PREP 2012 Final Scientific Program

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Opinions expressed by individuals presenting abstracts and workshops are not necessarily the opinions of the PREP 2012 Symposium
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WELCOME TO PREP 2012

On behalf of the Organizing Committee, we welcome you to Boston and PREP 2012, the 25th International Symposium, Exhibit & Workshops on Preparative and Process Chromatography. We are especially happy to join you in celebrating a quarter century of PREP Symposia driving the field of preparative and process chromatography.

Chromatography continues to play a critical role in chemical and biochemical manufacturing from the gram scale to the multiple ton scale. PREP 2012 brings you a rich scientific program addressing the most recent developments in this field. The oral program includes keynote sessions on Industrial Protein Chromatography, on Large Scale Columns, on Continuous Chromatography, and on Preparative Supercritical Fluid Chromatography. Plenary sessions will address Biomolecular and Bioprocess Modeling, Chromatography of Large Bioparticles, Monoliths and Membrane Chromatography, Bioprocess Applications, and New Developments and Optimization of Continuous Chromatography. More specialized parallel sessions will address Chiral Resolution, Stationary Phases, Fundamentals and Theory, Modeling and Design, and Green Processes. Posters will be on display over a two-day period allowing you interact extensively with the presenters.

PREP 2012 also includes a vibrant Workshop Program as well as an extensive Vendor Exhibit. The Workshop Program includes three Sunday training courses on Preparative Chromatography for Biopharmaceuticals, Preparative Chromatography for Intermediates and APIs, and Bringing Pharmaceuticals and Biomolecules to Market, as well as two vendor workshops providing in-depth coverage on chromatography products, processes, and technology. The Exhibit Program includes companies who are leaders in the preparative and process chromatography arena through which you can become familiar with the latest commercial advances in the field. We invite to take advantage of all of these unique training and educational opportunities and to interact with vendors and providers of chromatography media, equipment, processes, and services.

Many people need to be thanked for making PREP 2012 possible. Firstly, we would like to thank the members of the Scientific Advisory Committee and of the Industrial Advisory Committee for their help in planning and promoting the Symposium and reviewing the abstracts. Secondly, we would like to thank our industrial sponsors, who have provided very generous financial support. Thirdly, we would like to thank all of the vendors for participating in the Exhibit and Workshop programs, as well as all of the people who have submitted so many excellent abstracts. Finally, we would like to thank you for attending PREP 2012. We very much hope you will enjoy the meeting and take home with you a wealth of information that will enable you to solve today’s separation problems and be prepared for the future of preparative chromatography.

Giorgio Carta
PREP 2012 Chair

Georges Guiochon
PREP Symposium Series Chair
Unique chromatography selectivities deliver economy and performance at every scale

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**GENERAL INFORMATION**

**VENUE**
Hyatt Regency Cambridge (Overlooking Boston, MA)
575 Memorial Drive, Cambridge, MA 02139-4896, USA
Phone 617-492-1234

**BADGES**
A name badge must be worn by each registered participant and accompanying person in order to gain admittance to the meeting and social gatherings.

**REGISTRATION**
If you need assistance, please come to the Symposium Registration Desk located in the Courtyard Reception Area, lobby level.

**EXHIBITS**
The exhibition is an important component of the meeting, so please take the time to thank all the exhibitors for their support of the program by visiting the booths located in President’s Ballroom C-D.

**EXHIBIT HOURS**
- Sunday: 6:00 PM – 8:00 PM
- Monday: 10:00 AM – 6:00 PM
- Tuesday: 10:00 AM – 6:00 PM

**RECEPTION**
Sunday, July 15, 2012 @ 6:00 PM – 8:00 PM
Located in the Exhibit Hall (President’s Ballroom C-D, lobby level)

**FREE VENDOR WORKSHOPS**
Free vendor workshops will take place on Monday and Tuesday. Seating is limited, so please sign up at the sponsor’s booth located in the exhibit hall as soon as possible

- **Monday 12:45 – 2:00 pm**
  Free Vendor Workshop Sponsored by Pall Life Sciences
  A Novel Salt Tolerant Anion Exchanger for Flexible Process Development
  Meeting Location: William Dawes Room *(light lunch will be provided)*
  Must register with Pall Life Sciences in order to attend as seating is limited

- **Monday 12:45 – 2:00 pm**
  Free Vendor Workshop Sponsored by Gilson and Phenomenex
  Increase Your Preparative Chromatography Productivity with New Versatile Instrument, Fraction Collection Control and Innovative Pre-packed Column Technology
  Meeting Location: Crispus Attuck Room *(light lunch will be provided)*
  Must register with Gilson or Phenomenex in order to attend as seating is limited

- **Tuesday 2:45 – 2:00 pm**
  Free Vendor Workshop Sponsored by Agilent Technologies
  LEWA Introduces Process Chromatographic Systems
  Meeting Location: William Dawes Room *(light lunch will be provided)*
  Must register with LEWA in order to attend as seating is limited

- **Tuesday 12:45 – 2:00 pm**
  Free Vendor Workshop Sponsored by LEWA
  Must register with LEWA in order to attend as seating is limited

**MESSAGES**
Located near the meeting rooms will be a board for messages as well as for posting notices of positions available and positions wanted.
POSTER SESSIONS

– SETUP: ALL posters in Poster Session I and Poster Session II should be put up on poster boards on Monday between 8:30 a.m.-1:00 p.m. and left on the poster boards for participants to view throughout the day on Monday and Tuesday.

– Poster boards are labeled with the number corresponding to the abstract number in the Final Program book (see Author Index located on pages 61-64).

– Posters with program numbers in the P-100 series are presented during Poster Session I. Authors presenting posters in Poster Session I are requested to be in attendance at their poster board during Poster Session I that takes place on Monday at 2:00-3:30 p.m.

– Posters with program numbers in the P-200 series are presented during Poster Session II. Authors presenting posters in Poster Session II are requested to put up their posters on Monday morning and to be in attendance at their poster board during Poster Session II that takes place on Tuesday at 2:00-3:30 p.m.

– TEARDOWN: All posters must be removed after the end of Tuesday’s Poster Session II, between 3:30-6:15 p.m. (anything remaining after 6:15 p.m. on Tuesday will be discarded).

POSTER COMPETITION

Poster presentations are a very important component of the PREP Symposia. In order to acknowledge their contribution to the field and high standards of the symposium, awards will be offered to the best posters at PREP 2012. All presented posters will be evaluated on the basis of scientific content, clarity of presentation, and layout. The Poster Judging Committee will have final say in the selection of the Prize Winners. At least two committee members will read each poster and top posters will be read by at least four committee members. If a poster author does not want his/her poster considered for a poster award, they must notify the Symposium Manager before 4:00 p.m. on Monday, July 16.

Wednesday, July 18

Presentation of awards to winners of the Best Poster Competition will take place at 9:40 a.m. on Wednesday morning following the last talk in Session 10. The awards will be presented by Dr. Kathleen Mihlbachler, Poster Session Chair. The winners are encouraged to be present, but it is not mandatory to be present to win.

AUTHOR INDEX

The Author Index is located on pages 61-64 in the Final Program book. “L” preceding the abstract number = Lecture “P” preceding the abstract number = Poster
Workshop 1: Preparative Chromatography for Biopharmaceuticals

Lecturers: Giorgio Carta, University of Virginia
Alan K. Hunter, MedImmune
Alois Jungbauer, BOKU, Vienna

This workshop will focus on the theory and practice of biomolecule chromatography. Since mass transfer and the structure of the stationary phase influence deeply chromatographic performance, the main emphasis is on describing adsorption/desorption kinetics in single and multicomponent systems and determining the relationship between stationary phase properties and process performance. The latest advances in stationary phase developments will be reviewed along with methods for their experimental characterization. Design and optimization strategies for capture and high-resolution applications will be discussed.

Topics

Adsorption equilibrium and transport in single and multicomponent systems;
Stationary phases for small and large biomolecules; Design and optimization for capture and high-resolution steps

Lecturers

Giorgio Carta received his Ph.D. in Chemical Engineering from the University of Delaware in 1984. Since then he has been a professor in the Department of Chemical Engineering at the University of Virginia, where his research focuses on transport phenomena and bioseparations. He regularly organizes professional courses on various aspects of bioseparations, including a course on protein chromatography development and scale-up together with Alois Jungbauer.

Alan Hunter received his Ph.D. in Chemical Engineering from the University of Virginia in 2002 and is currently Principal Scientist in the Process Biochemistry Group at MedImmune. Prior to joining MedImmune he held positions of Process Engineer at Cambrex Bio Science Baltimore, and of Senior Principal Scientist at Pfizer Biologics. Dr. Hunter has broad biotechnology experience and expertise in areas including process development for large-scale cGMP manufacture of biologics, recombinant biopharmaceutical purification, and technology transfer and scale-up.

Alois Jungbauer is the head of protein technology and downstream processing at the Department of Biotechnology of the University of Natural Resources and Applied Life Sciences in Vienna (Austria). For more than 20 years, Professor Jungbauer has worked in biochemical engineering, with a focus on bioseparation, where he has published widely and holds 15 patents. For over 10 years, he has organized a biennial professional course in protein chromatography focused on mass transfer, dispersion, and scale-up.
Workshop 2: Preparative Chromatography for Intermediates and APIs

Lecturers: Olivier Dapremont, Ampac Fine Chemicals
Kathleen Mihlbachler, New Jersey Institute of Technology;
Larry Miller, Amgen

This workshop will focus on development of method for the preparative purification of small molecules for the pharmaceutical industry. After an introduction of the theory, optimization and practice of prep HPLC, SMB and SFC for small molecule separations, the instructors will present practical approaches to the development of preparative separation through a series of examples. The attendees will learn valuable information and techniques to apply in the laboratory and at manufacturing scale to increase throughput and performance.

Topics

Prep HPLC batch - Theory, optimization and practice; SMB - Principle and technology; SMB - Examples and applications; SFC - Theory, equipment and examples

Lecturers

Dr. Dapremont has worked on the development of SMB technology since 1992. He is Director of Process Technologies at AMPAC Fine Chemicals where his role encompasses the development of SMB separations using multiple SMB units ranging from 4.6 mm to 1 m in diameter as well as developing continuous processes for the manufacturing of APIs.

Dr. Mihlbachler has worked in the field of separation technology with an emphasis on process chromatography for more than 15 years. As a Sr. Researcher at BMS, Eli Lilly and Pfizer, she worked on the development, scale-up and manufacturing of purification/separation processes for chiral and non-chiral compounds, peptides and proteins, in particular to implement continuous processes. Since the fall of 2011, Dr. Mihlbachler has taught in the Department of Chemical, Biological and Pharmaceutical Engineering at New Jersey Institute of Technology. She is also an Independent Consultant in the area of preparative chromatography and related separation technologies.

Dr. Miller is a Principal Scientist in the Discovery Analytical Sciences group at Amgen in Cambridge, MA. He graduated with a B.S. degree from the University of Illinois in Urbana-Champaign in 1984 and a M.S. from Roosevelt University in 1990. In 1984 he joined Searle in suburban Chicago as an analytical chemist. He spent twenty years performing small molecule achiral and chiral purifications at the mg to multi-kg scale, utilizing preparative HPLC, steady state recycle (SSR) and simulated moving bed (SMB) chromatography. While at Searle/Pharmacia, Larry managed a group of ten scientists within a purification center of excellence providing purification support to three R&D sites. In 2004 Larry joined Amgen where he is responsible for discovery and early development purification support utilizing preparative SFC and HPLC.
**Workshop 3: Bringing Pharmaceuticals and Biomolecules to Market (and keeping them there)**

Lecturers: Lois Ann Beaver, LAB Enterprises
Joan Newburger, Johnson & Johnson Consumer Products Worldwide

This workshop will focus on Quality by Design, Quality Systems Management, Science-based Risk Management, Process Analytical Technology and Continuous Process Improvement as tools that can be incorporated into basic drug development processes. We will examine the relationship between good drug development science, quality and regulatory flexibility, with emphasis on application to the biopharmaceutical industry. Particular attention will be placed on the practice of Quality by Design. A practical study of how the integration of quality and risk management is used in an application to bring a mAb to market will be presented. In addition, we will address the challenges for technology and regulation created by the emerging biosimilars industry along with the current status of internationally developed guidance.

**Topics**

Regulatory aspects, QbD, Process Analytical Technology, Quality System Management, Biosimilars, Marketing

**Lecturers**

After a multi-decade career with the U.S. Food and Drug Administration, in 2009 Lois Ann Beaver founded a regulatory consulting group, LAB Enterprises. While working in FDA’s Office of the Commissioner, Lois served on the FDA Pharmaceutical Quality Council that conceived and developed quality by design; led activity for FDA’s joining the international Pharmaceutical Inspection Cooperation Scheme; worked as liaison with international organizations such as WHO, most recently focusing on biosimilars; managed the export program for international investigations of unapproved pharmaceuticals; was associate director for international harmonization working on international best practice and standardization activities such as the ICH; was US Coordinator for Veterinary ICH; and served as delegate to the APEC (Asia Pacific Economic Cooperation) Life Sciences Innovation Forum. Lois also led international projects on anti-counterfeiting of medical products and good manufacturing practices in pharmaceutical firms in emerging countries, and established a pharmaceutical information center in Cairo.

Dr. Newburger has worked in pharmaceutical development for over 25 years at Wyeth Ayerst, Bristol-Myers Squibb and, currently, Johnson & Johnson. During the first half of her career, she led separations groups supporting medicinal, formulations and process chemists. For the past 7+ years, Dr. Newburger has led cross-functional teams, including clinical, CM&C, toxicology, regulatory and commercial members, having the responsibility of developing and implementing strategies for all Phases of prescription drug development and post-Approval support.

**SYMPOSIUM REGISTRATION**

Location: Courtyard Reception Area, lobby level

**WELCOME RECEPTION AND EXHIBITION OPEN**

**Sponsored in part by Pall Life Sciences**

Location: President’s Ballroom C-D, lobby level
Monday, July 16, 2012

7:45 am  Symposium Registration Open
Location: Courtyard Reception Area, lobby level

10:00 am-6:00 pm  EXHIBITION OPEN
Location: President’s Ballroom C-D, lobby level

8:20 – 8:30 am  WELCOME & OPENING REMARKS
Meeting Location: President’s Ballroom A

1. Keynote: Industrial Case Studies in Protein Chromatography
Chair: Giorgio Carta, University of Virginia, Charlottesville, VA, USA
Meeting Location: President’s Ballroom A

8:30 – 8:50 am  L-101  A Quality by Design (QbD) Approach for Purification Process Development of a Therapeutic Protein. Bee Lin Cheang, Ying Yang, Benjamin Simeone, Chenny Nian Yun Chen, Eric Tang, Daniel DaSilva, Yong Wang, Dave Nichols, Shire HGT, Lexington, MA, USA

8:50 – 9:10 am  L-102  Evaluation of Novel Mixed Mode Chromatographic Resins for Aggregate Removal in Monoclonal Antibody Purification. Wai Keen Chung, Zhu Min, Steven Timmick, David Robbins, Medimmune, Gaithersburg, MD, USA; University of Delaware, Newark, DE, USA


9:30 – 9:50 am  L-104  Controlling Process Parameters for Scale-up of a Pepsin Enzymatic Cleavage Step for F(ab’)2 Production. Michelle Butler, Marc Wong, Anjali Srivastava, Kim Kaleas, Genentech, S. San Francisco, CA, USA

9:50 – 10:10 am  L-105  Biomanufacturing Platform Application of Counter Current Chromatography System to Continuously Capture Proteins from Perfusion Cell Culture. Veena Warikoo, Rahul Godawat, Kevin Brower, Sujit Jain, Konstantin Konstantinov, Frank Riske, Genzyme Corporation, Framingham, MA, USA

10:10 am – 10:40 am  Break in the Exhibit Hall
Location: President’s Ballroom C-D, lobby level

2. Biomolecular and Bioprocess Modeling
Chair: Owen R. T. Thomas, University of Birmingham, UK
Meeting Location: President’s Ballroom A

10:40 – 11:10 am  L-106  Use of Molecular Simulations to Understand and Predict Multimodal Ligand-protein Interactions. Siddharth Parimal, Melissa Holstein, James Woo, Shekhar Garde, Steven Cramer, Rensselaer Polytechnic Institute, Troy, NY, USA

11:10 – 11:30 am  L-107  Mechanistic Investigations of Protein Transport in Traditional and Polymer-Modified Media. Steven J. Traylor, Brian D. Bowes, Steven M. Timmick, Abraham M. Lenhoff, University of Delaware, Newark, DE, USA

11:30 – 11:50 am  L-108  Isolating of Monoclonal IgG from CHO Supernatant by IEC and HIC. Adsorption Behavior and Kinetic Effects. Wojciech Marek, Renata Muca, Sylwia Wos, Wojciech Piatkowski, Dorota Antos, Rzeszow University of Technology, Rzeszow, POLAND
11:50 am – 12:10 pm  L-109  Predicting the Performance of Capture Chromatography by using Small Scale Experiments. Kazunobu Minakuchi, Hiroyuki Nagaoka, Noriko Yoshimoto, Shuichi Yamamoto, Yamaguchi University, Yamaguchi, JAPAN

12:10 -12:30 pm  L-110  Development of a Model to Maximize Productivity of Protein A Affinity Chromatography and a Strategy for Resin Selection. James Pallikal, Min Zhu, David Robbins, MedImmune LLC, Gaithersburg, MD, USA

12:30 – 2:00 pm  PAUSE, EXHIBITS, POSTERS, FREE VENDOR WORKSHOPS

12:45 – 2:00 pm  Free Vendor Workshop Sponsored by Pall Life Sciences  
A Novel Salt Tolerant Anion Exchanger for Flexible Process Development  
Meeting Location: William Dawes Room (light lunch will be provided)  
Must register with Pall Life Sciences in order to attend as seating is limited

12:45 – 2:00 pm  Free Vendor Workshop Sponsored by Gilson and Phenomenex  
Increase Your Preparative Chromatography Productivity with New Versatile Instrument, Fraction Collection Control and Innovative Pre-packed Column Technology  
Meeting Location: Crispus Attuck Room (light lunch will be provided)  
Must register with Gilson or Phenomenex in order to attend as seating is limited

2:00 – 3:30 pm  POSTER SESSION - I  
Chair: Kathleen Mihlbachler, New Jersey Institute of Technology, Newark, NJ, USA

3.  Keynote: Large Scale Columns  
Chair: Igor Quinones-Garcia, Shire, Lexington, MA, USA  
Meeting Location: President’s Ballroom A

3:30 – 3:50 pm  L-111  Understanding Pressure-flow Behavior During Chromatography Scale-up for Biopharmaceutical Manufacturing. Jessica Prentice, Steven Evans, Diemchi Vu, Gisela Ferreira, David Robbins, Process Biochemistry Department MedImmune, Gaithersburg, MD, USA

3:50 – 4:10 pm  L-112  Monitoring the Packed Bed Behavior of a Compressible Resin at Process Scale. Igor Quinones-Garcia, Eric Mueller, Ting Yang, Shire, Lexington, MA, USA


4:30 – 4:50 pm  L-114  The Impact of Variations in Column Packing on a Multicolumn Chromatography Process. Marc Bisschops\textsuperscript{1}, Mark Brower\textsuperscript{2}, \textsuperscript{1}Tarpon Biosystems, Worcester, MA, USA; \textsuperscript{2}Merck Research Laboratories, Rahway, NJ, USA

4A.  Chiral Resolution  
Chair: Attila Felinger, University of Pecs, Hungary  
Meeting Location: President’s Ballroom A

5:00 – 5:20 pm  L-115  Novel Chiral Selectors and Chiral Stationary Phases for High Performance Enantioseparation. Timothy T.Y. Tan, Nanyang Technological University, Singapore, SINGAPORE

5:20 – 5:40 pm  L-116  Overview of Current Good Manufacturing Practice (CGMP) Regulations for Dietary Supplements. Saleh Turujman, U.S. Food and Drug Administration, College Park, MD, USA
Monday, July 16, 2012

5:40 – 6:00 pm  L-117 Mechanistic Studies of Enantioselective Interactions of a Polysaccharide Sorbent with Benzoin and Ethyl Lactate. Hung-Wei Tsui, Nien-Hwa Linda Wang, Elias I. Franses, Purdue University, West Lafayette, IN, USA

4B. Stationary Phases - I
   Chair: Shuichi Yamamoto, Yamaguchi University, JAPAN
   Location: Paul Revere, lobby level

5:00 – 5:20 pm  L-118 Salt Tolerant Anion Exchanger for Flexible Process Development. Rene Gantier\textsuperscript{1}, Magali Toueille\textsuperscript{2}, Jerome Champagne\textsuperscript{2}, \textsuperscript{1}Pall Life Sciences, Northborough, MA, USA; \textsuperscript{2}Pall Life Sciences, Cergy, FRANCE


5:40 – 6:00 pm  L-120 Cost Effective Approaches for the Capture and Purification of Biosimilars. Steve Burton, Sharon Williams, Dev Baines, ProMetic BioSciences Ltd., Cambridge, UK

Tuesday, July 17, 2012

8:00 am  Symposium Registration Open
   Location: Courtyard Reception Area, lobby level

10:00 am-6:00 pm  EXHIBIT OPEN
   Location: President’s Ballroom C-D, lobby level

5. Keynote: Continuous Chromatography
   Chair: Olivier Dapremont, Ampac Fine Chemicals, Rancho Cordova, CA, USA
   Meeting Location: President’s Ballroom A

8:20 – 8:40 am  L-201 Development and Optimization of Continuous Chromatography for the Production of a Chiral API by Varicol. Yeung Chan, James Falabella, Olav Lyngberg, Bristol-Myers Squibb, New Brunswick, NJ, USA

8:40 – 9:00 am  L-202 Purification of PEGylated Proteins using a Continuous 2-column Chromatography Process (MCSGP). Thomas Müller-Spath\textsuperscript{1}, Guido Stroehlein\textsuperscript{2}, Lars Aumann\textsuperscript{3}, Massimo Morbidelli\textsuperscript{3}, \textsuperscript{1}ETH Zurich/ChromaCon AG, Zurich, SWITZERLAND; \textsuperscript{2}ChromaCon AG, Zurich, SWITZERLAND; \textsuperscript{3}ETH Zurich, Zurich, SWITZERLAND


9:20 – 9:40 am  L-204 Countercurrent Tangential Chromatography for Purification of Monoclonal Antibodies. Oleg Shinkazh\textsuperscript{1}, Andrew Zydney\textsuperscript{2}, \textsuperscript{1}Chromatan Corporation, State College, PA, USA; \textsuperscript{2}Penn State University, State College, PA, USA

9:40 – 10:10 am  Break in the Exhibit Hall
   Location: President’s Ballroom C-D, lobby level
6. **Keynote: Discovery and Pharmaceutical Product Development**  
Chair: Christopher Welch, Merck & Co., Inc., Rahway, NJ, USA  
Meeting Location: President’s Ballroom A

10:10 – 10:30 am  
L-205 **Addressing Purification Challenges in Pharmaceutical Discovery and Early Development.** Larry Miller, Amgen, Cambridge, MA, USA

10:30 – 10:50 am  
L-206 **Productivity in Medicinal Chemistry: A Lean Sigma Approach to Centralized Purification.** Harold Weller, Bristol-Myers Squibb Company, Cambridge, MA, USA

10:50 – 11:10 am  
L-207 **Achiral Stationary Phase Classification: Enabling the use of SFC in High Throughput Environments.** Ray McClain¹, Matt Przybycieł², ¹Merck, Princeton, NJ, USA; ²ES Industries, West Berlin, NJ, USA

11:10 – 11:30 am  
L-208 **From Purification to Plating: Novel Software Development to Manage the Data Deluge.** William Leister, Chris Louer, Chris LeClair, National Institutes of Health, Rockville, MD, USA

7. **Chromatography of Large Bioparticles**  
Chair: Jeff Salm, Pfizer, Boston, MA, USA  
Meeting Location: President’s Ballroom A

11:30 – 11:50 am  
L-209 **Quality by Design (QbD) for Vaccines.** Dicky Abraham, Merck & Company, West Point, PA, USA

11:50 am – 12:10 pm  
L-210 **Chromatographic Separation of Bionanoparticles and Biological Superstructures by Monoliths.** Alois Jungbauer¹, Petra Gerster², Nikolaus Hammerschmidt³, ¹ACIB, Vienna, AUSTRIA; ²BOKU, Vienna, AUSTRIA

12:10 – 12:30 pm  
L-211 **Protein and VLP Adsorption on POROS 50HS.** Yige Wu¹, Dicky Abraham², Jared Simons², Sarah Hooson², Giorgio Carta¹, ¹University of Virginia, Charlottesville, VA, USA; ²Merck & Co. Inc., West Point, PA, USA

12:30 – 2:00 pm  
**PAUSE, EXHIBITS, POSTERS, FREE VENDOR WORKSHOPS**

12:45 – 2:00 pm  
**Free Vendor Workshop Sponsored by Agilent Technologies**  
Meeting Location: Crispus Attucks Room  
*(light lunch will be provided)*  
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12:45 – 2:00 pm  
**Free Vendor Workshop Sponsored by LEWA**  
LEWA Introduces Process Chromatographic Systems  
Meeting Location: William Dawes Room  
*(light lunch will be provided)*  
Must register with LEWA in order to attend as seating is limited

2:00 – 3:30 pm  
**POSTER SESSION - II**  
Chair: Kathleen Mihlbachler, New Jersey Institute of Technology, Newark, NJ, USA
8A. Fundamentals and Theory
Chair: Andreas Seidel-Morgenstern, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, GERMANY
Meeting Location: President’s Ballroom A

3:30 – 3:50 pm
L-212  Multilayer Adsorption in Liquid Chromatography. Attila Felinger, Peter Vajda, University of Pecs, Pecs, HUNGARY

3:50 – 4:10 pm
L-213  Modeling Adsorption with the Gillespie Stochastic Algorithm. John Michael¹, Jillian Epstein¹, Choolwe Mandon¹, A.C. Dias-Cabral², Marvin Thrash³, ¹Miami University, Oxford, OH, USA; ²University of Beira Interior, Covilha, PORTUGAL; ³Central State University, Wilberforce, OH, USA

4:10 – 4:30 pm
L-214  A Holistic View on Optimization of Preparative Liquid Chromatography – Importance of Column Properties and Design. Torgny Fornstedt¹, Patrik Forssen¹, Joergen Samuelsson¹, Joakim Hogblom², ¹Karlstad University, Karlstad, SWEDEN; ²Eka Chemicals AB, Bohus, SWEDEN

4:30 – 4:50 pm
L-215  Bypass Chromatography: An Improved Process for Reduced Purity Requirements. Jani Siitonen¹, Tuomo Sainio¹, Arvind Rajendran², ¹Lappeenranta of Technology, Lappeenranta, FINLAND; ²Nanyang Technological University, Singapore, SINGAPORE

8B. Stationary Phases - II
Chair: Alois Jungbauer, BOKU, Vienna, AUSTRIA
Location: Paul Revere, lobby level

