratio required [3]. This study also did a systematic optimization of SMB superstructure formulation and proposed Generalized Full Cycle strategy which was found to have significant improvement over existing strategies. Nevertheless, the operating strategies were not validated by experiments. In this study, we perform the experimental validation of JO and GFC operating strategies using the Semba Octave chromatography system. A prediction-correction algorithm has been implemented in order to resolve the model mismatch [4]. The ternary mixture consists of maltose, glucose and fructose as the least retained, intermediate and the most retained component, respectively. We demonstrate that these operating strategies have significant improvement over existing ternary operations as they lead to a higher throughput and meet the product performance at the same time. We also present a Pareto plot of the optimal throughput that can be attained versus the desired purity of intermediate eluting component. References [1] T. Masuda, T. Sonobe, F. Matsuda, and M. Horie. Process for fractional separation of multi-component fluid mixture. US Patent No. 5,198,120, 1993. [2] V. G. Mata and A. E. Rodrigues. Separation of ternary mixtures by pseudo-simulated moving bed chromatography. Journal of Chromatography A, 939:23–40, 2001. [3] G. Agrawal and Y. Kawajiri. Comparison of various ternary simulated moving bed separation schemes by multi-objective optimization. Journal of Chromatography A, 1238:105–113, 2012. [4] J. Bentley and Y. Kawajiri. Prediction-correction method for optimization of simulated moving bed chromatography. AIChE Journal, 00:00–00, 2012.

P-211  A Novel Silica Base for High Performance Stationary Phases. Imre Sallay1, Keiji Koyanagi2, 1DAISO Co. Ltd., Osaka, JAPAN; 2DAISO Co. Ltd., Amagasaki, Hyogo, JAPAN

Peptides and peptide related APIs are already dominating the blockbuster drugs and their popularity is expected to continue. A major application is diabetes where the number of patients is snowballing due to obesity and another growth factor is the increasing exposure to insulin products of a widening range of population in the emerging countries. Another driving force is the development of several biosimilar insulin products. Answering the need of this expanding market is especially challenging if we consider that almost all generic peptide manufacturing companies are newcomers to the process scale chromatography and have very little experience. Silica based stationary phases are optimal for high performance stationary phases however the fact is that silica is easy to damage if the user is un-experienced. A completely new, re-designed from scratch silica backbone have been created in view of need for extra mechanical strength and pH durability. Here we report on a new silica stationary phase backbone (bare silica gel) development for the future intended use in defined segments of general peptide and the insulin manufacturing industry. The new silica gel displays the highest mechanical strength, highest pH resistance. The pore size, particle size and porosity have been optimized for the peptide related applications. We report the methods we have made the relevant measurements for common reference, because so far no standard measurement methods have been employed in comparing bulk silica based stationary phases.


A platform approach to purification process development is being widely adopted in the biopharmaceutical industry due to its proven savings in cost, time and resources. We further define our MAb purification platform by incorporating HTP automation technology at key points in the development. An operating space for optimal buffer conditions was first determined using HTP automation to increase our product and process knowledge prior to small scale experiments. This knowledge allowed for rapid identification of the best conditions for capturing our target molecule. HTP automation was also utilized for resin screening identifying the optimal wash and elution conditions for polishing steps to achieve the desired purity levels at small scale. The results obtained using HTP automation translated to the process scale observations very accurately. This has significantly minimized the efforts and time on process scale optimization to achieve target purity and recovery. In summary, HTP automation has allowed for rapid process development of an early phase molecule and further defined the operating space in the platform that is applicable to the pipeline.
P-213  Characterization of a Novel High Capacity Weak Anion Exchange Resin. Chinlun Huang, J. Kevin O'Donnell, Tosoh Bioscience LLC, King of Prussia, PA, USA

Ion exchange resins with increased selectivity and capacity are now in great demand. A new high capacity weak anion exchange resin was recently introduced, and this poster will focus on this resin and its ability to purify proteins and other biomolecules. The high capacity weak anion exchange resin was developed by Tosoh Corporation and utilizes the well-known TOYOPEARL® HW-65 size exclusion resin as the base bead. The base resin is chemically modified with diethylaminoethanol groups to provide a high level of anionic binding sites, increasing the resin's capacity and selectivity in downstream purification steps. The new DEAE-type resin effectively separates proteins even at very high capacity. When loaded to 96 g/L protein, the resin was able to separate ovalbumin from trypsin inhibitor. Also as part of this study, solutions of up to 50 g/L β-lactoglobulins were loaded onto the column. The new resin was compared to other commercially available anion exchange resins and demonstrated excellent selectivity under increasing loading conditions. The separation of β-lactoglobulins is particularly impressive since β-lactoglobulin A and B differ in only one amino acid residue. An aspartic acid in variant A is substituted for a glycine in Variant B.

P-214  High Throughput Whey Protein Separation at Pilot Scale with CIM Monoliths. Linda Voswinkel, Ulrich Kulozik, TU München, Freising, GERMANY

Whey proteins are recognized as highly valuable nutrients and nutraceuticals, yet it is known that the commercial potential is not fully achieved. Regarding food technological and physiological aspects, many components have unique characteristics, if present in an isolated form. The minor whey proteins Lactoperoxidase (LPO) and Lactoferrin (Lf) are applied because of their antimicrobial effects and the latter for improved iron bioavailability. From a technofunctional point of view, Caseinomacropeptide (CMP) and β-lactoglobulins (β-lg) are applied in order to stabilize food systems. CMP is an important protein source for phenylketonuria (PKU) patients and β-Lg is eliminated from whey for better digestibility as baby food. Thus, isolated fractions of whey proteins have an added value compared to concentrated whey products such as WPC and WPI. For the separation of food proteins a highly economical process has to be applied since the added value is lower than for biopharmaceuticals and APIs. Since high throughput chromatography, e.g. stationary phases based on membranes and monoliths, is being improved continuously over the last decades, the efficiency and handling of the process is very attractive for dairy industry right now. In the present study Convective Interaction Media CIM® columns from 8 to 800 ml have been employed for the isolation of all whey proteins in cheese and acid whey with ideal upscale behavior. Whey was microfiltered at 0.1 µm pore size with crossflow filtration and – where necessary – pH was adjusted to binding conditions. Afterwards, using strong anion and cation exchangers, all proteins were bound and eluted subsequently. For proteins with highest value purities of > 99 % have been reached, but baseline separation implies high buffer consumption. In favor of a faster process and lower buffer consumption some proteins can be eluted simultaneously, according to the targeted specifications. Other efforts made to develop a cost-efficient process are minimized equilibration which is less necessary due to a high sample load and the reuse of elution buffer, additionally resulting in higher protein concentration. Furthermore, the minimally required frequency of CIP will be investigated, which is a calculation of loss of binding capacity vs. downtime and detergents. Finally an overview will be given on the process time, chemical consumption per gram protein as well as protein purity and recovery for seven whey proteins.


