Meeting Report: 27th International Symposium, Exhibit and Workshops on Preparative and Process Chromatography

The **27th International Symposium, Exhibit & Workshops on Preparative and Process Chromatography PREP2014** was held in Boston, Massachusetts, on July 20-23, 2014 at the Westin Boston Waterfront Hotel. The symposium was attended by 300 people from 20 different countries (70% US, 30% non-US). 20% of the participants were from academia and 80% from industry. More than 45 pharmaceutical, biotechnology, and fine chemical companies were represented along with 37 chromatography media, equipment and technology suppliers.

PREP2014 sponsors were **Agilent Technologies**, **American Elements, Amgen**, **Ampac Fine Chemicals**, **Genentech**, **Grace, GlaxoSmithKline**, **Lewa Nikkiso America, MedImmune**, **Merck & Co., Inc., Pall Corporation, Pfizer, Shire**, and **YMC America, Inc.**

The Symposium and Exhibit were managed by **Janet Cunningham of BARR ENTERPRISES.**

Four pre-conference **Training Workshops** were offered: (1) **Preparative Chromatography for Biomolecules**, (2) **Preparative Chromatography for Intermediates and APIs**, (3) **Continuous Chromatography for Downstream Processing of Biomolecules**, and (4) **Regulatory Fundamentals and QbD Tools to Bring Biomolecules to Market**. The four workshops were attended by a total of about 80 people.

The **PREP2014 Exhibit program,** included 22 instruments, equipment, and media suppliers:

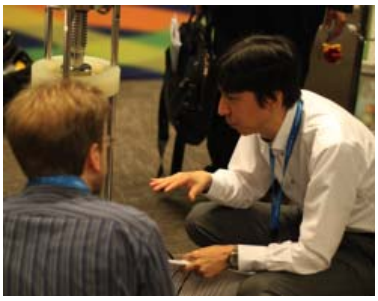
Advion
Agilent Technologies
AkzoNobel/Kromasil
Bio-Rad Laboratories
ChromWorks
DAISO Fine Chem USA
Fuji Silysia Chemical SA
Genevac, a SP Scientific Co.
Grace
Knauer
Labomatic Instruments
LEWA-Nikkiso America
Life Technologies
Mitsubishi Chemical
Novasep
Omnisep
Pall Life Sciences
Purolite
Semba Biosciences
Suzhou Nanomicro Technology
YMC America
Zeochem

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. Six lunchtime **Vendor Workshops** were also presented sponsored by **Grace**, **Knauer**, **LEWA-Nikkiso America**, **Life Technologies**, **Pall Life Sciences**, and **Purolite**.

The **PREP2014 Scientific Program** included 65 oral papers in 5 keynote sessions, 4 plenary sessions, and 8 parallel sessions, as well as 60 poster presentations.

The opening keynote session on **Industrial Case Studies in Protein Chromatography** included talks by Amgen, Genentech, MedImmune, Merck & Co., Inc., and Pfizer. A presentation by A. Hunter (MedImmune) introduced the use of **custom-made camelid antibodies as ligands for novel molecule purification.** The following presentation by B. Tangaraj (Amgen) illustrated how **HTS techniques can be leverage to select effective resin candidates** for Bi-ScFv purification. Next, J. Hou (Merck & Co., Inc.) described efforts to **replace protein media with less expensive membrane-based tools** to purify antibodies using Natrix membranes. A **mAb purification strategy called "overload and elute"** was introduced next by J. Patch (Genentech) based on the use of mixed-mode anion exchange for conditions where both target product and impurities bind strongly and competitively and are eluted selectively. The session concluded with a paper presented by W. Daniels (Pfizer) on **fouling mechanisms in a protein capture step for mAb purification** quantified through microscopic and nanoscopic imaging tools. The presenter showed how the experimental techniques developed

to characterize the fouled resin were also used to develop and validate the efficacy of improved cleaning protocols.



A second keynote session was dedicated to **Advancing Technologies in Support of Small Scale Purifications for Medicinal Chemistry, Drug Discovery and Development.** J. Kiplinger (Averica Discovery Services) described their **Target Isolation™ strategy** to purify medicinal chemistry products using alternate RP-HPLC and SFC tools. E. Streckfuss (Merck & Co., Inc.) described a **high-throughput workflow to isolate compounds generated by late-stage functionalization**. He reported that 20,000 compounds were isolated in his department in 2013 alone. D. Dunstan (Novartis) described the **use of SFC in small-scale prep to support medicinal chemistry** as a technique orthogonal to RP-HPLC. K. Hettiarachchi (Theravance BioPharma) illustrated a custom built **dual-mode SFC/RP-HPLC system for mass-directed purification** of medicinal chemistry compounds. Finally, M. Ventura (Amgen) described how they have addressed the **challenges of product recovery from SFC separations including solvent make-up strategies**.