3:30 – 3:50 pm
L-216  Beaded Chromatography Matrices with Multiple Layers and Functions. Thomas Willett¹, Kritsandchalee Karnchanasri¹, James Walsh², Eirini Theodosiou³, Owen R. T. Thomas¹, ¹University of Birmingham, Birmingham, UK; ²University of Liverpool, Liverpool, UK; ³Loughborough University, Loughborough, UK

3:50 – 4:10 pm
L-217  Capillary-Channeled Polymer (C-CP) Fibers: Flexible Stationary Phases for Protein Separations and Diagnostics. R. Kenneth Marcus, Abby Shaddock-Hewitt, Zhengin Wang, Clemson University, Clemson, SC, USA

4:10 – 4:30 pm
L-218  Enthalpic and Entropic Driving Forces in Protein Adsorption in Hydrophobic Interaction and Reverse Phase Chromatography. Rebecca J. Desch, Jungseung Kim, Stephen W. Thiel, University of Cincinnati, Cincinnati, OH, USA

4:30 – 4:50 pm
L-219  Nuvia cPrime a New Novel Mixed-mode Chromatography Media for Process Purification of Bio-macromolecules. Jiali Liao, Hong Chen, Yueping Xu, Kin Lam, Xuemei He, Sherif Hanala, Mark Snyder, Russ Frost, Bio-Rad Laboratories, Inc, Hercules, CA, USA
Tuesday, July 17, 2012

9A. Modeling and Design

Chair: Dorota Antos, Rzeszow University of Technology, Rzeszow, POLAND
Meeting Location: President’s Ballroom A

5:00 – 5:20 pm  L-220  Optimal Binary Separation Schemes using Simulated Moving Bed Superstructure. Balamurali Sreedhar, Yoshiaki Kawajiri, Georgia Institute of Technology, Atlanta, GA, USA


5:40 – 6:00 pm  L-222  Modeling of Protein Purification by Hydrophobic Interaction Chromatography for Column Design and Process Optimization. Mark Henry Kamga, Haewoo Lee, Seongkyu Yoon, University of Massachusetts, Lowell, MA, USA

9B. Green Processes

Chairs: Lois Ann Beaver, LAB Enterprises, Rockville, MD, USA and Joan Newburger, Johnson & Johnson Consumer Products Worldwide, Skillman, NJ, USA
Location: Paul Revere, lobby level

5:00 – 5:20 pm  L-223  Evaluating Preparative Chromatography Against the “Twelve Principles of Green Chemistry”. Olivier Dapremont1, Kathleen Mihlbachler2, 1AMPAC Fine Chemicals, Rancho Cordova, CA, USA; 2New Jersey Institute of Technology, Newark, NJ, USA

5:20 – 5:40 pm  L-224  Green Liquid Chromatography using Subcritical Water as the Mobile Phase. Yu Yang, East Carolina University, Greenville, NC, USA

5:40 – 6:00 pm  L-225  Complex Fitness Diagrams: Downstream Processing of Biologicals. Edwin N. Lightfoot Jr., University of Wisconsin, Madison, WI, USA

Wednesday, July 18, 2012

8:00 am  Symposium Registration Open
Location: Courtyard Reception Area, lobby level

10. Monoliths and Membranes

Chair: Massimo Morbidelli, ETH Zurich, SWITZERLAND
Meeting Location: President’s Ballroom A

8:20 – 8:40 am  L-301  Scale-up of Convective Affinity Chromatography Based on Validated Physical Model. Simone Dimartino1, Cristiana Boi2, Giulio C. Sarti2, 1University of Canterbury, Christchurch, NEW ZEALAND; 2University of Bologna, Bologna, ITALY

8:40 – 9:00 am  L-302  Application of the Chromatographic Monoliths to Estimation of Adsorbed Layer Thickness. Ales Podgornik2, Mark R. Etzel1, Tina Sutar2, Nika Lendero Krajnc2, Ales Strancar2, 1Department of Food Science University of Wisconsin, Madison, WI, USA; 2BIA Separations, Ljubljana, SLOVENIA
9:00 – 9:20 am
L-303 Application of Membrane Adsorption Chromatography to Peptide Fractionation. Elena Leeb, Ulrich Kulozik, Seronei Cheison, Technische Universitaet Muenchen, Freising, GERMANY

9:20 – 9:40 am
L-304 A Comparative Study of Fast Flow Whey Protein Fractionation with Membrane- and Monolith-based Ion Exchange Chromatography. Linda Voswinkel, Ulrich Kulozik, TU Munchen, Freising, GERMANY

9:40 – 9:50 am
PRESENTATION OF AWARDS TO WINNERS OF THE BEST POSTER COMPETITION
Poster Sessions Chair: Kathleen Mihlbachler, New Jersey Institute of Technology

9:50 – 9:55 am
Invitation to PREP 2013: July 14-17, 2013 in the Westin Boston Waterfront Hotel
www.PREPsymposium.org

9:55 – 10:00 am
Invitation to ISPPP 2013: July 17-19, 2013 in the Westin Boston Waterfront Hotel
www.ISPPP.org

10:00 – 10:10 am
Pause

11. Bioprocess Applications
Chair: Abraham Lenhoff, University of Delaware, Newark, DE, USA
Meeting Location: President’s Ballroom A

10:10 – 10:30 am
L-305 Non-Platform Purification Process Development for an Ig-fusion Protein. Shujun Sun, Bill Daniels, Jason Cormier, Dan Lacasse, Chris Gallo, Pfizer, Andover, MA, USA

10:30 – 10:50 am
L-306 Strategies to Control the Concentration of a Charge Variant during Renaturation and Purification of a Recombinant Protein from E. coli Inclusion Bodies. Thomas Linke, Guoling Xi, Chris Thompson, Alan K. Hunter, MedImmune, Gaithersburg, MD, USA

10:50 – 11:10 am
L-307 Simple Quantification of Elution Profiles to Determine Chromatographic Column Performance, Product Quality Characterization, and Process Improvement Opportunities in Commercial Scale Biopharmaceutical Production. Lakshmi Pathange, Bayer HealthCare, Berkeley, CA, USA

11:10 – 11:30 am
L-308 High Throughput Process for Downstream DoE Study. Yiming Yang, Shire HGT, Lexington, MA, USA

12. Continuous Chromatography New Developments, Modeling and Optimization
Chair: Arvind Rajendran, Nanyang Technological University, SINGAPORE
Meeting Location: President’s Ballroom A

11:30 – 11:50 am
L-309 Isolation of Intermediately Eluting Target Compounds Out of Quasi–ternary Mixtures by SMB Chromatography. Jadwiga Nowak1, Dorota Antos2, Andreas Seidel-Morgenstern1, 1Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, GERMANY; 2Rzeszow University of Technology, Rzeszow, POLAND

11:50 am – 12:10 pm
L-310 Parameter Space Design of Peptide Chromatographic Purification Processes. David Getaz1, Guido Strohlein2, Alessandro Butte3, Massimo Morbidelli1, 1Institute for Chemical and Bioengineering ETH Zurich, Zurich, SWITZERLAND; 2ChromaCon AG, Zurich, SWITZERLAND, 3Lonza AG LES R&D - DSP Technologies, Wallis, SWITZERLAND
Wednesday, July 18, 2012

12:10 – 12:30 pm  L-311  High-Purity and High-Yield Separations of Three Amino Acids in a Tandem SMB: Rapid Standing Wave Design and Column Dynamics. George Weeden¹, Lei Ling², Chim Chin², Nien-Hwa Linda Wang¹. ¹Purdue University, West Lafayette, IN, USA; ²PureVision Technology, Ft. Lupton, CO, USA

12:30 pm  Pause

13. Keynote: Supercritical Fluid Chromatography (SFC) - I
Chair: Lois Ann Beaver, LAB Enterprises, Rockville, MD, USA
Meeting Location: President’s Ballroom A

2:00 – 2:20 pm  L-312  Isotherm Determination of Enantiomers on a Chiral Stationary Phase using Supercritical Fluid Chromatography. Wade Mack II, University of South Florida, Tampa, FL, USA

2:20 – 2:40 pm  L-313  Experimental Study of Injection Methods in Preparative Supercritical Fluid Chromatography. Yun Dai, Geng Li, Keng hong Goh, Ximena Aristizabal, Arvind Rajendran, Nanyang Technological University, Singapore, SINGAPORE


3:00 – 3:20 pm  L-315  CO₂ Recycling Study Utilizing Mixed Solvents on Preparative Supercritical Fluid Chromatography. John Whelan II, John Baugher, Ziqiang Wang, Waters Corporation, New Castle, DE, USA

14. Keynote: Supercritical Fluid Chromatography (SFC) - II
Chair: Georges Guiochon, University of Tennessee, Knoxville, TN, USA
Meeting Location: President’s Ballroom A

3:20 – 3:40 pm  L-316  New Advances in Prep SFC Chromatography at Merck Research Laboratories. Lisa Nogle, Ray McClain, Tin-Yau Chan, Jimmy DaSilva, Merck Research, Rahway, NJ, USA

3:40 – 4:00 pm  L-317  Expanding the Versatility of SFC through New Enhancements to MS Directed, Open Bed, Fraction Collection Systems. Steven Zulli, Jonathan Jones, Ziqiang Wang, Chuping Luo, Timothy Martin, Waters, New Castle, DE, USA

4:00 – 4:20 pm  L-318  Utility of Accurate Measurements of the CO₂ Mass Flow Rate in Supercritical Fluid Chromatographic Operations. Abhijit Tarafder, Georges Guiochon, University of Tennessee, Knoxville, TN, USA

4:20 – 4:40 pm  L-319  Importance of Kinematic Viscosity in Understanding Pressure Drop Phenomena in Supercritical Fluid Chromatographic Operation. Georges Guiochon, University of Tennessee, Knoxville, TN, USA

4:40 pm  Adjourn
P-101 Continuous Chromatographic Separation of Proteins with the Carousel Multicolumn Setup. Roman Bochenek, Wojciech Marek, Wojciech Piatkowski, Dorota Antos, Rzeszow University of Technology, Rzeszow, POLAND

P-102 Continuous Synthesis and Purification Through Direct Combination of a Flow Reactor and Simulated Moving Bed Chromatography. Zoltan Horvath1, Alexander G. O’Brien2, François Lévesqué2, Ju Weon Lee1, Peter H. Seeberger2, Andreas Seidel-Morgenstern1, 1Max-Planck-Institute for Dynamics of Complex Technical Systems, Magdeburg, GERMANY; 2Max-Planck-Institute of Colloids and Interfaces, Potsdam-Golm, GERMANY

P-103 Eliminating Tedious Fraction Transfer of Purified Natural Products via Automated Fraction Pooling of Lycopene and β-Carotene in Various Tea and Tomato Peel Extracts using a Manual Purification System. Mark Muncey1, Seth Hanson1, Megan York1, Toni Hoffhine1, Genevieve Gingras2, 1Gilson, Middleton, WI, USA; 2Collaborated, Quebec City, CANADA

P-104 HiScreen™ Prepacked Columns Designed for Process Development. Maria Björner, Annika Forss, Anna Heijbel, Katarina Öberg, GE Healthcare, Uppsala, SWEDEN

P-105 Novel Bioprocess Affinity Resins for the Capture of Antibody Fragments. Peter Lindahl, Bjoern Noren, Carina Engstrand, Maria Ersoy, Linus Laurin, Tomas Nyhammar, GE Healthcare Lifesciences, Uppsala, SWEDEN

P-106 Protein A Functionalization of Polypropylene (PP) Capillary-Channeled Polymer (C-CP) Fibers for the Purification of Immunoglobulin G (IgG). Abby J. Schadock-Hewitt, Zhengxin Wang, R. Kenneth Marcus, Clemson University, Clemson, SC, USA

P-107 Isolation of a Natural Product from a Complex Mixture: an Application of Preparative SFE and SFC to the Natural Product Purification Workflow. John McCauley1, Harbaksh Sidhu2, Rui Chen1, 1Waters Corporation, New Castle, DE, USA; 2Waters Corporation, Pittsburgh, PA, USA

P-108 Direct Capture of Alpha-amylase from CHO Cell Culture Supernatant using the Novel Salt Tolerant Anion Exchange Sorbent HyperCel STAR AX. Rene Gantier1, Magali Toueille2, Jerome Champagne2, 1Pall Life Sciences, Northborough, MA, USA; 2Pall Life Sciences, Cergy, FRANCE

P-109 Whey Protein Fractionation with Membrane Ion Exchange Chromatography at Pilot Scale. Linda Voswinkel, Ulrich Kulozik, TU München, Freising, GERMANY

P-110 Identifying Key Chromatographic Resin Attributes Associated with Process Efficiency. Karl McCann1, Yvonne Vucica1, Danni Wang2, Russ Frost2, Joseph Poblete2, Joseph Bertolini1, 1CSL Biotherapies, Broadmeadows, Australia; 2Bio-Rad Laboratories, Hercules, CA, USA

P-111 Thermodynamic Effects of Surface Curvature on Protein Adsorption. Amina Darwish, Rebecca J. Desch, Stephen W. Thiel, University of Cincinnati, Cincinnati, OH, USA

P-112 Mobile Phase Modifier and Temperature Effects in Multimodal Chromatography. Steven M. Cramer, Melissa Holstein, Siddharth Parimal, Shekhar Garde, RPI, Troy, NY, USA

P-113 Flow Microcalorimetry Analysis of Lysozyme Adsorption on Carboxymethyl Cellulose. Diogo Gomes1, Marvin Thrash2, A.C. Dias-Cabral1, 1University of Beira Interior, Covilha, PORTUGAL; 2Miami University, Oxford, OH, USA
P-114 **Study of a Model Racemic Steroid Mixture: Separation of a and b-estradiol by Flash Chromatography.** Brian Goolsby, Kendra Cox, Hitachi High Technologies America, Dallas, TX, USA

P-115 **Direct Protein Capture and Contaminant Removal from Undiluted Feedstocks.** Yamuna Dasarathy¹, Sylvio Bengio², Magali Touelle², Jerome Champagne¹, René Gantier¹, ¹Pall Life Sciences, Northborough, MA, USA; ²Pall Life Sciences, Cergy, FRANCE

P-116 **Novel Matrix Assisted Refold of Therapeutic Relevant Proteins and Peptides Produced with NPro Autoprotease Fusion Technology (NAFT).** Rene Funke, Sascha Keller, Gerlind Stoller, Sandoz GmbH, Kundi, AUSTRIA

P-117 **The Importance of Introducing Semi-Preparative Screening in Method Development - A Case Study.** Robert Fredriksson, Kristina Hallman, Akzo Nobel Separation Products, Bohus, SWEDEN

P-118 **Modeled Simulated Moving Bed Purification of 2-hydroxybutyric Acid.** Anthony Grabski¹, Shuvendu Das², Bruce Thalley¹, Jay Yun³, Alla Zilberman¹, Soo-Il Kim³, Mani Subramanian², Robert Mierendorf¹, ¹Sembia Biosciences, Inc., Madison, WI, USA; ²University of Iowa, Iowa City, IA, USA; ³ChromWorks, Inc., Burlington, MA, USA

P-119 **Comparison of Various Ternary Simulated Moving Bed Schemes by Nonlinear Programming.** Gaurav Agrawal, Yoshiaki Kawajiri, Georgia Institute of Technology, Atlanta, GA, USA

P-120 **Cation Exchange Chromatography Challenges in Balancing Binding Capacity Versus Resolution.** Inkwon Han, Sunitha Kandula, Edward Glowacki, David Roush, Thomas Linden, Merck Research Laboratories, Union, NJ, USA

P-121 **Investigating the Mechanisms of Protein Sorption and Transport in Cross-Linked Cellulosic Ion Exchangers.** James Angelo, Harun Koku, Abraham Lenhoff, University of Delaware, Newark, DE, USA

P-122 **Comparing Bulk Silica Phases.** Imre Sallay, Keiji Koyanagi, Daisco Co., Ltd., Osaka, JAPAN

P-123 **Chiral HPLC Methods for the Enantioseparation of Proton-Pump Inhibitors using Polysaccharide-based Chiral Stationary Phase in Normal-phase, Polar Organic and Reversed-phase Elution Modes.** Liming Peng, Marc Jacob, ThuyLinh Tran, Tivadar Farkas, Phenomenex, Torrance, CA, USA

P-124 **Cost-effective Purification of Peptides and Proteins using a Novel Hybrid Reversed-phase Packing Material.** Ernest Sobkow¹, Noriko Shoji, Chie Yamashita², Takatomo Takaï², Masakatsu Omote², Naohiro Kuriyama, ¹YMC America, Inc., Allentown, PA, USA; ²YMC Co., Ltd, Kyoto, JAPAN

P-125 **Characterization of a New Mixed-mode Resin.** Koji Nakamura, Yuji Kubo, Hiroyuki Moriyama, Tosoh Corporation, Yamaguchi, JAPAN

P-126 **Purification of a Synthetic Peptide using Multi-column Chromatography (Contichrom & MCSGP).** Guido Stroehlein¹, Thomas Mueller-Spaeth¹, Olav Lyngberg², Derek Maclean³, ¹ChromaCon AG, Zuerich, SWITZERLAND; ²Bristol-Myers Squibb, New Brunswick, NJ, USA; ³KAI Pharmaceuticals, South San Francisco, CA, USA

P-127 **instrAction® Mixed Mode Screening Kit - Stationary Phases for Preparative Chromatography as an Access to the instrAction® Phase Technology and Phase Library.** Martin Welter, instrAction GmbH, Mannheim, GERMANY

P-128 **The Effects of Medical Oxygen Therapy on Renal Ischemia/Reperfusion Injury.** Muzaffer Oztosun, Emin Ozgur Akgul, Erdinc Cakir, Tuncer Cayci, Bulent Uysal, Ayhan Ozcan, Taner Ozgurtas, Ahmet Guven, Ahmet Korkmaz, Gulhane Military Medical Faculty, Ankara, TURKEY

P-129 **Novel Biomarkers of Renal Scar Formation in Acute Pyelonephritis in Rats.** Yasemin Gulcan Kurt, Bahadir Caliskan, Ahmet Guven, Emin Ozgur Akgul, Bulent Uysal, Bilal Altan, Ayhan Ozcan, Orhan Bedir, Ilhami Surer, Ahmet Korkmaz, Gulhane Military Medical Faculty, Ankara, TURKEY
Tuesday Poster Presentations

Tuesday Poster Session Times: 2:00 – 3:30 PM

All posters are to remain on the poster boards for viewing all day Monday and Tuesday.

P-201 Development of an Affinity Ligand for IgG Purification by Engineering a Human Fc Receptor. Teruhiko Ide1, Toru Tanaka2, Kouta Hatayama2, Yoshiharu Asaoka1, Hidetaka Kobayashi1, Koji Nakamura1, ´Tosoh Corporation, Ayase, JAPAN; 2Sagami Chemical Research Institute, Ayase, JAPAN

P-202 Capture of an Acid-sensitive Recombinant Protein from E. coli Lysate using Mixed-Mode Chromatography. Magali Toueille, Jerome Champagne, Sylvio Bengio, Rene Gantier, Pall Life Sciences, Cergy, FRANCE

P-203 Determination of Pore Size Distributions in Capillary-channeled Polymer (C-CP) Fiber Stationary Phases by Inverse Size-exclusion Chromatography (ISEC). Zhengxin Wang, R. Kenneth Marcus, Clemson University, Clemson, SC, USA

P-204 Purification of Natural Products using Inverted Chromatographic Process Design. Dominik Bergs1, Axel Delp2, Matthias Joehnck2, Georg Martin2, Gerhard Schembecker3, 1TU Dortmund University, Dortmund, GERMANY; 2Merck KgA, Darmstadt, GERMANY; 3Lanxess GmbH, Leverkusen, GERMANY

P-205 A Novel Mustang® XT Device for Scale Up/down Optimization of Membrane Chromatography Purification Steps. Aleksandar Cvetkovic1, Iann Rance2, Rene Gantier1, Pall Life Sciences, Northborough, MA, USA; 2Cytheris SA, Issy Les Moulineaux, FRANCE

P-206 Improving Resolution and Mass Capacity in Preparative Liquid Chromatography for Natural Product Isolation. Jo-Ann Jablonski1, Rui Chen2, 1Waters Corporation, Milford, MA, USA; 2Waters Corporation, New Castle, DE, USA

P-207 Dynamic Control of Buffer Variability by In-Line Conditioning for Process Chromatography. Roger Nordberg, Enrique Carredano, Martin Hall, Elenor Strandberg, Tomas Karlsson, Henrik Sandegren, Karol Lacki, GE Healthcare, Uppsala, SWEDEN

P-208 Tailored Surfaces for Affinity Selection of Human Cells. Tina Sutar1, Oliver Goodyear2, Ales Podgornik3, Owen Thomas3, Mark Cobbold2, Eirini Theodosiou4, 1Loughborough University, Leicestershire, UK; 2University of Birmingham Edgbaston, West Midlands, UK; 3COBIK, Solkan, SLOVENIA

P-209 Countercurrent Tangential Chromatography for Purification of Monoclonal Antibodies. Oleg Shinkazh1, Andrew Zydney2, Boris Napadensky3, AchyuTa Teella2, 1Chromatan Corporation, State College, PA, USA; 2Penn State University, State College, PA, USA

P-210 Adsorption Behavior of Proteins on Temperature-responsive Resins. Izabela Poplewska, Renata Muca, Wojciech Piatkowski, Dorota Antos, Rzeszow University of Technology, Rzeszow, POLAND

P-211 Macroporous and Nonporous Polymer Beads for Protein Analysis and Purification. Michael Lu, BioChrom Labs Inc., Terre Haute, IN, USA

P-212 Strategy for Chiral Chromatographic Separation of Pharmaceutical Enantiomers in Semi-preparative or Preparative Scale. Danhua Chen, Celgene, San Diego, CA, USA

P-213 New Generation of HPLC Silica-Based C18 Column for Both Highly Acidic and Basic pH: The SiliaChrom™ SB C18 and SiliaChrom™ XT C18. Francis Mannerino1, Vincent Bédard2, David Dubé2, François Béland2, 1SiliCycle Inc., Chicago, IL, USA; 2SiliCycle Inc., Quebec, Quebec, CANADA

P-214 Evaluation of High-Throughput Purification Platforms for Rapid, Milligram-Quantity Affinity Purification. Matthew Petroff, Jessika Feliciano, Jennifer Pollard, Hong Li1, David Pollard, Merck, Rahway, NJ, USA; 1Merck, Union, NJ, USA
P-215 Use of Amine Additive in the Sample Diluent in a Supercritical Fluid Chromatography (SFC) Purification. Natascha Bezdenejnih-Snyder, Valerie Hoesch, Nancy DeGrace, AstraZeneca, Waltham, MA, USA

P-216 New Core Bead Medium Enables Group Separation and High Productivity Purification of Large Targets such as IgM and Influenza Virus. Anna Heijbel, Patrik Adielsson, Anna Åkerblom, Fredrik Elwinger, Fredrik Larsson, Tobias Söderman, Eric Routher1, John Schreffler1, Eric Wiltsie1, Xun Zuo1, GE Healthcare, Uppsala, SWEDEN; 1Morphotek, Exton, PA, USA

P-217 New Ion-exchange Doped MATERIALS for Peptide Purification. Rushd Khalaf1, David Gétaz1, Susanna Bernardi2, Nicola Forrer3, Massimo Morbidelli1, 1ETH, Zurich, SWITZERLAND; 2Politecnico di Milano, Milan, ITALY; 3Zeochem AG, Uetikon am See, SWITZERLAND

P-218 Step-Induced pH Gradients Generated with Mixed Resin Beds for Protein Separations. Tarl Vetter, Giorgio Carta, University of Virginia, Charlottesville, VA, USA

P-219 Preparing Aqueous Plant Extract for Chromatographic Purification of Biopharmaceutical Proteins. Johannes Buyel1, Jürgen Drossard2, Rainer Fischer3, 1RWTH Aachen University, Aachen, GERMANY; 2Fraunhofer Institute for Molecular Biology and Applied Ecology, Aachen, GERMANY

P-220 Analysis of Gradient Elution of an Adenovirus Bioreactor Bulk on an Ion-exchange Membrane Adsorber. Piergiuseppe Nestola1, Cristina Peixoto2, Patricia Roch2, Paula M. Alves1, Manuel J.T. Carrondo1,2, Jose P.B. Mota3, 1IBET&ITQB-UNL, Oeiras, PORTUGAL; 2IBET, Oeiras, PORTUGAL; 3FCT&UNL, Caparica, PORTUGAL

P-221 Optimal Simulated Moving Bed Chromatography Design for Minor Actinide Separations. Balamurali Sreedhar1, David Hobbs2, Yoshiaki Kawajiri1, 1Georgia Institute of Technology, Atlanta, GA, USA; 2Savannah River National Laboratory, Aiken, SC, USA

P-222 Development of an Improved Separation to Enable the High Throughput Purification of 1000 Chemically Modified Cyclosporins. Jack Thornquest, Preston Absher, Thomas Bullock, James Montgomery, Clare Murray, Scynexis, Inc., RTP, NC, USA

P-223 Optimized Purification Techniques for Peptides. Janine Sinck1, Reno Nguyen2, 1Grace, Allentown, PA, USA; 2Grace, Hesperia, CA, USA

P-224 Highly Porous Polymeric Adsorbents Applied in Polishing and Separation Processes in Biopharma. Luc Froment1, Ksenia Sochilina2, Vladimir Sochilin2, Alessandra Basso1, 1Purolite Lifetech - UK, Llantrisant, South Wales, UK; 2Sintez of Polymer Sorbents, Moscow, RUSSIA

P-225 Modeling Competitive Adsorption Isotherms in Gradient Elution Nonlinear Reversed Phase Liquid Chromatography. Dennis Åsberg, Martin Enmark, Joergen Samuelsson, Torgny Fornstedt, Karlstad University, Karlstad, SWEDEN

P-226 Separation Workflow of Acidic Compounds by COOH Silica and SO3H Silica. Mitsuhiro Kamimura1, Tomio Yamakoshi1, Kazunori Nobuhara1, Tim O'Mara2, 1Fuji-Silysia Chemical Ltd, Kasugai, Aichi-ken, JAPAN; 2Fuji Silysia Chemical SA, Milwaukee, WI, USA

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Lecture and Poster Abstracts

$L = lecture \quad P = poster$
A Quality by Design (QbD) Approach for Purification Process Development of a Therapeutic Protein. Bee Lin Cheang, Ying Yang, Benjamin Simeone, Chenny Nian Yun Chen, Eric Tang, Daniel DaSilva, Yong Wang, Dave Nichols, Shire HGT, Lexington, MA, USA

The aim of purification process development is to establish a robust manufacturing process to consistently deliver process and product with intended performance and quality. A quality by design approach was employed during purification process development of a therapeutic protein for treatment of a rare human genetic disorder. For each of the purification unit operations, a quality risk assessment using failure mode, effect, and criticality analysis (FMECA) was performed. Risks associated with the operational parameters of the purification step were identified, assessed, and prioritized. A design of experiment (DOE) study was subsequently performed to evaluate the effect of selected operational parameters and their potential interactions. Additional confirmation runs further supported the DOE model and the established design space. The establishment of design space and appropriate manufacturing controls based on risk assessment and DOE study will reduce manufacturing risk and maintain quality build-in by design.