Recently, the use of Nuclear Magnetic Resonance (NMR), molecular dynamics (MD) and multimodal (MM) chromatography has revealed the presence of a preferred binding face on Ubiquitin for multimodal interactions. In the present study, single molecule force spectroscopy was employed to study the energetics of face specific binding of Ubiquitin to self-assembled monolayers (SAMs) of MM ligands. Cysteine mutations were performed on specific sites on Ubiquitin to facilitate face specific covalent attachment of the protein onto a thiol reactive surface. This provided complete control over the orientation of the protein exposed to solution. Force measurements were performed on the exposed protein face using a force probe functionalized with SAMs of MM ligands. By calculating area under the force-distance curves, binding energies were obtained between the multimodal ligands and the specific binding face of Ubiquitin at a molecular level. Force measurements were carried out at different retract velocities and the data was extrapolated to very low velocities to obtain equilibrium.
values of free energy. This data is employed to obtain molecular level insight into protein binding energetics. Kinetics of protein and MM ligand interactions has also been investigated using quartz crystal microbalance (QCM). QCM was used to obtain real time mass adsorption and desorption data of proteins binding to SAMs of MM ligands that mimic a chromatographic resin surface, in a continuous flow mode. A systematic approach has been employed to extract kinetic rates of protein adsorption and desorption by investigating the binding behavior of proteins under different fluid phase conditions in order to delineate the fundamental reasons for MM protein selectivity. The targeted use of these two techniques in concert with ongoing simulations work in our lab, are advancing the state of knowledge of protein interactions in MM adsorptive systems.

**P-216 Peptide-based Affinity Adsorbent with High Binding Capacity for Antibody Purification.** Amit D. Naik1, Takaaki Terasaka2, William Kish1, Stefano Menegatti1, Ruben Carbonell1, 1North Carolina State University, Raleigh, NC, USA; 2Fuji Siysia Chemical Ltd., Nagoya, JAPAN

Polyclonal antibodies (pAbs) derived from serum of human and other mammalian species are widely used as biopharmaceuticals and in diagnostics. The large scale production of pAbs comprises of ethanol fractionation, salt/caprylic acid precipitation, ion exchange and hydrophobic interaction chromatography. These multiple purification steps decrease the overall yield of pAbs and, therefore there is a need to introduce highly specific affinity chromatography step in the purification process. Protein A affinity chromatography which is the industrial standard for purification of monoclonal antibodies is not commonly used for pAb purification due to high cost of Protein A and its inability to capture all classes of IgG. We have developed a linear hexapeptide ligand-HWRGWV which specifically binds the IgG via its Fc region. The peptide coupled to a polymethacrylate resin has been used to purify monoclonal antibodies from CHO cell culture supernatant and plant extract with purity of 95% and yield of 85%. Unlike Protein A, this peptide resin can be regenerated with NaOH (0.1 - 0.5M) solutions. Here we demonstrate the applicability of HWRGWV- resin to capture and purify polyclonal IgG of different species such as human, llama, mouse, rabbit and goat. The polyclonal human IgG purified by the peptide resin comprised of all subclasses in original proportion, unlike the IgG obtained by Protein A purification. The peptide-resin was able to purify polyclonal IgGs from mouse serum, llama serum, rabbit serum and goat serum with purity and yield of 90 % ± 2 and 80 % ±3. This performance of the peptide resin was similar or better than that obtained with Protein A resin. The work also describes the enhancement of antibody binding capacity of HWRGWV resins using various coupling chemistries, base matrices and spacer arms. Two different chemistries were examined for coupling the peptide ligand and its variants to polymethacrylate and cross-linked agarose-based resins. The effects of spacer arms with branched and linear functionalities were studied. Using optimized coupling conditions and spacer arm, an IgG specific peptide based affinity adsorbent was developed with an equilibrium IgG binding capacity of 75 mg/ml and a dynamic binding capacity at 5 minute residence time of 60 mg/ml.

**P-217 Selective Metalloproteinase Adsorption from Viper Venom.** Amina Darwish1, Sharath Krishna2, Stephen Thiel1, 1University of Cincinnati, Cincinnati, OH, USA; 2Central State University, Wiblerforce, OH, USA

Viper venom is a complex mixture including a number of proteins with possible therapeutic applications. Different surfaces were prepared to adsorb a metalloproteinase selectively from this mixture, by grafting different amino acids on an amine-functionalized silica surface. Arginine, glycine, and aspartic acid were selected based on previously identified biological interactions with the metalloproteinase. Single point batch adsorption experiments were conducted with the venom solution and the prepared adsorbents. The venom solution was composed of dried venom dissolved in 50mM phosphate buffer with pH 7.5 at 37oC. UV absorbance at 280nm was used quantify the total amount of adsorbed protein. A casein assay was used to determine the amount of metalloproteinase adsorbed. Selectivity was determined as the ratio of the fraction of the metalloproteinase adsorbed to the fraction of the total protein adsorbed. Aspartic acid functionalized silica was found to have the highest selectivity for the metalloproteinase, and arginine functionalized silica was found to have the lowest selectivity.

**P-218 Optimization of Continuous Downstream Process in Biopharmaceutical Manufacturing.** Ketki Behere1, Jay Yun2, Seongkyu Yoon1, 1University of Massachusetts, Lowell, MA, USA; 2Chromworks, Burlington, MA, USA

The biopharmaceutical industry is constantly evolving with increasing consumer and regulatory demands. The need to make faster, more competent and economic products which will compete with the next generation biosimilars has been the motivation to develop an efficient multi-column process for the purification of recombinant proteins. A continuous counter-current chromatography presents an opportunity to work with smaller volumes, optimum resin and buffer usage, less hold up time all of which contribute to the stability of the proteins. A theoretical model was developed and optimized for single-column HIC chromatography for a recombinant protein. The results obtained were then simulated for the multi-column counter-current chromatography. The data was obtained using the Langmuir theorem and can be used to develop an efficient
downstream continuous process. This simulation study was conducted to optimize the multi-column counter-current chromatography for a variety of recombinant proteins.

P-219  Investigation and Optimization of Macro-Prep® High Q used in hlgG Polishing Step. Danni Wang, Joseph Pobolet, Russ Frost, Bio-Rad Laboratories, Hercules, CA, USA

The properties of Macro-Prep High Q (MPHQ), a polymeric micro-porous strong anion exchange resin used for a polishing step in human γ-Globulin (hlgG) purification processes, were investigated to optimize the impurity levels and the hlgG yield in the product. The effects of resin properties, such as particle size, ionic capacity, and pore structure (pore volume, pore area, and pore diameter) were studied. The chromatographic parameters, such as sample loading concentration, time, and flow rate were also studied. The results showed that pore structure was the key attribute which affected the performance of the resin. In order to control the pore structure of the resin, the polymerization conditions for the resin synthesis were investigated and optimized. A MPHQ resin with optimized pore structure was designed and manufactured which showed balanced performance on both the impurity level clearance and the hlgG yield.

