Meeting Report: 30th International Symposium, Exhibit and Workshops on Preparative and Process Chromatography

The **30th International Symposium, Exhibit & Workshops on Preparative and Process Chromatography** was held in Philadelphia, PA, on July 16-19, 2017 at the Loews Hotel. The symposium was attended by more than 320 people from 21 different countries (66% US; 81% industry). More than 50 pharmaceutical, biotechnology, and fine chemical companies were represented along with 50 chromatography media, equipment and technology suppliers. The PREP2017 sponsors were:

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The Symposium and Exhibit were managed by **Janet Cunningham, Barr Enterprises**.

Two half-day pre-conference **Training Workshops**, led by teams of experts in the field, were offered: **(1) Preparative Chromatography for Biomolecule Purification by Batch and Continuous Processes**; and **(2) Preparative Chromatography for Purification of APIs, Peptides, and Oligonucleotides by Batch Chromatography, SMB, and SFC**.

Two morning tutorials provided specialized instruction in more practical aspects of preparative chromatography focusing on **Tips, Tricks, and Troubleshooting Analytical and Overloaded Prep Chromatography**, taught by C. Mazza (AkzoNobel) and T. Yan (Pfizer), and **Practical Concepts on Process Characterization and Validation of Biopharmaceuticals based on QbD Principles** taught by G. Ferreira (MedImmune)

The **Exhibit program,** included 30 equipment, media, and technology suppliers:

3M Separation Science Division
Ace Glass Inc.
Agilent Technologies
AkzoNobel/Kromasil
Bio-Rad Laboratories
Biotage
DAISO Fine Chem USA
Essential Life Solutions
GE Healthcare Life Science
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The exhibit provided ample opportunities to get acquainted with the very latest in stationary phases, systems, modeling software, and equipment for small, medium, and large-scale prep chromatography. Eight **Vendor Workshops** were also presented sponsored by **Agilent Technologies**, **AkzoNobel/Kromasil**, **Bio-Rad Laboratories**, **DAISO Fine Chem USA**, **GE Healthcare**, **Purolite**, **Thermo Fisher Scientific**, and **YPSO-FACTO.**

The **Scientific Program** included 72 oral papers divided in 4 keynote sessions, 5 plenaries, and 8 parallel sessions, as well as 90 posters.

The opening keynote session **Industrial Case Studies in Protein Chromatography**, organized and chaired by A. Hunter (MedImmune), included five talks that provided opportunities to learn about the latest challenges and solutions for the industrial purification of

biopharmaceuticals. S. Kandula (Merck & Co.) opened the session describing the rapid development and commercialization of **Keytruda, the first immunotherapy mAb to reach the market.** Her presentation highlighted the downstream processing challenges that were overcome through high-resolution chromatography media and continuous process/product quality monitoring. M. Wendeler (MedImmune) addressed **separation challenges encountered in the production of antibody-drug conjugates,** which were solved through a combination of orthogonal chromatographic steps using both traditional resins and monoliths. K. Goklen (GSK) provided an in-depth **comparison of repetitive batch chromatography (RBC) and multicolumn systems (MCC)** for mAb capture in a biopharmaceutical manufacturing environment. The presenter noted that, in practice, an optimized RBC process can be more advantageous and less complex than MCC.

J. Angelo (BMS) discussed the **impact of dextran sulfate on chromatographic processing** addressing the underlying mechanisms affecting protein transport and binding in the stationary phase as a function of resin architecture. In the final talk in the session, S. Fisher (Genentech) focused on the **clearance of HCPs that co-purify with the target mAb with emphasis on phospholipase B-like 2 (PLBL2)**. As explained by the presenter, multivariate studies guided the development of optimized wash and polishing steps to effectively clear PLBL2.

The second keynote session organized and chaired by T. Yan (Pfizer) focused on **preparative chromatography in drug discovery, development, and manufacture**. This session continues to be a popular feature of the PREP symposia addressing the ever important field of small and medium scale prep HPLC. J. DaSilva (Merck & Co.) described the combined **use of reverse phase HPLC and ion exchange chromatography to deliver 40 g quantities of an API** in the proper salt form for improved stability in IV formulations. G. Rosse (Dart Neuroscience) described a highly integrated **facility using SFC-MS to support the production of ~10,000 medicinal chemistry compounds per month.** The presenter indicated that replacing HPLC with SFC resulted in $300k/year in solvent cost savings. J. Szeliga (Pfizer) addressed the column **loadability of charged compounds in preparative RP-HPLC.** Theoretical calculations of the ionization constants of complex APIs and intermediates were shown to be useful in predicting optimum mobile phase compositions for their purification. M. Juza (Corden Pharma) addressed efforts to **overcome the deficiencies of simple UV detection by incorporating MS detection in prep-scale HPLC.** The presenter indicated that introducing hyphenated systems resulted in better-quantified and higher purity products. T. Yan (Pfizer) gave the last talk of the session discussing the use of **SFC to obtain baseline enantiomeric resolution of amino acids and small peptides used as building blocks for ADC linkers**. The presenter highlighted the benefits of polysaccharide-coated phases for these separations.