Evaluation of Novel Mixed Mode Chromatographic Resins for Aggregate Removal in Monoclonal Antibody Purification. Wai Keen Chung1, Zhu Min1, Steven Timmick2, David Robbins1, Medimmune, Gaithersburg, MD, USA; 1University of Delaware, Newark, DE, USA

Mixed mode chromatography offers increased operation flexibility due to the multiple types of protein-resin interactions available. Two novel mixed mode resins were evaluated for their aggregate removal performance and compared with two commercially available mixed mode resins. By employing a robotic high throughput screening approach with 96 well resin plates, process variables such as pH, salt species and concentration were optimized for maximum binding capacity on all four resins. The elution conditions (including use of additives such as urea, arginine and glycine) were subsequently optimized for the removal of aggregates (mostly dimer) through DOE approaches. Lab scale column runs were performed for verification. Both new resins were found to effectively remove aggregates. As compared to the commercial mixed mode resins, one resin was found to have a wider range of operating conditions that met targets for yield and purity. The other resin had a binding capacity (114 mg/mL) high enough to be considered as a capture column. The distinct advantages of each experimental resin allows for optimization to meet various purification process development targets.

An Approach for Scale Down Modeling of Ceramic Hydroxyapatite Media Lifetime during Process Development. Brian Williamson1, Amanda Ebner1, Ronald Keener, III1, Shawn Cao1, Esteban Freydell2, Olivier Kaltenbrunner1, Amgen, Thousand Oaks, CA, USA; 2BioRad, Hercules, CA, USA

Ceramic Hydroxyapatite (CHA) is a multimodal chromatographic media widely used in the pharmaceutical industry for the purification of biomolecules. CHA is calcium phosphate in the form of sintered hydroxyapatite crystals. Hence, CHA has a modest stability at acidic conditions. This modest stability may lead to unpredictable packed bed lifetime, especially under acidic conditions. To monitor CHT stability, diagnostic tools that can assess changes in the media’s physical and chemical properties as a function of the process solutions are needed. This study presents the application of Dynamic Image Analysis (DIA) to monitor CHT particle damage, changes in solidity, and changes in morphology of the CHT media as a function of its position in the bed, the operational pH and the phosphate and calcium feed concentrations. Moreover, this work presents a correlation between changes in particle solidity data and the mechanical behavior of CHT measured by uniaxial confined bulk compression (UCBC).

Controlling Process Parameters for Scale--up of a Pepsin Enzymatic Cleavage Step for F(ab')2 Production. Michelle Butler, Marc Wong, Anjali Srivastava, Kim Kaleas, Genentech, S. San Francisco, CA, USA

F(ab')2 production posed some unique process development challenges since a pepsin enzyme was required to cleave the starting full length antibody to the desired F(ab')2 product. For the cleavage, both a pepsin solution and a pepsin resin process were evaluated. The cleavage efficiency of the pepsin enzyme was found to be dependent on pH, contact time, protein concentration and temperature. The set point and range for these parameters was established for the scale-up process.

Biomanufacturing Platform Application of Counter Current Chromatography System to Continuously Capture Proteins from Perfusion Cell Culture. Veena Wankoo, Rahul Godawat, Kevin Brower, Sujit Jain, Konstantin Konstantinov, Frank Riske, Genzyme Corporation, Framingham, MA, USA

In the current environment of rapidly fluctuating market demands, biotechnology companies need to develop innovative solutions for flexible and cost effective manufacturing processes. These solutions include both the implementation of new technologies and relevant organizational structures. We have integrated perfusion cell culture with the continuous capture of target proteins and view this system as a potential universal biomanufacturing platform that meets the current needs for flexibility, high product throughput and low cost. In this study, we demonstrate the successful application of a 4-column periodic counter-current chromatography system (PCC) for continuous capture of target proteins from perfusion bioreactors. The platform application of the PCC continuous capture technology is demonstrated by two case studies; a non-antibody protein captured by conventional media and a monoclonal antibody (MAb) captured by affinity media. The PCC unit operation resulted in elimination of harvest hold time due to continuous and direct processing of the harvest from bioreactor and up to two times faster process (higher flow rates and lower residence time) which results in higher volumetric productivity and comparable product quality for relatively unstable proteins such as enzymes. Additionally, the critical quality attributes (CQAs) of the captured proteins were comparable to the eluate from current lower productivity perfusion or fed-batch cell culture and batch chromatography. Overall, these results demonstrate the technical and economic benefits of the PCC continuous capture platform technology and mark a significant step towards implementation of continuous processing in the biotech industry. Additionally, this study also highlighted the significance of efficient upstream and downstream organizational integration required for innovation and advances in biomanufacturing.
Use of Molecular Simulations to Understand and Predict Multimodal Ligand-protein Interactions. Siddharth Parimal, Melissa Holstein, James Woo, Shekhar Garde, Steven Cramer, Rensselaer Polytechnic Institute, Troy, NY, USA

Molecular dynamics (MD) simulations have been employed to establish a fundamental understanding of how multiple interactions work together to create binding selectivity in chromatographic systems. MD simulations were performed to quantitatively investigate the interactions between a series of increasingly complex ligands having different combinations of electrostatic and hydrophobic moieties. These simulations were also used to evaluate the existence and the strength of synergism between different interactions, and to use this knowledge to identify the key contributors to synergism present in multimodal (MM) ligand-protein systems. The results were used to develop a new technique for mapping proteins based on their binding affinity to ligands with different interactions. This approach employed the evaluation of strength and size of electrostatic and hydrophobic regions on a protein surface, and their overlap with each other, to identify high affinity regions. This was used to make predictions of protein retention behavior in MM systems, and to understand avidity effects on these resins. The identification of high affinity regions was also used to make predictions of protein retention as a response to changes in temperature. Simulations were also performed to quantify the effect of mobile phase modifiers in modulating the different types of interactions in MM systems. The results obtained from these simulations were used to refine our identification of high affinity sites in the presence of modifiers, and predict retention behavior for different protein-ligand-modifier systems. The knowledge base created using these simulations can be used to select appropriate combinations of MM ligands and modifiers to achieve unique selectivities for challenging protein separations.

Mechanistic Investigations of Protein Transport in Traditional and Polymer-Modified Media. Steven J. Traylor, Brian D. Bowes, Steven M. Timmick, Abraham M. Lenhoff, University of Delaware, Newark, DE, USA

Understanding and modeling transport mechanisms in chromatographic media remains a key scientific challenge and obstacle to rational process design. Simplified models do not always capture trends in apparent diffusivity as a function of protein concentration and ionic strength, as well as in cases of multicomponent displacement and desorption. Additional challenges include the difficulty of experimentally measuring independent contributions to overall transport rates, and of generalizing transport behavior between the variety of media types now widely in use. Traditional confocal microscopy methods have greatly advanced our knowledge of intraparticle uptake profiles, but fall short of providing a full mechanistic description of uptake mechanisms. Here we explore the use of two additional confocal microscopy methods to directly estimate protein rates of mobility both on the pore surface and in the pore fluid. Combining these techniques with traditional methods of measuring transport is expected to provide an improved understanding of transport mechanisms, especially in the cases of polymer-modified media relative to conventional adsorbents. Polarization-sensitive confocal microscopy presents the most direct method for quantifying protein rotational mobility on the surface. The faster uptake often seen in polymer-modified than in conventional media may be due to higher mobility of adsorbed protein in the former adsorbents. Polarization microscopy experiments at low salt reported little difference in the mobility of bound protein over the short time scale of the experiment. We will report results of additional experiments, including as a function of salt concentration, to identify possible differences in mobility. Fluorescence recovery after photobleaching (FRAP) observations may be performed over a much longer time scale and have provided an excellent means of estimating pore and surface transport rates. FRAP has been performed on several resins to explore the effect of polymer modification and ionic strength on surface transport. Proteins on conventional and polymer-modified resins with open porous networks exhibit rapid re-equilibration after bleaching, while proteins in confined, polymer-filled networks re-equilibrate much more slowly. We will present further comparisons of the effect of protein size, as well as pH, ionic strength and resin type on surface exchange rates. We will also discuss mathematical comparisons between surface and pore recovery rates, and surface and pore diffusion parameters. In addition to its usefulness for independently measuring pore and surface diffusivity parameters, we believe this work will lead to a greater understanding of multicomponent transport in polymer-modified media.

Isolating of Monoclonal IgG from CHO Supernatant by IEC and HIC. Adsorption Behavior and Kinetic Effects. Wojciech Marek, Renata Muca, Sylwia Wos, Wojciech Piatkowski, Dorota Antos, Rzeszow University of Technology, Rzeszow, POLAND

Pharmaceutical applications enforce high demand for purity of antibodies produced. Therefore, chromatography as a highly-selective separation method is often a key operation in the production technology. Typically, affinity chromatography with Protein A is used; however, key disadvantages of this operation include cost and resin stability. As an alternative a combination of different chromatographic techniques can be employed. In this work the isolation of the monoclonal antibody IgG1 from CHO supernatant by coupling ion-exchange, IEC, and hydrophobic interaction chromatography, HIC, is analyzed. Prior to the process design the adsorption behavior of the target protein was investigated. A procedure for the quantification of adsorption isotherm of the target protein eluted in a multicomponent protein mixture was developed and experimentally verified in case of both chromatographic techniques. Additionally, the kinetic effects resulting from slow mass transport and conformational changes of IgG were investigated. In case of both IEC and HIC the mass losses of the target protein were observed occurring under conditions of strong adsorption, i.e., for IEC at low concentration of salt in the elution buffer and at high salt concentration in case of HIC. Moreover, peak splitting was observed during gradient elution, which resulted in additional yield reduction. On the basis of experimental investigations the model of the process dynamics has been developed and used for analysis of the separation mechanism in the IEC as well as HIC column. On the basis of the predictions the strategy for the process realization has been suggested. The strategy comprised two steps in which the CHO supernatant was directly injected into IEC column to capture monoclonal IgG. The captured fraction was further purified using HIC column. In both purification stages the multi-injection technique was employed to increase the mass loading and avoid the operation of buffer exchange between the stages [1]. 1. Marek W., Piątkowski W., Antos D., „Multiple-injection technique for isolating a target protein from multicomponent mixtures”, J. Chromat. A, 1218 (2011), 5423–5433

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Predicting the Performance of Capture Chromatography by using Small Scale Experiments. Kazunobu Minakuchi, Hiroyuki Nagaoka, Noriko Yoshimoto, Shuichi Yamamoto, Yamaguchi University, Yamaguchi, JAPAN

Chromatography is the main purification unit operation for biopharmaceuticals. However, it is still time-consuming and laborious to find proper operating and/or chromatography conditions by tuning various variables such as sample load, flow-rate and column dimension. The mobile phase must also be carefully examined as the separation and/or the binding capacity is strongly influenced. Protein A chromatography is now an essential unit operation as the first capture step in monoclonal antibody (Mab) separations. As both Mab and protein A are quite expensive, it is desirable to scale down experiments by using a small column and or a small-scale batch uptake setup. Such small scale experiments are also preferred for the capture by ion-exchange chromatography. As for the batch uptake, 96-well filter micro-plates can reduce the gel (resin) and the liquid volumes markedly. On the other hand, it is still not easy to predict the smallest column size, which can provide reliable data for the scale-up. In this study, the batch adsorption experiments by using a 96-well filter plate with various protein A gels were carried out to determine the isotherm. Accurate transfer of the gels into each well was found to be critical and the transfer method must be carefully developed based on the properties of individual gels. After we established the suitable method, the isotherms were successfully determined. In most cases the working liquid volume was 0.1-0.2 mL and the gel volume 0.005-0.010 mL. The mixing of the well was important to assure the complete mixing of the gel suspension. Although we confirmed that the scale-down to ca. 0.005 mL gel/well is possible, it was difficult to obtain the transient adsorption (uptake) curve precisely. It was suitable for the adsorption isotherm measurements or the static binding capacity (SBC). As for the column studies, a proper packing method is the key issue for scale-down. For most protein A gels columns of 1 mL bed volume (0.5 cm diameter and 5 cm bed height) were packed properly. Further scale down was attempted. A packed bed of 0.2 mL (bed height 2 cm) was possible with silica gels while it was rather difficult with agarose gels. A simple method for predicting the dynamic binding capacity (DBC) was proposed, which uses a correlation between the DBC/SBC and a dimensionless variable udp2/DeZ (u: mobile phase velocity, dp: particle diameter, De: effective diffusion coefficient, Z :column bed height). The correlation was reasonable with the molecular diffusion coefficient as De. More precise correlation was obtained when the stationary phase diffusion coefficient determined from non-binding pulse response experiments was used as De.

Development of a Model to Maximize Productivity of Protein A Affinity Chromatography and a Strategy for Resin Selection. James Pallikal, Min Zhu, David Robbins, MedImmune LLC, Gaithersburg, MD, USA

Recent progress in cell culture process development for the manufacturing of mAb products has resulted in potential process bottlenecks in downstream purification, including throughput and capacity of the Protein A affinity chromatography used for product capture. Due to the ligand size and mass transfer properties, there may be a limit to increases in binding capacity that can be achieved through improved design of the chromatography media. Therefore, it is worthwhile to consider whether column capacity and throughput can also be enhanced through optimization of operating conditions. Ghose And Cramer (2004) previously proposed an approach in which the loading time required to reach a column capacity is minimized by using two distinct flow rates during the column load step. The current work builds on this approach by developing a loading strategy that employs multiple flow rates in order to further increase capacity and throughput. By reducing the flow rate during the course of column loading, residence time can be increased as the column nears saturation. This increases the effective load capacity of the column with only modest increases in cycle time. As Ghose And Cramer (2004) recommend, instead of optimizing flow rates to achieve a fixed target capacity, the approach presented here considers the overall throughput for this process step, taking into account additional variables such as bioreactor titer, step yield, total cycle time, and pressure limits. A model was developed to select load flow rates and target protein loads that maximize throughput. It allows for column overloading as an option to increase overall productivity though there may be a modest decrease in recovery yield. The model was then used to assess differences between Protein A resins in their potential maximum throughput by accounting for differences in mass transfer and pressure-flow characteristics. This strategy can be used to increase productivity of the capture step and alleviate bottlenecks through a combination of resin selection and optimization of process conditions. It also permits selection of different optimal conditions that would be appropriate for individual manufacturing facilities with different equipment and operational constraints.

Understanding Pressure-flow Behavior During Chromatography Scale-up for Biopharmaceutical Manufacturing. Jessica Prentice, Steven Evans, Diemchi Vu, Gisela Ferreira, David Robbins, Process Biochemistry Department MedImmune, Gaithersburg, MD, USA

Pressure drop across chromatography beds employing compressible media is an important consideration in biopharmaceutical manufacturing. High pressure drops may impose significant operational limitations, including flow dynamics. These problems become more of a concern with increasing scale. A small scale laboratory model has been developed to predict pressure-flow behavior of media at larger scales. In our study, model predictions using 1 cm diameter columns agreed with observed pressure drops in packed beds at scales up to 80 cm diameter. Theory predicts that this behavior should hold with further increases in scale. However, unpredictably high pressures were observed when the same media was used in a 140 cm diameter column. We describe an investigation into factors inherent to large scale which could affect the accuracy of the model prediction, such as packing methods, packing flow velocity and slurry determination technique. These variables could result in over- or uneven compression of the packed bed, and thus have significant effects on pressure-flow behavior at manufacturing scale. These nonidealities in packing were then shown at small scale to have pressure-flow behavior consistent with observations at large scale. These results emphasize the need for additional large-scale considerations to support a consistent, scaleable chromatography result.
L-112  Monitoring the Packed Bed Behavior of a Compressible Resin at Process Scale. Igor Quinones-Garcia, Eric Mueller, Ting Yang, Shire, Lexington, MA, USA
Quality packing methods for large scale chromatography columns are a critical component of successful manufacturing. This case study pertains to a large scale capture column that utilized a compressible resin over multiple cycles. The column studied was packed in a way that was designed to ensure a consistent packing process. The packing procedure for the large scale column was developed using columns with a smaller radius and successfully scaled-up. The behavior of the packed bed at full scale was evaluated from run to run by monitoring the residence time distribution (RTD). The RTD characteristics were based on conductivity measurements and calculated using statistical moments. Values of the statistical moments calculated immediately after column packing and based on pulse inputs were compared to values obtained from process-related step transitions before and after every run. Process transitions associated with pre-sanitization and storage steps were used. When operating at a higher superficial velocity, bed headspace and higher pressure drops developed over the initial runs, eventually leading to channeling. Surprisingly, the highly compressed bed managed to re-structure itself to a significant extent after channel formation. The relationships between the RTD characteristics, step recovery and intermediate purity were also investigated.

Greater adoption of single-use and disposable systems in biomanufacturing and development is going to require improved downstream device innovation in particular for pre-packed chromatography columns. We developed and evaluated a disposable, pre-packed and pre-qualified column platform MediaScout MaxiChromAC suited for the purification of biopharmaceutical compounds such as mAbs and vaccines in preclinical, clinical phase I and II studies as well as full scale manufacturing depending on scale and tier of the fermentation process. The innovative design of the column allows direct connection to state-of-the-art disposable chromatography systems as well as the packing of a broad range of commercial available chromatography media of different particle sizes including rigid, semi-rigid and soft polymeric backbones. Transparent column housing allows top to bottom bed inspection during the chromatographic workflow. This outline is showing a complete packing evaluation for a 14.1 L pre-packed MaxiChromAC column including HETP and As measurements prior and after ground and aircraft transportation. Further more a case study for a protein purification process was performed and compared to standard stainless steel equipment.

L-114  The Impact of Variations in Column Packing on a Multicolumn Chromatography Process. Marc Bisschops, Mark Brower1, 2, Tarpon Biosystems, Worcester, MA, USA; 2Merck Research Laboratories, Rahway, NJ, USA
Continuous multicolumn chromatography is gaining more and more interest as a viable option for biopharmaceutical manufacturing of therapeutic proteins and monoclonal antibodies. One of the technologies that are currently gaining momentum is the BioSMB™ Technology. This is a fully disposable continuous multicolumn chromatography process. One of the critical process parameters in multicolumn chromatography processes is the BioSMB™ Technology. The effects were assessed experimentally by using two sets of columns with Protein A chromatography, one of which had significantly larger variations in packing than the other. Both sets were used to capture a monoclonal antibody from clarified cell supernatant. Various column configurations have been tested under different process conditions to evaluate the impact of column variations and to develop mitigation strategies to cope with these. This work has resulted in acceptance criteria for banks of (prepacked) columns to be used in combination with the BioSMB™ Technology. The experimental results were backed up with numerical process simulations that allow translating the knowledge to other multicolumn applications.

L-115  Novel Chiral Selectors and Chiral Stationary Phases for High Performance Enantioseparation. Timothy T.Y. Tan, Nanyang Technological University, Singapore, SINGAPORE
Our research group has previously reported a series of single isomer, cationic b-CDs bearing imidazolium substituents on the C6 position, which displayed good chiral discrimination to racemic dansyl amino acids. However, strong UV absorption by the imidazolium moiety interfered with analyte detection. To circumvent the said detection issue, a family of single isomer cationic CDs, namely py-CDCl, dhppy-CDCl, N-CH3-py-CDCl, N-CH2CH2OH-py-CDCl and 2-CH2OH-py-CDCl have been prepared by introducing different functionalized pyrrolidinium (py) moieties onto C6 position of b-CDs. These cationic single isomer CDs were employed as chiral selectors in CE for the enantioseparation of a series of anionic and amphoteric acids (negative charged analytes). All the cationic CDs exhibited good enantioseparation ability towards the enantiomers studied based on inclusion complexation, enantioselective electrostatic interaction as well as hydrogen bonding. The parent py-CDCl (absence of substituents on the pyrrolidinium ring) exhibited the best enantiomeric separation efficiency towards the racemic analytes tested. Enantioseparation efficiency diminished as CDs with bulky substituents on the pyrrolidinium ring were introduced. The results have shown that the chiral recognition ability of these CDs could be influenced by different alky substituent groups on the pyrrolidinium ring mainly on the basis of steric hindrance. Our group has also prepared a series of novel b-CD chiral stationary phase (CSPs) via “click” chemistry and applied them in capillary electrochromatography (CEC) for enantioseparation of neutral racemic compounds such as aryl alcohols and flavonoids. Efficient separation and good resolution were achieved using these novel CSPs. The effects of pH value, buffer concentration, applied voltage, concentration of organic modifier and analyte structure on the enantioseparation were also investigated. Application of sub-micron silica CSPs in CEC is favourable as CEC capillary allows for the use of very small particles without back-pressure...
consideration, and hence resulting in enhanced efficiency. We have also reported a series of porous and mesoporous sub-1 and sub-2 micron CSPs, which has demonstrated high efficiency enantion separation on ultra-high pressure chromatography.

L-116 Overview of Current Good Manufacturing Practice (CGMP) Regulations for Dietary Supplements. Saleh Turuiman, U.S. Food and Drug Administration, College Park, MD, USA
The Dietary Supplement Health and Education Act of 1994 (DSHEA) was enacted “to amend the Federal Food, Drug, and Cosmetic Act to establish standards with respect to dietary supplements, and for other purposes.” DSHEA requires a dietary supplement to be prepared, packed, or held under conditions that meet current good manufacturing practice (CGMP) regulations. CGMPs for dietary supplements were published on June 25, 2007. CGMP compliance is currently mandatory for all dietary supplement manufacturers. The CGMP final rule requires that proper process controls be in place before the start of manufacturing to ensure that the dietary supplements are processed and manufactured in a consistent manner as to identity, purity, strength, and composition. The talk will provide highlights of the CGMP final rule, and what an FDA CGMP inspection of a dietary supplement manufacturer entails, with a brief discussion of the importance of identity, process controls and specifications in CGMPs.

L-117 Mechanistic Studies of Enantioselective Interactions of a Polysaccharide Sorbent with Benzoin and Ethyl Lactate. Hung-Wei Tsui, Nien-Hwa Linda Wang, Elias I. Franses, Purdue University, West Lafayette, IN, USA
Derviatized amylose or cellulose sorbents show substantial interactions and enantioselectivities (S) for a variety of solutes. Understanding the mechanisms of such interactions is crucial for designing effective chromatographic separations and finding effective sorbents. We focus on understanding systems of benzoin (B) and ethyl lactate (EL) with amylose tris(S)-α-methylbenzylcarbamate, or AS. The retention factors, kR and kS, and enantioselectivities (S= kR/kS) were measured at 25°C for 0.5-10 vol. % IPA (isopropyl alcohol) in n-hexane as a mobile phase. S varied between 1.8 and 1.4 for benzoin, and between 1.20 to 1.24 for ethyl lactate. In addition, the quantities, kR, kS, and S were measured for pure n-hexane as the mobile phase, to ensure that the AS-solute combinations are relevant to the chromatographic data. They were 106, 49.6, and 2.13 for B, and 10.7, 7.89, and 1.36 for EL. Our previous X-ray diffraction data showed that n-hexane does not affect the polymer structure or its hydrogen-bonding (H-bond) state. Molecular Dynamics (MD) simulations support this conclusion. Infrared (IR) spectra showed enantioselective H-bonding interactions of R- or S-benzoin with AS (Tsui et al., J. Phys. Chem. B, 2011). Literature IR data by Wirtz et al. (Langmuir, 2003) also showed enantioselective H-bonding interactions of AS with R- and S- ethyl lactate. DFT (Density Functional Theory) simulations were used to model the side chain-side chain interactions, and side chain-benzoin and side chain-ethyl lactate interactions. They were also used to predict fairly well the shifts in the IR wavenumbers caused by the H-bonds of benzoin. Then MD simulations were used to model a single 12-mer helical polymer chain. The predicted polymer structure shows H-bonding strengths which are comparable to the ones inferred from the IR spectral analysis. MD simulations predict various potentially enantioselective cavities, two of which are sufficiently large to accommodate a benzoin or ethyl lactate molecule. Then “docking” studies with MD, or MC (Monte Carlo), or MC/MD hybrid simulations were done to probe AS-B and AS-EL interactions. For one specific cavity, the simulations predict substantial enantioselectivities for B and EL, which for the R-enantiomers are due to two H-bonds, (AS) CO ... OC (R-enantiomer) for the S-enantiomers, because of steric hindrance, there is only one H-bond, of the OH groups with polymer CO groups.

L-118 Salt Tolerant Anion Exchanger for Flexible Process Development. Rene Gantier1, Magali Touelle2, Jerome Champagne2, 1Pall Life Sciences, Northborough, MA, USA; 2Pall Life Sciences, Cergy, FRANCE
A new salt tolerant anion exchange sorbent named HyperCel™ STAR AX was launched recently by Pall to facilitate the capture of proteins from moderate to high conductivity feedstocks by limiting dilution requirements. Binding capacity studies using pure model proteins highlighted the ability of the sorbent to efficiently capture proteins in large ranges of conductivity and pH even at low residence time (<2 min). Good selectivity at high conductivity was also demonstrated for the sorbent. A purification process for HSA from neat plasma was developed as a working model to evaluate the performance of this new sorbent. In the context of this application, a two-step process including the HyperCel STAR AX sorbent as capture step followed by a cation exchange step was developed. Operating conditions were optimized by using design of experiment (DoE) and high throughput screening technique using AcroPrep™ Advance 96-well filter plates, followed by transfer to column to evaluate the performance of the process in dynamic mode. A two-step streamlined process without any pH or conductivity change was thus developed, yielding efficient purification performance. The HyperCel STAR AX sorbent showed high capacity capture of HSA directly from undiluted plasma in contrast with a conventional DEAE rigid agarose sorbent which required at least 3-fold dilution to provide efficient capture. The dynamic binding capacity obtained using the HyperCel STAR AX sorbent was very robust within a large range of conductivities, therefore allowing flexible process design. We also demonstrated a good performance of the sorbent used in Flow-Through mode for the separation of contaminants from target proteins such as Monoclonal Antibodies or other recombinant proteins directly from non processed feedstocks. Finally, cost of goods analysis using the data generated during the development of the above mentioned applications showed that the salt tolerance of the sorbent allows significant savings compared to standard non salt tolerant anion exchange sorbents. Altogether these data illustrate the functional and economical performance of the new salt tolerant sorbent HyperCel STAR AX and demonstrate the benefits of such a sorbent allowing the development of more streamlined and cost effective processes.
L-119 **Affinity Platform for the Purification of Antibody Fragments.** Gustav Rodrigo, Tomas Nyhammar, Peter Lindahl, Carina Engstrand, GE Healthcare Lifesciences, Uppsala, SWEDEN

Monoclonal antibodies are typically purified with a platform approach where capture using affinity chromatography with Protein A has become the industry standard. However, for antibody fragments there is not yet a corresponding solution. Here we will discuss purification challenges for antibody fragments and will present several affinity chromatography media alternatives suitable for purification of antibody fragments of different subclasses, size, and structure. Application examples showing screening and optimization of elution conditions, binding capacities, yield and ligand leakage data will be presented. We will also present data from a purification process, from capture to polishing, for antibody fragments allowing for an industrial platform approach.