P-220  Polishing of Monoclonal Antibodies in Bind/elute Mode using Capto™ MMC ImpRes. Bengt Westerberg, Kristina Nilsson-Välimaa, Anna Heijbel, Peter Hagwall, Patrik Adielsson, GE Healthcare, Uppsala, SWEDEN

A rapid procedure to establish a robust second step in bind/elute mode for the purification of a MAb using Capto MMC ImpRes is shown. The procedure includes screening of binding capacity in 96-well format, verification in column format and optimization of elution conditions employing DoE. The results from optimization of the loading conditions show high yield of MAb monomers, as well as good clearance of aggregate, host cell protein (HCP), and leached protein A.

P-221  Higher Throughput Purification Method to Support the Screening of Bioreactor Process Conditions. Hemanth Kaligotla, Douglas Gunzelmann, Mei-Huei Jang, Yong Wang, Igor Quinones-Garcia, Dave Nichols, Shire, Lexington, MA, USA

Purifying cell culture harvest through an entire purification train is a resource intensive process for the purposes of screening the impact of upstream bioreactor conditions on product quality attributes. The objective of this study was to determine the feasibility of a higher throughput (HT) purification method to support cell culture development. A membrane adsorber chromatography capture step followed by a single column chromatography step was developed as a HT purification method. A single step HT purification using membrane adsorber was initially evaluated. The membrane adsorber provided the ability to directly load dilute harvest material foregoing the resource intensive ultrafiltration/diafiltration capture method. It also provided nearly complete capture of the protein of interest in the elution fraction, thus conserving the product heterogeneity generated in the bioreactor while enabling higher process throughput. Upon further development, a chromatography step was added to the membrane adsorber process to generate a final pool with adequate purity for analytical testing. The two-step HT purification train produces sufficient amounts of protein for analytical testing with the required purity (95% by Capillary Electrophoresis-Sodium Dodecyl Sulfate). This HT process reduced the purification effort required to support cell culture development from one week to less than two days.

P-222  Comparison of Protein A Affinity Resins for Monoclonal Antibody Purification. Zhuo Liu, Abhinav Shukla, KBI Biopharma, Durham, NC, USA

Purifying cell culture harvest through an entire purification train is a resource intensive process for the purposes of screening the impact of upstream bioreactor conditions on product quality attributes. The objective of this study was to determine the feasibility of a higher throughput (HT) purification method to support cell culture development. A membrane adsorber chromatography capture step followed by a single column chromatography step was developed as a HT purification method. A single step HT purification using membrane adsorber was initially evaluated. The membrane adsorber provided the ability to directly load dilute harvest material foregoing the resource intensive ultrafiltration/diafiltration capture method. It also provided nearly complete capture of the protein of interest in the elution fraction, thus conserving the product heterogeneity generated in the bioreactor while enabling higher process throughput. Upon further development, a chromatography step was added to the membrane adsorber process to generate a final pool with adequate purity for analytical testing. The two-step HT purification train produces sufficient amounts of protein for analytical testing with the required purity (95% by Capillary Electrophoresis-Sodium Dodecyl Sulfate). This HT process reduced the purification effort required to support cell culture development from one week to less than two days.
P-223  Modification of an ÄKTA Making Automation of Multi-step and Complex Protein Purification Processes Possible. Peter Tiainen, Ditte Skibstrup, Jais Rose Bjelke, Haleh Ahmadian, Novo Nordisk A/S Protein Purification Technology, Malov, DENMARK

Automation of protein purification processes is unfortunately not always straightforward. One challenging example is when a protein requires a complex purification process with multiple steps, and utilization of delicate and/or intricate gradients further dictates the need for precise performance control. Processes where pH and/or conductivity need to be adjusted between column operations are additionally problematic to fit into an automatic setting. To our knowledge only simple multi-step automated purification processes have previously been presented, here we showcase a solution on how to accommodate the above mentioned challenges in an automated approach. An ÄKTAexplorer was modified to accommodate complex purification processes with full elution control making it possible to automate various column combinations. The modifications enable multi-step purification processes where elution from one column can be directed onto a second column either directly, or after passing through a buffer exchange column. The latter enables adjustment of parameters like conductivity and/or pH between the individual column steps, without jeopardizing full process control. The presented setup is very flexible, enabling automation of long and complex purification processes, and by reducing the need for operator attendance, it increases the purification throughput and cost-effectiveness significantly.

P-224  Characterization of Antibody Aggregation and Unfolding on Cation Exchange Chromatography Media. Jing Guo, Erik Fernandez, John O’Connell, Giorgio Carta, University of Virginia, Charlottesville, VA, USA

Cation-exchange chromatography plays a significant role in the purification of monoclonal antibodies (mAb). Recent evidence suggests that certain cation-exchangers can cause unfolding and aggregation of these antibodies. In this study of a model mAb on the resin Fractogel SO3 from EMD Millipore, dynamic light scattering (DLS) was used to determine the size and reversibility of the species in the two different peaks obtained when the protein is adsorbed at low ionic strength, held on the resin for more than one day, and then gradient-eluted at higher ionic strengths. Then hydrogen-deuterium exchange mass spectrometry (HXMS) was used on peptides from the digested species to determine solvent accessibility of a significant fraction of the residues. One species was apparently the monomer because its size and solvent accessibility were essentially identical to those of the set of peptides obtained from single species obtained from a solution that had not contacted the resin. On the other hand, a significant number of residues of the more retained and larger species, presumably aggregates, showed solvent accessibility that was statistically different from the monomer. Some residues had greater solvent exposure, likely representing partially unfolded regions. Other residues had greater protection, suggesting regions involved in aggregate formation. Reversibility of the aggregation was indicated by DLS measurements showing decreasing size of the larger species with time. The poster will describe the materials, methods, and results, as well as suggestions for further measurements to increase HXMS coverage and provide more quantitative description of aggregation formation, dissociation and structure.

