Meeting Report: 29th International Symposium, Exhibit and Workshops on Preparative and Process Chromatography

The **29th International Symposium, Exhibit & Workshops on Preparative and Process Chromatography** was held in Philadelphia, PA, on July 17-20, 2016 at the Loews Hotel. The symposium was attended by more than 300 people from 20 different countries (71% US; 81% industry). More than 45 pharmaceutical, biotechnology, and fine chemical companies were represented along with 48 chromatography media, equipment and technology suppliers.

PREP2016 sponsors were:

Ampac Fine Chemicals
Avantor Performance Materials
Bristol-Myers Squibb
Genentech
GlaxoSmithKline
GE Healthcare
Kromasil
MedImmune
Merck & Co., Inc.
Pfizer
Shire
YMC America
Ypso-Facto

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

Three 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**; **(2) Preparative Chromatography for Purification of APIs**, **Peptides, and Oligonucleotides by Batch Chromatography, SMB, and SFC**; and **(3) Regulatory Fundamentals, QbD and DOE for Biopharmaceuticals**.

New this year was a Monday morning **tutorial entitled Tips, Tricks, and Troubleshooting Analytical and Overloaded Prep Chromatography**, taught by C. Mazza (AkzoNobel) and T. Yan (Pfizer) that covered critical factors to be considered when scaling up and optimizing preparative HPLC.

The **Exhibit program,** included 22 equipment, media, and technology suppliers:

Agilent Technologies
AkzoNobel/Kromasil
Avantor Performance Materials
Bio-Rad Laboratories
CC Biotech LLC
DAISO Fine Chem USA
Essential Life Solutions
Itochu Chemicals America
JASCO
JNC America
JSR Life Sciences
Kaneka
NOVASEP
PIC Solutions
Purolite Life Sciences
Quantum Analytics
Semba Biosciences
Separation Methods Technologies
SP Scientific - Genevac
Thermo Fisher Scientific
YMC America
Ypso-Facto

The exhibit provided ample opportunities to get acquainted with the very latest in stationary phases, systems, software, and equipment for small, medium, and large-scale prep chromatography. Seven **Vendor Workshops** were also presented sponsored by **AkzoNobel/Kromasil**, **Bio-Rad Laboratories**, **DAISO Fine Chem USA**, **GE Healthcare**, **Itochu America/Mitsubishi Chemical Corporation**, **Purolite**, and **Thermo Fisher Scientific**.

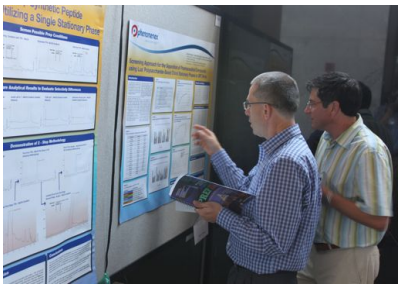
The **Scientific Program** included 70 oral papers in 5 keynote sessions, 5 plenaries, and 8 parallel sessions, as well as 72 posters.

The opening keynote session on **Industrial Case Studies in Protein Chromatography**, co-chaired by G. Carta (U. Virginia) and A. Hunter (MedImmune), included talks by Merck & Co., Bristol-Myers Squibb, Genentech, AbbVie, and MedImmune. R. Chmielowski (Merck) opened the session by describing the development of **a semi-continuous and integrated platform for mAb purification from perfusion and high-titer batch culture.** Z. Chen (Bristol-Myers Squibb) described a detailed investigation of **on-column aggregation of an Fc-fusion protein** suggesting mitigation strategies based on both mobile and stationary phase engineering. K. Lazzareschi (Genentech) described a

new approach to ensure **attainment of critical quality attributes based on process linkage assessment** along with several real-life examples. Natraj Ram (AbbVie) introduced new **pH-gradient elution approaches for improved mAb purification** by linking in series a pH-gradient generation column to a separation column. Several application examples were shown. A. Hunter (MedImmune) concluded the session by discussing challenges encountered in the **purification of recombinant polyclonal antibodies (rpAbs)**. The presenter concluded that, compared to CEX, HIC and MMC resins are often found to be better suited to attain aggregate removal without separating the rbAbs monomers from each other.

