# SPICA 2008

ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

12th International
Symposium on
Preparative and
Industrial
Chromatography and
Allied Techniques

# Book of Abstracts

September 28 to October 1, 2008
ETH Zurich, Switzerland

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# Proceedings

We thank ELSEVIER for publishing the proceedings of SPICA 2008 in the Journal of Chromatography A and for their kind support of the event.

# Index

# Scientific Program

## *Sunday, September 28, 2008*

13:00 – 20:00 Registration

### Workshops 1, 2 & 3

14:00 – 15:30 ***Preparative HPLC. Phase Screening, Method Optimization and Process Development,***
**M. Schulte, Merck KGaA, Darmstadt, Germany**

***Toolbox to Understand Simulated Moving Beds***
**M. Mazzotti[1] and A. Rajendran[2], ETH Zurich, Switzerland[1], Nanyang Technological University, Singapore[2]**

***Supercritical Fluid Chromatography***
**O. Ludemann – Hombourger[1], G. Terfloth[2], Novasep Process, Pompey, France[1], GlaxoSmithKline, King of Prussia, USA[2]**

15:30 – 16.00 Coffee Break

### Workshops 4, 5 & 6

16:00 – 17:30 ***From Leads to Production – How Chromatography is Scaled Up***
**J. Strube[1], R. Ditz[2], S. Boecker[3], Clausthal University of Technology, Germany[1], Merck KGaA, Darmstadt, Germany[2], Bayer Technology Services GmbH, Leverkusen, Germany[3]**

***Bioseparation: Removal of Adventitious Agents, DNA and Endotoxins***
**A. Jungbauer, University of Natural Resources and Applied Life Sciences, Vienna, Austria**

***Continuous Chromatography of Biomolecules***
**M. Morbidelli[1], L. Aumann[1,2], G. Stroehlein[1,2], T. Mueller – Spaeth[1], ETH Zurich[1], Switzerland, ChromaCon AG, Zürich, Switzerland[2]**

17:30 – 18:00 Break

### Welcome Reception

18:00 – 20:00 Dozentenfoyer, ETH Zurich

## *Monday, September 29, 2008*

08:15 – 09:15 Registration

09:15 – 09:30 Opening Address

### Session – Materials I

09.30 – 10:00 (0126) **W. Lindner, University of Vienna, Austria**

***Keynote Lecture:*** *Chromatographic Selectivity as a Key Parameter to be Adjusted via the Stationary and Mobile Phases*

10:00 – 10:15 (1117) **E. Francotte, P. Richert, G. Diehl, D. Huynh and J. Priess, Novartis, Basel, Switzerland**

*A New Generation of Powerful Chiral Stationary Phases for HPLC, SFC, and SMB*

10:15 – 10:30 (1012) **S. R. Mukai, S. Murata, K. Onodera, A. Eguchi, Y. Yao and T. Masuda, Hokkaido University, Sapporo, Japan**

*Synthesis of Monolithic Columns Equipped with a Hierarchical Pore System of Micro/Mesopores and Macropores Using the Ice Template Method*

10:30 – 11:00 Coffee Break

### Session – Solid-Solute Interactions

11:00 – 11:30 (0142) **B. Sellergren, University of Dortmund, Germany**

***Keynote Lecture:*** *Affinity-based Separations Using Imprinted Polymers*

11:30 – 11:45 (1134) **C. Frech[1], E. Todorova[1], H. Graalfs[2], A. Schwämmle[2] and M. Jöhnck[2], Hochschule Mannheim - University of Applied Sciences, Germany[1], Merck KGaA, Darmstadt, Germany[2]**

*Direct Capture of Antibodies from Fermentation Broth Using a New Multi-modal High Salt Binding Ion Exchange Material*

11:45 – 12:00 (1081) **T. Tarman and A. Jungbauer, University of Natural Resources and Applied Life Sciences, Vienna, Austria**

*Adsorption Properties of DNA on Charged Surfaces*

12:00 – 12:15 (1087) **A.M. Liddy, G. Wynn-Jones, E. Theodosiou and O. R. T. Thomas, The University of Birmingham, Edgbaston, United Kingdom**

*Bilayered SEC-IEC adsorbents for improved separation of bioparticulate nanoplex products*

12:15 – 12:30 (1061) **M. Salvalaglio, V. Busini, L. Zamolo and C. Cavallotti, Politecnico di Milano, Italy**

*Molecular Dynamic Investigation of the Interaction of Supported Affinity Ligands with Monoclonal Antibodies*

12:30 – 14:00 Lunch Break

## Session – Materials II

14:00 – 14:30 (0033) **S. Böcker and M. Lohrmann, Bayer Technology Services GmbH, Leverkusen, Germany**

***Keynote Lecture:*** *Challenges in Packing of Process Columns*

14:30 – 14:45 (1036) **E. Ndocko[1], R. Ditz[2] and J. Strube[3], Bayer Technology Services GmbH, Leverkusen, Germany[1], Merck KGaA, Darmstadt, Germany[2], Clausthal University of Technology, Germany[3]**

*Material Design Strategy for Chromatographic Separation Steps in Bio-Recovery Downstream Processing*

14:45 – 15:00 (1047) **F. Oehme, K. Kaiser, J. Lenz and J. Peters, Bayer HealthCare AG, Wuppertal, Germany**

*"Mixed-mode" Chromatography Materials in Downstream Process Development*

15:00 – 15:15 (1089) **J. Souquet[1], H. Liu[1], J. Liu[1], X. Fan[1], J. Liddell[2], P. Levison[3], E. Theodosiou[1] and O. R. T. Thomas[1], The University of Birmingham, United Kingdom[1], Avecia Biologics Ltd, Billingham, United Kingdom [2], Pall Europe Ltd, Portsmouth, United Kingdom [3]**

*Tracking the Movement of Individual Adsorbent Particles in Expanded Beds*

15:15 – 15:30 (1075) **E. Yilmaz, C. Widstrand, A.-K. Wihlborg and A. Rees, MIP Technologies AB, Lund, Sweden**