P-225  Optimization of a Filter Train for the Downstream Purification of Recombinant Pharmaceutical Proteins Produced in Tobacco Leaves. Johannes Felix Buyel, Rainer Fischer, RWTH Aachen University, Aachen, GERMANY

Clarification is the most important context-specific processing step for the purification of pharmaceutical proteins expressed in plants because it must remove particulate contaminants only found in plant tissues (such as fibers and components of the cell wall). The development of clarification steps that comply with good manufacturing practice is necessary to make plant-based production platforms competitive. Disposable filters are preferred for small and medium scale processes because the upfront investment and risk of contamination are low. However, the selection of an ideal filter train can be challenging because a vast number of different filters are commercially available. We previously developed a filter train to process 200 kg of transgenic tobacco leaves based on bag filtration followed by three depth filters and a final membrane filtration step. Here we scaled down the original process to 2 kg in order to optimize clarification while demonstrating the efficient extraction of a human monoclonal antibody (2G12) and the fluorescent protein DsRed from transgenic tobacco leaves, achieving the same target protein concentrations as the larger-scale process. We tested a range of bag filters and depth filters alone and in combination to develop the most efficient clarification process. Filter capacity, filtrate turbidity and filter costs as well as the protein recovery were used to assess the performance of the tested filter materials which were challenged with extracts from different tobacco varieties and related species grown either in soil or rockwool. The most suitable filter materials were identified and tested in a 50 kg pilot scale.
Common challenges in the construction of porous media for bioseparations are to achieve rigid media which does not exhibit significant non-specific adsorption while also offering fast process flow rates and higher column beds. Highly cross-linked agarose media offers superior mechanical properties and is the basematrix of choice for affinity, IEX and multimodal media. Hydrophobic interaction chromatography (HIC) media on highly cross-linked agarose was developed for early stage purification. A new generation HIC media optimized for polishing applications will be presented. Separation and material characteristics effecting productivity will be shown together with application results.

P-227 Design and Characterization of High Throughput 8 mL Radial Flow Monolithic Chromatographic Column. Janže Jancar¹, Ales Podgornik², ¹Bia Separations, Ajdovscina, SLOVENIA; ²COBIK, Ljubljana, SLOVENIA
One of the main impediments for more frequent use of radial flow operating chromatographic columns is changing of a mobile phase linear velocity while it passes through a bed. Consequently for porous particulate supports, matrix efficiency varies by mobile phase position within the bed and overall performance when applying real samples, is difficult to predict. This problem is not present when the monolithic supports are used, since it was demonstrated that their chromatographic properties are flow unaffected even at the extreme linear velocity. This, together with lower pressure drop of a radial design [1], is the motivation that new monolithic column was designed in a radial flow operating mode. When novel radial flow chromatographic housing containing improved flow distribution system was combined with high-throughput monolithic material, a new semi preparative column was obtained. New column enables work at extremely high flow rates up to 70 CV/min at moderate back pressure of only 20 bars. This was achieved by optimization of monolith dimensions with the height of 55 mm, inner diameter of 6.0 mm and thickness of 4.5 mm. In this work new approach for radial column housing design will be described together with its characterization. Pulse response experiments at a different flow rates up to 70 CV/min were carried out. Relatively high RSD was observed, being however consequence of the HPLC system. A close inspection of data revealed constant decrease of peak height for increase of the flow rate what was ascribed to the limitation of data collecting capability of detector. The peaks are actually so sharp that the maximum of the peak can be missed between two consecutive data point acquisitions [2]. Performance of developed column was further tested by investigating its chromatographic properties. Separation of standard protein mixture at various flow rates (up to 50 CV/min) was performed. In this range no significant change in resolution was observed by changing the flow-rate. Similar conclusion can be made from the dynamic binding capacity measurements performed by loading BSA on the monolithic column at different flow rates. Despite a very high flow-rate, pressure drop vs. linear velocity curve is straight, demonstrating that no compression of monolith. Such column design allows also refill of the monolith by easily taking out a used monolith and exchanging it with a new one. To investigate how reproducible such a procedure can be, column was disconnected from the HPLC system, outlet cover was unscrewed from the column, monolith was taken out than placed back into the same housing, outlet cover was again tightened and column was reconnected to the HPLC system. The entire procedure was completed within 2 minutes. After each refill pulse response test procedure at the flow rate of 30 CV/min was performed and number of theoretical plates (N) was calculated. RSD of N was calculated to be 4.3 % which is comparable value to the one obtained for the consecutive injections performed at constant flow-rate described above. Obviously column filling procedure has no detectable influence on column performance therefore refilling of monolithic columns can be considered as extremely reproducible, easy and fast. Finally new monolithic column was used for separation of protein aggregates from a monomer protein. For this purpose we used lyophilized protein BSA, which is known to contain aggregates [3] and dissolved it in a loading buffer. BSA dimers and aggregates interact slightly stronger with the anion exchange matrix; therefore they can be separated by implementation of salt gradient. Separation was performed at extremely high flow rate of 70 CV/min and completed within 46 seconds. Such short time indicates possibility to implement this type of column for in-process control during preparative aggregate removal but it can also be used for removal itself since the separation was efficient as indicated. References: [1] A. Podgornik, M. Barut, I. Mihelič, A. Štrancar, In: Švec F, Tennikova TB and Deyl Z (eds) Monolithic materials: preparation, properties, and applications, Elsevier, Amsterdam, 2003, p. 77. [2] R. Hahn, A. Jungbauer, Anal. Chem. 72 (2000) 4853. [3] A.K. Hunter, G. Carta, J. Chromatogr. A 937 (2001) 13.

P-228 Protein Processing Figures of Merit and Novel Ligand Chemistries Employing Capillary-Channeled Polymer (C-CP) Fiber Stationary Phases. R. Kenneth Marcus, Zhengxin Wang, Abby Shadock-Hewitt, David Jeffcoat, Clemson University, Clemson, SC, USA
Capillary-channeled polymer (C-CP) fiber stationary phases have demonstrated a number of very positive characteristics relevant to downstream processing of proteins. In physical terms, fibers packed into column structures provide very high permeability, while also promoting very efficient mass transfer to/from the fiber
The extruded polymer fibers have very low porosities \((rp = \sim 2 \text{ nm})\) as determined by inverse size exclusion chromatography (ISEC) measurements. In practical terms, proteins/polypeptides having molecular weights of \(>10,000 \text{ Da}\) experience virtually no van Deemter C-term broadening. As such, analytical-scale separations can be performed at linear velocities of up to 100 mm sec\(^{-1}\) without sacrifice of chromatographic efficiency. Current studies are aimed at discerning differences between the optimum fiber packing and flow velocities for analytical and preparative applications. To this end, dynamic binding capacities and process throughput and yield characteristics will be presented for nylon 6 fibers operating in an ion exchange mode. The melt-extrusion of C-CP fibers from simple thermopolymers (polypropylene, polyester, and nylon) means that the primary materials costs are very low. A more salient feature is the fact that these base polymers present very different surface chemistries, both in terms of their native states as well as the acceptance of surface modifications. As suggested above, nylon 6 is an excellent surface for weak anion/cation exchange separations. The surface is easily modified using simple triazine chemistries under ambient conditions. Polyester provides for a more hydrophobic surface having aromatic character, which can also be used for weak cation exchange. Finally, polypropylene provides for solely hydrophobic interactions between solutes and the fiber surface. This interaction allows for very robust affixing of capture ligands through adsorption. As such, chemistries can be affected by simply passing the ligands through the assembled column. This concept, and practical figures of merit, will be demonstrated through the common approach of the adsorption of protein A to the fibers for the capture of IgG as well as for an extremely novel, yet powerful use of head-modified PEG-lipid ligands. These commercially available phospholipids can have a wide variety of head groups, including amines and carboxylic acids, succinyl/thiophenyl groups, polydentate metal ligands, and high-selectivity agents such as biotin. Demonstrations of the latter strategy will be supplemented with fundamental measurements of surface loading and ligand robustness using a FITC-labeled PEG-lipid. It is believed that the physical and chemical attributes of C-CP fiber columns can have substantial impact in the realm of preparative protein separations.