L-120 **Cost Effective Approaches for the Capture and Purification of Biosimilars.** Steve Burton, Sharon Williams, Dev Baines, ProMetic BioSciences Ltd., Cambridge, UK

Non-antibody proteins represent 65% of the bio-therapeutics market and include growth factors, hormones, cytokines, plasma proteins, therapeutic enzymes and protein vaccines. Whilst Protein A has provided a useful affinity capture platform for monoclonal antibody purification, equivalent platforms for non-antibody molecules have proved elusive. Synthetic ligands, obtained through the use of computational chemistry and ligand library screening can be targeted to almost any protein. Such ligands provide a means of capturing and purifying non-antibody proteins and are also very robust and can be reused for many purification cycles. Ligands may be generated by screening general ligand libraries, modelling known binding compounds or identifying potential ligand binding sites on the protein of interest and modelling complementary binding ligands in silico. Techniques such as molecular docking provide useful tools for predicting how potential ligands might interact with a particular region of a protein and virtual screening algorithms can be used to pre-select potential ligand candidates for synthesis and in-vitro screening. The application of synthetic ligand design and screening techniques will be discussed in relation to the development of affinity purification platforms for albumin-fusion proteins and transferrin-fusion proteins. In both cases novel affinity adsorbents were developed which were applied successfully to the capture and purification of generic groups of proteins comprising variable regions and conserved regions with common structural motifs/binding sites.

L-201 **Development and Optimization of Continuous Chromatography for the Production of a Chiral API by Varicol.** Yeung Chan, James Falabella, Olav Lyngberg, Bristol-Myers Squibb, New Brunswick, NJ, USA

Facing pressure within the pharmaceutical industry to reduce time to market, preparative chromatography has increasingly been used in discovery and early development for rapid delivery of enantiomeric pure drug candidates. For kilogram scale supplies, multi-column continuous chromatography approach such as Simulated Moving Bed (SMB) and VarioCol is often the method of choice for production. The case study presented here describes the use of VarioCol to expeditiously manufacture a chiral API for the clinical studies. As the candidate compound progress rapidly through different clinical stages, the challenges encountered as well as the development and optimization strategies are discussed.

L-202 **Purification of PEGylated Proteins using a Continuous 2-column Chromatography Process (MCSGP).** Thomas Müller-Spath1, Guido Stroehlein2, Lars Aumann2, Massimo Morbidelli3, ETH Zurich/ChromaCon AG, Zurich, SWITZERLAND;3ChromaCon AG, Zurich, SWITZERLAND;3ETH Zurich, Zurich, SWITZERLAND

PEGylation of therapeutic proteins is a common modification used in biopharmaceutical drug production, aimed mainly at improving the pharmacokinetics of the drug. From the PEGylation reaction, generally a mixture of proteins PEGylated to a different extent is obtained, including multi-PEGylated species, di-PEGylated species, mono-PEGylated species and non-PEGylated protein. Typically only the mono-PEGylated species are desired and the other PEGylation reaction products have to be removed as impurities. Size-exclusion chromatography has been suggested for this purpose but is economically not attractive for preparative purification due to low throughput. In adsorption chromatography, the PEG-species behave similarly and, consequently, the desired PEGamer species can be purified only with low yield using single column adsorption chromatography. In contrast, using continuous countercurrent chromatography (MCSGP) with adsorption packing materials, the desired PEG-species can be isolated with high yield and purity simultaneously. In this presentation, a case-study for the isolation of the mono-PEGylated protein species on anion-exchange is shown. The purification is done using a recently developed 2-column embodiment of the MCSGP process. The principle of the 2-column process is demonstrated and the performance with respect to single column batch chromatography is discussed. Moreover, the advantages of the 2-column embodiment of MCSGP are discussed with respect to the “classical” MCSGP columns with 3 columns or more.

L-203 **Periodic Counter Current Packed Bed Chromatography for Continuous Capture Step.** Karol Lacki, Helena Skoglar, Eric Grund, GE Healthcare Bio-Sciences AB, Uppsala, SWEDEN

Purification of a biological product frequently involves an early capture step that allows major impurities to be washed away. Performing a capture step by packed bed chromatography has several advantages such as reduced process volumes and improved yields. The packed bed format also simplifies resin handling, in particular procedures for cleaning, storage and reuse. The main disadvantage of this mode of operation is that utilization of available binding capacity of the chromatography resin is suboptimal. To circumvent this drawback, the capture step can be operated in a continuous manner by dividing the single column into a number of smaller columns that can be connected in series and, when needed, can also be operated independently. This column arrangement enables what is usually referred to as the Simulated Moving Bed (SMB), or Periodic Counter Current (PCC) operation. However, despite the fact that multi-column and multi-zone continuous chromatography is well adopted in other industries, its use within bioprocessing is rather limited due to high entry barrier associated with complex hardware and not yet address regulatory concerns. One version of continuous chromatography that could have a lower entry barrier into the bioprocessing field is the three- or four-column periodic counter current (3C/4C-PCC) technology. This technology requires simpler hardware compared to a typical SMB arrangement, which in turn reduces capital and...
maintenance costs. The 3C/4C-PCC operating principle allows continuous feed loading with discrete collection of the purified product and offers close to 100% resin capacity utilization. In this work we provide an analysis of 3C/4C-PCC technology. The study is based on both theoretical considerations and experimental results. From the theoretical perspective, several factors are considered, including the optimal number of columns in each of the so-called operating zones, the effect of product concentration in feed streams and the duration/strength of wash and elution steps. The theoretical analysis is used to provide guidelines regarding applicability of the 3C/4C-PCC system for different separation tasks. Practical verification uses capture of monoclonal antibodies with a commercially available Protein A resin, MabSelect SuRe as an example. In addition, the process control aspect will be discussed briefly. Examples of control strategies and results from experiments performed on a custom-modified ÄKTA™-explorer chromatography system capable of operating according to 3C/4C-PCC principles will be shown. Robustness of the different strategies will also be compared.

L-204  **Countercurrent Tangential Chromatography for Purification of Monoclonal Antibodies.** Oleg Shinkazh, Andrew Zydney, Chromatan Corporation, State College, PA, USA; *Penn State University, State College, PA, USA

Countercurrent Tangential Chromatography (CTC) is a new column-free purification and capture technology that holds great promise for purification of high-value recombinant proteins like monoclonal antibodies. CTC can provide a scalable, disposable, and continuous unit operation that overcomes many limitations of packed bed chromatography. CTC replaces the stationary phase of a packed column with a moving slurry that is continuously pumped through several cascades of static mixers and hollow fiber membrane modules. Experimental studies were performed purifying serum and monoclonal IgGs using a custom-made small diameter Protein A resin and 0.2 µm pore size Spectrum hollow fiber membrane modules. Chromatographic operations of binding, elution, washing, and equilibration were performed directly on the moving slurry. The buffers required for each operation were introduced in a countercurrent direction to the slurry flow, providing significant increases in operational throughput when compared with conventional column performance. CTC provided high-resolution antibody purification at very low pressures (< 10 psi) with excellent protein recovery. In addition, CTC is no longer limited by the constraints of packed columns, allowing the use of smaller chromatography beads with improved binding kinetics and throughput. These results clearly demonstrate the potential of using Countercurrent Tangential Chromatography for low-cost antibody purification.

L-205  **Addressing Purification Challenges in Pharmaceutical Discovery and Early Development.** Larry Miller

Amgen, Cambridge, MA, USA

Over the past years the molecules being synthesized for pharmaceutical evaluation have become more complex. This molecular complexity is designed to increase potency and/or selectivity, improve PK properties as well as define IP space. To synthesize these molecules multi-step synthetic schemes are often required. The quantity of final product to be generated is small (< 50 mg) and in many cases the reaction conditions have not been optimized. Many of these compounds contain one or more chiral centers. The end result is a reaction mixture that may contain the desired product at low levels and require both achiral and chiral purification to generate material with the purities required for biological testing. In addition the use of automated synthetic equipment increases the number of compounds that are being prepared in a medicinal chemistry laboratory. For the above reasons purification has become a bottleneck in the medicinal chemistry process. An efficient analytical and purification process is required to eliminate this bottleneck. This talk will discuss the approaches employed in Amgen's chemistry and purification laboratories to address the many purification challenges needed to support discovery and early development pharmaceutical research. These approaches make use of open access purification equipment for use by medicinal chemists and specialized purification equipment (prep HPLC and prep SFC) and processes used by separation specialists. Multiple examples of these techniques will be presented.

L-206  **Productivity in Medicinal Chemistry: A Lean Sigma Approach to Centralized Purification.** Harold Weller

Bristol-Myers Squibb Company, Cambridge, MA, USA

Drug discovery is driven by an iterative optimization cycle of Design – Synthesize – Test – Redesign that leads to an eventual drug candidate. A significant bottleneck, and expense, of the Synthesis component is in purification and processing of final compounds for biological testing. The author will describe a new comprehensive Lean Sigma optimized process to purify final drug candidates by preparative LCMS, including drydown, QC analysis, assignment of a company code, initial distribution, and transfer to the company's central repository for biological analysis and storage. This fully elaborated process, optimized to maximize speed, yield and recovery, has now been implemented enterprise wide including three geographical locations. The presentation will provide quantitative metrics on the original performance gap identified, a description of the key hardware, software, and process elements that were implemented, and a description of ultimate success metrics - which suggest that this project has had significant positive impact on medicinal chemistry productivity.

L-207  **Achiral Stationary Phase Classification: Enabling the use of SFC in High Throughput Environments.** Ray McClain, Matt Przybycień, Merck, Princeton, NJ, USA; *ES Industries, West Berlin, NJ, USA

SFC is increasingly being used for semi-preparative, achiral separations in high-throughput purification groups supporting drug discovery. Selection of an appropriate stationary phase is important for success, and typically requires empirical screening. In an attempt to eliminate screening of multiple columns, we have developed a computational method of evaluating various stationary phases based on analyte class. Specific recommendations from the model will be summarized, and several semi-preparative achiral SFC separations will be used to illustrate the importance of rapidly identifying the proper stationary phase to be used for semi-preparative separations without screening. The need for a universal, achiral SFC phase will be highlighted and this models ability to help with this task will be shown.
L-208  **From Purification to Plating: Novel Software Development to Manage the Data Deluge.** William Leister, Chris Louer, Chris LeClair, National Institutes of Health, Rockville, MD, USA

There is an enormous amount of data generated in a medicinal chemistry lab. Small scale preparative purifications can be done quickly. Keeping track of data in a fast paced lab can be overwhelming. Our software, S.M.A.R.T., is a seamless data management tool to organize and store all of the data generated in our lab. Data stored through the software include LC, LC/MS, TOF/MS, NMR, Biological and Sample Disposition. The software is scalable to include new data streams. The search capabilities allow end users to view all data generated through a single portal.

L-209  **Quality by Design (QbD) for Vaccines.** Dicky Abraham, Merck & Company, West Point, PA, USA

Recent FDA and ICH guidance has focused on the need to implement quality systems based on relevant data and an enhanced understanding of the manufacturing process. Companies have initiated work to integrate the concepts of Quality by Design (QbD) into their development and subsequently, demonstrate that they have a strong understanding of the product's and the process's characteristics. • The QbD approaches support the development of the systematic accumulation of product and process understanding that is a major pillar of the vaccine product life cycle. In addition, these efforts will ensure robust and consistent processes, expedited development and facilitate favorable regulatory review. • Case studies will be presented to demonstrate how some of the tools can be applied to vaccine through their lifecycle.

L-210  **Chromatographic Separation of Bionanoparticles and Biological Superstructures by Monoliths.** Alois Jungbauer1, Petra Gerster2, Nikolaus Hammerschmidt2, 1ACIB, Vienna, AUSTRIA; 2BOKU, Vienna, AUSTRIA

Bionanoparticles such as viruses, virus like particles (VLP), protein-DNA complexes or biological superstructures such as protein nanotubes, and electrospun nanofibers of hybrid materials (polymers, ceramics, metals, biomolecules) are interesting compounds for nanoelectronics and nanomedicine. Except the viruses and virus-like particles, these structures cannot be produced and purified in large scale. Enabling technology is not available in the production and in the separation of these products. The initiation of the complex formation to form the superstructures is well understood but not the termination. Consequently a mixture of superstructures with different sizes and surface charges is generated. This is a challenge for the separation of this structure which range from 50-800 nm. Monoliths are tailored chromatographic systems for the separation of such mixtures. With a free channel diameter of 2000 to 5000 nm these structures migrate through the chromatographic bed. Separation is achieved in ion exchange mode with salt gradients. Separation examples are shown for virus-like particles and protein superstructure such as protein fibres and protein-coated nanoparticles. The problem of flow entrapment and how to solve it will be addressed. Then an outlook will be given how to improve selectivity for these novel and promising compounds.

L-211  **Protein and VLP Adsorption on POROS 50HS.** Yige Wu1, Dicky Abraham2, Jared Simons2, Sarah Hooson2, Giorgio Carta1, 1University of Virginia, Charlottesville, VA, USA; 2Merck & Co. Inc., West Point, PA, USA

The adsorptive and chromatographic behavior of lysozyme, IgG and thyroglobulin, and that of human papilloma virus (HPV) virus-like-particles (VLPs) are studied for POROS 50HS, a large-pore cation exchanger. Protein radii ranged from 2.3 to 8.5 nm while the VLPs had radius of 60 ±5 nm. POROS 50HS consists of spherical particles with a bimodal distribution of pore sizes. Porosities and pore sizes were obtained from transmission electron microscopy and by inverse size exclusion chromatography, both techniques giving results consistent with a bimodal pore size distribution, with gigapores about 500 nm in radius and small pores about 11 nm in radius. Mass transfer studies were conducted both under non-binding conditions, through HETP measurements as a function of mobile phase velocity, and under strong binding conditions, through confocal laser scanning microscopy (CLSM) using fluorescently labeled proteins and VLPs. For non-binding conditions, intraparticle transport is controlled by diffusion in the macropores at lower reduced velocity, but becomes convection dominated at higher reduced velocity. For strong binding conditions, IgG adsorption is controlled by macropore diffusion even at 1000 cm/h flow velocity and is consistent with the shrinking core model. However, VLP adsorption is confined to a thin layer near the external surface of the particles with little apparent penetration in the particle pores. Although unaggregated VLPs can theoretically access the particle gigapores and do so for non-binding conditions, their aggregation on the surface is apparently responsible for their nearly complete exclusion from the interior of the particle under binding conditions. For these conditions, intraparticle convection appears to play no role, since the pores become essentially clogged with aggregated VLPs. On the other hand, it is apparent that the substantial surface roughness of the POROS 50HS particle contributes significantly to the VLP binding capacity giving higher binding than could be expected for particles with a smooth surface.

L-212  **Multilayer Adsorption in Liquid Chromatography.** Attila Felinger, Peter Vajda, University of Pecs, Pecs, HUNGARY

Multilayer adsorption is often observed in liquid chromatography. Multilayer adsorption is typically observed when the solubility of the analyte in the mobile phase is limited. In overloaded, nonlinear chromatography, the characteristic shape of the concentration band shows the effect of multilayer adsorption. As it is the case with other isotherms that we use to model adsorption at liquid-solid surfaces, the BET isotherm was also adapted from gas-solid systems to liquid-solid adsorption. For the sake of simplicity, an infinite number of adsorbed layers are envisioned when the BET is employed in nonlinear liquid chromatography. On our study we show the advantage of modifying the original BET equation. We have extended the BET model to conditions in such the respective layers of adsorbates contain fewer and fewer solute molecules. Physically that model corresponds to fractal surfaces or to the concept that limited number of solvent molecules are occupying some parts of the adsorbed layers. The novel isotherm equation gives a better fit to experimentally determined isotherms in reversed phase liquid chromatography. The extent of solvent adsorption is often characterized by measuring excess isotherms. We have shown that in reversed phase liquid chromatography, the number of the adsorbed layers of the molecules of the organic modifier can be determined by fitting a finite-layer version of the BET equation to the raw isotherm data obtained with frontal analysis.

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This work focuses on the development of a mathematical model that describes the binding equilibrium of large biological macromolecules. The Gillespie Stochastic Algorithm was used to simulate single component isotherms. This method has been successfully used to simulate reversible protein binding onto DNA active sites. The Gillespie approach was selected because it has the potential flexibility to model single-component and multi-component protein isotherms on a variety of adsorbents. In this initial phase of the project, the forward and reverse binding interactions between the protein and the adsorbent were simulated using mass action kinetics. The forward and reverse binding constants were estimated from Langmuir isotherm data. Our results indicated relatively good agreement between the experimental and simulated isotherms. Our anticipated goal is that our work will lead to a robust method capable of simulating single and multicomponent isotherms for biological molecules interacting with a variety of adsorbents. References: 1. Miami University, Department of Chemical and Paper Engineering, Oxford Ohio 45056. 2. CICS-UBI – Health Sciences Research Centre and Department of Chemistry, University of Beira Interior, 6200 – 001 Covilha, Portugal.

Several important studies – based on firm theory - have been made on the different parameters of importance in chromatographic optimizations during the recent years. In this presentation we take an holistic view by investigating i) the relative importance of the operational parameters and column properties and ii) how these effect the optimal column design. For optimization we used an advanced global response surface method combined with local gradient methods. As a model system we used the practical problem of purification of one or both optical isomers of a racemate. In the first part, we investigated the relative importance of stationary phase characteristics such as: (i) the retention factor, (ii) the selectivity, (iii) the capacity, (iv) the efficiency and (v) the maximum allowed pressure. In the second part we investigated how the optimal column design for a of preparative separation problem is affected by column properties, such as particle size, and optimization constraints, such as required yield. The investigation showed – in contrast to what is generally believed – that the capacity of the stationary phase is of minor importance, instead the maximum allowed pressure is one of the most important properties. Moreover, smaller size packing materials always shows much lower solvent consumption. Rules of thumbs, derived from the holistic optimizations, will be given for industrial preparative settings in the batch mode.

Chromatography is mostly used as a purification step where the goal is usually to obtain very high purities, e.g.> 99%. Most design methods and studies in the literature address this issue. However, there are are important separations, e.g., production of high fructose corn-syrup in food industries, production of chiral agrochemicals, where only a partial enrichment of the purity is required. Design methods for such schemes are fairly limited in the literature. This presentation will address two aspects related to the use of chromatography for reduced purities. Firstly, we will discuss design methods based on equilibrium theory of chromatography which yield explicit expressions to calculate the injection volume and the cut-times to obtain exact target purities from the chromatographic unit. While the design equations are straightforward for linear isotherms, they are not so for non-linear isotherms owing to the competitive nature of the isotherm. Secondly, we introduce a simple process called "Bypass chromatography", which is practically implemented in the manufacture of high fructose corn-syrup. The basic idea of this process is transport of the feed and over-purify it and mix it with portion of the feed to meet the target purity. This simple step provides two additional degrees of freedom which can be used to improve the process performance. We derive explicit design equations using the equilibrium theory of chromatography to identify the degree of over-purification that is required in the column that will improve the productivity compared to the classical approach of meeting the target purity from the column alone. These equations also allow determining the productivity enhancement that can be achieved by bypass chromatography. It is also shown theoretically that the maximum productivity is always achieved when the less retained component is collected at 100% purity and the more retained component is collected at a purity typically less than 100%. The results from the equilibrium theory calculations are confirmed using numerical simulations.

New types of beaded chromatography media featuring two or more distinct functional regions spatially separated from one another within the same support bead, can be exceptionally useful purification tools. The present study concerns the simplest multi-layered multi-functional support design one can envisage, namely one featuring two different functionally characterised layers – an inert outer size excluding layer and inner ion exchange functionalised core. The bi-layered SEC-IEC support architecture enables efficient separation of large biological entities (e.g. plasmids, viruses, VLPs, IgMs) from smaller chemically very similar contaminants in a ‘one column-one bead’ process that combines size exclusion and ion exchange adsorption, and also renders expanded bed adsorption of protein targets from trickly crude feedstocks relatively ‘problem-free’. In this presentation, we describe recent progress on the development of bi-layered, bi-functional supports which closely approach the ideal design brief of a thin inert outer size excluding layer, freely accessible to smaller components, but not larger entities, surmounting a fully functionalised adsorptive core. Specifically we shall report on the use of: ‘bottom-up’ microwave-assisted and viscosity enhanced ‘reaction-diffusion balancing’ techniques, which permit different functionalities to be built into underivatized SEC matrices as discrete ‘onion-like’ layers; and new ‘top-down’ cold plasma methods, which allow us to
selectively modify the exteriors of commercial adsorbents, either by shaving ligands away and/or burying them beneath a thin polymer grafted layer. This work is supported by the BBSRC-EPSRC Bioprocessing Research Industry Club (BRIC) grant number BB/F004982/1.

L-217  **Capillary-Channeled Polymer (C-CP) Fibers: Flexible Stationary Phases for Protein Separations and Diagnostics.** R. Kenneth Marcus, Abby Shaddock-Hewitt, Zhengim Wang, Clemson University, Clemson, SC, USA

While the evolution of chromatographic stationary phases applicable in “small molecule” separations has been on a steady incline in terms of performance, improvements in the separation of proteins and other macromolecules have been on a much lower slope. This is not so surprising as the challenges of poor mass transfer and the potential for multitudinous protein-surface interactions are in direct conflict with the physical/chemical characteristics of small molecule separation media. Polymeric stationary phases of various format offer certain advantages in protein separations as their surface chemistries can be better tailored to affect specific, reversible protein interactions. Poor/limited porosities work to advantage in terms of minimizing mass transfer limitations, of course at the expense of thermodynamic binding capacities. Implementation of polymer phases in physical forms that allow effective fluid flow and solute-surface transport are thus a topic of high interest. We describe here the physical attributes and application of novel-shaped polymer fibers, termed capillary-channeled polymer (C-CP) fibers, as efficient stationary phases for protein separations and diagnostics. C-CP fibers are extruded from typical textile materials, including polypropylene, polyester, and nylon-6, in a manner that affects eight parallel channels along the fiber axis. This structure provides capillary action to the level of moving water across a polypropylene surface. When packed into column formats (from single fibers to standard columns), the channel legs interdigitate to essentially form parallel open channels of single-micron dimension. As a result, very efficient mass transfer (and separations) can occur at linear velocities of >30 mm sec⁻¹. In addition to the chemistries of the base polymers, straightforward derivatization can be performed to provide added selectivity. The use of C-CP fibers will first be introduced in terms of analytical scale separations, that will be projected to preparative-scale characteristics. Surface derivatization will be illustrated from the point of view of simple acid/base chemistries, and a powerful, generalized approach based on the use of PEG-lipid chemistries. It is believed that the C-CP fiber platform, as a whole, has promise for separations ranging from microfluidics, to solid phase extraction and clinical diagnostics, and preparative chromatographies.

L-218  **Enthalpic and Entropic Driving Forces in Protein Adsorption in Hydrophobic Interaction and Reverse Phase Chromatography.** Rebecca J. Desch, Jungseung Kim, Stephen W. Thiel, University of Cincinnati, Cincinnati, OH, USA

Many biomolecule separation schemes include a reverse phase process step involving a stationary phase modified with nonpolar alkyl chains. Complex interfacial interactions between analyte, mobile phase, and stationary phase determine the degree of adsorption and retention time. These hydrophobic interactions stem from hydrogen bonding as well as London, Keeson, and Debye forces. Hydrophobic interactions are commonly modified to improve separations by inclusion of organic solvents or salts in the mobile phase, as in reverse phase chromatography or hydrophobic interaction chromatography, respectively. Flow microcalorimetry was used to measure the enthalpy of lysozyme adsorption onto a C18 mesocellular foam silica stationary phase in the presence of 0.01 M acetate buffer (pH 5.2) mixed with varying ethanol and sodium sulfate concentrations. In all cases, a tailing exotherm was followed by a small desorption endotherm. A maximum net enthalpy of lysozyme adsorption was observed in the presence of 5% ethanol; adsorption enthalpy was attenuated in the presence of concentrated ethanol or sodium sulfate. Simpler systems were also investigated to isolate interactions between select components involved in reverse phase chromatography. These results can be explained by considering the fundamental phenomena involved in the adsorption process. Improved thermodynamic models thus obtained can be used to design more efficient reverse phase and hydrophobic interaction chromatographic separations.

L-219  **Nuvia cPrime a New Novel Mixed-mode Chromatography Media for Process Purification of Biomacromolecules.** Jiali Liao, Hong Chen, Yueping Xu, Kin Lam, Xuemei He, Sherif Hanala, Mark Snyder, Russ Frost, Bio-Rad Laboratories, Inc, Hercules, CA, USA

Nuvia cPrime is a new mixed-mode chromatography media. The multifunctional ligands, incorporated onto the surface of a rigid macroporous polymeric base matrix, facilitate binding of incoming bio-macromolecules via charge and hydrophobic interactions, as well as hydrogen bonding. Nuvia cPrime exhibits good dynamic binding and low backpressure at high flow rates required for downstream processes. Protein binding to Nuvia cPrime is tolerant to pH and a broad range of conductivity. In a DOE study, optimal binding and elution conditions were developed for a series of proteins with isoelectric points between 4.8 and 9.3. Results will be presented demonstrating binding capacity is maintained over a broad range of linear flow rate and load compositions. Binding capacity and protein selectivity were not affected, following 800 hours storage in 1M sodium hydroxide. The interactions between immobilized ligands and target molecules can be modulated by buffer salt composition/concentration and pH. Taking advantage of the broad-range salt tolerance exhibited by this media, and knowledge of the protein isoelectric point and stability, examples of target protein purification will be presented. Target proteins can be effectively purified by coupling two mixed mode purification steps using Nuvia cPrime and ceramic hydroxyapatite. Following purification on Nuvia cPrime, the eluted fraction can be applied directly, without buffer exchange, to ceramic hydroxyapatite for further purification.
L-220 **Optimal Binary Separation Schemes using Simulated Moving Bed Bed Superstructure.** Balamurali Sreedhar, Yoshiaki Kawajiri, Georgia Institute of Technology, Atlanta, GA, USA

Simulated moving bed (SMB) and related technologies are widely known to outperform batch chromatographic processes in binary separations. In a traditional SMB implementation, the valve switching operation mimicking bed movement is done in a synchronous manner along with constant mobile phase velocity in each zone. It has been found out that by exploiting the additional degrees of freedom involved in relative valve positioning, switching and zone mobile phase velocity, the separation performance could be drastically improved. In this work, we experimentally investigate the higher performance capabilities of the superstructure SMB schemes proposed in an earlier study[1] by optimally using all degrees of freedom available. The experimental system chosen was the separation of glucose and fructose on an eight column configuration packed with Dowex Monosphere 99Ca ion-exchange resin with water as mobile phase. Both conventional and superstructure SMB processes were implemented using Octave 100 Chromatography System module from Sembia Biosciences, Inc. The parameters measured from single column pulse experiments (bed porosity, Henry's and mass transfer coefficients) were used as an initial guess for a Predictor-Corrector (PC) technique proposed recently[2] to arrive at optimal experimental SMB operation. We used a simplified PC method based on minimizing least squares to find parameters with higher experimental predictability. The mathematical optimization required for finding optimal configurations and parameter re-estimation were based on a theoretical implementation[1] using an efficient interior point solver. Using the optimal conventional SMB configuration as a basis, the higher performance potential of more advanced schemes exploiting the additional degrees of freedom are experimentally demonstrated.