*Facilitated Extractions with Selective Resins - the Use of MIPs in Separations and Analysis*

15:30 – 16:00 Coffee Break

## Session – Processes I

16:00 – 16:30 (0145**) J.B. Lenfers and M. Lögers, Bayer Schering Pharma, Wuppertal, Germany**

***Keynote Lecture:*** *Present and Future Technology Trends in API Development*

16:30 – 16:45 (1131) **N. Mavroudis[1], P. Bongers[1], J. Koek[1], S. Dubbelman[1] and M. Mazzotti[2], Unilever Food and Health Research Institute, Vlaardingen, the Netherlands[1], ETH Zurich, Switzerland[2]**

*Retention of Amino-Acids in Ion Exchange/Exclusion Chromatography*

16:45 – 17:00 (1039) **C. Wenda, A. Rajendran and M. Amanullah, Nanyang Technological University, Singapore**

*Optimized Preparative Supercritical Fluid Chromatography: Enantioseparation of Flurbiprofen*

17:00 – 17:15 (1022) **U. Emde, M. Dietz, H. Reubold and H. Pflug, Merck KGaA, Darmstadt, Germany**

*Sample Solubility - Important Parameter for Successful Preparative Chiral Chromatography*

17:15 – 17:30 (1016) **J. Priess and E. Francotte, Novartis, Basel, Switzerland**

*A Versatile Solubility Screening Platform for Preparative Chromatography: Methodology and Applications*

17:30 – 17:45 (1071) **T. Fornstedt[1], P. Forssén[1], R. Arnell[2], M. Kaspereit[3] and A. Seidel-Morgenstern[3, 4], Uppsala University, Sweden[1], AstraZeneca AB, Södertälje, Sweden[2], Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany[3], Otto-von-Guericke-University, Magdeburg, Germany[4]**

*Utilisation of Peak Shape Tuning to Optimize Preparative Batch Chromatography*

17:45 – 18:00 (1018) **Kramarz , W. Piatkowski and D. Antos, Rzeszów University of Technology, Poland**

*Altering Efficiency of Hydrophobic Interaction Chromatography by Combined Salt and Temperature Effects*

## Poster Session I

18:00 – 20:00 Poster Presentations

(2002) **N. Bian[1], N. Soice[1], C. Wang[1], K. S. Cheng[1], S. Ramaswamy[1], and K. Beyzavi[2], Millipore Corporation, Bedford, USA[1], Millipore Corporation, Conssett, United Kingdom[2]**

*Resin Optimization for Affinity Chromatography Media*

(2008) **R. M. Dias and C. C. Santana, State University of Campinas, Brazil**

*Chiral Chromatographic Separation of o,p'-Dichlorodiphenyldichloroethane (Mitotane) in Semi-preparative Columns of o,o'-bis[4-Tert-butylbenzoyl]-n,n'-diallyl-l-tartadiamide and Separation Regions for a Simulated Moving Bed*

(2013) **K. Hallman, B. Kofoed-Hansen and D. Börjesson, Eka Chemicals AB, Bohus, Sweden**

*Important Aspects when Designing Chiral Preparative Separations Using Coated Polysaccharide Stationary Phases*

(2019) **J. Nowak[1], I. Poplewska[2], D. Antos[2] and A. Seidel-Morgenstern[1,3], Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany[1], Rzeszów University of Technology, Poland[2], Otto-von-Guericke-University, Magdeburg, Germany[3]**

*Adsorption Behavior of Sugars versus their Activity in Single and Multicomponent Liquid Solutions*

(2020) **F. Torrens[1] and G. Castellano[2], Universitat de València, Spain[1], Universidad Católica de Valencia San Vicente Mártir, València, Spain[2]**

*Binding of Water-Soluble, Globular Proteins to Anionic Model Membranes*

(2023) **T. Keller[1], N. Forrer[2] and L. Jelinek[1], Zeochem AG, Uetikon, Switzerland[1], ETH Zurich, Switzerland[2]**

*A New Silica Gel from Zeochem AG for Preparative HPLC: ZEOsphere*

(2027) **M. M. H. El-Sayed and H. A. Chase, University of Cambridge, United Kingdom**

*Cation-exchange Adsorption of the two Major Whey Proteins*

(2029) **J. Li and H. A. Chase, University of Cambridge, United Kingdom**

*Characterization and Evaluation of Amberlite XAD7HP for Use in the Expanded Bed Adsorption of Flavonoids*

(2032) **V. Brochier, T. Robin, V. Ravault and A. Schapman, Pall BioSepra, Cergy, France**

*Lab-scale Optimization and Scale-up of two Purification Steps Using a Mixed-mode and a Novel Ion Exchange Sorbent*

(2034) **K. Morishita[1] ,T. Matsutomi[1], J. Kadoya[1] and G. Krautz[2], Daiso Co., Ltd., Osaka, Japan[1], Daiso Fine Chem GmbH, Düsseldorf, Germany[2]**

*New Process Scale Stationary Phase for Peptide Purification*

(2040) **C. Wenda and A. Rajendran, Nanyang Technological University, Singapore**

*Supercritical Fluid Chromatography Enantioseparation of Flurbiprofen: Determination of Non-linear Isotherms*

(2042) **C. Boi[1], S. Dimartino[1], G. C. Sarti[1], S. Hofer[2] and W. Lindner[2], Università di Bologna, Italy[1], University of Vienna, Austria[2]**

*Influence of Different Spacer Arms on A2P Affinity Membranes for Human IgG Capture*

(2044) **L. Joron [1], J. Fisher[2], A. Gehris[2] and J. Maikner[2], Rohm and Haas-Advanced Biosciences, Chauny, France[1], Rohm and Haas–Advanced Biosciences, Philadelphia, USA[2]**

*An Economical Approach to Upstream Decolorization*

(2046) **L. Joron [1], J. Fisher[2], A. Gehris[2] and J. Maikner[2], Rohm and Haas-Advanced Biosciences, Chauny, France[1], Rohm and Haas–Advanced Biosciences, Philadelphia, USA[2]**