**P-229 Versatile use of Mixed-mode Sorbent for Intermediate Purification of a Fab Fragment.** Karl Rogler, Rene Gantier, Pall Corporation, Northborough, MA, USA

Mixed-mode chromatography has been demonstrated to be a very useful alternative technique to ion-exchange (IEX) and hydrophobic interaction chromatography (HIC) for intermediate and polishing steps in downstream processing of monoclonal antibody. The versatility of mixed-mode chromatography sorbents, coming from the combination of ionic and hydrophobic interactions, makes them usable for extended type of purification applications including the emerging antibody fragments. In this study we used different mixed-mode sorbents for intermediate purification of a Fab fragment from E. coli periplasmic extract after a first capture step using cation-exchange (CEX) chromatography. High-throughput screening (HTS) on 96-well filter plates was employed to identify optimum conditions for the Fab purification in both bind/elute and flow through modes. Column based performance was then evaluated for Dynamic Binding Capacity (DBC), purity, and yield of recovery. While some sorbents achieved a high binding capacity, Fab purity and yield of recovery in bind and elute mode, other sorbents provided similar purification performance when loading up to 150 mg of Fab per ml of sorbent in flow through mode. Overall, this study gives insight on the multipurpose applicability of mixed-mode sorbents and their ability to be used efficiently as a practical Fab purification step.

**P-230 Overcoming the Yield Limitation of Thermodynamically Constrained Bioreactions by Integration of Biocatalysis and SMB.** Nina Wagner, Andreas Bosshart, Matthias Bechtold, Sven Panke, ETH Zurich, Basel, SWITZERLAND

Biocatalysis has matured into an established technology in the field of fine chemistry due to its high selectivity, absence of side reactions and the ability to operate under ambient temperature and pH conditions. However, a considerable set of highly attractive enzyme reactions (e.g. C-C bond formations by aldolases or isomerizations) shows an unfavorable position of the thermodynamic equilibrium impairing their implementation on industrial scale \([1]\). The yield limitation can be overcome by a continuous in-situ product removal process using simulated moving bed (SMB) technology for the separation of substrate and product \([2]\). In the scope of this work we present an integrated process setup for the production of the rare sugar D-psicose which can be obtained directly by epimerization from readily available D-fructose employing the enzyme D-tagatose epimerase (DTE). Specifically, we demonstrate a number of milestones toward a fully optimized integrated process: (i) the proof of principle (ii) model-based characterization of all involved units (iii) optimization of the stand-alone SMB unit and (iv) model-based simulation of the integrated process. The principal feasibility of the process concept was demonstrated by a fully integrated process on lab-scale consisting of an 8-column SMB based on calcium-substituted ion exchange material, an enzyme membrane reactor (EMR) retaining DTE and a nanofiltration (NF) device for concentration of the fructose-rich SMB raffinate before recycling to the EMR. It was operated for several days and provided 97% yield \([3]\). Next, a detailed characterization of all involved unit operations was conducted by model-based experimental analysis. In case of the chromatographic process the inverse method was used to populate parameters of different chromatography models. Model discrimination was

P-231  **High Recovery and Purity Peptide Purification.** Pierre Penduff1, Jeff Blacker2, Andreas Tei1, Ronald Guilliet3, Helmut Schulenberg-Schell1, 1Agilent Technologies, Waldbronn, GERMANY; 2Agilent Technologies, Stoughton, MA, USA; 3Agilent Technologies, Middelburg, NETHERLANDS

This poster describes the workflow to successfully deploy a purification process on a bio-inert preparative system after identification, optimization of the method, and column load study on the analytical system. The poster describes an optimized solution for bio-purification from analytical flows up to 100 mL/min, covering from small injection volumes up to hundreds of milliliters. By using Agilent Load & Lock (dynamic axial compression column technology) packed with Agilent Prep C18 or PLRP-S bulk media for reversed phase chromatography, as well as PL-SAX and PL-SCX bulk media for ion exchange chromatography, the preparative system exhibits complete flexibility in regarding biomolecules purification workflow. For optimum purification results the entire instrument described in this application is biologically inert, since interaction with metallic surfaces can cause peak tailing or sample degradation. Pump heads, mixing chamber, injection valve, capillaries and fittings are made of ceramic or PEEK material to ensure the reliability and the performance of the purification process. On this system a scale-up from analytical to preparative systems was performed on a crude synthetic decapeptide sample mixture: After completion of the reaction mixture conversion, the target peptide peak and its corresponding molecular ion and fragments were identified on a Agilent 1260 Infinity Bio-inert Quaternary LC system coupled with an Agilent 6130 Single Quadrupole MS detector. After determination of the target molecule elution area, an optimized focused gradient method based on time windows was applied in order to increase resolution and sample purification load on the analytical column. Applying method transfer from the analytical bio-inert LC system to the preparative bio-inert LC system, the purification was performed on a two inches preparative column. Purity and recovery of the successful purification are shown.

P-232  **Development and Implementation of a Higher Capacity Anion Exchange Chromatography Step for a Late Stage Protein Purification Process.** Bee Lin Cheang, Chenny Chen, Vishal Lal, Mei-Huei Jang, Igor Quinones-Garcia, Dave Nichols, Shire HGT, Lexington, MA, USA

A process optimization initiative, utilizing quality by design (QbD) approach, to increase production throughput will be presented. An early phase manufacturing process for a non-mAb protein therapeutic utilized a Q Sepharose Fast Flow (Q FF) resin. The load capacity of this column limited potential scale-up and created concerns regarding buffer consumption and processing time. To enhance scalability and facility fit, resin scouting was initiated. Batch mode resin scouting was performed to identify promising candidates with higher binding capacity and equal or better selectivity. Subsequent linear and step gradient separations were performed to identify the optimal operating conditions. A preliminary full factorial experimental design with the three most important factors was conducted on the top resin candidate to optimize the column operating conditions. Side-by-side confirmation runs through the entire purification train were then performed using Q FF and the new resin to compare process performance and product quality. The new chromatography step was characterized using a sequence of fractional factorial and central composite designs including multiple operational variables. These designs allowed the impact of input variables to be quantified on process performance (e.g. yield) and product quality, thus identifying the design space. Using the identified models, Monte Carlo simulations were then performed to establish in-process ranges for operating and performance parameters. The new resin was subsequently implemented for a late stage purification process operated at a larger scale.