The second keynote session, co-chaired by O. Dapremont (Ampac) and K. Mihlbachler (Lewa) was dedicated to **Continuous Chromatography.** In general, this was a popular theme explored by many of the oral and poster papers throughout the Symposium. M. Morbidelli (ETH Zurich) described a **continuous manufacturing approach to produce therapeutic proteins** by integrating continuous upstream culture with continuous capture and continuous polishing. Based on the analysis of multiple case studies, the author demonstrated the ability of continuous processing to improve on the Pareto compromise inherent with batch processes. J. Mota (IBET, Portugal) introduced a new process concept for **continuous purification of viral vectors** based on a single column system with recycling through a mobile phase accumulation device. T. Huuk from the Hubbuch group at KIT, Germany, described a comprehensive **modeling strategy to convert a single-column batch process into 3- and 4-column periodic countercurrent processes** for more efficient capture demonstrating large improvements in productivity with optimally designed PCC systems. J. Lee (Max Planck Institute, Magdeburg) addressed the development of **model predictive control systems of SMB chromatography** based on simplified process models designed to improve operational robustness. In the final talk, R.-M. Nicoud (Ypso-Facto, Nancy, France) offered a thought provoking perspective on **rationally comparing different affinity chromatography processes for antibody capture** using process schemes of varying efficiency and complexity based on effective process modeling guided by experimental measurements to achieve reproducible and defined quality attributes.

The third keynote session chaired by T. Yan (Pfizer) focused on **preparative chromatography in drug discovery, development, and manufacture**. This session has become a regular feature of the PREP symposia addressing the ever important field of small and medium scale prep HPLC. R. McClain (Merck & Co.) presented on the use of **liquid carbon dioxide as a green alternative to organic solvents in prep and analytical HPLC** including several examples that demonstrated the effectiveness of this approach. T. Hochdorfer (Pfizer) discussed the use of **DOE for the design of process-scale HPLC** and urged the audience to implement this tool to develop optimum operating conditions. R. Buco (Shimadzu) presented the latest **instrumentation advances allowing HPLC and SFC to be conducted in a single integrated device including in-line SFE**. The final paper by J. Preston (Phenomenex) focused on **chiral prep HPLC as a tool to accelerate early drug development** including numerous examples to demonstrate strategies for method selection, optimization, and sample introduction techniques.



The fourth keynote session, co-chaired by L. Beaver (LAB Enterprises) and J. Edelman (Washington Chromatography Discussion Group) focused on **supercritical fluid chromatography (SFC)**. F. Gritti (Waters) highlighted the potential importance of **viscous heat generation in LC and SFC columns** and the ensuing radial gradients and reduced efficiency in non-adiabatic columns. The presenter showed that large improvements in performance can be obtained by placing the column in a vacuum housing that renders the column essentially adiabatic. M. Przybyciel (ES Industries) introduced **chemometric and molecular diversity modeling approaches to predict retention and selectivity** for various stationary phases in SFC, thereby reducing the amount of experimentation required. J. Boni (Novasep) discussed the use of **Gas Expanded Liquid Chromatography (GELC)** as an alternative to HPLC and SFC. According to the presenter, solubilizing CO2 in a liquid eluent allows operation well under 100 bar

while retaining many of the advantages of SFC. J. Hill (Waters) discussed the **scale-up of gradient elution SFC** and an approach to convert an analytical scale SFC gradient separation into an isocratic bulk purification method.

The last paper in the session presented by T. Fornstedt (Karlstad U., Sweden) discussed **peak-distortion effects in SFC caused by adsorption of the co-solvent** for both linear and overloaded chromatographic conditions. An important result discussed by the presenter is that the elution profiles for the same solute can be Langmuirian, anti-Langmuirian or distorted, dependent on (a) the co-solvent fraction and the adsorption strength of the solute relative to the additive and (b) the retention of the solute relative to the additive system peak.

The last of the keynote sessions co-chaired by O. Dapremont (Ampac) and M. Jacob (Phenomenex) included three papers dedicated to **preparative purification of peptides**. G. Sambeth (Bachem) highlighted **challenges and achievements in the prep scale purification of peptide APIs by RP-HPLC**. The presenter emphasized the importance of understanding the relevant physiochemical processes combined with state-of-the-art equipment for the effective scale-up from milligram to multi-kilogram scale. F. Lime (AkzoNobel/Kromasil) introduced **chemically stable organic/inorganic composite silica materials for HPLC of peptides and proteins**. Detailed analyses of the effects of high pH operation used for CIP on stability of RP-HPLC stationary phases were presented along with its impact on the separation of basic anti-depressants. The final paper by G. Krautz (Phenomenex) discussed the **impact of the loading factor on prep HPLC of peptides**. Case studies illustrating non-linear effects including sample displacement and tag-along were presented.

