Meeting Report: 25th International Symposium, Exhibit and Workshops on Preparative and Process Chromatography

The **25th PREP International Symposium, Exhibit & Workshops on Preparative and Process Chromatography** was held in Cambridge, Massachusetts on July 15-18, 2012. The symposium was attended by more than 240 people from 25 different countries with two-thirds from the US and one third from outside the US. 21% of the participants were from academia and 79% from industry. More than 30 pharmaceutical, biotechnology, and fine chemical companies were represented. Meeting sponsors were **Agilent Technologies**, **American Elements**, **Amgen**, **Ampac Fine Chemicals**, **Genentech**, **GlaxoSmithKline**, **Lewa, MedImmune**, **Pall Corporation, Shire**, and **YMC America, Inc.**

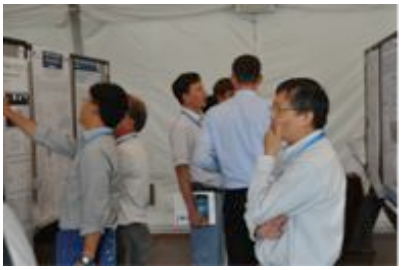
The **Exhibit program,** including 18 equipment and media suppliers, provided an opportunity to view the very latest in stationary phases, systems, and equipment for small, medium, and large-scale prep chromatography. Three training **Workshops** were offered the Sunday before the Symposium addressing prep chromatography for biomolecules, preparative chromatography for APIs, and regulatory and marketing aspects of biopharmaceuticals. The **Scientific Program** included 64 oral papers distributed between 5 keynote sessions, 6 plenary sessions, and 6 parallel sessions, as well as more than 60 posters. The Symposium and Exhibit were managed by Ms. Janet Cunningham, Barr Enterprises.

The opening keynote session on **Industrial Case Studies in Protein Chromatography** included talks by Shire, MedImmune, Amgen, Genentech, and Genzyme Corporation. A presentation by B. Cheang (Shire) described a comprehensive **QbD** approach for purification process development for therapeutic proteins based on **DOE**. The following presentations by MedImmune, Amgen, and Genentech provided further examples of applications of both DOE and physical models highlighting the optimization of **aggregate clearance in mAb purification with mixed-mode resins** (W. Chung, MedImmune), scaled-down models to examine the **stability of ceramic hydroxyapatite media** (B. Williamson, Amgen), and control of an **enzymatic cleavage step in F(ab')2 production** (M. Butler, Genentech). The final presentation in this session by R. Godawat (Genzyme Corporation) provided an assessment of **periodic countercurrent adsorption applied to protein capture**.

In the following plenary on **Biomolecular and Bioprocess Modeling**, Prof. S. Cramer (RPI) discussed the synergistic use of **molecular modeling and NMR measurements** to understand and predict ligand-protein interactions with multimodal resins. A lecture from Prof. A. Lenhoff's group (Univ. of Delaware) presented by S. Traylor introduced new **FRAP-based experimental measurements and models to describe protein transport in polymer-grafted ion exchangers** at high protein loadings and in multicomponent systems. A lecture by Prof. D. Antos (Rzeszow Univ., Poland) described the **integration and detailed modeling of IEC and HIC in the purification of a monoclonal antibody**, concluding that unfolding on the resin surface needs to be taken into account for a proper description of the process. Prof. S. Yamamoto (Yamaguchi Univ., Japan) discussed the experimental **accuracy of high-throughput screening (HTS) experiments** used to design capture columns and introduced simple but effective **physical models to correlate the data and arrive at optimum designs**. The final lecture in the session presented by J. Pallikal (MedImmune) described a DOE-based model to **optimize resin selection and throughput in mAb purification.**

A second keynote session on **Large Scale Columns** including presentations by MedImmune, Shire, Atoll, and Tarpon Biosystems covered several critical aspects related to columns used in manufacturing. A paper by J. Prentice (MedImmune) described the use of a **physical model to correlate and predict the pressure-flow behavior in columns up to 80 cm diameter**. I. Quinones-Garcia (Shire) reported on the use of **continuous monitoring techniques to predict the run-to-run stability of large-scale columns** based on RTD measurements using mobile phase transitions during various process steps. T. Shroeder (Atoll)

described the **performance and storage/shipping stability of pre-packed columns** up to 15 L in volume. The final paper in this session presented by M. Bisschops (Tarpon Biosystems) offered an analysis of the **impact of column-to-column variations on multicolumn chromatographic processes** for biomolecule purification.