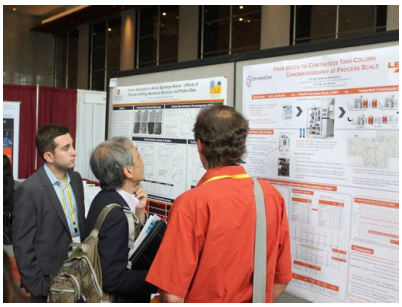
The last two keynote sessions were dedicated to **Continuous and Integrated Chromatographic Processing**. These sessions were chaired by I. Quinones-Garcia (Mersana Therapeutics) and by Olivier Dapremont (Ampac Fine Chemicals). The first of these sessions focused on biopharmaceuticals and featured and opening talk by L. Beaver (LAB Enterprises). The presenter offered an **up-to-date perspective on continuous manufacturing of pharmaceuticals** in terms of improved process efficiency and product quality. Included in the talk was a review of existing and emerging partnerships between government, academic, industrial, and scientific associations focused on advancing research in the field.

L. Auman (ETRH Zurich and ChromaCon) introduced **control strategies based on UV-detection for capture-SMB and multicolumn polishing processes (MCSGP)**. D. Sauer (ACIB Vienna) presented a **sophisticated, multi-detector system for the on-line monitoring of drug product quantity, purity, and potency**. The presenter demonstrated the approach by developing a statistical model to control the purification of Fibroblast Growth factor-2 using in-line UV/Vis, ATR/FTIR, Light Scattering, RI, and Fluorescence detector signals. N. Vecchiarello from the Cramer group at RPI introduced a

novel strategy for the in-silico development of integrated purification processes. The presenter demonstrated the approach for three commercial biological products. This first session concluded with a presentation by J. Hummel (Pall Life Sciences) describing a **BioSolve-based cost model for continuous and integrated downstream processing of biopharmaceuticals at the clinical and commercial scale**.

The second of the two keynote sessions on continuous and integrated processing shifted the focus toward small molecules. F. da Silva (Max Planck Institute, Magdeburg) described the **design of an 8-zone SMB process based on the equilibrium theory and ChromWorks™ software simulations**. S. Tie from the group of Y. Kawajiri at Georgia Tech provided a comparison of **esterification and transesterification reactions conducted in simulated moving bed reactors for the production of glycol ether acetate**. The presenter described a detailed model with kinetic parameters obtained from batch reactions and reactive chromatography experiments to optimize the SMBR design. G. Lodi (Politecnico di Milano) expanded on the SMB process by introducing a **four-column I-SMB scheme for the recovery of sugars from lignocellulosic hydrolysates**. The stationary phase in this case works based on the Donnan ion exclusion principle, which was incorporated into a model to numerically simulate the I-SMB performance. J. Mota (LAQV@REUIMTE, Lisbon) presented a proof-of-principle study of antibody capture by protein A aimed at validating a **one-column with recycle analog of three- and four-column SMB systems**. The approach uses a plug-flow device to accumulate and temporarily store eluted fractions that are then recycled. The presenter showed that the single-column process could achieve the same purity and productivity as the analogous SMB systems. A paper by F. Gritti (Waters Corporation) concluded the session with a detailed analysis of **recycling chromatography for separations with extremely low alpha-values**, such as the separation of deuterated benzene isomers. The presenter noted that the effects of local pressure on the local retention factor are significant and need to be taken into account for an accurate prediction of performance.