*Rapid Removal of Detergents from Protein Solutions Using Polymeric Reversed Phase Resins*

(2051) **S. Katsuo, O. Kartachova and M. Mazzotti, ETH Zurich, Switzerland**

*Measurement of Adsorption Isotherms of Tröger's Base Enantiomers for Chromatography-Crystallization Combined Process Design*

(2052) **K. Wrzosek, M. Gramblicka and M. Polakovic, Slovak University of Technology, Bratislava, Slovakia**

*Equilibrium and Kinetics of IgG Adsorption on a Strong Cation-exchanger*

(2053) **I. Tatárová[1], M. Polakovic[1] and R. Fáber[2], Slovak University of Technology, Bratislava, Slovakia[1], Sartorius Stedim Biotech, Göttingen, Germany[2]**

*Investigation of Bimodal Pore Structure of Sartobind Q Membrane Adsorbent Using Different Experimental Techniques*

(2067) **R. Marti[1], S. Höck[1] and M. Juza[2], ZHAW Zürcher Hochschule für Angewandte Wissenschaften, Winterthur, Switzerland[1], Siegfried Ltd., Zofingen, Switzerland[2]**

*Chromatographic Purification of Reference Standards – a Case Study*

(2068) **H. Gumm[1], M. Hamdan[2] and W.-R. Jueterbock[1], Sepiatec GmbH, Berlin, Germany[1], GlaxoSmithKline SpA, Verona, Italy[2]**

*Implementation of a New Strategy for Rapid Enantiomer Screening*

(2078) **K. Vanková, M. Gramblicka and M. Polakovic, Slovak University of Technology, Bratislava, Slovakia**

*Adsorption Equilibria of Fructose, Glucose, Sucrose and Fructooligosacharides on a Cation-exchange Resin*

(2079) **A. Damtew[1] and A. Seidel-Morgenstern[1,2], Otto-von-Guericke-University Magdeburg, Germany[1], Max Planck Institute Dynamics of Complex Technical Systems, Magdeburg, Germany[2]**

*Analysis of the Optimal Shape of Gradients for Systems Characterized by Complex Adsorption Isotherms*

(2083) **P. Dimitrova and H-J. Bart, TU Kaiserslautern, Germany**

*Modified Ligand Exchange Chromatography for Amino Acids Separation*

(2084) **M. A. Snyder, Bio-Rad Laboratories, Hercules, USA**

*Rapid, Efficient Packing Methodologies for Ceramic Hydroxyapatite*

(2085) **L. J. Cummings, Bio-Rad Laboratories, Hercules, USA**

*Managing pH Excursions in Hydroxyapatite Columns Associated with Changes in NaCl Concentration Mobile Phase Concentration*

(2088) **H. Liu, E. Theodosiou and O. R. T. Thomas, The University of Birmingham, Edgbaston, United Kingdom**

*Chromatographic Materials Displaying "Smart" New Functions".*

(2091) **J. Horak and W. Lindner, University of Vienna, Austria**

*Introduction of a Fast and Widely Applicable Analytical Screening Method for the Design of New Materials for Antibody Purification*

(2092) **H. St-Laurent, L. Tremblay, P. Couture, G. Gingras and F. Béland, SiliCycle Inc., Quebec, Canada**

*Novel Fluorous Phase for the Separation of Organic Compounds*

(2093) **A. Rajendran, Nanyang Technological University, Singapore**

*Direct Estimation of Competitive Langmuir Isotherm Parameters from Overlapping Bands of Binary Injections*

(2098) **A. Tarafder and M. Morbidelli, ETH Zurich, Switzerland**

*Effect of Multiple Acidic Buffers on the Retention of Ionogenic Molecules*

(2099) **B. Bloedorn[2], M. Omote[1], K. Morishita[1], N. Shoji[1], and N. Kuriyama[1], YMC Co. Ltd., Ishikawa, Japan[1], YMC Europe GmbH, Dinslaken, Germany[2]**

*Newly Developed High Strength and Chemically Stable Silica Gel Based Preparative Reversed Phase Packing Materials*

(2102) **B. Bloedorn[2], M. Omote[1], K. Morishita[1], N. Shoji[1], and N. Kuriyama[1], YMC Co. Ltd., Ishikawa, Japan[1], YMC Europe GmbH, Dinslaken, Germany[2]**

*New Generation of Semi Prep column by YMC*

(2112) **A. Törncrona, J. Ekeroth, B. Kofoed-Hansen and S. Winkel-Pettersson, Eka Chemicals AB, Bohus, Sweden**

*Optimization of Silica Based RPLC Materials for Peptide Purification*

(2114) **J. N. Kinkel[1], A. Zucker[2] and P. Koeppel[1], Ohm-University of Applied Sciences, Nürnberg, Germany[1], Biontis GmbH, Geesthacht, Germany[2]**

*New Polar Phases for Preparative HILLIC Applications*

(2116) **B. Gutiérrez[1,2] and C. Minguillón[1,2], Institute for Research in Biomedicine, Barcelona, Spain[1], University of Barcelona, Spain[2]**

*Enantioselective Membranes Containing L-Proline-derived Chiral Carriers*

(2118) **L. Charles and T. Kaiser, Fuji Silysia Chemical S.A., Le Mont-sur-Lausanne, Switzerland**

*Orlistat – a Successful Approach to an Efficient Preparative Liquid Chromatography Purification Method*

(2120) **J. Saar[1], R. T. Nguyen[2] and B. Denoulet[3], Grace Davison Discovery Sciences, Worms, Germany[1], Grace Davison Discovery Sciences, Hesperia, USA[2], Grace Davison Discovery Sciences, Lokeren, Belgium[3]**

*Optimize the Performance of Denali RP Media for Preparative Purification by Using MODcol SpringColumn and MultiPacker Technology*