Two parallel sessions on **Chiral Resolution** and **Stationary Phases** and the first of two **poster sessions** concluded the first day of the symposium. The session on **Chiral Resolution** provided examples of newly developed **chiral selectors** (Prof. T. Tan, Nanyang Technol. Univ., Singapore), an overview on **cGMP regulations** (S. Turujiman, US FDA), and mechanistic studies, based on detailed molecular models, of **enantioselective interactions on polysaccharide adsorbents** (Prof. E. Franses, Purdue Univ.). The session on **Stationary Phases** (the first of two) focused on **salt-tolerant ion exchangers** for protein purification (R. Gantier, Pall Life Sciences), new **affinity ligands for the separation of antibody fragments** (T. Nyhammar, GE Healthcare), and custom-based **affinity ligands developed with the aid of molecular modeling and extensive libraries** for capture and purification of non-antibody proteins and biosimilars (S. Williams, ProMetic BioSciences).

The second day of the symposium opened with a keynote session on **Continuous Chromatography**. Y. Chan (Bristol-Myers Squibb) presented a case study on the **development and optimization of continuous SMB chromatography** of a chiral API with a Varicol scheme. G. Stroehlein (ChromaCon) introduced **a new continuous two-column process (MCSGP)** and its application to the chromatographic purification of a mono **PEGylated protein** from un-PEGylated and multi-PEGylated components. K. Lacki (GE Healthcare) described **three- and four-column periodic countercurrent processes (PCC) for protein capture** with Protein A adsorbents, the enabling technology and its performance and robustness. Finally, a paper presented by O. Shinkazh (Chromatan Corporation) described a mAb capture process where small **adsorbent beads are continuously re-circulated through a countercurrent tangential flow filtration system**. In this approach, small particles were shown by the author to improve binding kinetics and throughput.

Applications of prep LC in **Discovery and Pharmaceutical Product Development** were discussed in the next keynote session. L. Miller (Amgen) provided an extensive overview of how his company addresses **purification challenges in pharmaceutical discovery and early product development** through application of prep HLPC and SFC. H. Weller (Bristol-Myers Squibb) focused on improving productivity through a **Lean Sigma approach to centralized purification** of leading drug candidates through LCMS, QC analysis, and data management. R. McClain (Merck & Co., Inc.) described an **approach to classify and select achiral stationary phases enabling the use of SFC in high-throughput semi-prep scale** supporting drug discovery. The final paper in the session presented by W. Leister (NIH) provided an overview of a **new software tool (S.M.A.R.T.) to manage the large set of data** generated in high-throughput prep purifications in **medicinal chemistry**.

The next plenary addressed the use of **chromatography for the purification of large bioparticles**, such as viruses and VLPs. A paper presented by D. Abraham (Merck & Co., Inc.) offered an overview of **quality by design (QbD) for vaccines** including several case studies demonstrating applications in production. The next paper, presented by Prof. A. Jungbauer (BOKU, Vienna), discussed the **separation of large virus-like particles and protein super-structures using monoliths** with numerous examples of successful applications at the lab and prep scales. The final paper in the session, presented by Prof. G. Carta (Univ. of Virginia), examined the **mechanism of protein and VLP adsorption in a large-pore cation exchanger** using both macroscopic and microscopic measurements based on CLSM and mass transfer models.

The morning program was followed by the second **poster session** and by four parallel

sessions. A session on **Fundamentals & Theory** discussed the occurrence of **multilayer adsorption** in LC columns (Prof. A. Felinger, Univ. of Pecs, Hungary), modeling LC column efficiency with **stochastic algorithms** (Prof. M. Thrash, Central State Univ.), holistic **optimization of preparative chromatography** (J. Samuelsson, Karlstad Univ., Sweden), and a new **by-pass chromatography scheme** to improve overall process efficiency for reduced purity requirements (Prof. R. Rajendran, Nanyang Technol. Univ., Singapore).

The second session dedicated to **Stationary Phases** introduced new beaded **chromatography matrices having a multilayer architecture** for selective purification of proteins and bioparticles (Prof. O. Thomas, Univ. of Birmingham, UK), novel **capillary channeled polymeric fibers** as a platform to develop both micro- and prep-scale columns with a variety of functionalities (Prof. K. Marcus, Clemson Univ.), the analysis of **enthalpic and entropic driving forces in protein adsorption** on reverse phase stationary phases (R. Desch, Univ. of Cincinnati), and a new **mixed-mode chromatographic matrix** for process scale purification of biomolecules based on large-pore polymeric beads (J. Liao, Bio-Rad Laboratories).

The last two sessions of the day were dedicated to **Modeling & Design** and to **Green Processes**. The session on **Modeling & Design** addressed the development of **optimum SMB processes for binary separations** along with an experimental verification of the model (B. Sreedhar, Georgia Tech), model-based **methodologies for protein purification process development** illustrated for a monoclonal antibody produced by hybridoma cell culture (B. Nfor, Delft Univ. of Technology, Netherlands), and **mechanistic modeling of monomer/aggregate resolution** by HIC (M. Kamga, Univ. of Massachusetts - Lowell).

In the session on **Green Processes** Prof. K. Mihlbachler (NJIT) presented an approach to evaluate the environmental impact of prep chromatography for pharmaceutical and API purification based on the "**Twelve Principles of Green Chemistry**". This presentation was followed by a paper on using **subcritical water as the mobile phase in HPLC** (Prof. Y. Yang, East Carolina Univ.) and by a paper presented by Prof. E. Lightfoot (Univ. of Wisconsin) on **using rational strategies to develop more efficient purification processes** by replacing traditional packed-bed chromatography with membranes and countercurrent systems.