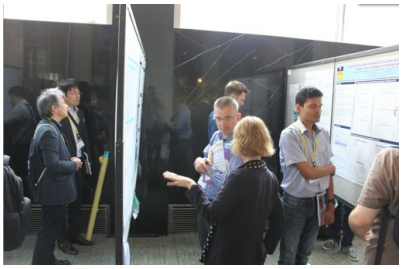
Two sessions were dedicated to mechanistic understanding and modeling of chromatographic processes. In the first of these two sessions, presented as a plenary and chaired by D. Roush (Merck & Co.), O. Khanal from the Lenhoff group at the U. Delaware, presented an interesting **study using confocal microscopy on protein adsorption on filter aids and depth filters**. J. Robinson (RPI) discussed the use of **structure-activity relationships (QSAR) to predict retention of model proteins and Fab variants on multimodal AEX and CEX resins.** She emphasized how molecular properties can be tuned based on this strategy to develop bioproducts with enhanced manufacturability. J. Diedrich (Forschungszentrum Juelich) introduced a new **model to describe protein binding in tentacle-type ion exchange resins.** The model postulates the existence of multiple binding states with protein surface interactions described by an extended version of the SMA model. The presenter indicated that model predictions are consistent with complex elution peak shapes observed experimentally for mAbs.



G. Wang (KIT) described the **calibration of mechanistic models of chromatography using artificial neural networks (ANN)** and its application to predict a three-component protein separation at low loadings. The last paper in the session by H. Luo (MedImmune) generated a lot of discussion. The presenter provided very explicit **evidence of the formation of two-liquid phases during mAb elution from protein A columns**. The two phases were responsible for turbidity observed in the eluate and for high backpressure in subsequent filtration steps. Liquid-liquid-phase separation (LLPS) was observed for a variety of conditions and different mAbs, but could be avoided or mitigated by appropriate choice of elution conditions.

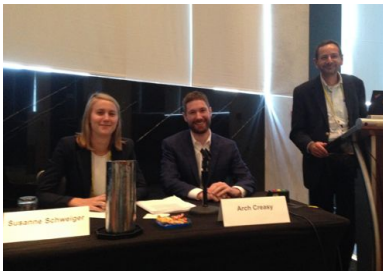
The second of the mechanistic understanding and modeling sessions, presented as a parallel

session and chaired by M. Schmidt (Genentech), included contributions from D. Antos (Rzeszow U., Poland) dealing with detailed **moment analysis techniques to scale-up protein chromatography**, by A. Matlschweiger (BOKU, Vienna) dealing with the phenomenon of **partial pore blocking during adsorption of mixtures of proteins that differ greatly in size**, by C. Frech (U. Appl. Sciences, Mannheim) dealing with the **prediction of the separation of mAb charge variants based on SMA and Donnan potential models**, and by K. Behere (U. Massachussetts, Lowell) dealing with the **impact of process cycling (including CIP) on antibody adsorption isotherms on a protein A resin**.



Three plenary sessions held on Wednesday were dedicated to non-traditional stationary phases, non-traditional applications, and process modeling. The first of these sessions was dedicated to monoliths and alternatives to packed beds and was chaired by M. Hearn (Monash U.). In the opening paper, C. Sanchez-Trasvina from the Rito-Palomares group at Tecnologico de Monterrey, Mexico, introduced **novel PEGylated monoliths that exhibit unique selectivity for the separation of PEGylated proteins**. T. Kuobo (Kyoto U.) described a variety of applications of **new "spongy" monoliths for high-throughput IgG capture by protein A** characterized by high speed, selectivity, and recovery. C. Fee (U. Canterbury, New Zealand) described recent **advances in the design and manufacture of 3-D printed chromatographic columns**. The approach allows for the creation of rationally designed geometries that maximize surface area and minimize pressure drop. The presenter indicated that **full-scale columns, suitable for manufacturing applications, will be achievable in the near future** with the rapid progress expected in 3-D software and hardware technologies. J. Hester (3M Company) illustrated recent **improvements in adsorptive membrane technology for both protein A capture and polishing including new bio-inspired ligand chemistries**. The last paper in this session presented by K. Marcus (Clemson U.) described the **modification of capillary channeled fibers with protein A ligands for IgG capture**.

The next plenary session focused on **applications of chromatography to virus, VLPs, and cells** and was co-chaired by A. Creasy (U. Virginia) and S. Schweiger (BOKU, Vienna). Both are current PhD students and did an excellent job introducing the speakers and moderating a lively discussion. The first paper by M. Brown (US FDA) focused on the **mechanisms of viral clearance in multimodal AEX columns**. The paper discussed whether the characteristic binding mechanism observed for isolated virus is retained when dealing with complex mixtures. P. Perreira-Aguilar (BOKU, Vienna) discussed the use of **polymer-grafted AEX resins for the purification of HIV-1 gag VLPs**. Although VLP binding was shown to be likely limited to the resin bead surface, the resin binding capacity, combined with high selectivity, was sufficient for process development. R. Silva (IBET, Oerias) reviewed progress made in the application of **non-traditional chromatographic techniques to purify large biomolecules and bioparticles**. Of particular interest was the use of core-shell bead technology (implemented with a CaptoCore prototype) to purify human mesenchymal stem cells in expanded bed mode. K. Schnorf (Sartorius Stedim) described new **modular cassette-type adsorptive membranes for the bind &elute purification of adenovirus, VLPs, and large proteins**. The new format was validated at scales up to 20 L of membrane volume. The last paper in the session, presented by S. Joseph from the Thomas group at U. Birmingham, described the development of **synthetic protein-based nanoparticles (PNP) as models for virus and virus-like particles**. The successful development of PNPs with defined size and pI was demonstrated matching the characteristics of actual adenovirus, human papilloma virus, and poliovirus.