(2122) **J. Saar[1], R. T. Nguyen[2], S. Anderson[3], W. Luo[3] and B. Denoulet[4], Grace Davison Discovery Sciences, Worms, Germany[1], Grace Davison Discovery Sciences, Hesperia, USA[2], Grace Davison Discovery Sciences, Deerfield, USA[3], Grace Davison Discovery Sciences, Lokeren, Belgium[4]**

*Vydac MS RP-HPLC Columns Provide Unique Selectivity and High Recovery for Peptide and Protein Separations*

(2124) **A. Sousa[1], F. Sousa[1], D. M. F. Prazeres[2] and J. A. Queiroz[1], Universidade da Beira Interior,**

**Covilhã, Portugal[1], Instituto Superior Técnico, Lisboa, Portugal[2]**

*Histidine Affinity Chromatography of Homo-oligonucleotides: The Role of Multiple Interactions on Retention*

(2137) **J. Priess, C. Valente, G. Diehl and E. Francotte, Novartis Institutes for Biomedical Research, Basel, Switzerland**

*Evaluation of Chiral Stationary Phase Packed AXIA HPLC Columns*

## *Tuesday, September 30, 2008*

### Young Scientist Buffet Breakfast

07:00 – 08:15 (0149) **G. J. Terfloth, GlaxoSmithKline, King of Prussia, USA**

*How to Work in Industry*

### Session – Continuous Chromatography I

08:30 – 09:00 (0141) **O. Werbitzky, U. Altenhöhner and F. Quattrini, Lonza, Visp, Switzerland,**

***Keynote Lecture:*** *Large Column Operation, Challenges in the Downstream Processing of APIs*

09:00 – 09:15 (1050) **S. Katsuo and M. Mazzotti, ETH Zurich, Switzerland**

*Comparison of Performance between Improved and Conventional Simulated Moving Bed (SMB) Process*

09:15 – 09:30 (1054) **M. Kaspereit[1], R. Arnell[2], P. Forssén[3], A. Seidel-Morgenstern[1,4], T. Fornstedt[3] and A. Kienle[1,4], Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany[1], AstraZeneca AB, Södertälje, Sweden[2], Uppsala University, Sweden[3], Otto-von-Guericke-University, Magdeburg, Germany[4]**

*Theoretical Analysis of Continuous Chromatography with Adsorbing Additives*

09:30 – 09:45 (1049) **J. M. M. Araújo, R. R. C. R. Rodrigues and J. P. B. Mota, Universidade Nova de Lisboa, Portugal**

*Automated Iterative Refinement of Adsorption Isotherm Models for Simulated Moving Bed Operation Based on Cyclic Steady State Data*

09:45 – 10:00 (1060) **E. Valery, W. Majeswsky and C. Morey, Novasep SAS, Pompey, France**

*Making Multi Columns Chromatography Simpler and Faster*

10:00 – 10:15 (1065) **P. Scherpian, D. Schlinge and G. Schembecker, Technische Universität Dortmund, Germany**

*Design Method for Closed-Loop and Steady-State Recycling Chromatography*

10:15 – 10:45 Coffee Break

## Session – Processes II

10:45 – 11:15 (0144) **W. Kuhne, Roche Diagnostics GmbH, Penzberg, Germany**

***Keynote Lecture:*** *New Perspectives in the Downstream Processing of Large Molecules*

11:15 – 11:30 (1119) **T. M. Pabst[1], G. Carta[1], A. Hunter[2] and N. Ramasubramanyan[2], University of Virginia, Charlottesville, USA[1], Pfizer Inc., St. Louis, USA[2]**

*Advances in Chromatofocusing with Unretained Buffers for Challenging Preparative Protein Separations*

11:30 – 11:45 (1004) **B. K. Nfor[1], T. Ahamed[1], G. W. K. van Dedem[1], L. A. M. van der Wielen[1], E. J. A. X. van de Sandt[2], M. H. M. Eppink[3] and M. Ottens[1], Delft University of Technology, the Netherlands[1], DSM Anti-Infectives B.V., Delft, the Netherlands[2], Biotechnology Operations N.V. Oss, the Netherlands[3]**

*Modeling-based Rational Protein Purification Process Synthesis Methodology*

11:45 – 12:00 (1057) **P. A. J. Rosa, A. M. Azevedo, I. F. Ferreira and M. R. Aires-Barros, CEBQ Instituto Superior Técnico, Lisbon, Portugal**

*Optimisation of Affinity-enhanced Purification of Antibodies Using Aqueous Two-Phase Extraction*

12:00 – 12:15 (1127) **P. Wood[1], L. Janaway[1], P. Hewitson[2], S. Ignatova[2] and I. Sutherland[2] , Dynamic Extractions Ltd, Berkshire, United Kingdom[1], Brunel University, Uxbridge, United Kingdom[2]**

*An 18 Litre Process Scale High Performance Counter-current Chromatography Centrifuge*

12:15 – 12:30 (1135) **K. Kaczmarski[1] and G. Guiochon[2,3], Rzeszów University of Technology, Poland[1], The University of Tennessee, Knoxville, USA[2], Oak Ridge National Laboratory, USA[3]**

*Preparative Extraction by Chromatography of Organic Pollutants from Water Streams*

12:30 – 14:00 Lunch Break

## Session – Membranes

14:00 – 14:30 (0146) **G. Belfort, Rensselaer Polytechnic Institute, Troy, USA**

***Keynote Lecture:*** *Synthetic Membrane Filtration: Breakthroughs and Needs*

14:30 – 14:45 (1003) **M. Kreuß and U. Kulozik, Technische Universität München, Freising-Weihenstephan, Germany**

*Development of a Preparative Ion Exchange Process for the Separation of Individual Fractions of Caseinomacropeptide Using Membrane Adsorption Chromatography*

14:45 – 15:00 (1066) **P. van Beijeren, D. Plassmann and P. Kreis, Technische Universität Dortmund, Germany**

*IEX Membrane Adsorbers for Capturing of Proteins: Model Development, Model Validation and Scale up*

15:00 – 15:15 (1041) **C. Boi[1], V. Busini[2], C. Cavallotti[2] and G. C. Sarti[1], Università di Bologna, Italy[1], Politecnico di Milano, Italy[2]**