P-233  **Discovery and Development of Universal Fc Binders for Antibody Purification.**  Marc Arnold1, Holger Bittermann2, Thomas Neumann2, 1Novalix, Heidelberg, GERMANY; 2Graffinity Pharmaceuticals, Heidelberg, GERMANY

Therapeutic antibodies are mainly purified by established platform processes including a Protein A affinity capture step. Although this protein ligand is accepted as the current gold standard for antibody purification it also has some severe limitations like high costs, low chemical stability and leaching. Consequently, there is a demand for alternative chromatography material for the purification of mAbs. Through a unique SPR screening platform we have identified and developed small molecule affinity ligands for cost efficient affinity purification of antibodies. For the identification of the binders, several humanized therapeutic antibodies were screened against a unique and diverse library of > 116k compounds immobilized on gold chips via linkers. A tissue culture
supernatant from CHO cells served as a control to identify unspecific binders. Observed antibody specific hits were then coupled to NHS-Sepharose 4 FF for chromatographic evaluation. Generic binding to the Fc region of IgG1, IgG2 and IgG4 antibodies could be demonstrated. Best ligands were further characterized by high selectivity, high capacity and fast binding kinetics comparable to Protein A. Due to the extraordinary high chemical stability and low cost of the developed ligands they might represent an attractive Protein A replacement.

P-234  Purification of a Common Light Chain Bispecific Antibody using Contichrom (CaptureSMB and MCSGP)  Thomas Muller-Spath1, Nicole Ulmer1, Guido Strohlein1, Michael Bawand1, Linda Kaldenberg-Hendriks2, Lex Bakker2, John de Kruijf2, Mark Throsby2, ChromaCon AG, Zurich, SWITZERLAND; 1Merus B.V., Utrecht, NETHERLANDS
A human bispecific light chain IgG antibody (AB) was purified from PER.C6 cell culture harvest with high yield and high purity using a three-step chromatographic downstream process. The purity of AB in the harvest with respect to the parental antibodies AA and BB was 40%. The downstream process comprised a Protein A capture step, a first polishing step using MCSGP cation-exchange chromatography and a second polishing step using mixed-mode chromatography. The key to successful purification of AB was the operation of the first polishing step with Contichrom® in MCSGP mode that enabled a step yield of 87% AB and with less than 1% AA, BB main isof.

P-235  Characterization of a New High Capacity and Alkaline Stable Protein A Resin. Koji Nakamura, Satoshi Fujii, Shigeru Nakatani, Tosoh Corporation, Yamaguchi, JAPAN
Therapeutic monoclonal antibodies (mAbs) have become the major product class in the biopharmaceutical industry, and they are expected to continue to be a major source of new therapy for the next decade. In the past 10 years, expression levels of mAbs using Chinese hamster ovary cells have dramatically increased at levels up to 10 g/L. This increased upstream productivity has subsequently required the resin with high binding capacity. To meet such demand, we have developed a new high capacity and alkaline stable protein A-Toyopearl. Toyopearl is a hydrophilic polymer based resin and has been used for purification of proteins. A recombinant alkaline stable protein A was immobilized onto Toyopearl which has a large, 1000 angstrom pore size. The static binding capacity of human IgG was 80 mg/mL and the dynamic binding capacity was about 70 mg/mL at a residence time of 5 min. In this poster, basic properties of the new protein A Toyopearl, purification of monoclonal antibody from cell culture supernatants and a CIP study with sodium hydroxide solution will be presented.

P-236  SFC and Normal Phase Chiral Chromatography with Pharmaceutical Compounds to Demonstrate Scaling Analytical to Prep While Balancing Purity, Yield, and Through-Put. Marc Jacob1, Michael McCoy1, P. Sky Countrryan1, William Farrell2, J. Preston1, 1Phenomenex, Torrance, CA, USA; 2Pfizer, San Diego, CA, USA
There is little doubt among chromatographers that packed columns with eluent carrying samples are complex systems. HPLC has been extensively studied since the late 1960’s. There have been numerous theoretical models developed to describe, explain, and predict the results of chromatographic experiments. It is important to remember that the typical goal of chromatography is to separate compounds from each other. The most straight forward way to evaluate separation is to calculate the Resolution between two peaks of interest. Peak width will have a large impact on Resolution. A VanDeemter curve is a typical way to find the optimum flow-rate for a chromatographic system. The optimized flow-rate will provide narrower peaks resulting in the most Resolution. If achiral chromatography doesn’t seem complicated enough, chiral chromatography introduces an entirely different level of complexity. However, the goal is still Resolution and it will be better with narrower peaks.

SFC has become a very effective chiral chromatography technique. One reason chiral SFC in particular has become so prevalent is the relationship between normal phase and SFC chiral methodologies. It is often considered straightforward to switch between Normal Phase and SFC. The use of preparative chiral chromatography has also increased significantly over the past 5-10 years and SFC has been a significant driver for this increase. It is well know that chromatography can be directly scaled from very small columns to very large columns when the eluent composition remains consistent. The work presented in this poster will address the relationship between normal phase and SFC chiral methodologies at the analytical and prep scale for several pharmaceutical related compounds. The impact on Resolution at both scales due to flow-rates and
eluent compositions will be evaluated and compared between SFC and Normal Phase. The effect of prep column hardware along with resulting purity and through-put from related SFC and Normal Phase purification methodologies will also be evaluated.

P-237  **Modeling, Isotherm Determination, and Simulation of Nonlinear Chromatography using ChromWorks Software.** Reid Erwin¹, Baochun Shen², Jay Yun³, Yoshiaki Kawajiri¹; ¹Georgia Institute of Technology, Atlanta, GA, USA; ²Kunming Medical University, Kunming, CHINA; ³ChromWorks Inc., Burlington, MA, USA