The third keynote was dedicated to **Continuous Chromatography**. R.-M. Nicoud presented an enlightening **comparison of batch and continuous chromatography** concluding that, when practical operability constraints are considered, the relative efficiency of these systems depends on the particle size used. J. Blehaut (Novasep) described the design, construction, and operation of a **facility for the large-scale purification of omega-3 fatty acids** by combining single column and SMB technology. The next paper presented by A. Seidel-Morgenstern (Max-Planck Institute, Magdeburg, Germany) discussed options to **purify the malaria drug artemisin produced continuously in a photocatalytic flow reactor** using a 3-zone SMB with an additional regeneration zone. B. Sreedhar (Georgia Tech) provided a detailed **model-based analysis of SMB coupled with crystallization for the separation of enantiomers** at high purity and high productivity. Finally, J. Mota (Requimte/CQFB & IBET, Portugal) described a new **"Relay SMB" process**. According to the presenter, the R-SMB process requires less desorbent and is more productive than the standard SMB under conditions of finite column efficiency.

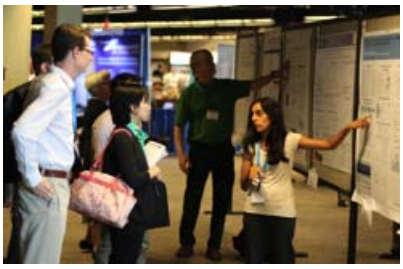
The fourth keynote session was dedicated to the **Process Scale Purification of Peptides.** A. Butte (ETH Zurich) addressed the role of both doping (ion exchangers) and main (RP) ligands on interactions with peptides. A mathematical model was presented to describe these interactions and predict the effects of modifier concentration, counter-ion concentration, and doping amount on retention. U. Altenhoner (Lonza) discussed the **advantages of chromatography in the production of highly active small molecules and peptides**. According to the presenter, process design is the most important factor to reduce risks in the production of highly active substances and that preparative chromatography is one of the key technologies for this purpose. B. DeHoff (Corden Pharma) addressed the specific challenges encountered in peptide purification and discussed the relative **advantages of various chromatographic approaches in terms of purity, yield, and throughput**. The last paper in the session, presented by J. Preston (Phenomenex), describe the use of a **single silica column to carry out a multiple-step peptide purification**. The selectivity at each step changed based on changes in mobile phase pH or choice of organic solvent. The presenter illustrated the strategy using the separation of various commercially significant synthetic peptides including Bivalirudin.

The last keynote session was dedicated to **Preparative Supercritical Fluid Chromatography (SFC)**. F. Kamarei (Univ. Tennessee) illustrated the **modeling of overloaded band profiles in SFC** taking into account the effect of pressure variation along the column on retention**.** The next speaker, M. Enmark (Karlstad University, Sweden), addressed the **scalability of preparative SFC** based on extensive experimental measurements and provided guidelines for reliable scale-up of chiral SFC. A. Tarafder (Waters) discussed the use of **rule-based approaches to scale-up SFC**. The author proposed matching the average pressure in the different scale column as a criterion in addition to the standard rules for scaling HPLC. M.-T. Liang (I-Shou University, Taiwan) described the

use of **SFC-SMB to purify the natural product Tanshinone IIA** suggesting that the approach is an effective greener alternative for this as well as future botanical drugs. Finally, G. Cox (PIC Solution) discussed an approach termed "**extraction-injection" or "X-Injection" to overcome sample injection challenges in SFC**.

Molecular and Process Modeling was the subject of an exciting plenary addressing some of the latest advances in this field. K. Srinivasan (RPI) presented empirical and molecular dynamic simulation approaches to **understand the thermodynamic basis of selectivity of multimodal resins**. P. Satzer (BOKU, Vienna) addressed conformational changes exhibited by proteins upon binding on the surface of nano-particles. Various models were present to explain the effects of surface curvature on protein unfolding. J. Angelo (U. Delaware) presented a study on the **elution behavior of proteins from polymer grafted stationary phases** based on a combination of chromatography and confocal microscopy studies. D. Antos (Rzeszow U., Poland) described a detailed **model to simulate protein folding in different chromatographic and non-chromatographic systems**. The model, validated using alpha-lactalbumin as a test case, showed the advantages and disadvantages of different matrix assisted and non-matric assisted refolding strategies. The final paper in the session, presented by F. Gritti (Univ. of Tennessee), provided a **model to describe adsorption on RPLC stationary phases that are doped with positively charged surface ligands**. The model, originally introduced by Uwe Neue who recently passed away, could successfully describe the overloaded elution profiles of different analytes and could be used to understand the effectiveness of these stationary phases.

The next plenary addressed **Monolith and Membrane Chromatography**. S. Podgornik (U. Ljubljana, Slovenia) illustrated the **connection between the mechanical properties of monoliths and their pressure-flow curves** through a model based on empirically determined porosity and pore size. C. Teepakorn (U. Lyon, France) used **computational fluid dynamics (CFD) to describe flow and dispersion in ion exchange membranes**. According to the presenter, the CFD model coupled with a description of the kinetics of protein binding could successfully predict the breakthrough curves of BSA. The final paper in this session, presented by P. Jorjorian (Gallus Biopharmaceuticals), described the use of **disposable, membrane chromatography systems as a platform for mAb purification**. According to the presenter, using hydrogel-based membranes provides large economic advantages compared to traditional chromatography columns.