*Understanding Ligand-Protein Interactions in Affinity Membrane Chromatography for Antibody Purification*

15:15 – 15:30 (1125) **F. Sousa[1], D. M. F. Prazeres[2], J. A. Queiroz[1], Universidade da Beira Interior, Covilhã, Portugal[1], Instituto Superior Técnico, Lisboa, Portugal[2]**

*A New Amino Acid-based Affinity Chromatography Approach to Supercoiled Plasmid DNA Purification on Arginine-agarose*

15:30 – 16:00 Coffee Break

## Special Symposium

**"Present and Future of Chromatography Purification", Chair: A. Jungbauer**

16:00 – 18:00 (0143) **K. Larson, AstraZeneca AB, Södertälje, Sweden**

*The Compination of SMB Chromatography, Slurry Wash and Batch Chromatography for the Resolution and Purification of a Racemate*

(0140) **E. Freund, CarboGen-Amcis, Aarau, Switzerland**

*SMB: A Flexible and Quick Tool for Chiral Separations in Early Development - A Case Study*

(0148) **B. Schenkel, Novartis Pharma AG, Basel, Switzerland**

*Peptides and Macrolides*

(0147) **F. Vix, F. Hoffmann-La Roche AG, Basel, Switzerland**

*Process Optimization for Peptide Purification*

(0129) **O. Lyngberg, Bristol-Myers Squibb, New Brunswick, USA**

*Development Challenges for Chromatographic Purification Process of an early development 11-Aminoacid polypeptide program*

(0110) **J. Davies, Lonza Biologics, Slough, United Kingdom**

*Does Chromatography have a place in the Future of the Manufacture of Monoclonal Antibodies?*

(0138) **H. Kornmann, Merck-Serono Biotech Center, Fenil-sur-Corsier, Switzerland**

*Evaluation of Alternative Technologies for the Capture of a Recombinant Protein*

## Poster Session II

18:00 – 20:00 Poster Presentations

(2005) **F. Hassaine-Sadi, L. Sadoun and H. Bouchabou, University of Sciences and Technology Houari Boumediene, Algiers, Algeria**

*Treatment of Wastewater Containing Toxic (Chromium) and Heavy (Cadmium) Metals: Extraction with Oxyde tri-n-octyl Phosphine*

(2010) **M. Zandian and A. Jungbauer, University of Natural Resources and Applied Life Sciences, Vienna, Austria**

*Engineering of a scFv Affinity Column for Separation of Cytokines*

(2011) **J. Nti-Gyabaah, M. J. Iammarino and D. J. Roush, Merck and Co. Inc., Rahway, USA**

*A Potential Paradigm Shift in Downstream Chromatographic Purification of Monoclonal Antibodies*

(2014) **E. Freund[1], J. Lill[1] and M. Juza[2], CarboGen-Amcis, Aarau, Switzerland[1], Siegfried Ltd., Zofingen, Switzerland[2]**

*The Chromatographic Isolation of Reference Standards – a Case Study*

(2017) **J. García Palacios[1], M. Kaspereit[1] and A. Kienle[1,2], Max-Planck-Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany[1], Otto-von-Guericke University, Magdeburg, Germany[2]**

*Systematic Study of Production Processes Integrating Chromatographic Separation and Isomerisation Reactions*

(2021) **M.-H. Chuang and M. Johannsen, Hamburg University of Technology, Germany**

*Sub-/Supercritical Fluid Chromatography with Propane*

(2024) **M. Onsberg, M. Degerman and B. Nilsson, Lund University, Sweden**

*Determining Optimal Operating Conditions for Preparative Purifications with Varying Feed Composition*

(2026) **K. Westerberg, M. Degerman and B. Nilsson, Lund University, Sweden**

*Determination of Robust Pooling in Preparative Chromatography*

(2028) **J. Sempere, R. Nomen, E. Serra, K. Cuevas, O. Pou, J. Menacho and S. Martínez, Institut Químic de Sarrià , Barcelona, Spain**

*Simulation and Optimization of two and three Components SMB Separations*

(2030) **J. Li and H. A. Chase, University of Cambridge, United Kingdom**

*The Use of Expanded Bed Adsorption (EBA) to Purify Flavonoids from Ginkgo Biloba L.*

(2031) **K. Eriksson, K. Lacki and H. J. Johansson, GE Healthcare, Uppsala, Sweden**

*Rapid Development of Monoclonal Antibody Downstream Processes for Production of Clinical Phase I/II Material: a Case Study*

(2035) **A. Rajendran, Nanyang Technological University, Singapore**

*Equilibrium Theory Based Design of Simulated Moving Bed Processes under Reduced Purity Requirements: Linear Isotherms*

(2037) **R. C. R. Rodrigues, J. M. M. Araújo and J. P. B. Mota, Universidade Nova de Lisboa, Portugal**

*Robust Design and Operation of two- and three-Column Compact SMB Process for Binary and Ternary Separations*

(2038) **C. Grossmann[1], G. Ströhlein[1,2], M. Morbidelli[1] and M. Morari[1], ETH Zurich, Switzerland[1], ChromaCon AG, Zürich, Switzerland[2]**

*Development of a 'Cycle to Cycle' Control for the MCSGP-Process for a Monoclonal Antibody Variant Separation*

(2043) **J. Nowak[1], D. Antos[2], Y. Kawajiri[1,3] and A. Seidel-Morgenstern[1,4], Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany[1], Rzeszów University of Technology, Poland[2], Georgia Institute of Technology, Atlanta, USA[3], Otto-von-Guericke-University, Magdeburg, Germany[4]**

*Theoretical Investigation of Separation of a Ternary Mixture by Simulated Moving Bed Chromatography*

(2045) **J. M. M. Araújo, R. C. R. Rodrigues and J. P. B. Mota, Universidade Nova de Lisboa, Portugal**