A model-based rational methodology for protein purification process development that addresses the challenge of selecting the most optimal purification scheme from several possible alternatives was developed and illustrated in this work [1]. In this approach, different purification schemes for a given separation problem are synthesized and evaluated based on technoeconomic performance obtained by model-based optimization of the integrated processes using validated column models. The methodology involves five main sequential steps; purification task definition, resin selection and model parameters acquisition, purification flowsheets synthesis, optimization problem formulation and performance metrics definition, flowsheets optimization and evaluation. A key aspect of the methodology is that purification schemes are evaluated under their ideal or best working conditions, hence the evaluation is unbiased. Furthermore, the number of evaluated flowsheets is kept to the minimum by a systematic elimination of the least feasible process options. The methodology is generic, i.e. it can be used to develop purification processes for therapeutic proteins, food proteins and industrial enzymes, and its implementation is straightforward. The methodology was illustrated by synthesizing a process for the purification of monoclonal antibody from crude hybridoma cell culture supernatant using four non-affinity chromatographic methods. The necessary model parameters were acquired by multidimensional fractionation and characterization of the crude protein mixture [2]. Acknowledgement This project is financially supported by the Netherlands Ministry of Economic Affairs and the BE-BASIC partner organizations (www.be-basic.nl) through BE-BASIC, a public-private NWO-ACTS programme (ACTS = Advanced Chemical Technologies for Sustainability). References [1] B.K. Nfor, T. Ahamed, G.v. Dedem, P.D.E.M. Verhaert, L.A.M.v.d. Wielen, M.H.M. Eppink, E.J.A.X.v.d. Sandt, M. Ottens, Biotechnol. Bioeng. submitted (2011a). [2] B.K. Nfor, T. Ahamed, G.v. Dedem, P.D.E.M. Verhaert, L.A.M.v.d. Wielen, M.H.M. Eppink, E.J.A.X.v.d. Sandt, M. Ottens, Biotechnol. Bioeng. submitted (2011b).

L-222 **Modeling of Protein Purification by Hydrophobic Interaction Chromatography for Column Design and Process Optimization.** MarkHenry Kamga, Haewoo Lee, Seongkyu Yoon, University of Massachusetts, Lowell, MA, USA

Despite the use of Hydrophobic Interaction Chromatography (HIC) in the purification of a wide variety of protein therapeutics, the mechanism of separation of protein monomer/aggregate species within the HIC column is complex and still not fully understood. Several attempts have been made to develop a comprehensive model to explain the separation process including the solvophobic, preferential interaction and homogenous diffusion/ competitive Langmuir binary isotherm. A mechanistic model was developed based on competitive binary Langmuir kinetics as the rate-limiting step and a mass balance in the liquid phase was used to predict protein/aggregate distribution in the column under binding and elution conditions. The mechanistic model developed successfully predicted the binding and elution curves for both monomer and aggregates. Optimum amount of resin necessary for a specification, flow rate, and ideal binding and elution points can be estimated using the mechanistic model. This could result in significant savings within the biopharmaceutical industry by reducing the process development time and optimizing resin use in chromatography.

L-223 **Evaluating Preparative Chromatography Against the “Twelve Principles of Green Chemistry”.** Olivier Dapremont, Kathleen Mihlbachler, AMPAC Fine Chemicals, Rancho Cordova, CA, USA; New Jersey Institute of Technology, Newark, NJ, USA

Implementing preparative chromatography into pharmaceutical process schemes is not only economically efficient but also environmentally responsible. We will evaluate the Preparative Chromatography technology against the “Twelve Principles of Green Chemistry” [1]. These principles ask researchers to develop intrinsically safe and energy efficient processes that maximize the raw material consumptions and generate the least amount of waste with a minimal number of process steps. Preparative chromatography provides a powerful tool to achieve these objectives. Due to the existing extensive analytical chromatography experience of chemists and engineers the technology can be applied to the isolation of chemical entities and their related impurities from mg quantities to multi tons. Furthermore, we will discuss examples showing how pharmaceutical processes can benefit from preparative chromatography. For these examples, process waste was reduced

L-224  **Green Liquid Chromatography using Subcritical Water as the Mobile Phase.**  **Yu Yang**, East Carolina University, Greenville, NC, USA

When pure water is used as the mobile phase in reversed-phase liquid chromatography, this green chromatographic separation technique is called subcritical water chromatography (SBWC). Obviously, the main advantage of subcritical water chromatography is the elimination of hazardous and expensive mobile phase organic solvents such as methanol and acetonitrile required in traditional reversed-phase liquid chromatography. In addition, separation efficiency, selectivity, and resolution can be optimized by temperature in subcritical water chromatography. SBWC also offers fast analysis and temperature-programmed elution. It is very encouraging that recently industry started paying attention to this economical and green SBWC technique. Topics that will be addressed in this presentation include characteristics of subcritical water; analytes separated by subcritical water chromatography; columns suitable for subcritical water chromatography; analyte detection in subcritical water chromatography; and SBWC method developments.

L-225  **Complex Fitness Diagrams: Downstream Processing of Biologicals.**  **Edwin N. Lightfoot Jr.**, University of Wisconsin, Madison, WI, USA

Our concern here is development of complex processes and specifically with modifying laboratory procedures that are unsuitable for large-scale operations. We use as our example process scale chromatography for downstream processing of high-value biologicals such as therapeutic proteins. Scale-up is complicated here because commercial acceptability is normally based upon staying with an accepted laboratory procedure rather than by product analysis. This constraint has led to the use of process-scale chromatography in spite of its basic commercial scale unsuitability. Our development procedure can be visualized as moving from one optimum, for a chromatography based separation, to a second optimum for commercial operations. This is seldom easy even in the absence of the above constraint. Success will require a careful analysis of our problem as well as heuristic modification. We choose as example that common situation in which the first and most costly major process step is simple saturation of a chromatographic column with suitably clarified solute feed to the separation chain, a process commonly known as capture. This is done at a high enough solute concentration that the solute front moving down the column can be successfully visualized as moving without significant change in shape: a constant-form front. The bulk of the column is then either empty of solute or acting as a simple but very expensive storage vessel. It follows then that it can be converted to a simple steady counterflow by moving the adsorbent upward at a speed just sufficient to make the separation zone stationary and eliminating both the storage and unused zones. In the developmental stages of our process, speed and flexibility are of primary importance. Here chromatographic procedures are optimum. In actual commercial production however capital, materials, and labor costs take precedence. For example high capital costs, and operating pressures and slow intra-particle equilibration within the adsorbent particles, become important. We must then shift our attention to second optimum. This second optimum simply represents substituting a large-scale continuous counterflow separation for chromatography. Only one step in the series need be changed, and it is by far the most expensive under production conditions. Moreover making such a substitution is mathematically equivalent in it essential features to the optimized laboratory process. The remaining problem is essentially hydrodynamic: the chromatographic particles, originally used in a stationary granular bed, must now be capable of controlled motion in a suspension. Their selectivity must be essentially unchanged, but their fluid mechanical properties can be novel. It is quite possible that their diffusional properties can be much improved. These problems will be discussed.

L-301  **Scale-up of Convective Affinity Chromatography Based on Validated Physical Model.**  **Simone Dimartino¹, Cristiana Boi², Giulio C. Sarti²**, ¹University of Canterbury, Christchurch, NEW ZEALAND; ²University of Bologna, Bologna, ITALY

One of the most important unit operations in the biotechnology industry is represented by affinity chromatography. A significant effort has been devoted recently to develop convective media columns, packed with affinity membrane or monoliths, to overcome the known drawbacks of traditional packed bead columns (high pressure drop, slow mass transfer through the diffusive pores and strong dependence of the binding capacity on flow rate). A crucial tool required to promote their application in large scale processes is represented by a validated simulation tool suitable to predict the process performance at all scales. This work presents a validated physical model simulating convective affinity chromatography processes, including all the three step of adsorption, washing and elution. The model accounts for all the relevant physico-chemical process factors: axial convection, longitudinal dispersion in the micro-porous matrix, affinity binding, dispersion and delay times associated to the extra-column circuit. All model parameters have a precise physical meaning and their value can be obtained independently. A detailed analysis of the experimental data indicates that a bi-Langmuir binding kinetics is required for a correct process description up to the saturation of the stationary phase. For model validation an extensive set of experimental data with affinity membranes and monoliths has been used. A very good agreement is achieved with experimental data at all operating conditions. The model also predicts the chromatographic cycle of IgG1 capture from a cell culture supernatant based only on data obtained for pure IgG solutions. The model has been finally applied for different scale-up calculations for membrane adsorbers, varying area and column thickness. The results have been compared with a simpler and frequently used lumped model; interestingly, both models which can describe equally well the lab scale process, but on the contrary they lead to important differences in the scale-up calculations, for which the physically based model is more reliable.
Porous monoliths are still widely studied topic and implemented in many areas of analytics and purification. They consist of a single piece of porous material with highly interconnected open channels resulting in convection to be predominant transport mechanism. Adsorption of target molecules takes place on the channel walls. As a consequence, pore diameter decreases, and causes an increase of the pressure drop on the monolith. Monolith porosity also decreases when the mobile phase cannot flow inside the adsorbed layer. Based on these assumptions one can derive mathematical equation to calculate adsorbed layer thickness from pressure drop measurements. The equations require data about monolith porosity and pore diameter. Monolith porosity can be measured rather accurately using various methods like determination of average residence time with injection pulse under non-binding condition or by weighing the wet and dry monolith. On the other hand, determination of the pore diameter is complicated because pores are not uniform and results can be highly dependent on the measurement method. This problem limits general applicability of the equation for estimation of adsorbed layer thickness. To improve robustness of the equation we derived several new mathematical equations assuming different microscopic structures of the monolith. It was concluded that meaningful values for adsorbed layer thickness can be obtained regardless of the assumed microscopic structure, leading to the conclusion that there is a methodology to estimate adsorbed layer thickness without assuming any particular microscopic structure. On this basis, we were able to derive an equation for determination for adsorbed layer thickness where no information about the pore structure was needed, and neither was there a need for accurate determination of pore size. The equation was validated using latex spheres and also with proteins of different known size. The proposed method creates some unique features like estimation of adsorbed layer thickness from the nano- to micro-scale range under various experimental conditions and the on-line, instantaneous study of changes in the thickness of the adsorbed layer during chromatography simply from the pressure drop. Because of that, it seems to be powerful tool for adsorption studies.

To date processes to obtain biofunctional peptides out of natural sources as well as the enrichment of their quantities are not feasible. This is in spite of the fact that different milk proteins are known to release functional peptides after enzymatic hydrolysis. In particular β-Lactoglobulin (β-Lg), the major whey protein, is a precursor of several biofunctional peptides with different beneficial properties like hypocholesterolemic activity as well as angiotensin-I converting enzyme (ACE)-inhibitory activity. Enzymatic hydrolysis results in a mixture of different peptides, so that further fractionation processes are necessary in order to obtain the specific peptides for any target application. For these peptides to be applied in the food industry, it is important that they can be separated and/or concentrated at industrial scale. Nowadays, the fractionation of such a complex mixtures is realized by chromatographic methods based on the physicochemical properties of the peptides. Since conventional bead-based chromatographic columns have several disadvantages like low flow rates and high back pressure which make them difficult for scale-ups, membrane adsorption chromatography (MAC) was utilized in this study because of the relative ease in scale up. At first the fractionation process to separate tryptic β-Lg hydrolysat was developed at laboratory scale and built up as a two-step purification procedure using an anion and a cation MAC module (Sartobind® Q and Sartobind® S). Optimal process parameters were investigated for each MAC module, including sample concentration, applied buffer systems and operating pH. Additionally various isocratic and step wise salt- and pH-gradients were tested. Optimal process conditions were determined, using 0,03 mol/l phosphate-buffer at pH 7 for the anion exchange process. This gave seven individual fractions in the first fractionation process step. In the second step the eluent of the anion exchange process was further separated using a cation exchanger and 0,03 mol/l phosphate buffer (pH 3). With a step-wise NaCl-gradient for both fractionation processes a total of 14 fractions were produced. Within six fractions enrichment of the bactericide peptides f(92-100) and f(78-83), the hypocholesterolemic peptide f(78-83) and the ACE-inhibitory peptides f(9-14) and f(142-148) was achieved. In the second step the optimal process parameters were successfully transferred to pilot scale using flow rates of up to 5 l/min, making this technique highly interesting for industrial fractionation of biofunctional peptides.

The isolation of major and minor whey proteins from acid and cheese whey will provide economic benefits compared to concentrated whey products due to the applicability of fractions according to each specific property. Proteins are added to foods for technological and/or biofunctional reasons, therefore they should be produced in food grade. The separation of up to eight proteins can be realized using ion exchange chromatography since this method offers a high specificity for separating proteins with similar properties in terms of size and surface characteristics. For a future application of the fractionation process at large scale some drawbacks of column chromatography must be overcome. The material should provide long-term performance and be CIP-able, sample pretreatment to remove particles and lipophilic substances has to be minimized and the consumption of buffers/ salts must be reduced as far as possible. The major drawback, which is a slow process due to diffusional transport and high back pressure, is already eliminated by the invention of membrane and monolith chromatography. Therefore, the use of the two types of stationary phases has been investigated for the fractionation of whey proteins from fresh whey. By adjusting pH-value and ionic strength different proteins interact with the same stationary phase and will be eluted separately by rising ionic strength. For the fractionation of whey proteins a process has been developed using acid whey (Voswinkel und Kulozik, 2011). The sample contains α-Lactalbumin, β-Lactoglobulin, Bovine Serumalbumin, Lactoferrin and Lactoperoxidase. Cascaded anion and cation exchanger membranes (Sartobind®nano Q and S, Göttingen, Germany) were used for the two-step-fractionation of the six proteins. Buffers and chemicals are sodium acetate and sodium chloride, all food grade. The process has been transferred to CIM® disk monolithic columns (BIA separations, Villach, Austria) also combining anion and cation exchanger stationary
phases. The separation efficiency in terms of resolution, protein concentration in the products as well as recovery of each protein fraction is compared between membrane and monolithic stationary phases. The main impact factors on binding and elution, ionic strength and pH-value of the sample and buffers, are investigated related to the type of stationary phase. Results show that both chromatographic media give similar results, the separation is more dependent on sample properties than on the stationary phases and flow rates. References: Voswinkel, L. & Kulozik, U. (2011). Fractionation of whey proteins by means of membrane adsorption chromatography. Procedia Food Science. 900–907.

L-305 Non-Platform Purification Process Development for an Ig-fusion Protein. Shujun Sun, Bill Daniels, Jason Cormier, Dan Lacasse, Chris Gallo, Pfizer, Andover, MA, USA

An Ig-fusion protein has been expressed in mammalian cell culture and captured from the clarified conditioned media by Protein A (ProA) chromatography. The resultant Protein A elution pool contains 10-25% high molecular mass species (HMMS) comprised of both multimers and dimers. The two-column [Protein A and anion-exchange (AEX)] platform purification process in use at Pfizer works well for almost all antibody projects, but in this case, the anion-exchange polishing step in the platform purification process was unable to remove the HMMS to acceptable levels without seriously compromising product yield. Early formulation studies also suggested the molecule was sensitive to freeze/thaw in certain buffers, as well as exhibiting instability at high protein concentrations under a range of pH conditions. As a result, a 3-column (Protein A, AEX, and ceramic hydroxyapatite) purification process was developed. The AEX step was optimized to provide maximum HMMS removal while maintaining the step’s capacity to clear virus. The ceramic hydroxyapatite chromatography step was developed to specifically remove dimeric HMMS to acceptable levels. The in-process stability of the molecule was controlled by shortening the load hold times for several of the process pools. Generation of HMMS during the ultrafiltration step was minimized by using optimized formulation buffer and operating conditions.

L-306 Strategies to Control the Concentration of a Charge Variant during Renaturation and Purification of a Recombinant Protein from E. coli Inclusion Bodies. Thomas Linke, Guoling Xi, Chris Thompson, Alan K. Hunter, MedImmune, Gaithersburg, MD, USA

The manufacture of a biologically active, recombinant fusion protein expressed in microbial cells requires renaturation from isolated inclusion bodies (IBs) and purification of the refolded protein by a multi-column process. The biological activity of the purified fusion protein is inversely correlated with the concentration of a charge variant in the final product. Here we demonstrate that the concentration of the charge variant in the final product can be controlled both in the denatured and in the refolded state. In order to obtain proteins from IBs in active soluble form, they must be first solubilized, often under denaturing and reducing conditions. Analytical ion exchange chromatography showed that the pH of the inclusion body solubilization buffer directly impacted the generation of the fusion protein charge variant. Lowering the pH of the solubilization buffer by 1.5 pH units reduced the concentration of the charge variant greater than 3-fold. Other product quality attributes such as monomer purity and fragment concentrations were not affected; however, it reduced the refold titer by nearly 50% and the amount of protein that could be recovered in the subsequent purification steps. Optimization of solubilization resulted in reducing the concentration of the charge variant while maximizing refolded product yield. Preparative anion exchange chromatography in gradient elution mode can also be used to control the concentration of the charge variant in purified fusion protein product. Separation of the charge variant from intact fusion protein took place within a narrow range of conductivities, underscoring the need for high resolution anion exchange resins. Concentrations of the charge variant were reduced more than two-fold in the anion exchange product pool compared to that in the load. Separation of the charge variant from the intact fusion protein by linear gradient elution was shown to be robust at multiple scales.

L-307 Simple Quantification of Elution Profiles to Determine Chromatographic Column Performance, Product Quality Characterization, and Process Improvement Opportunities in Commercial Scale Biopharmaceutical Production. Lakshmi Pathange, Bayer HealthCare, Berkeley, CA, USA

In commercial scale biopharmaceutical production, generally chromatographic elution profiles are evaluated and compared visually with standard reference profiles to qualitatively determine if the product and impurity profiles comply. The qualitative review is cumbersome and requires qualified personnel to interpret the chromatographs. In addition, qualitative review cannot detect subtle changes in chromatographic behavior as it cannot be compared with previous or historical process data. To overcome these shortcomings, a simple parameter matrix consisting of maximum elution UV peak height, post column conductivity, pre/post filter pressure was developed to quantify chromatographic elution profiles. These quantitative parameters values are entered and tracked by manufacturing personnel, on the production floor, for each manufacturing lot. By constant monitoring and continuous assessment, significant process performance issues such as column packing integrity, column underperformance, chromatographic skid issues, product profile variations, product quality characterization and process improvement opportunities were identified.

L-308 High Throughput Process for Downstream DoE Study. Yiming Yang, Shire HGT, Lexington, MA, USA

High throughput process for downstream DoE study Yiming Yang, Xiaomin Yang, Judy Miao, Hua Qiang, James Stout, Dave Nichols Purification Process Development, Shire Human Genetic Therapies

The Design of Experiment (DoE) approach is used to build design space in the development of purification processes for enzyme replacement therapies. A DoE strategy was developed for process development to improve efficiency and design space exploration. A high throughput screening (HTS) platform with Atoll Mini-Columns was used to screen all the process parameters that may affect the protein recovery and impurity clearance. The HTS methodology was adapted to an automated system. The results combined with the process knowledge gained from previous development studies were used to define the significant process factors for a downstream column operation. These significant factors were confirmed with scale down column runs to define parameter ranges and optimal conditions. The strategy significantly reduces scale down
Isolation of Intermediately Eluting Target Compounds Out of Quasi–ternary Mixtures by SMB Chromatography. Jadwiga Nowak¹, Dorota Antos², Andreas Seidel-Morgenstern³, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, GERMANY; ²Rzeszow University of Technology, Rzeszow, POLAND

Simulated Moving Bed (SMB) chromatography is a well-established and widely applied technique for the separation of binary or pseudo-binary mixtures. A common separation problem arises when out of a multi-component mixture a target compound of intermediate adsorption strength has to be purified. To overcome the limitation of conventional SMB systems, not to be able to perform center-cut separations, several modifications have been proposed [e.g. 1–5]. Based on results of a detailed theoretical study evaluating different model separation problems, an 8-zone SMB unit with internal recycle was found to be capable to isolate efficiently an intermediately eluting target compound out of a ternary mixture. An advanced SMB system allowing the separation according to this 8-zone SMB scheme was designed and built in our laboratory. For the experimental validation of the new process concept suggested, a mixture of three cycloketones (cyclopentanone, cyclohexanone and cycloheptanone) was selected as a model separation problem. The adsorption isotherms of the cycloketones were determined experimentally in methanol-water mixtures on LiChropret®RP-18 columns (25-40μm) and fitted to the Langmuir isotherm model. Systematic SMB experiments carried out were prepared in a systematical theoretical study of the separation problem under linear and nonlinear conditions. The work to be presented shows the successful purification of cyclohexanone as the target intermediate component out of a ternary mixture of cycloketones using the SMB concept applied. Results of several systematic experiments, designed based on the equilibrium theory [6], demonstrate the possibilities to predict and improve the productivity and to prevent product losses. The separation of more concentrated feed mixtures is also discussed, including the influence of feed compositions. [1] P.C. Wankat, Ind. Eng. Chem. Res. 40 (2001) 6185 [2] L.C. Kessler, A. Seidel-Morgenstern, J. Chromatogr. A 1126 (2006) 323 [3] J.S. Hur, P.C. Wankat, Ind. Chem. Eng. Res. 45 (2006) 1426 [4] M. Ando, M. Tanimura, M. Tamura, US Patent 4,970,002, 1990 [5] M.azzotti, S. Jermann, S. Katsuo, Submitted to: Org. Process Res. Dev. (2012). [6] G. Storti, M. Mazzoli, M. Morbidelli, S. Carra, AIChE J. 39 (1993) 471.

Parameter Space Design of Peptide Chromatographic Purification Processes. David Getal, Guido Strohlein, Alessandro Butte, Massimo Morbidelli, Institute for Chemical and Bioengineering ETH Zurich, Zurich, SWITZERLAND; ²ChromaCon AG, Zurich, SWITZERLAND; ³Lonza AG LES R&D - DSP Technologies, Wallis, SWITZERLAND

Nowadays, Quality by Design is a major concern in pharmaceutical process development. The interest of pharmaceutical companies towards Quality by Design arose since the publication of the PAT initiative [1] and the ICH Q8 and Q9 guidelines [2,3]. In this presentation, the design of a polypeptide purification process using chromatographic modeling will be discussed. Firstly, the model calibration and the Pareto optimization of the process will be presented. Then, a method to extend the design space will be discussed. This method is based on the determination of the critical process parameters (CPP) depending on the position in the design space and on the determination of correlated effects between them. The selection of the limiting critical quality attribute (CQA) in a process containing multiple CQA will be discussed and the sensitivity of the limiting CQA towards process disturbances will be assessed. Finally, an experimental verification of the results obtained by modeling will be presented. The approach presented in this presentation can be used to validate a purification process and to ensure the process and product quality according to FDA regulations. References: [1]Guidance for Industry PAT – A Framework for Innovative Pharmaceutical Manufacturing and Quality Assurance, US Food and Drugs Administration, US Department of Health and Human Services, Rockville, U. S. 2004. [2] ICH, ICH harmonised tripartite guideline: Pharmaceutical development Q8, 2005. [3] ICH, ICH harmonised tripartite guideline: Quality Risk Management Q9, 2005.

High-Purity and High-Yield Separations of Three Amino Acids in a Tandem SMB: Rapid Standing Wave Design and Column Dynamics. George Weeden, Lei Ling, Chin Chin, Nien-Hwa Linda Wang, Purdue University, West Lafayette, IN, USA; ²PureVision Technology, Ft. Lupton, CO, USA

Batch chromatography has been widely used for analytical applications. It is, however, less efficient for large scale production than simulated moving bed chromatography (SMB). SMB has not been widely used for the separation of mixtures with three or more components. A major barrier is the difficulty in SMB design, which involves specifying the length of each zone (the region between two ports), the flow rate in each zone, and the port velocity to achieve high product purity and high yield. Design by trial and error is costly and challenging. In our previous studies, a Standing Wave Design method (SWD) based on the concept of standing concentration waves was developed to overcome this barrier. The required zone flow rates and port velocity to achieve high efficiency separations were calculated readily from the adsorption isotherms and the mass transfer parameters. SWD reduces the number of optimization variables from nine to four for a four-zone SMB. In this study, we developed a Rapid Standing Wave Design method (RSWD), which does not require adsorption isotherms or mass transfer parameters. Apparent wave velocities were estimated from frontal loading and elution chromatograms. A difference between the port velocity and the wave velocity was used to focus a key concentration wave in an individual zone to control product purity, yield, and the split (distribution of the various components in the two products). RSWD was used in developing tandem SMB processes (two loops in series) for recovering three amino acids with high purity and high yield. RSWDs for various splitting strategies were developed and compared. A simulation tool based on a detailed rate model was used to verify the purities and yields targeted in the RSWDs. The designs were then tested with the data from a pilot SMB unit. The results showed that RSWD achieved high product purity and high yield (>97%). Close agreement was obtained between the rate model predictions and the experimental concentration profiles and effluent histories. Compared to the conventional SWD, the RSWD required fewer experiments in process development. Dynamic concentration profiles were highly dependent on the design methods and the splitting strategies. The desorption waves in the tandem SMB based on RSWD were more focused than those based on SWD, resulting in more solvent usage and a higher product dilution. The easy split should be done first in the tandem SMB to reduce solvent usage and product dilution.
The primary objectives of this research were to develop an effective separation process for enantiomers and determine their
isotherms on a chiral stationary phase using supercritical fluid chromatography. Development of a separation process of
Ibuprofen enantiomers using SFC was the first step. The Whelk O1 chiral stationary phase was employed as the substrate
for the separation of Ibuprofen enantiomers. The defined and optimized parameters that impact separation were utilized.
The adsorption isotherms from mass overload pulse introduction were determined. In addition, the isotherms from volume
overload bulk introduction were determined as well. Ibuprofen can be separated efficiently using the Whelk O1 CSP and
scCO$_2$ with 5% ethanol modifier. Linear range pulse experiments provide initial isotherms at temperature and pressure with
low solute amounts. FA and FACP isotherms provide saturation limits of solute loading on column at specific conditions;
pressure, temperature and concentration. The Scatchard plot yielded straight line of data meaning the Langmuir model is
applicable for this system. These isotherm data may be used for the scale up of a simulated moving bed system.