The two Tuesday-morning plenary sessions focused on **understanding and modeling biomolecule chromatography** (chaired by D. Roush, Merck) and on **innovative materials and processes for biochromatography** (chaired by K. Lazzareschi, Genentech). In the first of these two sessions, S. Banerjee from the Cramer group at RPI described enhanced **molecular modeling techniques to predict protein binding free-energies and elution in multimodal chromatography**. Predictions based on molecular modeling approaches designed for improved computational efficiency were compared with data obtained with MM ligands incorporated in self-assembled monolayers (SAMS). A. Lenhoff (U. Delaware) described the use of **X-ray scattering to probe the structure of grafted polymer layers in IEX media**. Combined SAXS and USAXS intensity profiles obtained for tentacle-type adsorbents showed a range of structural features that depended on ligand density, ionic strength, and counterion type demonstrating the ability to probe nanoscale-features with this powerful technique. D. Antos (Rzeszow U., Poland) discussed the **effects of isotherm non-linearity on the protein elution behavior in hydrophobic interaction and multimodal chromatography**. Especially noteworthy was the introduction of differential scanning fluorospectroscopy as a tool to probe conformational changes of proteins in the bound state. The presenter showed that the elution behavior observed is related to unfolding on the surface.
E. Hackemann (U. Kaiserslauten, Germany) outlined an interesting approach to **model protein adsorption on HIC resins as a function of mobile phase composition and pH for the case where mixed electrolytes are used**. While the approach presented is empirical, the model was able to predict single component isotherms over broad ranges of conditions.

In the next plenary focused on innovative materials and processes, A. Jungbauer (BOKU, Vienna, Austria) introduced **computational fluid dynamics approaches to predict the pressure profiles in monoliths**. The presenter showed predicted structures that could result in high hydraulic permeability. The next paper presented by C. Fee (U. Canterbury, New Zealand) introduced recent advances made in **3-D printing of stationary phases suitable for protein chromatography**. The presenter showed agarose-based structures printed with features on the order of 200 um, which is the resolution achievable with current technology. These features were shown to be effective for protein capture from solutions containing particulates and may serve as a substitute for EBA.

M. Franzreb (KIT, Germany) presented advances in the development of **thermally responsive stationary phases and equipment for temperature swing protein purification processes**. Using a Traveling Cooling Zone Reactor (TCRZ) system, the presenter illustrated the achievement of continuous bioseparation without alteration in mobile phase composition in a process completely driven by a temperature cycle. The last paper in the session, presented by C. Frech (U. Applied Sciences, Mannheim) retuned to the theme of modeling alternative process cycles for protein purification by developing an **approach based on the Donnan potential theory to describe the effects of salt concentration and pH on protein binding to weak CEX and AEX resins**.

The Wednesday program included three further plenary sessions focused on **chromatography for virus particles** (chaired by A. Jungbauer/G. Carta), **chromatography fundamentals** (chaired by J. Mota, IBET, Portugal), and **advances in CPC, CCC, and chiral chromatography** (chaired by Y. Kawajiri, GA Tech).

In the session on virus particles, M. Brown (US FDA) demonstrated the effective use of **high-throughput screening methods to optimize conditions leading to virus adsorption on selected AEX and multimodal resins** and to help identify the underlying binding mechanisms. Batch measurements were compared with column data to determine whether the HTS study results are scalable. R. Silva (IBET, Portugal) discussed **positive and negative modes of chromatography for the purification of virus-like particles using continuous multicolumn processes**. The presenter concluded that the best of the two alternative modalities needs to be selected by balancing complexity and required quality attributes. P. Marichal-Gallardo (Max Planck Institute, Magdeburg) addressed the **purification influenza virus particles obtained from cell-culture using hydrophilic membrane filters aided by non-ionic polymers**. The presenter showed that capture of the virus on the filter is caused by steric exclusion from the mobile phase driven by the non-ionic polymer. High recoveries where demonstrated by binding the virus in the presence of PEG and eluting it with a buffer without PEG. The last paper in this session presented by G. Gillam (Virginia Polytechnic Institute) discussed how the **heterogeneity of the charge distribution on the capsid proteins contributes to binding of the virus on IEX resins**.