*A Streamlined Two-Column SMB Process for Chiral Separation*

(2048) **C. Langel, C. Grossmann, M. Mazzotti, M. Morari and M. Morbidelli, ETH Zurich, Switzerland**

*Implementation of an Automated Online HPLC Monitoring System for SMB Processes*

(2056) **C. Frerick[1], P. Kreis[2] and A. Górak[2], Bayer Technology Services GmbH, Leverkusen, Germany[1], Technische Universität Dortmund, Germany[2]**

*Dynamic Discrete Event Modelling and Mixed Integer Optimisation of Protein Downstream Processes*

(2059) **J. Bléhaut, E. Lang and E. Valéry, Novasep SAS, Pompey, France**

*Preconceived Ideas about Preparative Chromatography*

(2062) **C. Morey[1], E. Valery[1], C. Bobier[1] and M. Bailly[2], Novasep SAS, Pompey, France[1], Laboratoire des Sciences du Génie Chimique, Nancy Cedex, France[2]**

*Integration of PAT (Process Analytical Technologies) in Continuous Chromatographic Processes: the Advanced Control System*

(2063) **M. Amanullah[1], S. Katsuo[2] and M. Mazzotti [2], Nanyang Technological University,**

**Singapore[1], ETH Zurich, Switzerland[2]**

*Design and Optimization of a Chromatographic-Crystallization Hybrid Process for the Separation of Tröger's Base Enantiomers*

(2064) **P. Scherpian, D. Schlinge and G. Schembecker, Technische Universität Dortmund, Germany**

*Choice of Process Concept in Preparative Chromatography*

(2070) **J.-L. Wu[1,2] , M. Minceva[1], Q.-J. Peng[2] and W. Arlt[1], University of Erlangen-Nuremberg, Germany[1], Jiangnan University, Wuxi, China[2]**

*Design and Optimization of a Pilot Scale Simulated Moving Bed Unit for Citric Acid Separation from Fermentation Broth*

(2074) **F. Grote[1], R. Ditz[2] and J. Strube[1], Clausthal University of Technology, Germany[1], Merck KGaA, Darmstadt, Germany[2]**

*Integrated and Model-based Bioprocess Development from Upstream to Downstream - and Beyond*

(2077) **T. Hellenkamp[1], P. Balling[2], M. Günther[2] and G. Schembecker[1], Technische Universität Dortmund, Germany[1], Inosim GmbH, Ammersbek, Germany[2]**

*Systematic Design of Downstream Processes – Modeling and Simulation*

(2094) **M. Bechtold, M. Füreder S. Makart and S. Panke, ETH Zurich, Switzerland**

*Integrated Operation of SMB, Biocatalysis and Cross-flow Filtration for the Production of Fine Chemicals*

(2095) **M. Bechtold, M. Füreder and S. Panke, ETH Zurich, Switzerland**

*SMB Design for Chiral Stationary Phases Showing an Analyte–related Memory Effect*

(2097) **R. Herry[1], J. Stevens[2], M. Crawford[2] and L. Roenneburg[2], Gilson International B.V.**

**Den Haag, The Nederlands[1], Gilson, Inc., Middleton, USA[2]**

*Automated Fraction Trapping of Purified Compounds from Preparative Chromatography*

(2101) **T. K. Tran, L. H. Phung, S. N. Xuan and V. T. Pham, Hanoi University of Technology, Vietnam**

*Dynamic Model for the Ultrasonic-assisted Extraction Process of Curcumin from Curcuma Longa L.*

(2104) **J. Hoogendoorn and D. Stadermann, Solvay Pharmaceuticals B.V., Weesp, the Netherlands**

*Comparison Batch vs SMB/Varicol for a Racemic Mixture and for a Complex Mixture*

(2105) **R. Chen and P. Ridgeway, Thar Instruments Inc., Pittsburgh, USA**

*Separation and Purification of Polar, Basic Pharmaceutical Relevant Compounds by MS Directed Preparative Supercritical Fluid Chromatography (SFC)*

(2106) **N. A. Vaidya, ChiroSolve Inc., San Jose, USA**

*Chiral Resolution for Full Spectrum of Racemates*

(2111) **H. J. Johansson, H. Tengliden and A. Grönberg, GE Healthcare Bio-Sciences, Uppsala, Sweden**

*Rapid Development of Cleaning-In-Place Protocols for Affinity Media*

(2113) **C. Birkner[1], U. Haering[1], J. N. Kinkel[2] and T. Gubbey[2], Roche, Diagnostics Division, Penzberg, Germany[1], Ohm-University of Applied Sciences, Nürnberg, Germany[2]**

*Batch and Continuous Liquid-liquid Partition Chromatography of Biopolymers in Aqueous/Ionic Liquid two Phase Systems*

(2115) **J. N. Kinkel, Ohm-University of Applied Sciences, Nürnberg, Germany**

*LLPC /SMB: The Continuous Separation of Biopolymers on Polyacrylamide Silica*

(2121) **J. Saar[1], K. Vind[2] and B. Denoulet[3], Grace Davison Discovery Sciences, Worms, Germany[1], Peak Biotech, Kvistgaard, Denmark[2], Grace Davison Discovery Sciences, Lokeren, Belgium[3]**

*The Use of High Quality Silica Media in Pilot and Process Scale Chromatography Using Custom Designed High Pressure Process LC Columns and Systems*

(2123) **M. Gramblicka and M. Polakovic, Slovak University of Technology, Bratislava, Slovakia**

*A Rational Approach to Modelling of Uptake Rate of Large Proteins by Porous Chromatographic Materials*

(2130) **M. Mazzotti, ETH Zurich, Switzerland**

*Non-classical Composition Fronts in Nonlinear Chromatography*

(2136) **S. Seraman, S. Ayothiraman, A. Rajendran and V. Thangavelu, Annamalai University, Tamilnadu, India**

*Optimization of Lovastatin Production by Monascus Purpures MTCC 369 Using Plackett-Burman and Central Composite Statistical Experimental Design*