Preparative supercritical fluid chromatography is becoming an important purification tool in the pharmaceutical industry. The
advantages, especially in terms of speed and reduced solvent consumption, make it particularly attractive compared to
traditional prep-HPLC. In prep-HPLC, the solutes to be separated are mixed in the mobile phase and injected at the column
inlet. However, in prep-SFC, since the mobile phase is typically a mixture of high-pressure CO$_2$ and an organic modifier, it is
not not straightforward to prepare a feed in a similar manner. Hence, very often the solutes are dissolved in the organic
modifier and introduced into the column. There have been two main injection modes that have been proposed in the
literature. The first one, "mixed stream injection", is similar to HPLC, in which the CO$_2$ and the modifier are mixed upstream
of the injection valve. The second method, "modifier stream injection", suggests that the injection be made into the modifier
stream and then mix with the CO$_2$ downstream of the injection valve. There have been discussions in the community about
which of these is preferable in a practical situation. In PREP2011, we introduced an analysis framework, based on the
equilibrium theory of chromatography, to study the the migration of bands in the two injection schemes. It was shown that for
the case of mixed stream injection, the modifier band introduced during the injection travels through the column thereby
leading the band broadening or distortion, in the case of the mixed stream injection, since the modifier composition is usually
moderate, the band is broadened owing to the time required to inject the same volume in terms of the mixed stream injection.
We also derived the conditions at which one of these methods would be preferable over the other. In PREP2012, we
continue this study, but now using experiments. The separation of flurbiprofen enantiomers on Chiralpak ADH with
CO$_2$+methanol is considered. In order to elucidate the important characteristics only experiments under linear conditions are
considered. Experiments were performed using different injection volumes and modifier compositions and the
chromatograms were measured. It was clear that large volume injections suffer significant band distortions in the case of
mixed stream injections. Experiments were also performed to highlight the loss of resolution with increasing injection
volumes. The Henry constants at different modifier compositions were measured experimentally and were used as model
inputs. Finally, the experimentally measured band profiles were described using both the equilibrium theory of
chromatography and by numerical simulation, which show a good match.

In our drug discovery purification laboratory, we routinely use supercritical fluid chromatography (SFC) which typically utilizes
supercritical carbon dioxide mixed with an organic solvent such as either methanol, ethanol or isopropanol and occasionally
acetonitrile or heptane for preparative purification of active pharmaceutical ingredients or intermediates. In a typical
preparative SFC process, the fractions containing the compounds of interest are most of the time the majority of organic
solvents eluted during the preparative chromatographic process. After recovering the compounds of interest from the organic
solvent modifiers by rotary evaporation, the resulting solvents collected are usually discarded without any further treatment.
We have developed a simple, fast and safe method to recycle the solvent using powdered activated charcoal. The talk will
describe the results from the adsorption studies and the method development process used for the extraction of small
amount of compounds that may carry over during the rotary evaporation process. The configuration of the HPLC instrument
to allow large volume injection will also be discussed. Finally, practical examples of samples that were purified using recycled
solvents in our labs will also be presented.

Supercritical Fluid Chromatography(SFC) is a useful tool for analytical and large scale separations. SFC is most often used
with carbon dioxide as a mobile phase and an organic modifier such as some type of organic alcohol. It has some significant
advantages over standard HPLC methods such as less pressure drop across the columns, faster column equilibration, faster
method development, higher efficiency separations and significantly less generation of hazardous waste. Some of the main
advantages for preparatory chromatography include solvent waste reduction, facilitated product recovery, lower solvent cost
and the possibility for recycling. The study that will be presented will discuss several mixed solvent studies done with CO$_2$
recycling and the effects on the resulting prep chromatography.
L-316  New Advances in Prep SFC Chromatography at Merck Research Laboratories. Lisa Nogle, Ray McClain, Tin-Yau Chan, Jimmy DaSilva, Merck Research, Rahway, NJ, USA
This presentation will include recent advances in SFC instrumentation and analyses used within global Merck Research Laboratories in support of drug discovery efforts - to include both achiral and chiral applications.

L-317  Expanding the Versatility of SFC through New Enhancements to MS Directed, Open Bed, Fraction Collection Systems. Steven Zulli, Jonathan Jones, Ziqiang Wang, Chuping Luo, Timothy Martin, Waters, New Castle, DE, USA
Several new design and software features are demonstrated which create extremely versatile preparative, semi-preparative and analytical scale SFC systems, utilizing mass directed, open bed fraction collection. These enhancements for the Waters Prep 15, 30, and 100 SFC MS Directed systems are the first SFC systems to have the method selectable capability to perform injections in either modifier (co-solvent) or combined stream modes, enabling the choice of the advantages of either injection mode, allowing method development and purification of a wider range of compounds. A novel mixing device improves performance through peak shape enhancement, especially useful to mitigate the negative effects of the sample diluent on peak shape in combined stream injection mode. The stacked injection module, which can also be configured for modifier or combined stream injections, allows high throughput purifications with repetitive injections and collections in a single run, with fractions repeatedly collected into a single vessel per compound. The newly implemented Stacked Injector Graphical Editor is an easy to use mouse click driven user interface which simplifies optimization of stacked injection and collection parameters, such as injection interval and total run time, supporting all FractionLynx Timed Events, such as fixed time window or peak threshold determined collections, and different collection thresholds (MIT’s) for each compound. The stacked injector can operate as automated and unattended, or can be controlled in real time for the optimization of injection parameters during a run. These systems utilize the MassLynx/FractionLynx software package, and feature open-bed automated sample handling and fraction collection, supporting Mass, UV, and ELSD directed purifications, and OpenLynx walk-up operation. These system’s analytical scale capabilities and the AutoPurify software enable automation of pre-purification sample screening and focused gradient selection, and purity determinations of collected fractions. Presented are examples and limitations of these new capabilities, and typical performance results.

L-318  Utility of Accurate Measurements of the CO₂ Mass Flow Rate in Supercritical Fluid Chromatographic Operations. Abhijit Tarafder, Georges Guiochon, University of Tennessee, Knoxville, TN, USA
Accurate measurements of the mass flow rate of the mobile phase are critical in the design of the operating conditions of chromatographic separations. This issue is more important for SFC than for HPLC separations, because liquid fluids are nearly incompressible, their mass flow rate is proportional to their volumetric flow rate and this ratio rarely changes with the operating pressure and temperature. So, in HPLC one can safely rely on the instrument during method development and there are easy ways to monitor the pump performance. In SFC, the situation is quite different. The volumetric flow rate may vary significantly along the column in certain pressure and temperature zones in which the compressibility of the mobile phase is high. Then it is necessary to know the mass flow rate of CO₂ in order to predict the volumetric flow rates. Contemporary practice in industry, however, is to select an operating zone in which the mobile phase compressibility is low, the use of an organic modifier making it even lower. Commercial instruments generally use reciprocating pumps and translate the desired flow rate input into a piston stroke frequency. This would be fine with an ideal fluid since then the pump accurately delivers a predetermined mobile phase volume, keeping constant the mass flow rate during operations. This does not work well, however, if (1) the inlet conditions of CO₂ change over time, (2) the pump head temperature varies, or (3) the high-pressure valves of the CO₂ pump leak. The properties of CO₂ may change when the CO₂ cylinder is changed, especially during winter, and the pump head temperature may vary over time. For all these cases, the density of CO₂ entering the pump cylinder will vary, but as the stroke volume and frequency do not change, the actual mass flow rate will fluctuate, uncontrolled by the instrument. The situation is still more serious if the pump outlet valve leaks. Due to the low CO₂ viscosity, the chances of leaking increase, especially at high back pressures. Even small a leak can affect the inflow of CO₂ to the pump and can considerably alter the mass flow rate, again uncontrolled by the instrument. The most serious consequence is that, in all these situations, there is no easy way to determine how correct the pump operation is. Using the results obtained from an accurate coriolis mass-flow meter, this presentation illustrates the effects of uncontrolled variations of the CO₂ mass flow rate on the measurement of critical parameters of the design of SFC separations and highlights the importance of online measurements of the CO₂ mass flow rate during method development.

L-319  Importance of Kinematic Viscosity in Understanding Pressure Drop Phenomena in Supercritical Fluid Chromatographic Operation. Georges Guiochon, University of Tennessee, Knoxville, TN, USA
In HPLC, the density of the mobile phase and its viscosity are practically constant along a column. In contrast, the compressibility of the mobile phase being significant or important in SFC, density and viscosity vary with the pressure along the column and, depending on the local pressures and temperatures, their variations may be important. The differential form of Darcy equation provides the relationship between the local pressure gradient, dP/dz, the mass flow rate, G, and the characteristics of the column and the mobile phase: dP/dz = - G / (K ρ A) where G is the mobile phase viscosity, ρ its density, and A the cross sectional area of the column (A=πε π Rc², with Rc the column diameter and ε its external porosity). Integration of this equation to calculate the pressure and the density drops along a column as a function of the mass flow rate was done using the viscosities and densities of pure CO₂ and of its mixtures with methanol recently provided by NIST. This method provided plots of the differences between the inlet and outlet pressures and densities as functions of the outlet pressure and of the temperature for 150 x 4.6 mm columns packed with particles of average size 5 and 10 μm and operated with mass flow rates of 3 to 5 g/min. Plots of the density and pressure along these columns will also be shown. Considering separately the viscosity and the density to interpret these curves cannot provide clear conclusions; at constant viscosity: the density varies markedly with the pressure and temperature and so does the viscosity at constant density. In contrast, the
interpretation of these plots is easy by considering the kinematic viscosity of the mobile phase, $k = \eta / \rho$. The use of only one parameter in the discussion permits clear conclusions, as illustrated by diagrams of the kinematic viscosity of pure carbon dioxide and of its mixtures with methanol at concentrations up to 40% as a function of the outlet pressure and the column temperature. These diagrams are useful to select the operation conditions.
Novel Bioprocess Affinity Resins for the Capture of Antibody Fragments. Peter Lindahl, Bjorn Noren, Carina Engström, Maria Ersoy, Linus Laurin, Tomas Nyhammar, GE Healthcare Lifesciences, Uppsala, SWEDEN

Different variants of classic monovalent antibody fragments (Fab, scFv Dab etc.) are now emerging as credible alternatives to monoclonal antibodies (mAbs). There are several different types of antibody fragments that can retain the targeting specificity of a full antibody but lack the Fc portion. Here we present new affinity chromatography resins for the capture of antibody fragments of different sizes. Features of these affinity chromatography media are presented along with application examples showing binding capacities and selectivity for several types of antibody fragments and cleaning in place methods and data will be presented.

Protein A Functionalization of Polypropylene (PP) Capillary-Channeled Polymer (C-CP) Fibers for the Purification of Immunoglobulin G (IgG). Abby J. Schaddock-Hewitt, Zhengxin Wang, R. Kenneth Marcus, Clemson University, Clemson, SC, USA

Capillary-channeled polymer (C-CP) fibers are investigated in this laboratory as high-performance liquid chromatography (HPLC) stationary phases for the separation of macromolecules. They are distinct due to eight capillary channels that run the entire length of each fiber. When packed into a column, the fibers interdigitate to form channels that allow for high linear velocities with low backpressures, while the essentially nonporous surface allows for highly efficient mass transfer because there is no diffusion in and out of the stationary phase structure. C-CP fibers wick fluids spontaneously along the length of their channels, making them highly efficient for fluid transport. Fibers currently studied are made from polypropylene (PP), polyester (PET), and nylon-6, and can be applied in reversed phase (RP), hydrophobic interaction (HIC), and mixed-mode ion exchange (IEX) chromatography. Affinity chromatography has recently been investigated by functionalization of PP C-CP fibers with polyethylene glycol surface ligands to develop an allelo-specific IMAC stationary phase. We present a similar approach here; modifying the PP C-CP fiber surface with protein A to afford a highly selective surface for immunoglobulin G (IgG) capture. Separation of IgG from human serum is of great interest due to its various clinical applications. The ever-increasing demand for purified IgG has put focus on the development of a fast, high yield, cost-effective purification method. Affinity chromatography with recombinant protein A, which binds IgG with high selectivity and yield, is a common approach. However, most commercially available protein A resins are very costly (~$150/5mg). C-CP fibers are an attractive alternative because they are low cost (~$0.025/5mg fiber with < 1mg of Protein A ($0.50/mg) needed for functionalization of a standard 4.6 x 250 mm column) and allow for tailored surface modification without compromising the fibers’ integrity. The total fiber cost of one standard-sized Protein A functionalized C-CP fiber column would be less than $10.00 USD. PP C-CP fibers are modified with Protein A by adsorption to the fiber surface. Optimized loading and binding conditions, binding capacity, and yield are determined. Future work will study the use of linker molecules between fiber surface and Protein A to give an even stronger binding of the capture protein. Modelling of IgG separation on the C-CP fibers is seen as the next step in moving towards preparative scale separations.

Isolation of a Natural Product from a Complex Mixture: an Application of Preparative SFE and SFC to the Natural Product Purification Workflow. John McCauley1, Harbaksh Sidhu2, Rui Chen1, 1Waters Corporation, New Castle, DE, USA; 2Waters Corporation, Pittsburgh, PA, USA

Natural products have been a highly productive source of drugs and for lead compounds for drug discovery and development. Enzymatically derived natural products are usually structurally diverse and sterically complex. Furthermore, they often are found in a complex biological matrix with large numbers of structurally similar compounds. In order to facilitate unambiguous bioassays, there has been considerable research effort towards pursuing efficient techniques for natural product isolation. Historically, a typical natural product purification scheme involves a solvent extraction step to fractionate the complex mixture and enrich the target-containing fraction, followed by preparative chromatography, often reiterative, until the target compound is isolated. Supercritical Fluid Extraction (SFE) has long been used in extracting neutraceuticals and bioactive compounds. Supercritical fluid chromatography (SFC) has previously been found to be highly useful in many stages of pharmaceutical research and development. Due to the solubilizing power, low viscosity and high diffusivity of supercritical CO2, the main solvent used in both SFE and SFC, protocols utilizing SFE and SFC enable selective sample enrichment, efficient preparative chromatography and fast post-collection processing under mild conditions. In the present study we will detail a supercritical fluid based workflow for the isolation of a natural product from its biological matrix. The total workflow involves an SFE process to enrich the target compound into a more tractable mixture, SFC analytical method development, leveraging the orthogonal selectivity of column chemistries available to separate the target from the complex matrix; and scale up to large scale preparative SFC for the production of the pristine target compound. The ease and predictability of the scale up from analytical to increasingly larger preparative systems and the implications on overall throughput will be discussed. The cost benefits of this workflow will also be commented upon. This supercritical CO2 based isolation workflow is expected to have a broad applicability in the natural products arena.

Direct Capture of Alpha-amylose from CHO Cell Culture Supernatant using the Novel Salt Tolerant Anion Exchange Sorbent HyperCel STAR AX. Rene Ganter1, Magali Toueille2, Jerome Champagnolo2, 1Pall Life Sciences, Northborough, MA, USA; 2Pall Life Sciences, Cergy, FRANCE

Direct capture of acidic proteins produced in different feedstocks (plasma, mammalian or bacterial cell culture) often requires dialysis or dialfiltration steps prior to loading on Anion Exchange sorbents. To facilitate the capture of such proteins from medium to high conductivity feedstocks without dilution requirement, Pall just launched a new salt tolerant anion exchange sorbent named HyperCel STAR AX. After having demonstrated robust capacity and selectivity of the HyperCel STAR AX sorbent in a wide range of conductivities using pure proteins, Alpha-amylose - an acidic protein widely used in the Food and...
Beverage and Therapeutic market - was chosen as a model protein to demonstrate the specific benefits of the sorbent in process development. Capture of alpha-amylase from a CHO cell culture supernatant was thus developed using the salt tolerant STAR HyperCel AX sorbent compared to a standard strong anion exchanger. Data highlighted an efficient capture of the protein by the HyperCel STAR AX sorbent directly from the ccs, without any operation to modify pH or conductivity of the feedstock. The dynamic binding capacity obtained with the salt tolerant sorbent under those conditions was as much as 5 times higher than with the conventional anion exchange sorbent. Cost of Goods analysis using the generated data showed that the salt tolerance of the sorbent provides it with an advantage in terms of process economics compared to conventional sorbents, as it allows development of streamlined process eliminating the requirement for initial dilutions.

P-109  **Whey Protein Fractionation with Membrane Ion Exchange Chromatography at Pilot Scale.** Linda Voswinkel, Ulrich Kulozik, TU München, Freising, GERMANY

The isolation of whey proteins from cheese and acid whey has successfully been developed at lab scale using membrane based ion exchange chromatography (MAC) (Voswinkel & Kulozik, 2011). Anion and cation exchangers are Sartobind® Q and S (Sartorius-Stedim Biotech, Göttingen, Germany) which are available from lab to pilot scale. The chromatographic media offer many advantages compared to conventional column chromatography such as higher flow rates and less sample pretreatment. Furthermore, the MAC-modules are able to CIP and scale-up is relatively simple. The lab scale process is now being transferred to pilot scale using a pilot plant with automated fraction collector for up to eight different proteins. Furthermore, it is equipped with a pH and conductivity sensor for adjusting the buffers and sample as well as a pH, conductivity and UV sensor at the outlet of the MAC-units for an automated fractionation. At lab scale the column volume (CV) is 3 ml and the flow rate is 4 CV/min. The construction is a porous membrane (3-5 µm) coiled around a solid core. The process stream is led tangentially across both sides of the membrane and is directed from top to bottom through the pores. At pilot scale the construction of the MAC-unit differs in that the stream is mainly led tangentially across the surface and does not necessarily pass through the pores. Due to the more open construction even higher flow rates can be realized (10 CV/min at a CV of 250 ml) but binding is not as readily as at lab scale. This can be explained by reduced interactions between proteins and ligands when only convective transport across the surface takes place and not through the pores. Therefore, binding and elution of target proteins are investigated dependent on the flow rate vs. recirculation mode. Furthermore, a relatively large dead volume and possible mixing and dilution within the module have to be considered. Since the process is highly dependent on accurate pH-values and ionic strength for binding and elution, mixed phases must be avoided. For this reason, emptying the MAC-unit before loading and between elution steps is realized by reversed rotational direction of the gear pump. The applicability of the whey protein fractionation process is evaluated by the purity and recovery of each protein fraction as well as process time and buffers salts consumption compared to the lab scale process.


P-110  **Identifying Key Chromatographic Resin Attributes Associated with Process Efficiency.** Karl McCann1, Yvonne Vucica1, Danni Wang2, Russ Frost2, Joseph Poblete2, Joseph Bertolini1, CSL Biotherapies, Broadmeadows, Australia; 2Bio-Rad Laboratories, Hercules, CA, USA

The majority of commercial plasma fractionators have incorporated a chromatographic polishing step into their IgG manufacturing process. Chromatographic purification of complex biologicals such as human plasma is dependent on a number of critical resin attributes. Understanding how these attributes affect the binding of various target molecules or specific impurity molecules assist with developing efficient chromatographic processes. MacroPrep HQ resin is used as a final polishing step in the Intragam P (IgG) manufacturing process at CSL Biotherapies. A range of MacroPrep HQ variant resins with differing bead size, pore size, pore area, pore volume and ionic capacity were prepared and assessed for their ability to remove a range of impurity proteins including IgA, IgM, α2-macroglobulin, transferrin and albumin from the Crude IgG preparation. The results showed that pore area and pore diameter were the key attributes which affected the binding capacity of the resin. The results also showed that BSA binding capacity of the resins did not correlate well with the binding capacity of the resin for the key impurities. The increased binding capacity afforded by selecting a resin with these optimum attributes meant that a 30% increase in protein loading could be achieved when compared to a standard MacroPrep HQ resin.

P-111  **Thermodynamic Effects of Surface Curvature on Protein Adsorption.** Amina Darwish, Rebecca J. Desch, Stephen W. Thiel, University of Cincinnati, Cincinnati, OH, USA

The effect of surface curvature on protein adsorption was studied using multilayer adsorption models. Lysozyme was adsorbed on porous silica surfaces with different surface curvatures (commercial silicas with 60Å and 500Å pore diameters). Different adsorption isotherm forms were observed even though the two silica adsorbents had identical surface chemistry. Even after accounting for the limited volume available within the pores, the adsorption data indicate that the equilibrium coefficients between the protein in the first layer and the solid are significantly influenced by surface curvature. This effect may be due to the number of accessible adsorption sites at each surface curvature. Based on the Gibbs free energy, enthalpy and entropy calculated from the experimental data, surface curvature had a significant effect on the chemical potential of the adsorbed lysozyme.
In this work a detailed investigation into the engineering of multiple weak interactions to create selective MM protein separation systems is carried out. This research seeks to determine what conditions are required to achieve selective separations of similar protein variants and to provide fundamental insight into the mechanisms underlying these separations. High-throughput adsorption studies were carried out to screen for the adsorption trends with a wide range of protein libraries, MM resins and fluid phase modifier (FPM) conditions. Protein pairs and MM ligand/FPM combinations exhibiting unique selectivities were then selected for detailed investigation by NMR and MD simulation. Solution NMR titration experiments were carried out with 13C/15N-labeled proteins and MM ligands in the presence of FPMs to examine changes in binding kinetics, association affinities, and ligand binding sites on the proteins. MD simulations were performed on increasingly complex systems to develop a new framework for the understanding, manipulation, and prediction of multiple weak interactions in MM systems in the presence of FPMs. In addition to using fluid phase additives to improve selectivity, we have also found that temperature can play an important role in determining protein binding affinity in MM systems. Isothermal and gradient chromatography experiments were carried out with a protein library and the proteins were found to exhibit a range of responses to changes in temperature, providing windows of selectivity for separating protein pairs. Interestingly, it was found that a protein's sensitivity to temperature was not directly correlated with its hydrophobicity but was dependent on both the electrostatic and hydrophobic properties of the protein and the relative position of the strongly charged and hydrophobic regions on the protein surface. Further, a thermodynamic analysis was carried out to quantify the relative entropic and enthalpic binding contributions to the interactions in multimodal systems. Finally, these results were used to develop a new technique for mapping protein surface properties to identify high affinity binding regions. The knowledge base created from these studies can be used to select appropriate combinations of MM ligands, modifiers and temperature to achieve unique selectivities for challenging protein separations. This work provides fundamental understanding of the nature of these interactions at the molecular level and provides insight into the design of MM ligands, the roles of synergy and the modulation of selectivity using FPMs and temperature which can be employed to address challenging problems in downstream bioprocessing.

This work illustrates the complexity of lysozyme adsorption on carboxymethyl cellulose. Flow microcalorimetry was used in conjunction with isotherm measurements and water release data to analyze the adsorptive process occurring in this case study. Isotherm data shows typical type 1 Langmuir isotherms, however, flow microcalorimetry data indicates that the heat of adsorption (\(\Delta\text{Hads}\)) is dependent upon surface coverage. At pH 8 the dependence of exothermic \(\Delta\text{Hads}\) on the protein surface concentration shows a decrease to a sharp minimum followed by an increase as the surface concentration increases further. Our initial expectation was that heat of adsorption would be less exothermic as surface concentrations increases. Moreover, at pH 5 where lysozyme molecule seems to be more fully charged, at all the analyzed surface coverage, the heat signal consists of two peaks, an endothermic peak followed by an exothermic peak. By using these data in conjunction with adsorption isotherms an explanation for the observed behavior will be advanced. This work underscores the complexity of the adsorptive process when macromolecules are involved.

Two stereoisomers of estradiol, 17a and 17b-estradiol, are used in widely different pharmacological applications. 17b-estradiol is an estrogen used in hormone replacement therapy, while 17a-estradiol has no estrogen activity and is instead used to treat hair loss. While these isomers can be synthesized selectively, they also offer a model system for investigation of separation properties of similar steroid compounds on a large scale. In this study, normal phase chromatography is used to take advantage of the steric differences between the isomers in their interaction with a silica stationary phase and a mobile phase consisting of dichloromethane and isopropanol. Separation is monitored by UV absorbance at 228 nm. This work describes the optimization and scale-up of this separation system from analytical scale, where baseline resolution is achieved, to separation of a racemic mixture of a- and b-estradiol by Flash Chromatography. Brian Goolsby, Kendra Cox, Hitachi High Technologies America, Dallas, TX, USA.

Anion exchange chromatography is a standard method for protein capture or impurity removal. Conventional sorbents using Q or DEAE functionalities have low binding capacity for proteins in a high salt medium. This necessitates biological feedstock to be diluted or diafiltered into a buffer aiding direct capture by ion exchange sorbents. In this poster we show that with a novel IEX sorbent, we can directly capture and purify proteins from cell culture, plasma, or other feedstock, with minimal sample treatment. This sorbent has high dynamic binding capacity at short residence time (>100 mg/mL BSA at 1 to 2 min residence time), it maintains binding capacity at conductivities up to 15 mS/cm; therefore, it allows direct feed processing without dilution or ultrafiltration / diafiltration (UF/DF). We discuss several applications including synergistic effect with Protein A sorbents for removal of host cell proteins (CHOP) in monoclonal antibody purification, Capture of an acidic protein (pI 3.5) and Purification of HSA from undiluted plasma.
P-116  Novel Matrix Assisted Refold of Therapeutic Relevant Proteins and Peptides Produced with NPro Autoprotease Fusion Technology (NAFT). Rene Funke, Sascha Keller, Gerlind Stoller, Sandoz GmbH, Kundl, AUSTRIA

By using the genetically engineered Npro autoprotease from Classical swine fever virus (CSFV) a technology platform was established to produce difficult-to-express therapeutic peptides and proteins in form of inclusion bodies in E. coli. Due to the nature of this fusion protein technology processing requires renaturation of the inclusion bodies, autoprotease cleavage and refolding of the released target molecule which is usually performed in batch mode. We first demonstrate the renaturation from solubilized Inclusion bodies of Npro autoprotease fusion proteins and peptides of therapeutic relevance using a new approach of chromatography. In the present study, we show, for at least two therapeutic model proteins, that the fusion protein can be bound to the column in a moderate conductivity range. During the elution into kosmotropic conditions the self-cleavage activity of Npro is used to release the fusion partner with an authentic N-terminus. In comparison to the classical batch renaturation of Npro fusions by rapid dilution in continuously stirred tank reactors we achieved with the new matrix assisted refold technique a significant increase in productivity.

P-117  The Importance of Introducing Semi-Preparative Screening in Method Development - A Case Study. Robert Fredriksson, Kristina Hallman, Akzo Nobel Separation Products, Bohus, SWEDEN

Preparative HPLC differs from analytical HPLC since it needs to be performed in the nonlinear part of the adsorption isotherm in order to maximize important parameters such as column loading and productivity. In preparative HPLC method development screening is commonly performed with analytical injections in order to establish and compare selectivity in different chromatographic systems. From the screening one or more candidate phases are selected for a closer preparative study with overloaded injections. Typically, the method development is concluded by analyzing the fractions from the preparative study, to establish important final parameters, such as purity, yield and productivity. However, our case study illustrates the risk of relying only on selectivity during preparative method development.

In this case one specific chromatographic system was nearly overlooked due to low selectivity. However, when performing overloaded injections, a favorable adsorption isotherm was seen which proved beneficial for the purification. This effect was not seen during the analytical screening process and illustrates the importance of introducing semi-preparative screening in the early parts of preparative HPLC method development.