The last day of the Symposium opened with a plenary on **Monoliths & Membranes.** Prof. G. Sarti (Univ. of Bologna, Italy) presented a **detailed physical model to describe IgG capture on monoliths and adsorptive membranes**. The author showed that properly accounting for thermodynamic and dispersion effects is critical for an accurate description of scaled-up units. A. Podgornik (BIA Separations) presented a generalized approach to **determine the thickness of the adsorbed layer in a monolith** based on pressure drop measurements. E. Leeb and L. Voswinkel, both from TU Munchen, Germany, presented the last two papers in the session devoted to **applications of membrane chromatography to peptide fractionation and to whey protein fractionation**, respectively.

The last two plenaries addressed **Bioprocess Applications** and **Continuous Chromatography, Modeling, & Optimization.** In the **Bioprocess Applications** session, S. Sun (Pfizer) described a **three-step purification process for an Ig-fusion protein** comprising Protein A for capture, AEX for initial purification, and ceramic hydroxyapatite for polishing. T. Linke (MedImmune) discussed the challenging problem of **removing a charge variant of a recombinant E. coli protein** formed during renaturation at high pH. The author showed that optimization of solubilization conditions and AEX could be used to reduce the undesirable variant. The last two papers by L. Pathange (Bayer HealthCare) and Y. Yang (Shire) discussed **continuous monitoring and assessment strategies for process control in commercial scale biopharmaceutical purification** and **DOE-based strategies coupled with high-throughput screening with mini-columns** to accelerate the definition of a proper parameter space in the purification of a biopharmaceutical for enzyme replacement therapy.

The session on **Continuous Chromatography, Modeling, & Optimization** began with a paper presented by Prof. A. Seidel-Morgenstern (Max Planck Institute, Magdeburg, Germany) on the **isolation of intermediately eluting**

target compounds by SMB chromatography. Prof. M. Morbidelli (ETH Zurich) presented an interesting **approach to define the proper parameter space for a peptide fractionation process** based on a detailed physical model together with an analysis of the sensitivity of the process to disturbances. The last paper in the session, presented by Prof. L. Wang (Purdue Univ.), described a **rapid method to design tandem SMB processes for ternary fractionation** based on a standing wave approach and dynamic simulations.

The PREP2012 program concluded with two keynote sessions on **Supercritical Fluid Chromatography** (SFC). These sessions addressed the complex behavior of supercritical CO_2 with and without modifiers. In the first session, a paper by W. Mack (Univ. of South Florida) discussed advances in the **determination of isotherms for enantiomers in SFC**. Prof. A. Rajendran (Nanyang Technol. Univ., Singapore) provided an extensive analysis, including new measurements, of different **sample injection strategies in prep SFC**. The next two papers, addressed the important problem of **recycling both CO_2 and organic modifiers in SFC** using either activated carbon (S. Thomas, Amgen) or special equipment (J. Whelan, Waters Corporation).

In the second SFC session, L. Nogle (Merck & Co., Inc.) described her company's comprehensive **strategy for high-throughput purification of pharmaceuticals and APIs using both chiral and achiral SFC** to support drug discovery. S. Zulli (Waters Corporation) then demonstrated how the versatility of **prep SFC can be enhanced by a new MS directed, open bed, fraction collection system**.

The last two papers presented by the Guiochon group addressed the reliability of flow control and the importance of kinematic viscosity in SFC. A. Tarafder (Univ. of Tennessee) showed very **accurate measurements of the actual flow rate of CO_2 in SFC systems** concluding that substantial variations can occur, directly impacting performance and reliability. Prof. G. Guiochon (Univ. of Tennessee) gave the final talk illustrating how **diagrams of kinematic viscosity as a function of column outlet pressure and temperature can be used to select proper operating conditions in SFC**.

Best Poster Award Winners

First place – Z. Horvath, Max-Planck Institute, Magdeburg, Germany "Continuous Synthesis and Purification through Direct Combination of a Flow Reactor and SMB"

Second place – B. Sreedhar, Georgia Tech, Atlanta, GA, USA, "Optimal SMB Design for Minor Actinide Separations"

Third place – S. Parimal, RPI, Troy, NY, USA, "Mobile Phase Modifier and Temperature Effects in Multimodal Chromatography

On behalf of the Organizing Committee and as PREP2012 Chair, we 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.

As this 25th PREP Symposium comes to a close and we recognize the legacy of a quarter century of PREP Symposia driving the field of preparative and process chromatography, we are pleased to announce that **PREP2013 will be held at the Westin Boston Waterfront Hotel, in Boston, MA, on July 14-17, 2013**. Program details will be posted in the near future at http://www.PREPsymposium.org.

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