Modeling, simulation, and design of Simulated Moving Bed (SMB) chromatography pose many challenges. Isotherm parameters must be obtained by analyzing chromatograms, which result in a complex mathematical model. This mathematical model must be solved numerically to predict and analyze the performance and process economics. Performing these tasks requires substantial effort, and often multiple computational packages are used. In this work, we employ ChromWorks, a computer-aided modeling and simulation software package for preparative and continuous chromatography. This software is capable of performing simulation, design, and analysis of various SMB processes, including isotherm parameter estimation and determining optimal operating conditions by simulation. There are a number of techniques implemented in ChromWorks. For isotherm determination, the Elution by Characteristic Points (ECP) method provides an initial guess of nonlinear isotherm parameters from multiple chromatograms of different injection volumes. This initial guess provides a good starting point for dynamic estimation, where rigorous dynamic model is fitted to experimental chromatograms to obtain isotherm parameters simultaneously with mass transfer and dispersion coefficients. Isotherm data can then be transferred to the process design interface which utilizes the triangle equilibrium diagram [1] to determine the optimal SMB operating conditions. This study presents application of ChromWorks to the following two case studies of nonlinear chromatography: (1) validation of isotherm modeling and finding operating conditions for SMB separation of cycloketones (cyclopentanone, cyclohexanone) in Bentley et al. [2] and (2) determination of Langmuir isotherm parameters for enantiomer separation of amino acids (phenylalanines). The nonlinear isotherm parameters were obtained successfully from chromatograms of single-column experiments. Furthermore, to design an SMB process, the trade-off of throughput maximization and desorbent minimization while achieving purity requirements was analyzed using the obtained model. References [1] Bentley, J., Sloan, C., & Kawajiri, Y. (2013). Simultaneous modeling and optimization of nonlinear simulated moving bed chromatography by the prediction-correction method. Journal of Chromatography A, 1280, 51–63. [2] Mazzotti, M., Storti, G., & Morbidelli, M. (1997). Optimal operation of simulated moving bed units for nonlinear chromatographic separations. Journal of Chromatography A, 769, 3-27.

P-238  **Effects of Particle Size on Protein and VLP Adsorption on Perfusion Media.** Yige Wu, Giorgio Carta, University of Virginia, Charlottesville, VA, USA

The adsorptive and chromatographic behavior of lysozyme, IgG and thyroglobulin, and that of human papilloma virus (HPV) virus-like-particles (VLPs) are studied for POROS 20HS, a large-pore cation exchanger with smaller particle size compared with POROS 50HS. Transmission electron microscopy shows that POROS 20HS has similar interior structures with POROS 50HS, that a broad distribution of pore sizes with 100-500 nm through-pores transecting a network of much smaller pores formed by aggregates of microgranules about 100 nm in size is presented. Dextran standards, proteins, and VLPs show size-exclusion behavior consistent with such a bimodal distribution of pore sizes. For non-binding conditions, the trends in HETP as a function of mobile phase velocity and molecular size are consistent with perfusion suggesting that intraparticle transport is controlled by diffusion in the macropores at lower reduced velocity, but becomes convection dominated at higher reduced velocity. Compare to POROS 50HS, POROS 20HS shows more intraparticle mass transfer enhancement due to smaller particle size. Intraparticle concentration profiles during transient adsorption are determined by confocal microscopy in batch and flow systems. The profiles are spherically symmetrical indicating a dominance of diffusion for smaller proteins (IgG) in both batch and flow systems but become highly asymmetrical and skewed in the direction of flow for thyroglobulin at 1,000 cm/h. Estimates of the convective enhancement of intraparticle transport for these conditions based on the confocal measurements are consistent with estimates of the intraparticle Peclet number and the models of Carta et al. (1992) and Liapis et al. (1995). Adsorption of VLPs, however, was still found to be confined to a thin layer on the outer surface of the particles as POROS 50HS, indicating that bound VLPs block access to the underlying pore network and suggesting that pores larger than those present on the resin studies are needed to take advantage of the effects of perfusion for the adsorption of large VLPs.
P-239  Re-Inventing the Packed Bed – Implications of 3D Printing for Chromatography. Conan Fee, Simone Dimartino, Suhas Nawada, University of Canterbury, Christchurch, Canterbury, NEW ZEALAND

The advent of 3D printing now enables us to overcome the traditional limitations of packed bed micro-structures that are imposed by slurry packing. The precise control of particle size, configuration and shape that 3D printing provides has significant implications both in terms of creating superior geometrical configurations and in terms of offering a greater theoretical understanding of non-idealities in packed beds. The most obvious hurdle that printing helps to overcome is the random close-pack limit of spheres (ε =0.36) because it enables us to create an ordered arrangement of particles, with a theoretical minimum of ε =0.26 for monodisperse spheres. Precise control of particle shape permits the creation of identical spheres and of specific non-spherical particle shapes such as tetrahedra or octahedra, which can be printed in packing configurations with controlled orientation to maximize the internal surface area of the packed bed. Indeed, the concept of “packing” may well be superseded by this technology, with which we can produce monoliths with precise control throughout the bed over the pore size, shape and alignment. For theoretical studies, a range of packed beds with random, semi-random and ordered configurations spanning a range of heterogeneity values can be printed and tested for eddy dispersion and other behaviors to validate simulations with exact physical replicas of the in silica models. Furthermore, 3D printing can enable the decoupling of porosity, specific surface area, tortuosity, and other characteristics as causal actors in the performance of packed bed. Additionally, the local performance along column walls can be studied because walls with embedded beads can be created to minimize porosity differences caused by limitations on particle placement near the column wall. In this poster, a number of proof-of-concept packed-bed columns are presented, showing the versatility of 3D printing and the potential for creating a new generation of chromatographic media. Furthermore, the role of 3D printing in chromatography can be expected to increase significantly as printing resolution improves over time.

P-240  Greater Loading Capacity and Resolution for Improved Process-scale Peptide Purification. Jochen Saar1, Reno Nguyen2, Chitra Sundararajan3, Scott Anderson4, Dennis McCreary4, Janine Sinck5, Grace, Worms, GERMANY; 2Grace, Hesperia, CA, USA; 3Grace, Hyderabad, INDIA; 4Grace Discovery Sciences, Deerfield, IL, USA; 5Grace, Columbia, MD, USA

Peptides and proteins are becoming increasingly popular for their potential use as therapeutic drugs. To earn and maintain a share in the fast-growing peptide market, peptide researchers and manufacturers are constantly trying to improve and optimize the various steps in peptide synthesis. One of the main bottlenecks in peptide synthesis is the purification step. Techniques such as FPLC and preparative HPLC are limited by small loading amounts, long separation times, poor recoveries and high costs. Here, we demonstrate that flash chromatography can be a powerful tool in the fast and efficient purification of a diverse range of peptides. A new wide pore C18 phase expands flash purification capabilities to peptides and proteins approaching 70000 MW, while providing better resolution based on the smaller particle size. We present data to show the benefits of higher loading and faster purifications in peptide purification. This rapid purification technique ensures less degradation of peptides and provides better recovery, yield and purity.