P-118  Modeled Simulated Moving Bed Purification of 2-hydroxybutyric Acid. Anthony Grabski¹, Shuvendu Das², Bruce Thalley¹, Jay Yun³, Alla Zilberman¹, Soo-II Kim³, Mani Subramanian², Robert Mierendorf¹, Sembia Biosciences, Inc., Madison, WI, USA; ²University of Iowa, Iowa City, IA, USA; ³ChromWorks, Inc., Burlington, MA, USA

Advantages of simulated moving bed chromatography (SMBC) over standard linear or batch processes include dramatically increased productivity, purity, and efficiencies in chromatography media and buffer utilization. However, complex equipment and multivariate SMBC process development has inhibited universal acceptance and application of this highly efficient purification technology. We have combined a bench-scale multicolumn chromatography system with a comprehensive simulation software toolbox to simplify the design, optimization and demonstration of continuous chromatography processes. The software intuitively combines stepwise numerical graphical outputs with flexible fine control options. The dynamic modeling employs the adaptive mesh refinement algorithm for spatial discretization to present robust and accurate simulations of well-known continuous chromatography processes such as SMB, Intermittent SMB, and Bio-chromatography in preparative and continuous operation modes. The automated eight column chromatography system performs SMBC and other continuous protocols using software activated pneumatic valves. Fluid flow is controlled by up to eight pumps that can be run individually or in any combination. The step-wise development of SMBC purification for the API precursor 2-hydroxybutyric acid from modeling through the demonstrated continuous purification will be presented.

P-119  Comparison of Various Ternary Simulated Moving Bed Schemes by Nonlinear Programming. Gaurav Agrawal, Yoshiaki Kawajiri, Georgia Institute of Technology, Atlanta, GA, USA

Cation Exchange Chromatography Challenges in Balancing Binding Capacity Versus Resolution. Inkwan Han, Sunitha Kandula, Edward Glowacki, David Roush, Thomas Linden, Merck Research Laboratories, Union, NJ, USA

Cation-exchange chromatography CEX can be a powerful tool in a platform scheme as a purification or polishing step for monoclonal antibodies (mAbs) in the downstream process. CEX has been demonstrated to clear host cell proteins, aggregates and protein A. CEX is often used in conjunction with AEX flow-through which removes DNA and other host cell residuals. There is literature precedent for this combination of steps as a generic purification scheme for monoclonal antibody products. One challenge with CEX is balancing capacity vs. resolution. In this study, separation efficiency was compared for two CEX resins with the same ligand but different binding capacities. The higher capacity resin had 75% higher DBC which potentially affords multiple process advantages (ex. fewer cycles, smaller processing volume). The best resolution of dimers from monomers was obtained at 30 mg/mL loading on the low capacity resin. Resolution of aggregates was a strong function of resin loading for both resins evaluated. Higher productivity was also obtained with the lower capacity resin. Ultimately the process parameters were optimized with the low capacity resin at the lab scale and confirmed upon scale-up at 500 L pilot scale runs. The results from this study emphasize the criticality of evaluating resolution in conjunction with binding capacity for CEX resins.

Investigating the Mechanisms of Protein Sorption and Transport in Cross-Linked Cellulosic Ion Exchangers. James Angelo, Harun Koku, Abraham Lenhoff, University of Delaware, Newark, DE, USA

High loading capacity and rapid separation of proteins are strongly desired traits of ion exchange materials utilized in the downstream purification of biopharmaceutical agents. The complex network of natural carbohydrate polymers that cellulose ion exchange resins provide incorporates an easily accessible microstructure with substantial binding capacity for biomolecules. The anion and cation exchange moieties of a commercially available cellulose material, HyperCel (Pall), were characterized by their adsorption capacity, protein uptake rates and retention at differing total ionic strengths. Adsorption isotherms and isocratic elution were used to assess the binding characteristics of four model proteins: lysozyme and lactoferrin on the anion exchange variety and β-lactoglobulin and BSA on the anion exchange variety. In kinetic measurements via batch uptake, rapid transport was observed in both resins even under strongly favorable binding conditions. Additional mechanistic insight was sought using confocal and transmission electron microscopy to gain a physical understanding of protein uptake profiles and pore architecture, respectively. Time–series confocal imaging of particle cross-sections elucidated the type of intraparticle transport in which fluorescently labeled protein traversed to the particle core. TEM imaging of thin sections of material allowed for visualization of the microporous structure, and can allow for quantitative determination of intraparticle porosity, accessible binding area and pore size distribution parameters.

Comparing Bulk Silica Phases. Imre Sallay, Keiji Koyanagi, Daisco Co., Ltd., Osaka, JAPAN

As more companies venture (though reluctantly) into the treacherous field of process scale HPLC purification, the question arises with increased frequency: Which stationary phase to use? Comparing HPLC columns available on the market is a popular topic, countless papers have been published on this interesting topic. However there is no reliable comparison for silica based bulk stationary phases. Here we report our effort to work out a set of parameters highly indicative for the users of the bulk stationary phases. Standardized test are presented to determine the usefulness of given stationary phases for a particular application: The most important application for plant scale RP HPLC is the insulin purification process. We describe the tests we found most relevant for this peculiar and most challenging application. The aim of this study is to start a constructive discussion and furthering efforts on similar tests concluding in contribution for the pharmaceutical industry, making life easier for chromatographers.

Chiral HPLC Methods for the Enantioseparation of Proton-Pump Inhibitors using Polysaccharide-based Chiral Stationary Phase in Normal-phase, Polar Organic and Reversed-phase Elution Modes. Liming Peng, Marc Jacob, ThuyLinh Tran, Tivadar Farkas, Phenomenex, Torrance, CA, USA

Proton-pump inhibitors (PPIs) are a group of drugs used for the treatment of stomach acid-related diseases due to their long lasting reducing gastric acid production. They are benzimidazole or imidazopyridine derivatives, typically weak bases (pKa1 = 3.8 - 4.5). Because PPI enantiomers potentially have different pharmacokinetic and pharmacodynamic profiles, there has been an interest in developing pure single enantiomer of PPIs for the chiral switch. Thus, there is a need for the chiral separation of PPIs drugs and the development of chiral separation methods for the enrichment of enantiomerically pure PPIs. In this presentation, we report effective chiral high performance liquid chromatography (HPLC) methods for the separation of various PPIs including lansoprazole, omeprazole, pantoprazole, rabeprazole and tenatoprazole using various polysaccharide based chiral stationary phases (CSPs) in normal-phase (NP), polar organic(PO) and reversed-phase(RP) elution modes. The results show that the selection of mobile phase additive has a dramatic effect on enantioselectivity and retention of benzimidazole related PPIs in NP and PO elution modes. Choline-containing polysaccharide-based CSPs and cellulose tris(3,5-dimethylphenylcarbamate) in particular provide the best enantioseparation of PPIs in RP, PO, and NP elution with resolution greater than 2 in most cases, and analysis times shorter than 10 minutes in PO and RP elution. Chiral HPLC methods for the enantioseparation of PPIs using polysaccharide-based CSPs are very effective and well suited for the chiral analysis and chiral preparative purification of such drugs.
P-124  
Ernest Sobkow,1 Noriko Shoji, Chie Yamashita2, Takamatu Takai2, Masakatsu Omote1, Naohiro Kuriyama, 1YMC America, Inc., Allentown, PA, USA; 2YMC Co., Ltd, Kyoto, JAPAN

Reverse phase chromatography has an important role in precise purification of high value-added products, such as pharmaceutical peptides or proteins. In this field, silica based packing materials have been widely used due to silica's high mechanical strength and cost-effectiveness. However, conventional silica-based packing materials have lower chemical stability under both strong acidic and strong alkaline conditions than the polymeric based materials. These limitations are significant during purification because acidic eluent and/or gel cleaning with alkaline solution are widely employed for peptide and protein separations. In order to overcome such drawbacks, mixed-mode resins have been developed. Mixed-mode resins have attracted attention due to its unique selectivity, and which can bind proteins under physiological conditions. However, available mixed-mode resins have lower binding capacity than IEC resins. In the capture step, throughput is very important. Also due to recent developments in upstream processing, protein expression levels have been increased. These require a resin with higher binding capacity under high flow-rate. Recently Tosoh has commercialized a new mixed-mode resin named TOYOPEARL MX-100M. The dynamic binding capacity for IgG of this resin was 90-100 mg/mL under physiological salt conditions, which was higher than protein A affinity resins. In this poster, characteristics of the resin and application to the purification of antibodies will be presented.

P-125  
Characterization of a New Mixed-mode Resin.  
Koji Nakamura, Yuji Kubo, Hiroyuki Moriyama, Tosoh Corporation, Yamaguchi, JAPAN

Hydrophobic interaction (HIC) and ion exchange chromatography (IEC) have been used for downstream processing in the biopharmaceutical industries. However, HIC needs lyotropic salts during binding, which leads to high production cost and environmental problems. In contrast, IEC needs to reduce conductivity of protein solution prior to the adsorption step, which also leads to high cost. In order to overcome such drawbacks, mixed-mode resins have been developed. Mixed-mode resins have attracted attention due to its unique selectivity, and which can bind proteins under physiological conditions. However, available mixed-mode resins have lower binding capacity than IEC resins. In the capture step, throughput is very important. Also due to recent developments in upstream processing, protein expression levels have been increased. These require a resin with higher binding capacity under high flow-rate. Recently Tosoh has commercialized a new mixed-mode resin named TOYOPEARL MX-100M. The dynamic binding capacity for IgG of this resin was 90-100 mg/mL under physiological salt conditions, which was higher than protein A affinity resins. In this poster, characteristics of the resin and application to the purification of antibodies will be presented.

P-126  
Purification of a Synthetic Peptide using Multi-column Chromatography (Contichrom & MCSGP).  
Guido Stroehlein1, Thomas Mueller-Spaeth1, Olav Lyngberg1, Derek Maclean1, 1ChromaCon AG, Zuerich, SWITZERLAND; 2Bristol-Myers Squibb, New Brunswick, NJ, USA; 3KAI Pharmaceuticals, South San Francisco, CA, USA

The poster presentation shows the advantages of using multi-column chromatography (Contichrom® & MCSGP) in terms of yield, purity and productivity improvements for the purification of a late-stage therapeutic, synthetic peptide. The purification was performed with batch and multi-column chromatography (MCSGP) in parallel. MCSGP is a versatile platform process for the purification of therapeutic peptides and proteins. A direct comparison of the performance of the batch and the MCSGP process in terms of yield, purity and productivity is presented, including experimental data of both processes.

P-127  
instrAction® Mixed Mode Screening Kit - Stationary Phases for Preparative Chromatography as an Access to the instrAction® Phase Technology and Phase Library.  
Martin Welter, instrAction GmbH, Mannheim, GERMANY

The instrAction Mixed Mode Screening Kit contains 12 mixed mode stationary phases for the development of preparative chromatographic applications. Combinations of different retention modes (lipophilic, polar, H-acceptor/donor, π-stacking, cationic/anionic, aromatic/hetero-aromatic interactions etc.) are implemented into individual phases, making the set of phases a unique approach to solve difficult preparative separation challenges. The instrAction phases present in the Mixed Mode Screening Kit base upon silica gel. The silica is coated with a linear polymer, which is covalently cross-linked to a stable three-dimensional network. The network exhibits amino groups, which are functionalised with different kinds of binding side residues, offering different modes of interaction. The binding side residues can be incorporated in different concentration (mmol/ml) and different ratios to each other. Usually two or three types of binding groups/binding modes are incorporated into the network. The instrAction phases of this Screening Kit are a sub-set of the instrAction phase library, containing more than 3000 different stationary chromatographic phases. The 12 mixed mode phases cover a broad range of possible combinations of interaction (e.g. anionic – lipophilic, cationic – lipophilic, polar – ionic etc.) useful for a broad spectrum of compounds. The phases with a pore size of 10 nm (100 Å) can be used for “small molecule” purification and peptide polishing up to a molecular weight of approximately 5000 - 7000 Da. The peptide particle size of 10 μm was chosen with respect to later large-scale use in preparative columns, limiting the backpressure to a range useful for manufacture. The instrAction mixed mode phases can be used together with all common chromatographic solvents, from hexane to aqueous buffer systems. There is no limitation concerning modifiers, like acetic acid, formic acid, TFA or buffer systems. The combination of different modes of molecular interactions lead to an unusual, often remarkable selectivity between closely related compounds depending on the mobile phase composition. This poster will show several examples of the characteristics of these screening kit phases to give an idea how the use of multimodal phases can offer a completely new purification strategy. Comparisons of the columns in different applications will illustrate where the strengths of these phases lie and how to use it. The screening kit offers the access to the entire instrAction Phase Technology – in form of from-the-shelf media or of a customised multimodal phase, fine-tuned and tailor-made for the individual separation task if necessary.
P-128  The Effects of Medical Ozone Therapy on Renal Ischemia/Reperfusion Injury. Muzaffer Ozlosun, Emin Ozgur Akgul, Erdinc Cakir, Tuncer Cayci, Bulent Uysal, Ayhan Ozcan, Taner Ozgurtas, Ahmet Guven, Ahmet Korkmaz, Gulhane Military Medical Faculty, Ankara, TURKEY

Introduction: This study was designed to investigate possible beneficial effect of ozone therapy (OT), known as an immunomodulator and antioxidant, on the renal function, morphology and biochemical parameters of oxidative stress in kidneys subjected to ischemia/reperfusion injury (IRI). Materials and Methods: Thirty male Sprague-Dawley rats were divided into three groups; Control, renal IRI, renal IRI+OT. IRI injury was induced by 60 min of bilateral renal ischemia followed by 6-h of reperfusion. After reperfusion, kidneys and blood were obtained for histopathologic and biochemical evaluation. Nitrate plus nitrite (NOx) concentrations levels by using ion chromatography, serum neopterin (NP) levels were determined by using a High Pressure Liquid Chromatography (HPLC) system with a fluorescence detector. Results: Renal IRI increased tissue oxidative stress parameters (lipid peroxidation, protein oxidation and nitrite plus nitrate) and decreased antioxidant enzyme activities (superoxide dismutase and glutathione peroxidase). All these parameters came to control values in the treatment group. Serum NP levels were increased in IRI group but decreased in the treatment group. Histopathologically, the injury score in the treatment group was significantly lesser than in the renal IRI group. Conclusions: Our results clearly showed that OT has beneficial effect to protect kidney against IRI. In addition, serum neopterin level can be used to detect degree of renal IRI.

P-129  Novel Biomarkers of Renal Scar Formation in Acute Pyelonephritis in Rats. Yasemin Gulcan Kurt, Bahadir Caliskan, Ahmet Guven, Emin Ozgur Akgul, Bulent Uysal, Bilal Altan, Ayhan Ozcan, Orhan Bedir, Ilhami Surer, Ahmet Korkmaz, Gulhane Military Medical Faculty, Ankara, TURKEY

Introduction: This time-course study was design to determine the effect of progression of acute pyelonephritis (APN) on oxidative/nitrosative stress parameters, serum procalcitonin and neopterin levels, and to correlate them with renal structure. Materials and Methods: Fifty rats were divided into five groups of ten rats each. Eight rats in each group underwent APN procedure. The other two rats in each group served as a sham-operated control group. APN was induced by injection of 0.1 ml freshly prepared E. Coli solution into the both kidneys. The rats in each group were evaluated after 1, 3, 7, 14 and 42 days of APN procedure, respectively. At the end of the experimental period, animals were killed via decapitation and trunk blood was collected. Both kidneys were harvested for antioxidant enzyme activity (superoxide dismutase (SOD) and glutation peroxidase (GPx)), oxidative stress parameters (tissue lipid peroxidation (MDA) and protein carbonyl content (PCC), nitrosative stress (NOx), hydroxyl prolin (HP) level and histopathologic examination. Nitrate plus nitrite (NOx) concentrations levels by using ion chromatography, serum neopterin (NP) levels were determined by using a High Pressure Liquid Chromatography (HPLC) system with a fluorescence detector, serum Procalcitonin (PCT) levels were measured by automated immunofluorescent assay. Results: Both Oxidative/nitrosative stress parameters and antioxidant enzyme activities increased progressively, but after 14th day markedly decreased. Serum procalcitonin level to evaluate the bacterial infections and serum neopterin level to evaluate immunologically induced oxidative stress increased progressively, particularly after 3th day, and then decreased after 14th day to control values. Tissue HP level was increased progressively throughout the experimental period suggesting increased scar formation which confirmed with histopathologic evaluation. Histopathologic evaluation also showed that injury score showed a correlation with biochemical evaluation. Conclusion: This study showed that there is a clear correlation between oxidative/nitrosative stress parameters and renal scar formation in APN. Serum NP and PCT levels and oxidative stress parameters in acute period can be used to determination of renal scar formation in chronic period.

P-201  Development of an Affinity Ligand for IgG Purification by Engineering a Human Fc Receptor. Teruhiko Ide, Toru Tanaka, Kouta Hatayama, Yoshiharu Asaoka, Hidetaka Kobayashi, Koji Nakamura, Tosoh Corporation, Ayase, JAPAN; Sagami Chemical Research Institute, Ayase, JAPAN

Recombinant monoclonal antibodies such as human immunoglobulin G1 (IgG1) are an important class of biological pharmaceuticals that are used to treat a wide variety of conditions, including infectious diseases, cardiovascular diseases, cancer, and autoimmune diseases. Production of monoclonal antibodies by using hybridoma technology or transgenic animals can be easily scaled up, but immunoglobulin purification from crude feedstock still poses several problems. The main difficulties are the low antibody concentrations in the cell culture supernatants or milk of transgenic animals and the large amounts of contaminating proteins. Traditionally, researchers and manufacturers have turned to Protein A as the principal antibody purification technology. However, increasing concerns about the limitations of Protein A, including leaching into the product, antibody denaturation, poor stability under harsh process conditions, and the nature of its biological source, have resulted in the need for a suitable alternative. We developed a new affinity ligand engineered from human Fcγ receptor I (hFcγRI). Human FcγRI recognizes the Fc portion of IgG1 and IgG3 with high affinity (KD value: 10–10 to 10–9 M). The extracellular domain of hFcγRI, which interacts with human IgG, was expressed as recombinant soluble hFcγRI (rFcR) in Escherichia coli. The amino acid sequence of rFcR was optimized by using a directed evolution technique in order to increase its stability towards both acidic and basic conditions. The optimized rFcR, which named mrFcR, was modified by a 71-amino-acid substitution of the sequences of the extracellular region of wild-type rFcR. The mrFcR was immobilized on Toyopearl chromatography media for use as a ligand for affinity chromatography. For purification of IgG1 the binding pH range of mrFcR resin was between pH 6 and 8—relatively independent of the salt concentration. Elution normally occurred at a pH range of 3 to 4. The static binding capacity of mrFcR resin was greater than 60 mg of pure human IgG1/ml settled resin. The dynamic binding capacity varied depending on several factors, such as target antibody and flow rate. The mrFcR is a promising ligand for affinity chromatography for antibody purification from complex feedstocks.
The benefits of the unique selectivity and chemical stability offered by mixed-mode chromatography for the purification of monoclonal antibodies (mAbs) have been demonstrated in several occasions. However, unique features of mixed-mode sorbents can also be exploited efficiently to purify “non-antibody” proteins produced in bacterial systems such as E. coli. A significant number of therapeutic proteins are produced in E. coli at large scale with high expression titers. The chromatography capture step of such recombinant proteins is highly challenging, since bacterial lysates contain a broad variety of contaminating protein species. An ideal protein capture step should be selective, have high capacity, and preserve the biological activity of the target protein. Pall’s Mixed-mode chromatography sorbents portfolio is composed of three sorbents- MEP, HEA and PPA HyperCel™ - carrying synthetic ligands immobilized on a robust and scalable matrix (HyperCel). The present study illustrates the utility of the mixed-mode sorbent HEA HyperCel - screened as the best mixed-mode candidate for this capture application - to efficiently an acid sensitive protein expressed in the periplasm of E. coli (recombinant Glutathione-S-Transferase or rGST). rGST was chosen as a model protein relatively difficult to purify due to its lability at acidic pH, limiting its elution conditions and separation from acidic endogenous contaminants from E. coli. Screening of mixed-mode and ion exchange sorbents was performed, and conditions for efficient capture step using HEA HyperCel sorbent were determined to protect the biological activity of rGST. HEA HyperCel mixed-mode sorbent was selected and showed a high selectivity for rGST (90% purity in one step), a capacity over 10 mg rGST/mL along with an efficient regeneration. A 300-fold scale-up of the separation performance on HEA HyperCel sorbent was achieved. This approach demonstrated the performance of mixed-mode sorbents used as capture step for one model protein produced in E. coli and could be extended to other E. coli protein expression models.

Capillary-channeled polymer (C-CP) fibers were being investigated as high performance liquid chromatography (HPLC) stationary phases for protein separations in our laboratory. Due to the eight channels running along the fibers, when packed into column, the flow channels extend the entire length of tube, allowing operation at high linear velocity (>30 mm/sec) and low backpressure (<2000 psi). The virtually nonporous surface of the fibers decreases the diffusion so that they have very fast mass transfer. What’s more, the costs of the fiber material are very low (<US$0.25 per 4.6 x 250 mm column). A unique feature of the C-CP fiber phases is the virtual lack of a chromatographic c-term for macromolecules, though there is a definite mass transfer term found for small organic molecules. To better understand the actual porosity present in C-CP fibers, inverse size-exclusion chromatography (iSEC) has been employed to determine the pore size distributions (PSD). Because of the advantages of mild experimental conditions, inexpensiveness, and convenience, iSEC is a very effective means for determining PSD under chromatographically relevant conditions. Polypropylene (PP) C-CP fibers were packed into fluoropolymer capillary column (0.8 mm i.d., 38.5 cm long) to achieve and interstitial (void) fraction of 0.60 (found to be optimal for protein separations). A set of ionic (metal cations) and molecular (uracil, small heterocycles, polypeptides, and proteins) probes with defined sizes was chosen to provide a range of hydrodynamic/viscous probe radii. UV-VIS absorbance was first performed on solutions after exposure to the fiber to test the potential for chemical adsorption of the test species to the fibers Only the samples which don’t react with fibers were then injected on-column to determine their respective partition coefficients Kd versus viscosity radius Rη. The assumption being that any amount of retention was due to entropic, rather than enthalpic, driving forces. The results indicate that Kd decreases with increasing Rη; as was hoped. Van Deemter plots are also generated for each of the test species to expose any c-term effects. Ultimately, figures of merit including H versus Rη, logMW versus Kd, and finally the calculated PSD of the column will be obtained. It is believed that this general method will be of use for different C-CP base polymers as well and different fiber extrusion conditions to better tailor the columns for protein separations.

The choice of the appropriate chromatographic system requires a lot of expertise. In general, the stationary phase is selected first and then the mobile phase is chosen. This approach often leads to challenging amounts of solvents due to the limited solubility of the target substances. Additionally, the use of solvent gradients cause waste streams consisting of solvent mixtures, which are hardly recyclable. This study deals with a possible inversion of the chromatographic process design. First the mobile phase is optimized in regard to solubility of the target component and second the stationary phase is selected. In this study, the extractant should also be used as eluent for the chromatography unit. In case of recovering natural products, the target substance needs to be extracted from a solid plant matrix. The selection of suitable extractants is achieved by a Microsoft Excel based selection tool. Suitable extractants are pre-selected based on physical and toxicological properties, the area of product application and solubility parameters, such as log Kow-values, Hildebrandt and Hansen parameters. Due to pre-selection the experimental effort and costs are reduced. With the pre-selected solvents, standardized laboratory experiments are carried out to collect data on extraction efficiency. The experiments are investigated regarding to yield and selectivity and costs. The outcome is the selection of the most desirable extractant and eluent, respectively for the combined process. To complete the chromatographic process the next step is the selection of the stationary phase. Several silica adsorbents, polymer adsorbents, and adsorber resins are tested. Another selection tool to exclude unsuitable adsorbents based on the prediction of interactions between the target molecule, the eluent and the stationary phase is used. To increase selectivity, a combination of different stationary phases connected in series with equal eluent shall be determined. The inversion of the chromatographic process design was carried out successfully for the purification of rebaudioside A from leaves of Stevia rebaudiana Bertoni.
P-205 A Novel Mustang® XT Device for Scale Up/down Optimization of Membrane Chromatography Purification Steps. Aleksandar Cvetkovic1, Iann Rance2, Rene Gantier1, 1Pall Life Sciences, Northborough, MA, USA; 2Cytheris SA, Issy Les Moulineaux, FRANCE

Membrane chromatography is now routinely implemented in many large scale biotech processes to remove contaminating host cell proteins, DNA and viruses at high flow rate. Pall developed the range of ready to use and scalable Mustang® XT membrane chromatography capsules (5 to 5000 mL) for purification process development from lab to industrial scale. A new small scale device, Mustang Acrodisc XT (0.86mL) was recently launched to complete that range of products. The evaluation of performance (dynamic binding capacity, pressure drop, peak asymmetry and flow pattern) of the new Mustang Acrodisc XT capsule is presented here. In addition, a scalability study was conducted on different Mustang devices including Acrodisc XT (0.86 mL), XT5 (5 mL) and XT140 (140 mL) capsules. A biotech process where Mustang Q membrane is used as an early contaminant removal step for the purification of a therapeutic recombinant protein was considered. The three different size capsules were used following a linear scalability rule. Almost identical chromatography elution, pressure drop, contaminant removal and product recovery patterns were obtained on all the three capsules which confirmed a good scalability between the devices tested. This demonstrated that the new Acrodisc XT capsule can be used as a scalable device at lab scale for early stage purification process development studies. This new device could therefore be considered for any scale down study such as a viral clearance.

P-206 Improving Resolution and Mass Capacity in Preparative Liquid Chromatography for Natural Product Isolation. Jo-Ann Jablonski1, Rui Chen2, 1Waters Corporation, Milford, MA, USA; 2Waters Corporation, New Castle, DE, USA

Natural product extracts are complex mixtures with many structural analogues and isomers. Preparative liquid chromatography is the most commonly used technique for the isolation of bioactive compounds, the purification of standards for use in analytical quantitation, as well as the isolation and identification of minor components. Successful preparative isolations require adequate resolution between adjacent peaks. Better resolution enables higher mass loading, which increases productivity and the efficiency of the purification process. In this study, we use peppermint as an example to illustrate different techniques for improving the resolution of compounds in natural product isolation. Peppermint is a plant cultivated worldwide and possesses a number of antioxidant polyphenolic compounds. Many of these are structurally related and are of medicinal interest. Different column chemistries will be examined to determine which provides the best resolution and peak shape for the components in the peppermint extract. In addition, a step-wise gradient focusing to enhance peak separation will be demonstrated. Finally, the at-column dilution (ACD) injection scheme will be compared with a conventional injection scheme for sample introduction. The improvement in loading capacity attributed to at-column dilution will be discussed. The techniques presented in this poster should have general applicability in natural product isolation.

