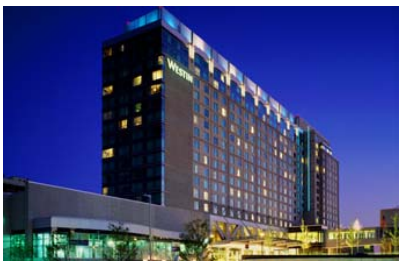
Meeting Report: 26th International Symposium, Exhibit and Workshops on Preparative and Process Chromatography

The **26th PREP International Symposium, Exhibit & Workshops on Preparative and Process Chromatography** was held in Boston, Massachusetts, USA on July 14-17, 2013 at the Westin Boston Waterfront Hotel. The Symposium was attended by approximately 300 people from 22 different countries with two-thirds from the US and one third from outside the US. 25% of the participants were from academia and 75% from industry. 38 pharmaceutical, biotechnology, and fine chemical companies were represented along with representatives from 37 chromatography media, equipment and technology suppliers. Meeting sponsors were **Agilent Technologies**, **Amgen**, **Genentech**, **GlaxoSmithKline**, **Lewa Nikkiso America, MedImmune**, **Pall Life Sciences, Pfizer, Shire**, and **YMC America, Inc.** The Symposium and Exhibit were managed by **Ms. Janet Cunningham, Barr Enterprises**.



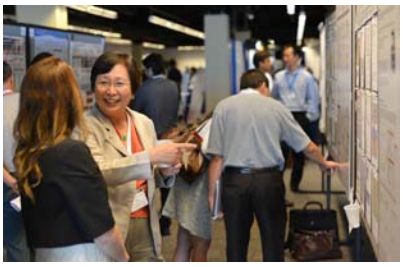
New this year was a joint day (Wednesday) with **ISPPP2013 - 33rd International Symposium and Exhibit on the Separation and Characterization of Biologically Important Molecules**, held in the same venue on July 17-19 and Co-Chaired by B. Boyes (Advanced Materials Technology and R. Orlando (Univ. Georgia), during which participants in either meeting had access to all oral and poster sessions and the exposition. This provided an excellent opportunity for exposure to scientific and technological advances in both preparative chromatography and biomolecular analysis.

The **PREP2013 Exhibit program,** including 21 equipment and media suppliers, provided ample opportunities to get acquainted with the very latest in stationary phases, systems, software, and equipment for small, medium, and large-scale preparative chromatography. Three **Training Workshops** were offered the Sunday before the Symposium addressing preparative chromatography for biomolecules, preparative chromatography for APIs, and regulatory and marketing aspects of biopharmaceuticals and were well attended. Four **Vendor Workshops** were also presented, two on Monday and two on Tuesday, sponsored by Agilent Technologies, Knauer, Lewa-Nikkiso America, and Pall Life Sciences.

The **PREP2013 Scientific Program** included 69 oral papers distributed between 5 keynote sessions, 5 plenary sessions, and 8 parallel sessions, as well as 90 posters. The opening keynote session on **Industrial Case Studies in Protein Chromatography** included talks by Amgen, Genentech, MedImmune, Pfizer, and Shire. A presentation by N. Aboulaich (MedImmune) described a **novel method to characterize HCP-mAb association** and understand the effects of mobile phase additives using a mAb crosslinked to an agarose matrix. The following presentation by K. Mani (Genentech) introduced a **general strategy for the characterization of the design space for an extraction process based on QbD** principles. A presentation by C. Chen (Shire) followed illustrating the **application of QbD/DOE to a high capacity AEX resin step** for a late-stage purification problem. S. Hernandez (Amgen) described the **use of small scale models for the optimization and life-cycle management** of industrial protein chromatography. The session concluded with a paper presented by T. Iskra (Pfizer) on **resin fouling mechanisms** in a flow through step for mAb purification quantified through microscopic and nanoscopic imaging tools. The presenter showed how a depth filter helped overcome the fouling problem.

A second keynote session was dedicated to **Increased Productivity and Reduced Costs in Small Scale Purification**. In this session, J. Twomy (Novartis) described an **open-access prep HPLC approach to facilitate handling large numbers of samples**. C. Kraml (Princeton University) discussed how to **build successful partnerships for**

effective outsourcing, while B. Khunte (Pfizer) described an **aggregated singletons strategy for automated purification workflow**. The session concluded with talks by J. Paulson (BMS) and C. Aurigemma (Pfizer) addressing the issue of **how to build a centralized purification service** and **how to use supercritical fluid chromatography (SFC) to increase workflow efficiency**.



The third keynote was dedicated to **Continuous Chromatography**. R.-M. Nicoud presented a stimulating perspective on **continuous chromatography and of its different modalities** as well as predictions on its future evolution. According to the presenter, the field has evolved from processes with large numbers of long columns to hybrid processes that use many fewer and much shorter columns for applications to chiral resolution and biomolecule purification. Current applications of **multicolumn chromatography systems for API purification** were then discussed by V. Pinilla (UCB Pharma) while B. Hritzko (BMS) addressed simulated moving bed **(SMB) use in early clinical development**. The session concluded with the presentation of a new use of SMB system for **continuous, matrix assisted refolding of a self-cleaving tagged fusion protein** by M. Wellhoefer (ACIB, Vienna).