P-241  New Wide Pore Media Improves Loading Capacity and Productivity of Peptide and Protein Purification by Flash Chromatography. N. K. Bopanna, Chitra Sundararajan, Melissa Wilcox, Janine Sinck, Reno Nguyen, Grace Discovery Sciences, Deerfield, IL, USA

Peptides and proteins are becoming increasingly popular for their potential use as therapeutic drugs. To earn and maintain a share in the fast-growing peptide market, peptide researchers and manufacturers are constantly trying to improve and optimize the various steps in peptide synthesis. One of the main bottlenecks in peptide synthesis is the purification step. Techniques such as FPLC and preparative HPLC are limited by small loading amounts, long separation times, poor recoveries and high costs. Here, we demonstrate that flash chromatography can be a powerful tool in the fast and efficient purification of a diverse range of peptides. A new wide pore C18 phase expands flash purification capabilities to peptides and proteins approaching 70000 MW, while providing better resolution based on the smaller particle size. We present data to show the benefits of higher loading and faster purifications in peptide purification. This rapid purification technique ensures less degradation of peptides and provides better recovery, yield and purity.

P-242  Methodical Guide for Stationary Phase Selection for Early-Discovery Peptide Analysis and Process-Scale Peptide Purification by HPLC. Oscar R. Rebolledo1, Imre Sallay2, Akira Sugisaki1, Junichi Kadoya2, 1DAISO Fine Chem USA, Inc., Torrance, CA, USA; 2DAISO Co., Ltd., Osaka, JAPAN

DAISOGEL® chromatographic packing materials for drug discovery and process-scale manufacturing provide unsurpassed performance and mechanical stability. The research and development of DAISOGEL® stationary phases started more than a quarter century ago, but it is still being refined and re-invented to keep it at the cutting edge of separation technologies. With ISO 9001 certified manufacturing of bare silica and GMP compliant bonding facilities, our reversed phase (RP) chemical modifications provide outstanding quantitative and qualitative information. The separation mode of choice among peptide manufacturers is C18/ODS/Octadecyl, however, C8/Octyl phases down to C1, Aminopropyl (APS), Phenyl, Cyano, and Diol, provide customized solutions to the most complex and challenging separations. Additional bonding types and bonding densities feature 100% aqueous solvent applications, extended acidic and alkali resistance, with high mass loadability. This, combined with exceptional mechanical strength and availability in various pore and particle sizes makes DAISOGEL® excellent for screening and process development of peptide and peptide-related API purifications. Answering the demand for customized solutions, here we demonstrate a methodical guide for stationary phase selection for analytical and preparative peptide purification. With superior durability, pH resistance, and suitable for Dynamic Axial Compression (DAC) columns, DAISOGEL® bridges the gap from drug discovery to process-scale manufacturing of peptides.
Development of a Strategy for Scaling SFC Methods: Applications for Preparative Chromatography. Christopher Hudalla, Abhijit Tarafder, Kenneth Fountain, Waters Corporation, Milford, MA, USA

It is well known that for separations using CO2 as the principal component of a mobile phase, analyte retention factors are influenced largely by the mobile phase density and temperature. Because of the high compressibility of CO2 under standard operating conditions, the density can change significantly with changes in pressure (under isothermal conditions) with retention factors decreasing with increasing mobile phase density (pressure). In addition, the selectivity and resolution of the analytes may be impacted as they respond differently to the same changes in mobile phase density. This can present a challenge when attempting to transfer a method between different column configurations that involve changes in column length or stationary phase particle size, which in turn alters the pressure (density) profile for the separation. This is best exemplified when analytical scale separations developed using UltraPerformance Convergence Chromatography (UPC2), employing sub-2µm stationary phases, are scaled up for preparative SFC conditions, employing 5µm particle size stationary phases. The difference in the density profiles across the column, between the analytical and the preparative system, will lead to very different chromatography unless the scale-up procedure is guided by a systematic approach. Here we present a strategy for scaling SFC separations between various column configurations. This will be demonstrated for the transfer of methods between analytical columns of different configurations, and more interestingly, for a method developed under analytical UPC2 conditions with subsequent scale up to preparative SFC conditions. This ability enables the rapid screening of methods on the faster analytical scale, with the direct transfer of the final method to preparative chromatography, resulting in significant savings in time and mobile phases.


As an extension of recent advances with our Zeta PlusTM encapsulated single-use depth filtration platform, we are developing advanced adsorptive anion exchange media for use in downstream purification processes. These media include a hybrid (adsorptive and size exclusion) purifier for particulate and soluble contaminant reduction prior to affinity chromatography and a single-use AEX chromatography membrane adsorber that exhibits high protein binding capacities as well as excellent tolerance to high ionic strength. This poster will present data from the development of these new materials. The hybrid purifier may provide an alternative to conventional depth filtration media for the removal of turbidity while also providing excellent host cell impurity removal. Optimized membrane adsorbers display dynamic binding capacities for bovine serum albumin (BSA) of greater than 100 mg protein/mL of membrane volume, LRV’s for virus of > 6 at 150 mM NaCl, and efficient removal of host cell proteins and DNA in multivalent buffers.

The Effects of General and Spinal Anesthetic Techniques on Endothelial Adhesion Molecules in Caesarean Section. Mehtap Honca1, Tarık Purtuloglu2, E.Ozgur Akgul2, Muzaffer Oztosun2, Tevfik Honca2, Ali Sizlan2, Mehmet Agilli2, Ibrahim Aydin2, F. Nuri Aydin2, Halil Yaman2, 1Kecioren Training and Research Hospital Clinic of Anesthesiology and Reanimation, Ankara, TURKEY; 2Gulhane School of Medicine, Ankara, TURKEY

Abstract Background: The different anesthetic methods and surgical trauma cause metabolic and inflammatory changes that correlate with the extent of tissue damage. The aim of this study was to evaluate a possible relation between maternal and fetal inflammatory modifications during general anesthesia (GA) and spinal anesthesia (SA) in healthy parturients undergoing elective caesarean section. Methods: Patients were randomly assigned to the general anesthesia (n=20) or spinal anesthesia (n=20) groups. Maternal and cord blood neopterin, sE-selectin and sL-selectin levels were measured in the GA and the SA groups. Neopterin concentrations were measured by high performance liquid chromatography. sE-selectin and sL-selectin levels were measured by ELISA method. Statistical analyses were performed by using SPSS 15.0 software. Results: Cord blood neopterin concentrations in the SA group were not different than those in the GA group, but maternal neopterin levels in the SA group were different than those in the GA group (p = 0.465 and p < 0.001, respectively). Maternal and cord blood levels of sE-selectin and sL-selectin were not different in both groups (p = 0.579 and p = 0.725, respectively). Similarly, the cord blood levels of sE-selectin and sL-selectin were not different than the maternal levels in both the SA and the GA groups (p = 0.273 and p = 0.725, respectively). We found an increased inflammatory process in fetal circulation due to anesthetic method. Conclusions: These results indicate the effects of general and spinal anesthetic techniques on serum sL-selectin, sE-selectin and neopterin levels in neonates and parturients undergoing elective cesarean section.