P-207 Dynamic Control of Buffer Variability by In-Line Conditioning for Process Chromatography. Roger Nordberg, Enrique Carredano, Martin Hall, Elenor Strandberg, Tomas Karlsson, Henrik Sandegren, Karol Lacki, GE Healthcare, Uppsala, SWEDEN

As compared to traditional buffer formulation procedures and simple dilution of buffer concentrates, buffer formulation for process scale chromatography and filtration can be done more effectively using the concept of In-line Conditioning. This method where buffer is formulated in line from stock concentrates is advantageous in decreasing cost, use of time, labor, space and increasing flexibility. In-line Conditioning pertains to and has been implemented for large scale chromatography systems and is even of interest for the large scale column perspective since its design may probably allow more controllable variability of buffer fronts inside a column. However, it is important to know the limitations of the formulated buffer in terms of error bars of the pH values. At the high concentration edge solubility, cost, and ionic strength may impose the limits whereas at low concentration the low buffer capacity is the most common limiting factor. For the pumps low flow rate of the concentrated stock solutions is necessary for high dilution factors but the error of the pumps increases as flow rate decreases under the specification level. In this presentation calculations of the dynamic range of the pH of the formulated buffer and comparison to process scale buffer formulation data will be shown.

P-208 Tailored Surfaces for Affinity Selection of Human Cells. Tina Sutar1, Oliver Goodyear2, Ales Podgornik3, Owen Thomas4, Mark Cobbold2, Eirini Theodosiou1, 1Loughborough University, Leicestershire, UK; 2University of Birmingham Edgbaston, West Midlands, UK; 3COBIK, Solkan, SLOVENIA

Central to the successful deployment of stem cells for tissue and organ replacement in man or for drug discovery and toxicology studies will be the development of new downstream processing technology for cellular products. The barriers to commercial-scale reproducible selection of human therapeutic cells should not be underestimated. At the present time there are no truly affordable, scaleable, cell enrichment devices on the market that are capable of selecting therapeutic cells. The clinical benchmark remains magnetic affinity cell sorting (MACS), a technique invented >30 years ago primarily designed for haematopoietic stem cell transplantation (H SCT). Magnetic methods for cell separation have been researched in the clinic for more than two decades. They are preferred by clinical scientists, easy to implement and have delivered starting results. To many bioprocessors, who see chromatography as the solution to all high-resolution separation problems, this trust in magnetic methods is misplaced. Unfortunately however, most chromatographic studies thus far have employed wholly unsuitable adsorbent materials, and have therefore not surprisingly yielded very poor results cf. MACS. In short, the Bioprocessing community will have a difficult time convincing clinical scientists of the merits of chromatographic methods, unless it is able to provide dramatically improved chromatographic materials, modules and systems specifically tailored for the purification of human therapeutic cells in the clinic.

Important considerations for the design of these are: ♣ the large size, extreme fragility and negligible diffusivity of human cells; ♣ the base matrix material – its biocompatibility, clinical acceptance and form (surface area, mass transfer, shear); and ♣ the functionalized surface – which should engineered both for highly selective adsorption and gentle efficient detachment
in viable form. In this first presentation from a multidisciplinary team composed of bioseparation scientists/engineers, bioconjugation chemists and clinical immunologists, we shall describe some of the unusual approaches we are using to develop generic scaleable selection systems for human therapeutic cells, with a specific emphasis on engineering clinically approved materials for reversible binding. This work is supported by BBSRC-EPSRC Bioprocessing Research Industry Club (BRIC) grant numbers BB/I017143/1 and BB/I017151/1, a Loughborough Development Funded Studentship, and COBIK (Slovenia).

P-209 Countercurrent Tangential Chromatography for Purification of Monoclonal Antibodies. Oleg Shinkazh1, Andrew Zydney2, Boris Napadensky1, Achyuta Teella2, Chromatan Corporation, State College, PA, USA; 2Penn State University, State College, PA, USA

Countercurrent Tangential Chromatography (CTC) is a new column-free purification and capture technology that holds great promise for purification of high-value recombinant proteins like monoclonal antibodies. CTC can provide a scalable, disposable, and continuous unit operation that overcomes many limitations of packed bed chromatography. CTC replaces the stationary phase of a packed column with a moving slurry that is continuously pumped through several cascades of static mixers and hollow fiber membrane modules. Experimental studies were performed purifying serum and monoclonal IgGs directly on the moving slurry. The buffers required for each operation were introduced in a countercurrent direction to the slurry flow, providing significant increases in operational throughput when compared with conventional column performance. CTC provided high-resolution antibody purification at very low pressures (< 10 psi) with excellent protein recovery. In addition, CTC is no longer limited by the constraints of packed columns, allowing the use of smaller chromatography beads with improved binding kinetics and throughput. These results clearly demonstrate the potential of using Countercurrent Tangential Chromatography for low-cost antibody purification.

P-210 Adsorption Behavior of Proteins on Temperature-responsive Resins. Izabela Poplewska, Renata Muca, Wojciech Piatkowski, Dorota Antos, Rzeszow University of Technology, Rzeszow, POLAND

Temperature-responsive polymers have recently gained interest as chromatographic media for purification of proteins under mild and environmental friendly conditions. The mechanism of separations is based on temperature-mediated cycles of adsorption-desorption, wherein a change of temperature is induced to alter adsorption properties of the resin. One of the most studied temperature-responsive polymers is poly(N-isopropylacrylamide) (PNIPA) and its derivatives. In this study different synthesis routes have been used to produce PNIPA based resins for chromatography of proteins. Hydrogels were synthetized in the suspension polymerization in presence of silica gel as a carrier or using the immobilization of the polymerization initiator (4,4'-azobis (4-cyanovaleric acid)) onto the amino functionalized agarose beds [1]. For both cases the influence of reagent concentration, i.e., cross-linking agent (N,N'-methylenebisacrylamide) and ionizing agent (sodium acrylate), on the properties of the resin has been analyzed. The course of the phase transition of the resin as a function of temperature and its influence on the column void volumes have been measured. Moreover, kinetics of the phase transition have been studied and of the resin response to the temperature change was quantified. The adsorption mechanism of the model proteins (lysozyme, lactoferrin, ovalbumin) on the PNIPA resins has been explored. The study indicated that the cycle of binding and elution involved the conformational changes of proteins on the adsorbent surface. Increase of temperature favored the protein unfolding and strong binding to the surface, whereas the temperature reduction – the protein folding and elution. The measurement of the adsorption isotherm at different temperatures showed that NIPA immobilized on the agarose bed exhibited reduced sensitivity to temperature changes compared to that obtained by polymerization in the presence of inert silica, which, however, was counterbalanced by improvement of the mechanical properties. [1] Maharjan P., De Silva K., Hearn Milton T.W., Jackson W. Roy, Woonton Brad. W., J. Chromatogr. A 1216 (2009) 8722

P-211 Macroporous and Nonporous Polymer Beads for Protein Analysis and Purification. Michael Lu, BioChrom Labs Inc., Terre Haute, IN, USA

Based on macroporous and nonporous polystyrene-divinylbenzene (PS-DVB) beads, three media separate proteins under analytical and large-scale preparative conditions. Macroporous PS-DVB beads separate proteins in reversed-phase mode, and two hybrid media based on macroporous and nonporous PS-DVB beads separate proteins in ion-exchange and hydrophobic interaction modes. BioChrom’s highly porous, macroporous PS-DVB beads separate proteins directly in reversed phase mode eluting with organic solvents and water under high-pressure chromatographic conditions (figure 1). With a macropore structure, high porosity and low hydrophobic surface area exclusive to BioChrom, the beads also separate high molecular weight of proteins and enzymes up to 290 kDa (figure 2). Poor peak shape and low recovery in ion-exchange and hydrophobic interaction chromatography eluting with buffer without any organic solvent are two common problems due to partial hydrophobic surface of media in large-scale purifications or in the separation of the strongly hydrophobic proteins such as lysozyme. To fix these problems, BioChrom applied a thick hydrophilic, biocompatible coating to the PS-DVB beads to prevent proteins from penetrating through the hydrophilic coating layer and interacting with the underlying hydrophobic surface of polymer beads. After an additional modification with a biocompatible soft gel, these hydrophilic polymer beads formed the foundation for two hybrid media that combine the speed and resolution of the rigid PS-DVB polymer yet with the high recovery and loading capacity of a biocompatible soft gel. Further immobilization of charged ligands such as quaternary amine produced an ion-exchange media (figures 3 & 4) and immobilization of hydrophobic ligands such as butyl functional groups created a hydrophobic interaction media (figures 5 & 6).
Chiral separation is one of the most challenging areas in separation science. Purity, yield and loading scale are important considerations in preparative chiral chromatography and historical information helps little because enantiomers with similar structure often cannot be separated using the same column and mobile phase conditions. The initial step is to obtain a good chromatographic method in analytical scale with high resolution. Once the analytical method is selected, the preparative or semi-preparative separation of enantiomers can be conducted with appropriate column size and flow rate. A systematic study has been completed and a chiral screening platform established in our lab with multiple columns and different mobile phase conditions using column and mobile phase switch equipment. For better efficiency, two automated HPLC systems were set up to handle different mobile phases, one for the normal phase mode and the other for reversed phase and polar organic phase modes. Pharmaceutical enantiomers were injected on the automated screening system to select the most favorable method. Over 90% of the enantiomers could be at least partially separated through the initial screening with either automatic system. Further optimization was conducted when needed. Examples will be presented where different method conditions have to be applied for the chiral separation of structurally similar compounds. In addition to columns and mobile phases, the additives and temperature are the important parameters in chiral separation and detailed results will be presented for the enhancement of resolution using additives and temperature. In case of limited separation, enantiomer purity (i.e. 95% EE) has to be achieved with the compromise of load or yield and examples will be provided for the selection of the triggering threshold for fraction collection.

Acidic and basic mobile phases have found widespread applications in the reversed-phase C18 HPLC separation of many important pharmaceutical, bio-analytical, forensic, food, and environmental compounds. Acidic and basic analytes often show peak shape, retention and selectivity changes when the mobile-phase pH is changed from neutral to either extremely acidic (pH=1.0) or basic (pH=11.0). In fact, by using appropriate pH conditions, one can obtain less tailing, better retention and resolution for the target compound. This is obtained by increasing retention and altering selectivity. However, by using silica-based C18 as stationary phase, there are some major limitations: low pH (less than 2) hydrolyses the C18 chains on the phase, while high pH dissolves the silica gel itself. Basically, the optimum pH range is 2.0 to 9.0 for all standards HPLC columns using C18 reversed phase chemistry. Recently, some hybrid packings have been developed to reinforce stability of the particle at a wider pH range, but this material is made from a chemical reaction of silica gel with a polymer giving an hybrid particle which is less efficient than pure silica-based stationary phases. In this poster, a complete study is presented in terms of stability of the SiliaChrom SB C18 and SiliaChrom XT C18 at low pH, like pH=1.0 using TFA. And also in aggressive alkaline conditions (pH=11.0) with a phosphate buffer at different salt concentrations. In order to see the impact of phosphate ions, another study has been made, without phosphate ion at pH=11.0, using an organic buffer (triethylamine). Finally, to fully characterize these new stationary phases some analyses using BET and SEM were performed to demonstrate the stability of these materials under extremely low and high pH.

Protein purification is the chief bottleneck limiting analysis of a fermentation broth or cell culture supernatant for its product protein's quality attributes. The application of high-throughput, 'microscale' protein purification techniques has the potential to increase the throughput of current purification protocols. Such application, however, poses key challenges that include high analytical material requirements and the need to accommodate samples from different stages of upstream development. This poster discusses work where the affinity capture step of a monoclonal antibody was adapted to fit micro-tip column (Phynexus phytip), resin plate, and ATOLL technology platforms. Microscale methods were developed by optimization of the key technique-specific parameters for each platform, with the goals of maintaining process yield and quality relative to the packed-column process. The formats were then directly compared by purifying a representative cell culture supernatant using both the microscale and conventional techniques. Product analysis using a full analytical battery, including size exclusion chromatography, CE-SDS Page, and glycan analysis, demonstrated that each technique generated product of equivalent purity across a wide range protein loadings. Additional considerations such as throughput, operational complexity, and robustness are also discussed.

The use of neutral modifiers to separate basic compounds in supercritical fluid chromatography (SFC) can result in poor peak shape and resolution. Basic additives, such as dimethylethylamine, can improve chromatography by minimizing the interactions between the compounds and the silanol groups present on the stationary phase. These amines may be difficult to remove post-separation, and a second purification may be needed for amine removal. In this study, dimethylethylamine was added to the sample diluent rather than the modifier in the chiral SFC purification of a proprietary AstraZeneca compound known to retain amine additives. The zwitterionic compound contained a functionalized pyrimidone and other aromatic substituents. We present an example in which the base improved the statistical method is selected, the preparative or semi-preparative separation of many compounds and other applications, however, poses key challenges that include high analytical, forensic, food, and environmental compounds. Acidic and basic analytes often show peak shape, retention and selectivity changes when the mobile-phase pH is changed from neutral to either extremely acidic (pH=1.0) or basic (pH=11.0). In fact, by using appropriate pH conditions, one can obtain less tailing, better retention and resolution for the target compound. This is obtained by increasing retention and altering selectivity. However, by using silica-based C18 as stationary phase, there are some major limitations: low pH (less than 2) hydrolyses the C18 chains on the phase, while high pH dissolves the silica gel itself. Basically, the optimum pH range is 2.0 to 9.0 for all standards HPLC columns using C18 reversed phase chemistry. Recently, some hybrid packings have been developed to reinforce stability of the particle at a wider pH range, but this material is made from a chemical reaction of silica gel with a polymer giving an hybrid particle which is less efficient than pure silica-based stationary phases. In this poster, a complete study is presented in terms of stability of the SiliaChrom SB C18 and SiliaChrom XT C18 at low pH, like pH=1.0 using TFA. And also in aggressive alkaline conditions (pH=11.0) with a phosphate buffer at different salt concentrations. In order to see the impact of phosphate ions, another study has been made, without phosphate ion at pH=11.0, using an organic buffer (triethylamine). Finally, to fully characterize these new stationary phases some analyses using BET and SEM were performed to demonstrate the stability of these materials under extremely low and high pH.
When large biomolecules such as IgM or viruses are purified, group separation with gel filtration is a technique typically used. Advantages using gel filtration are the high purity and the high recoveries that can be achieved, while a drawback is the relatively low productivity. Capto™ Core 700 is a chromatography medium (resin) from GE Healthcare designed with core bead technology. The core bead technology enables double functionality for Capto Core 700 - size exclusion and binding separation in one bead. The benefits of this technology include high loading, high flow rates with short residence times, and robust performance in a wide window of operations, securing high productivity and straightforward process design. Here we show the benefits offered from Capto Core 700 in purification examples of IgM, and influenza virus.

Nowadays, peptide APIs are of growing interest because of the huge potential and efficiency of those drugs to treat diseases. Reversed-phase liquid chromatography is the method of choice for peptide purification. However, the high cost of this process promotes the continuous development of new more efficient technologies. Recently, a strong focus on the development of stationary phases with bimodal chemical surface character has been made. In this presentation, the performance of several doped materials (ion exchange/ reversed phase) will be compared to conventional reversed-phase materials. It will be shown that doped materials display: an increased selectivity between closely eluting impurities and the main component; and an increased yield in preparative scale chromatography. The ease of application (from simple column substitution to small mobile phase modifications) will be discussed. The choice between cation or anion exchange as the second part of the doped material depending on peptide charge (positive or negative) is discussed and a series of industrially relevant examples are shown. In addition, the influence of the modifier and salt concentrations and of the stationary phase on peptide retention is described. A model depicting this influence is developed and briefly discussed.

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Plant leaves offer several advantages for the production of biopharmaceutical proteins, such as absence of human pathogens, low up-stream costs and the potential for large-scale production [1]. However, downstream costs can be high compared to other production platforms such as Chinese hamster ovary cells. This is because complete mechanical homogenization of leaf tissue is most often required for protein extraction resulting in high concentrations of host cell protein (HCP), DNA, secondary metabolites, such as phenolic compounds, an cell debris, including cell wall fragments, in the raw extract [2]. Filtration is frequently used to remove particles from raw extract prior to chromatographic separation steps because disposable technologies are available. Using these technologies eliminates the risk of cross-contamination that is present for multi-use components such as centrifuges. However, filter life time is often limited due to high particle burden in the feed stream and plant phenolic compounds both leading to rapid filter clogging. The severity of this problem can even increase if artificial growth supports such as rockwool are used instead of soil due to safety concerns. Here we present a design of experiments (DoE) approach to identify additives that improve filter life time but do not interfere with protein recovery and/or subsequent chromatographic purification steps. A wide spectrum of buffer pH and conductivities was tested to ensure additive efficacy under a broad range of process conditions and compatibility with various target proteins. The data were compiled into a regression model that was able to predict the additive effect in dependence of its concentration and other process parameters. As a result, filter life time was improved two-fold. Improvements were even better, more than four-fold, in case different additives were combined, thereby cutting the total downstream costs by more than 10%. References. 1. Fischer, R., et al., Plant-based production of biopharmaceuticals. Curr Opin Plant Biol, 2004. 7(2): p. 152-8. 2. Wilken, L.R. and Z.L. Nikolov, Recovery and purification of plant-made recombinant proteins. Biotechnology Advances, 2012. 30(2): p. 419-33.
Viral vectors and viral vaccines are playing an important role in novel pharmaceutical approaches. Although important results within the manufacturing processes have been achieved, several bottlenecks still remain. In particular, downstream operations need improvement to deliver better recovery yields with higher purity grades and within short time. Chromatography processes using membrane adsorbers are widely used for the purification of complex biopharmaceuticals such as viruses. Membrane adsorbers have several advantages: (i) the transport takes place predominately by convection, (ii) the binding is generally independent from the feed flow rate, and therefore high flow rates can be used, which decreases the time for purification, (iii) membrane-chromatography devices are generally easier for scale-up when compared with packed bed. However some issues still remain. In particular, when dealing with complex mixtures where proteins, DNA, and viruses are present, the determination of the multicomponent adsorption equilibrium becomes a challenge, and currently not amenable to precise theoretical treatment. Approaches such as the ideal adsorbed solution theory suitable for small molecules often fail for protein and viruses, due to their large size and complex interactions. Chromatography methods, including frontal analysis, isocratic elution and linear gradients, are often used to determine adsorption equilibria. Compared to small molecules, the determination of adsorption equilibrium for viruses and related impurities is complicated by the fact that broad bands are obtained due to diffusion limitations; on the other hand isocratic is very difficult to implement because of the extreme sensitivity of protein adsorption equilibrium to mobile phase composition. Gradient elution alleviates this difficulty to some extent. In the presented work, we implemented the Steric Mass Action (SMA) formalism to analyze the separation performance of an adenovirus bioreactor bulk on an ion-exchange membrane adsorbent by using linear gradient elution. For practical purposes, and due to the extreme complexity of the sample, which can reach up to several thousand components, the solutes were grouped based on their affinity to the surface. Therefore the description of our system can be reduced to a three component-system. Retention factors as a function of the mobile phase composition from linear gradient elution experiments were determined. This study provided important quantitative adsorption data for predicting the performance of anion-exchange chromatography of a very heterogeneous and multi-component system. The retention factors obtained will be used for design and implementing a simulating moving-bed based on membrane chromatography.

Development of an Improved Separation to Enable the High Throughput Purification of 1000 Chemically Modified Cyclosporins. Jack Thornquest, Preston Absher, Thomas Bullock, James Montgomery, Clare Murray, Scynexis, Inc., RTP, NC, USA

Cyclosporins are cyclic undecapeptides that exhibit a broad spectrum of biological activities. Cyclosporin A (CsA) is a powerful immunosuppressant and is the active ingredient of Sandimmun® and Neoral® for preventing organ transplant rejection, as well as Restatis® for treating the autoimmune disease dry eye. Besides immunosuppression, Cyclosporin A has activity in HIV, ischemia, muscular dystrophy, cancer, alopecia, and HCV. CsA exerts its activity by binding to two proteins sequentially to form a ternary complex. The first of these is a cyclophilin (Cyp), which is a cis-trans prolyl isomerase. The CsA-Cyp complex then binds to calcineurin (CN), a calcium-dependent serine/threonine phosphatase that promotes the synthesis of T cell lymphokines. CsA use in many indications is limited by immunosuppression, side effects, and unpredictable pharmacokinetics. Modification of selective residues alters the binding of a CsA analog to cyclophilin and/or calcineurin. It is possible to make modifications to CsA that enhance antiviral activity and reduce off-target activity. High performance liquid chromatography (HPLC) analysis of CsA and its derivatives can prove challenging. Their physiochemical properties often result in broad chromatographic peak shape which reduces the peak capacity of the separation, can mask impurities, and reduce the efficiency of fraction collection. The use of tetrahydrofuran (THF) as part of a reversed phase gradient has been shown to significantly improve the peak shape. An existing purification process based on the integration of LC-MS and reverse phase HPLC has been in use to purify >500,000 traditional small molecule compounds over ten years of operation. This system was utilized with novel chromatographic methodology incorporated a cyanogen stationary phase and a water/acetonitrile/THF mobile phase to deliver CsA derivatives with high purity, good recovery and rapid throughput. Additionally, our proprietary software, HEOS®, was used to track the samples from medicinal chemistry submission to delivery of final, isolated compound. These tools were used to create a library of over 1,000 cyclosporin derivatives, synthetically modified in regions of the molecule interacting with the binding domains of CypA and CN.
The demand for high purity peptides is increasing. Small synthetic peptides to large cellular produced peptides are being investigated for possible therapeutic benefits. Both can be difficult to purify to high levels, >98%, because of the very similar products, many times differing by only one amino acid. Optimized purification techniques are required to meet these high purity demands in an economical manner. Reversed-phase chromatography, because of its high resolving power, has been the technique of choice for achieving the high level of purity necessary in the pharmaceutical industry. For industrial purification, important consideration and selection of particle size, pore size, and stationary phase in relation to the peptide can optimize purification. We illustrate how the new Vydac® 150Å reversed-phase media is highly effective at purifying peptides with greater loading capacity and improved productivity compared to competitive media. The media has unique selectivity that can reveal peaks masked by other C18 phases and improves resolution of closely related peptides and impurities for higher purity target peptides. The bulk media incorporates bonded phase chemistries identical to those used in analytical and prep columns, thereby assuring economical method development and reliable scale-up for preparative and process purification. Media packed in dynamic axial compression MODcol® Spring® columns demonstrate high efficiency and extended lifetime.

Chromalite® PCG adsorbents and IEX resins are part of a range of Chromalite® products produced for Analytical and Preparative Chromatography. Here we will present some key applications of Chromalite® PCG1200, which is a rigid, highly porous highly crosslinked macroporous copolymer with a divinylbenzene matrix in microbeaded form. This product is optimal for hydrophobic interaction chromatography (HIC). The spherical porous microbeads show exceptional chemical and operational stability to extreme pH (1-14) and high pressures (up to 280 bar). The macroporosity ensures fast and efficient adsorption and separation of different kind of biomolecules as protein and peptides. Experience in microbead manufacture has provided a product characterised by quality, reproducibility and scalability, features that are particularly important for industrial applications where there are strict regulatory demands. Applications in insuling polishing and antibiotics purification will be presented.

Mobile phase gradients have been used in LC since the 1950s and are still an important programming technique in both analytical and preparative LC. By changing the elution strength of the solvent during the separation, substances with a wide range of retention factors can be separated much more effectively in gradient elution compared to isocratic elution. In overloaded chromatography there is also a peak sharpening effect when gradient elution is used due to the fact that the backside of the peak moves faster than the front. The purpose of this study was to determine the adsorption isotherms for the solutes in a binary mixture as a function of both solute and modifier concentration and use them to solve the Equilibrium-Dispersive model in order to predict band profiles under gradient elution. The modeling of band profiles and the optimization of preparative separations has largely been studied under isocratic conditions. When gradient elution actually has been taken into account, single component isotherms have, almost exclusively, been studied. The shape of the gradient and the behavior of the solutes are therefore, in practice, often determined experimentally with a “trial and error” approach which can be both time consuming and expensive. Recent studies have shown that a significant improvement in productivity can be achieved if the gradient shapes are designed carefully [1, 2]. By using computer simulations, different gradient shapes and other parameters such as the injection volume could be optimized without any additional experiments. The investigated substances were cyclohexanone and cycloheptanone, while the solvent consisted of methanol and water and the column was a Kromasil C18-column. The adsorption isotherms were determined at six modifier plateaus with the perturbation pulse method and the Equilibrium-Dispersive model was solved with orthogonal collocation on finite elements. We found that the modifier dependent competitive adsorption isotherms could be determined from their single component counterparts and that the Linear Solvent Strength Theory was adequate to describe the modifier dependence. Good agreement between simulated and experimental band profiles was observed. A contribution from the Fundamental Separation Science Group www.separationscience.se. References: [1] A. Damtew, B. Sreedhar, A. Seidel, Science Group Chem. A 1216 (2009) 5355. [2] Y. Shan, A. Seidel-Morgenstern, J. Chromatogr. A 1093 (2005) 47.

Chromatography methods have a wide application to purify organic compounds in many industrial fields. Silica gel has been used for the stationary phase in Normal-phase liquid chromatography (NPLC). Acidic compounds such as carboxylic acids and phenols exist as various ionic dissociations in mobile phase. For the reason of various ionic dissociations, the acidic compounds have some difficulties in the NPLC separation. Usually the addition of acid such as acetic acid and phosphoric acid to the eluent improves the separation to restrain ionic dissociations. However the separation method needs a process of removal for the acid component and it is a complicated process. In this study, silica gel was modified by a carboxyl group (COOH Silica) or sulfo group (SO3H Silica) on the surface and used the separation of acidic compounds. The modified silica gels were inhibited from ionic dissociations and provided efficient separation of acidic compounds. The separation is simplified and useful by using such modified silica gel. This study attempts to develop the separation workflow of acidic compounds by using COOH Silica and SO3H Silica.
Genotoxic impurities in drugs is a serious issue for the pharmaceutical industry. Therefore removal of these impurities is a crucial task during the active pharmaceutical ingredient (API) production process. Many APIs under development today are produced by multi step processes requiring extensive downstream purification techniques such as crystallization. Such processes include several steps which often result in loss of API and therefore increase the total cost of final product. API purification can also be accomplished via chromatographic or other methods using adsorbents packed in columns.(1-2)

Molecularly imprinted polymers (MIPS) are one type of selective adsorbents and are often termed artificial antibodies. This is due to the commonly used description in the scientific literature that MIPs are highly selective and can discriminate between target molecules with closely related structures and/or functionalities. Whilst working with MIPs, we have experienced that many MIPs exhibit a much broader selectivity profile than previously anticipated. A key element of this discovery is the observation that molecules with chemical and structural similarity to the template molecule are also able to bind selectively to the MIP. (3) In this study, we conducted screening experiments using MIP resins from our library to identify resins with the desired selectivity towards genotoxic aminopyridines. The screening process involves passing a solution of the analyte through SPE columns on a 96-well plate and analyzing the eluates on LC-MS and measuring the percentage of bound analyte. The background of the theory, the outcome and conclusions of our experimental efforts are outlined in this presentation. References: 1. Rouqueol, F.; Rouquerol, J.; Sing, K. Adsorption by Powders and Porous Solids: Principles, Methodology and Applications; Academic Press: New York, 1999. 2. Lee, C.; Helmy, R.; Strulson, C, Org. Process Res. Dev., 14 (4), 1021, 2010. 3. C. Widstrand, E. Yilmaz, B. Boyd, J. Billing and A. Rees, American Laboratory News, October, 2006.
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