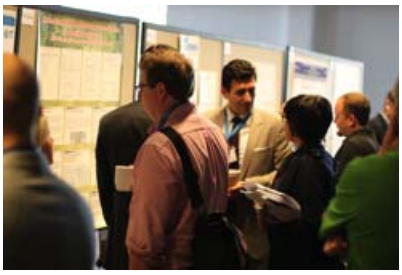


The next two plenary sessions covered **New Approaches for Biopurification** and **Improving Bioprocess Chromatography**. In the first of these sessions, R. Hahn (BOKU, Vienna) introduced the **use of microparticles** (1-2 µm in size, obtained by grinding inexpensive ion exchange resins) **for the rapid, direct and effective purification of proteins.** After adsorption, the microparticles are recovered by flocculation, which, in turn, can be enhanced by adding oppositely charged microparticles. A short movie of the adsorption and flocculation steps impressed the audience with its speed and simplicity. H.-T. Gan (Bioprocess Technology Institute, Singapore) described various **non-column, non-protein A processes for protein purification as alternatives to traditional chromatography columns**. O. Shinkazh (ChromaTan) provided updates on the development of a **countercurrent tangential chromatography system for the purification of antibodies**. In this system, the chromatography particles are kept in a slurry and undergo a full cycle of load, wash, elution, and regeneration as the slurry moves from one membrane stage to the other.

In the session on **Improving Bioprocess Chromatography** A. Cvetkovic (Pall Life Sciences) described the results of a study based on DOE and HTPD on **using membrane chromatography to purify influenza virus**. According to the presenter, membrane chromatography is a valuable alternative for the purification of influenza virus from clarified cell culture feedstock, allowing for faster, simpler and more cost efficient processing.

The last paper in these sessions presented by C. Huang (Bristol-Myers Squibb) provided the results

of an extensive **characterization of product-related impurities of an Fc-fusion protein and its use to design a robust downstream purification process**. The audience was especially impressed by the reported ability of this process to resolve isoforms based on their sialic acid content.



The **8 Parallel Sessions** covered a broad range of important topics in prep chromatography: **Purification Strategies and Processes for Biomolecules**; **Stationary Phases I & II**; **Resolution of Chiral Molecules and APIs**; **Modeling and Design of Chromatographic Processes**; **Continuous Chromatography for Bioseparations**; **DoE and QbD for Bioprocess Development**; and **Chromatographic Processes for Small Molecule Separations**.

The **Poster Sessions,** held on Monday and Tuesday afternoon and co-chaired by K. Mihlbachler (LEWA-Nikkiso) and A. Rajendran (U. Alberta), comprised 60 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 poster papers can be found in the PREP2014 Final Program at www.PREPsymposium.org. Winners of the **Best Poster Awards**, selected by an independent panel of judges, co-chaired by Mihlbachler and Rajendran, were recognized on Wednesday along with several other presenters who received honorable mention.

Best Poster Award Winners

First place winner – Mohan-Vivekanandan Poongavanam[1], L. Kisley[2], J. Chen[2], A. Mansur[2], Dominguez Medina[2], E. Kulla[2], M. Kang[2], B. Shuang[2], K. Kourentzi[1], S. Dhamane[1], C. Landes[2], R. Willson[1], "Mechanistic Insights into Protein Ion-exchange Adsorptive Separations using Single molecule, Super-resolution Imaging", [1]University of Houston, Houston, TX, USA; [2]Rice University, Houston, TX, USA

Second place winner – Yi Feng Lee[1], H. Graalfs[2], C. Frech[1], "Modeling of Dual Gradient Chromatofocusing in Ion Exchange and Multi-Modal Chromatography", [1]University of Applied Sciences Mannheim, Mannheim, Germany; [2]Merck KGaA, Darmstadt, Germany

Third place winner – Jing Guo, G. Carta, "On-Column Unfolding and Aggregation of a Glycosylated Monoclonal Antibody in Columns Packed with Different CEX Resins", University of Virginia, Charlottesville, VA, USA

Honorable mention

Sascha Keller, "The Benefits of Continuous Downstream Processing", Sandoz Biopharmaceuticals, Kundl, Austria

Fang Xia, H. Eastwood, K. Baucom, K. Gahm, D. Semin, "Multiple Chromatographic Approaches in Nano-particle Drug Substance and Intermediate Purification", Amgen Inc., Thousand Oaks, CA, USA

On behalf of the Organizing Committee and as PREP2014 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.

PREP2015 will be held at the Loews Philadelphia Hotel in Philadelphia, PA, USA on July 26-29, 2015. This meeting, celebrating 30 years of PREP symposia with a special session honoring Professor Georges Guiochon, will be followed immediately and in the same venue by **ISPPP2015 – 35th International Symposium and Exhibit on the Separation and Characterization of Biologically Important Molecules**, on July 29-31 . The common day will provide participants in either meeting had access to all sessions. **Program details will be posted at** www.PREPsymposium.org.

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