The next keynote session was dedicated to **QbD and DOE Strategies in Chromatography and Downstream Processing**. It was clear from the presentations in this session that DOE has become prominent in biopharmaceutical process development. Following a detailed and comprehensive description by G. Ferreira (MedImmune) of **how the fundamentals of QbD are applied to protein purification process development**, E. Mueller (Shire) presented a case study where DOE was used to define an **effective procedure for packing a highly compressible resin in large scale columns**. The final paper, presented by K. Lacki (GE Healthcare), focused on **QbD strategies based on DOE and Monte Carlo simulations** to address the issue of **lot-to-lot resin variability** by properly defining a robust design parameter space for process chromatography.

The final keynote session was devoted to **Preparative Super- Fluid Chromatography (SFC)**. G. Guiochon (Univ. Tennessee) illustrated the **fundamental differences between GC, HPLC, and SFC and introduced a rational approach to measure adsorption isotherms in SFC**. The presenter pointed out that several-fold improvements in productivity are possible with SFC. Papers presented by J. Samuelsson (Karlstad Univ.) and A. Tarafder (Waters) addressed key **equipment issues and the scale up of SFC**, while X. Yang (GSK) discussed the effects of **mobile phase modifiers and additives on SFC separation of pharmaceuticals**. The final paper, by J. Whelan (Waters) described the use of **computational fluid dynamics (CFD) models to design SFC product collectors**.

Biomolecular and Bioprocess Modeling were the subject of an exciting plenary addressing some of the latest advances in this field. S. Cramer (RPI) got things started by presenting empirical and molecular dynamic simulation approaches to **understand the selectivity of multimodal resins** with an interesting comparison of two multimodal ligands that contain essentially the same chemical moieties but exhibit very different selectivity for proteins. R. Khalaf (ETH Zurich) introduced a **first-principles based model to describe proteins IEX equilibrium**, while S. Traylor (Univ. Delaware) focused on **high-throughput measurements of single and multicomponent protein adsorption equilibrium and kinetics**.

The last two papers in this session presented by A. Werner (Kaiserslauter Univ.) and D. Antos (Rzeszow Univ.) focused on hydrophobic interaction chromatography providing, respectively, **thermodynamic models and column dynamics models to understand and predict protein-surface interactions and chromatographic separations in HIC**. Antos offered an interesting quantitative comparison of productivity attainable with multicolumn systems and counter-current approaches. Although the exact result seemed to depend on the system to which these processes are applied, carrousel systems seemed potentially more productive in this study.

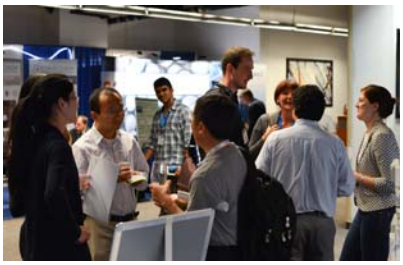
Three consecutive plenary sessions addressed **Bioprocess Applications** covering the application of **cold ethanol precipitation as a potential tool for mAb production** (A. Jungbauer, BOKU, Vienna), **high-throughput screening tools to define protein solubility maps** and select optimum buffer compositions (G. Barker, BMS), and additives to improve selectivity **in protein chromatography with multimodal media** (L. Wolfe, KBI Biopharma), **and with cation exchangers** (C. Frech, Mannheim Univ. Applied Science and S. Neumann, Roche Pharma). Several examples of continuous downstream processing for protein purification were also presented, including **multicolumn processes for IgG capture with protein A resins** (A. Grabski, Semba Biosciences), a **twin-column SMB system for capture applications** (M. Angarita, ETH Zurich), and an **integrated and fully continuous process to produce recombinant proteins** including both continuous upstream and downstream components (R. Godawat, Genzyme). The intense focus on these technologies seems to suggest that continuous bio-downstream processing is becoming a reality and may become common-place in the future. Other papers in these sessions, however, suggested that more traditional single column processes will continue to have advantages in terms of process simplicity, robustness, and predictability. S. Yamamoto (Yamaguchi Univ., Japan), illustrated a **practical approach to determine key rate and equilibrium parameters for antibody adsorption on protein A resins and to define operating conditions that maximize productivity** in single column operations. E. Schirmer (Eleven Biotherapeutics) demonstrated the successful **scale-up** (more than 100 fold) **of a multiple step chromatography process used to purify an IL-1 receptor antagonist** from E. coli, while J. Brand (Technische Univ., Munchen) illustrated the use of **ion exchange membranes to isolate egg-white proteins.**

The final plenary session was dedicated to **Chromatography of Large Biomolecules and Bioparticles**. P. Satzer (BOKU, Vienna) discussed the advantages of using a **SMB system to separate protein-loaded nanoparticles from the free protein** using SEC and presented a strategy to integrate resolution and protein recycling. M. Meininger (Max Planck Institute, Magdeburg) presented purification strategies for the **chromatographic purification of Enterovirus 71 VLPs**, while Y. Kurosawa (Hoya, Japan) introduced **ceramic hydroxyapatite for virus purification**. This session clearly demonstrated that preparative chromatography is also moving beyond molecular separations and into the field of nanoparticles opening up a range of opportunities for technological and scientific advances.