The last plenary session was chaired by A. Jungbauer (BOKU, Vienna) and included a number of papers on chromatographic process modeling and simulation. J. Buyel (Fraunhofer IME) discussed the application of the **SMA model to describe binding of Ribulose-1,5-biphosphate carboxylase/oxygenase** ("RuBisCO") in oligomeric state to IEX resins. S. Yamamoto (Yamaguchi U.) introduced a **model to describe flow-through chromatography (FTC) for the separation of monomer-dimer protein mixtures** based on the determination of binding parameters from LGE

experiments. The FTC design model was validated for the separation of BSA monomer-dimer mixtures. The model was also found to be helpful to understand the sensitivity of the separation to small changes in mobile phase composition. D. Pfister (Ypso-Facto) next introduced a simplified **approach to the design of single and multicolumn capture systems**. Based on the comprehensive design strategy introduced by the presenter, optimum operating conditions and process configurations could be identified including a variant of a two-column capture SMB system with a two-step load cycle that was shown to improve productivity.

T. Huuk (GoSilico GmbH) described **mechanistic modeling as an alternative to DoE-based strategies to identify an effective operating space**. The presenter introduced a practical example where mechanistic modeling was able to identify conditions where a protein could be purified with the desired purity and yield that were completely missed by DoE modeling. The final paper by A. Schultze-Jena (TNO and Wageningen U.) focused on **understanding and modeling the effects of viscosity on the chromatographic separation of prebiotics galacto-oligisaccharides**.

The **8 Parallel Sessions** covered a broad range of important topics in prep chromatography: **Bioprocesses, Stationary Phases for Bio-Applications, Protein A and Affinity Chromatography, Stationary Phases for RP-HPLC, Mechanistic Understanding and Modeling, Supercritical Fluid Chromatography, Column and Molecule-Surface Interactions,** and **Natural Product Applications and CPC.** The topics ranged from advances in the manufacture of chromatographic resins, to understanding the thermodynamics of antibody binging to modified protein A resins, to improved HPLC stationary phases for peptide purification, to theory and practice of SFC, to column packing visualization with X-ray nanotomography and CFD simulation, to neutron scattering to detect resin structure and understand protein-surface interactions, to modeling the chromatographic behavior of zwitterionic species, to modeling and large scale-equipment for CPC and countercurrent chromatography with applications to natural products.

The **Poster Program** sponsored by **Bristol-Myers Squibb** and chaired by D. Antos (Rzeszow U., Poland) and Attila Felinger (U. Pecs, Hungary) comprised a total of 90 posters presented in alternate days and covering a tremendous range of prep chromatography problems and solutions for fine chemicals, APIs, and biomolecules. Details on the content of the parallel and poster sessions can be found in the PREP2017 Final Program at www.PREPsymposium.org.

Best Poster Award Winners*

First place winner – presenter from Industry: A. Lou[1], G. Jarvas[2], M. Lies[1], A. Guttman[2], **Cindy Liang[3],** **"**Fast Glycan Labeling and Analysis: High-resolution Separation and Identification in Minutes." [1]SCIEX, Brea, CA, USA; [2]Horvath Csaba Laboratory, Debrecen, HUNGARY; [3]SCIEX, Framingham, MA, USA

First place winner – presenter from Academia: **Franziska Ortner,** C. Ruppli, M. Marco, "Description of Thermodynamic Equilibria between Adsorbed and Convective Phases under Nonideal Conditions." ETH Zurich, Switzerland.

*Full list of award recipients will be available at www.PREPsymposium.org. Photo of winner from academia (left) with Symposium Chair G. Carta and members of the judging committee A. Felinger, D. Antos, J. Mota, and F. Gritti.

To conclude, as PREP2017 Chair, I want to thank all of the sponsors, the exhibitors, the participants, the contributors, and the members of the Scientific and Industrial Advisory Committees for making this Symposium a success.

PREP2018: Dates, venue, and program details will be posted at www.PREPsymposium.org.

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