20:00 – 24:00 Gala-Dinner

## *Wednesday, October 01, 2008*

### Session – Continuous Chromatography II

08:30 – 09:00 (0139) **F. Kjell, Siemens, Ninove, Belgium**

***Keynote Lecture:*** *PAT Application and Architecture in Pharmaceutical Process Development*

09:00 – 09:15 (1058) **O. Ludemann-Hombourger, M. Holzer, L. David, H. Osuna-Sanchez and E. Valery, Novasep SAS, Pompey, France**

*Sequential Multi-Column Chromatography: New Disruptive Continuous Purification Technology for Downstream Processing*

09:15 – 09:30 (1076) **L. Aumann[1,2], T. Müller-Späth[1], G. Ströhlein[1,2] and M. Morbidelli[1], ETH Zurich, Switzerland[1], ChromaCon AG, Zürich, Switzerland[2]**

*Continuous Downstream Processing of Monoclonal Antibodies Using MCSGP with Cation-exchange Resins*

09:30 – 09:45 (1108) **M. Pennings[1], M. Bisschops[2], T. Ransohoff[2] and S. Fulton[3], Xendo Manufacturing B.V., Leiden, the Netherlands[1], Tarpon Biosystems Inc., Marlborough, USA[2], Biosystem Development, Middleton, USA[3]**

*BioSMB™, Continuous Chromatographic Purification with Disposable Columns and Membrane Adsorbers*

09:45 – 10:00 (1096) **Y. Kawajiri[1,2] and A. Seidel-Morgenstern[2,3], Georgia Institute of Technology, Atlanta, USA[1], Max Planck Institute of Dynamics of Complex Technical Systems, Magdeburg, Germany[2], Otto-von-Guericke-University, Magdeburg, Germany[3]**

*Optimization of 8-Zone Simulated Moving Bed Chromatography for Ternary Separation with Recycle Stream Enrichment*

10:00 – 10:15 (1107) **D. Horneman[1], M. Bisschops[1] and M. Guiseppin[2], Xendo Manufacturing B.V., Leiden, the Netherlands [1], Solanic B.V., Veendam, the Netherlands[2]**

*Continuous Recovery of Proteins Using Large-scale Multicolumn EBA Chromatography Technology*

10:15 – 10:45 Coffee Break

## Session – Manufacturing

10:45 – 11:15 **L. Beaver, Food and Drug Administration, Washington, USA**

***Keynote Lecture:****Pharmaceutical Process Design Considerations*

11:15 – 11:30 (1025) **M. Degerman, K. Westerberg and B. Nilsson, Lund University, Sweden**

*Robust Design and Process Validation of Preparative Chromatography*

11:30 – 11:45 (1073) **W. R. Leonard Jr., P. Sajonz and C. J. Welch, Merck & Co. Rahway, USA**

*Advances and Case Studies in the Use of Chiral Chromatography in Pharmaceutical Process Research*

11:45 – 12:00 (1109) **H. Hüttmann[1], A. Jungbauer[2], S. Zich[2] and M. Berkemeyer[1], Boehringer Ingelheim Austria GmbH, Vienna, Austria[1], University of Natural Resources and Applied Life Sciences, Vienna, Austria[2]**

*Industrial Crystallization of Recombinant Biopharmaceutical Proteins*

12:00 – 12:15 (2133) **C. Langel, C. Grossmann, M. Mazzotti, M. Morari and M. Morbidelli, ETH Zurich, Switzerland**

*'Cycle to Cycle' Optimizing Control of Simulated Moving Beds – Experimental Implementation for a Nonlinear Chiral Separation*

12:15 – 12:30 (1055) **T. Zhang, D. Colantuono, D. Robin, J.-M. Heym, V. Briand, M. Schaeffer and P. Franco, Chiral Technologies Europe, Illkirch, France**

*Efficient Combination of HPLC and SFC at Preparative Scale for the Separation of Enantiomers: Timings and Productivity*

12:30 – 12:45 Closing Remarks

12:45 – 14:00 Lunch

14:00 Start of SFC 2008

## Invited Lectures

**0033**

# Challenges in Packing of Process Columns

**Sebastian Böcker, Martin Lohrmann**

Bayer Technology Services GmbH, Leverkusen, Germany

## ABSTRACT

The demands on the quality of process chromatography system are high. Since high-quality adsorbents with small particles are more and more used, accordingly this has to be taken into account in the process chromatography.
Here the packing and the column performance are of great significance. To achieve high separation efficiency a homogeneous packing as well as the column construction and design play an important role.
A selection of the optimal packing technique for the particular adsorbent is a challenge. The complete packing procedure, starting with the preparation of the packing suspension up to the packing technique, has to be adjusted optimally to reach the best possible separation efficiency later in the process. Particularly when operating with low packing heights an excellent distribution of the inlet flow to the entire cross-sectional area of the packing is important and unnecessary holdup volumes in the cap and in the bottom of the column has to be avoided. A fluid distributor and collector have to be designed appropriately. Furthermore, when frequently packing and unpacking a user-friendly and timesaving handling is desirable.
This lecture compares the common packing procedures in the process chromatography as well as the technical design of chromatographic columns and assesses these in terms of the separation task.

**0110**

# Does Chromatography have a place in the Future of the Manufacture of Monoclonal Antibodies?

**Jim Davies[1], Martin Smith[1], Lee Allen[1]**

[1]Lonza Biologics plc, Slough, UK

## ABSTRACT

The increase in monoclonal antibody titres from mammalian cell cultures has been as rapid as it has been well documented. The impact of upstream improvements on the downstream processing and how this can limit the potential savings to the overall manufacturing costs has also been discussed in detail. The output of such models is varied but whilst the outputs change with scale and process needs, the trends are the same. Increased mass leads to increased chromatography cycles and / or column size, buffer requirements and intermediate product volumes, all of which can impact plant throughput and costs.

With many of these effects related to the reliance on chromatography to carry the bulk of the downstream process load, many companies and academic researchers are investigating alternative purification methods in an effort to solve some of these issues. This presentation will look at some of these alternatives and compare them to how chromatography resins have and are evolving to meet the needs of product mass flux in an effort to assess the role of chromatography in the next generation of monoclonal antibody purification processes.