The 8 parallel sessions covered a broad range of current topics in preparative chromatography including **modeling & design tools**, **theoretical and experimental advances in the description of adsorption isotherms and adsorption kinetics**, **new and improved stationary phases**, **chiral resolution**, and **purification strategies for mAbs, FaBs, recombinant proteins, and peptides**.

In these sessions, there was a lot of emphasis on **mAb purification** suggesting that even though many companies have adopted platform processes, technical and scientific challenges continue to exist, especially with regards to resin life time and process productivity. **Multimodal resins**, including **mobile phase additives**, also received a lot of attention for both mAb and non-mAb applications. Advances were also presented on **mechanistic aspects of chiral resolution** and on approaches to **overcome reaction equilibrium limitations by combining biocatalysis with SMB**. Advances in the **design, optimization, and control of SMB** systems were presented. It seems that we are moving toward **model predictive feedback control systems** which could extend use of SMBs for less stable systems. Several papers introduced advances in the development and characterization of **new and improved ligands**, especially for bio-chromatography. **Protease-resistant hexapeptide ligands** for IgG purification obtained by introducing non-natural amino acids were presented by S. Menegatti (NC State Univ.), while M. Arnold (Novalix) presented a **high-throughput**

approach for the discovery of selective small-molecule ligands based on large libraries for protein purification.



Multimodal ligands receive a lot of attention also in these sessions showing that they are becoming increasingly accepted. **Polymer grafted ion exchangers** were shown to have improved capacity and kinetic performance for both native and PEGylated proteins (M. Zhu, Univ. Virginia), while **hybrid silica matrices** were shown to have high pH stability for reversed phase preparative separations (F. Lime, Akzo Nobel/Kromasil).

Physical, mechanical, and structural properties of stationary phases also received a lot of attention. K. Brisack (Bio-Rad) illustrated approaches to **pack compressible resins in columns up to meter-scale diameter**. C. Fee (Univ. Canterbury, New Zealand) presented the thought-provoking idea of doing away with packed beds replacing them with **structures obtained by 3D printing**. Several prototypes were presented along with the suggestion that although we are not yet there in terms of feature size, printing equipment, and material properties, there is tremendous promise for the future as 3D printing technology is refined. In a different approach, K. Marcus (Clemson Univ.) provided an update on using **capillary channeled fibers** to construct packed beds.

The **Poster Sessions,** held on Monday and Tuesday afternoons and Chaired by K. Mihlbachler (NJIT), comprised 90 posters presented in alternate days and covering a tremendous range of preparative chromatography problems and solutions for fine chemicals, APIs, and biomolecules. A number of oral papers were also presented as posters providing outstanding opportunities for further discussion of what was presented orally. A complete list of the poster papers may be found in the PREP2013 Final Program located at: www.PREPsymposium.org.

Winners of the Best Poster Awards, selected by an independent panel of judges, were recognized on Wednesday along with several other presenters who received honorable mention.

Best Poster Award Winners:

First place – Kartik Srinivasan, S. Parimal, M. Sorci, G. Belfort, S. Cramer, Rensselaer Polytechnic Institute, Troy, New York, USA, "Fundamental Investigation of Protein Ligand Interactions in Multimodal Chromatography using Single Molecule Force Spectroscopy and QCM"

Second place – Monica Angarita, T. Mueller-Spath, P. Arosio, R. Falkenstein, W. Kuhne, M. Morbidelli, ETH Zurich, ChromaCon, Zurich, Switzerland, and Roche Diagnostics GmbH, Penzberg, Germany, "Anion Exchange Chromatographic Purification of Recombinant Apolipoprotein A-I in the Presence of Urea"

Third place – Petra Gerster, A. Durauer, A. Jungbauer, Univ. Natural Resources and Life Science, Vienna, Austria, "Flow Dependent Entrapment of Submicron Particles in Monolithic Media"

Honorable mention:

Jing Guo, E. Fernandez, J. O' Connell, G. Carta, Univ. Virginia, Charlottesville, Virginia, USA, "Characterization of Antibody Aggregation on Cation Exchange Chromatography Media"

Suhas Nawada, C. Fee, S. Dimartino, Univ. Canterbury, Christchurch, New Zealand, "Re-Inventing the Packed Bed – Implications of 3D Printing for Chromatography"

Jennifer Zhang, R. Caple, L. Conley, S. Ghose, Biogen Idec, RTP, North Carolina, USA, "Maximizing the Functional Lifetime of Protein A Resins"

On behalf of the Organizing Committee and as PREP2013 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.

In conclusion, we are pleased to announce that **PREP2014 will be held at the Westin Boston Waterfront Hotel, in Boston, MA, on July 20-23, 2014**. Program details will be posted in the near future at www.PREPsymposium.org.

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