In the next session on chromatography fundamentals, F. Ortner from the Mazzotti group at ETH Zurich introduced a **chromatography model taking into account the formation of two immiscible phases in the column**. Experimental results were also presented for the ternary system phenetole/methanol/water on a C-18 column. The model incorporating a thermodynamic description of liquid-liquid equilibria together with empirical adsorption isotherms was shown to be in very good agreement with the experimental results. A. Felinger (U. Pecs, Hungary) presented an investigation of the **chromatographic behavior of mefloquine on chiral zwitterionic columns as a function of the loading factor**. The presenter illustrated the importance of the buffer composition and the resulting interactions of ionic species with the stationary phase ligand on enantiomer separation. Peculiar peak shapes resulting from displacement of ionic species by the solute could be described by a detailed chromatographic model.

S. Yamamoto (Yamaguchi U., Japan) presented a general **approach to maximize the productivity of protein A processes used for antibody capture based on a simplified mechanistic model**. The presenter demonstrated a trade-off between productivity and buffer consumption as a function of the number of cycles used to process a given volume of feedstock in a set time. H.-K. Knutson from the Nilsson group at Lund U., Sweden, presented an approach for the **robust, multi-objective optimization of chromatographic processes** using the separation of rare-earth elements as a practical example. P. Vengsarkar from the Kawagiri group at GA Tech presented an approach for the **concurrent determination of adsorption isotherms and SMB design for multicomponent systems**. The presenter showed that more reliable predictions can be made if the isotherm parameters are updated based on SMB performance data.

In the last of the parallel sessions, L. Lorantfy (RotaChrom) described new equipment designs for **Centrifugal Partition Chromatography (CPC)**. Photographs of impressive large-scale units accompanied technical data related to the separation of zwitterionic species, peptides and model protein mixtures. The presenter suggested that such units could replace conventional RPC using two immiscible liquids instead of a gel. M. Knight (CC Biotech) continued on the two-liquid phase approach to chromatographic separation by describing new **spiral countercurrent chromatography units for preparative separations using aqueous two-phase extraction** including application to the separation of single-walled carbon nanotubes. The experimental data showed the different color fractions resulting from separating SWCNTs.

The final two papers addressed chiral separations. J. Kiplinger (Averica) illustrated the steps taken to develop **cost-effective approaches that merge consideration of technical aspects with current program goals, business needs, and scenario planning**. The presenter concluded that chromatography can be a viable production for single enantiomer products later in the drug development process than widely believed. K. Wrzosek from the Seidel-Morgenstern group at Max Planck Institute, Magdeburg, presented the last talk in the symposium on **coupling enzymatic racemization with enanatioselective chromatography can lead to high purity with high yield** by implementing an optimally timed strategy for product collection and racemate recycling.

The **8 Parallel Sessions** covered a broad range of important topics in prep chromatography: **Strategies and Processes for Biomolecule Purification**; **Stationary Phases I/II for small molecules and for biomolecules**; **Monoliths and Membrane Chromatography**; **New Developments in Affinity Chromatography**; **New Development and Applications of Continuous Chromatography**; **Column Characterization**; and **Advances in Chromatographic Modalities for Bioprocess Applications**. Details on the content of these sessions can be found in the PREP2016 Final Program at www.PREPsymposium.org.

The **Poster Program** sponsored by **Bristol-Myers Squibb** and chaired by D. Antos (Rzeszow U., Poland) comprised a total of 72 posters presented in alternate days and covering a tremendous range of prep chromatography problems and solutions for fine chemicals, APIs, and biomolecules. A complete list of the oral and poster papers can be found at www.PREPsymposium.org.

Winners of the **Best Poster Awards**, selected by an independent panel of judges, were recognized on Wednesday in two categories:

Best Poster Award Winners*

First place winner – presenter from Industry: **T. Tanaka**, N. Yamanaka, Y. Asaoka, M. Aoki, S. Nishiyama, S. Oe, T. Ide, "Efficient Separation of Antibodies based on Their Glycan Structures with Affinity Resin Coupling Engineered Fc Receptor", Tosoh Corporation, Ayase, Japan.

First place winner – presenter from Academia: **S. Nawada**, C. Fee, S. Dimartino, "A Comparison of Ordered Internal Column Morphologies Manufactured using 3D Printing", Univ. Canterbury, Christchurch, New Zealand and Univ. Edinburgh, UK.

*Full list of award recipients will be available at www.PREPsymposium.org. Photos of winner (left) with symposium chair and representatives of the judging committee, D. Antos and A. Podgornik.

On behalf of the Organizing Committee and as PREP2016 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.

PREP2017 will be held again at the Loews Philadelphia Hotel, in Philadelphia, PA, on July 16-19, 2017. Program details will be posted at www.PREPsymposium.org.

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