**0126**

# Chromatographic Selectivity as a Key Parameter to be Adjusted via the Stationary and Mobile Phases

**Wolfgang Lindner**

Department of Analytical Chemistry and Food Chemistry, University of Vienna, Austria

## ABSTRACT

The triumph of modern liquid chromatography, analytically but also on a preparative scale, is closely associated with the input of dedicated surface modifications of porous organic and inorganic polymer materials, and in particular of silica. In this course the selectivity term $\alpha$ to separate the wanted compounds from the potential interferences represents a key issue. However, $\alpha$ is reflected by the chemical nature of the target compound, the chemical characteristics of the modified sorption material and of the mobile phase composition employed. It is a troika characterized by (i) the chemical properties of the ligands/selectors (SO) immobilized onto the support material, (ii) by the chemical nature of the compound(s) to be selectively separated (the selectands, SAs) from other components, and (iii) by the chemical nature of the mobile phase. On a molecular level the type and magnitude of enforced intermolecular (SO-SA) interactions drive essentially the selectivity factors which can be very high considering (bio)affinity like sorption materials.

In this lecture focus will be given on the characteristics of modified surfaces to generate the dedicated selectivities. New trends will be highlighted as e.g. the development of "mixed mode" stationary phases by combining diverse SO-SA interaction sites in one ligand concept. Inter alia also enantioselective ion exchange chromatography as well as so-called hydrophilic interaction chromatography (HILIC) concepts will be discussed in the light of representative examples thus highlighting "the power of selectivity" in chromatography.

**0129**

# Development Challenges for Chromatographic Purification Process of an early development 11-Aminoacid polypeptide program.

**Olav Lyngberg[1], Francesca Quattrini[3], Ulf Altenhoener[3], Matthieu Giraud[3] , Claudio Mapelli[2], Rodney Parsons[1], Douglas Riexinger[2], Yan Zha[1].**

[1]Pharmaceutical Development, Bristol-Myers Squibb, One Squibb Drive, P.O. Box 191, New Brunswick, New Jersey, 08903,

[2]Molecular Biosciences, Bristol-Myers Squibb, One Squibb Drive, 206 Province Line Road, Princeton, New Jersey, 08540,

[3]Peptides and Oligonucleotides, Research & Development, Lonza ltd, Valais Work, CH-3930 Visp, Switzerland

## ABSTRACT

The chromatographic purification process development challenges for two 11-Amino acid containing peptide drug candidates entering Phase 1 clinical development is described. The presentation is focused on the development challenges of chromatographic purification and chemistry and chromatographic process interactions. Apart from the project specific hurdles tackled during development two are expected to be commonly applicable to many other peptide programs and will be presented here in more detail.

The first major hurdle to overcome during chromatographic process development was the need to efficiently remove diastereomeric peptide sequences formed by racemization of the amino acid chiral centers during the elongation process. These isomers showed very similar stationary phase affinities to the desired peptide and therefore posed a resolution challenge for the chromatographic purification. An interactive synthesis procedure optimization and separation efficiency assessment cycle was utilized for one compound to improve separation efficiency and robustness for selected impurities.

Another major hurdle originated in the necessity to isolate the product devoid of the salts used as mobile phase modifiers via a freebase desalting chromatographic step. The desalting chromatography was performed with high pH buffers of acetonitrile/water which lead to acetamide formation and contamination in the final product. To address this, a desalting step using MeOH in place of acetonitrile was developed.

**0138**

# Evaluation of alternative technologies for the capture of a recombinant protein.

**Henri Kornmann**

Merck-Serono Biotech Center, Fenil-sur-Corsier, Switzerland

## ABSTRACT

A case study of the development of an industrial capture step for a recombinant protein, which compares alternative technologies (custom-affinity ligand and aqueous 2-phases extraction) to conventional chromatography. The pros and cons of the various options will be discussed in the context of process productivity, scalability and cost-of-goods, with a high standard of quality objective as key decision criterion

**0139**

**Title:**

## PAT in pharma development:
## PAT application and architecture in pharmaceutical process development

**Presenting Author**


| | |
|---|---|
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### ABSTRACT

One of the biggest challenges facing pharma and biopharma development is how to use PAT and Quality by Design principles in development enabling faster process development and transfer to commercial manufacturing. This presentation reports on the application of PAT tools and the exploration of the design space in bio-reactor based process development studies, as well as in classical pharma downstream processes. Also the design of PAT architectures enabling the use of PAT tools in development and facilitating transfer to manufacturing will be discussed.

**0140**

CARBOGEN AMCIS

**30-May-2008, EFR**

# SMB: a flexible and quick tool for chiral separations in early development – a case study

**Freund, Ernst**

Carbogen Amcis AG, Aarau, Switzerland

## ABSTRACT

In the course of a customer project, the generation of the pure enantiomers of a spirocyclic intermediate was required. As the project was in an early development phase and both enantiomers were desired no synthetic efforts towards this aim have been undertaken, but chromatography was selected as the technique of choice. Multi kg amounts of pure enantiomers would finally be required and therefore a SMB feasibility study was performed.

However, the low solubility of the intermediate in most common organic solvents prevented the development of a productive SMB method. To overcome this restriction the two racemic APIs derived from the intermediate by one additional synthetic step were separated. This shows the flexibility of SMB separations. They might be performed at any step of a racemic synthesis.

Productive SMB methods for both racemic APIs were developed. The actual separations were challenging as both enantiomers of each API were required in >99 % a/a HPLC chiral purity. In addition the first separations were performed with small amounts of racemate with respect to the used equipment. To reach the target amount of pure enantiomers the usual optimization phase for SMB separations had to be kept as short as possible to avoid the generation of OOS material. High purities and yields of all the enantiomers could be reached quickly for both APIs by a rational choice of the starting parameters and systematic changes of the separation parameters.

**0141**

# Large column operation, challenges in downstream processing of APIs

**Oleg Werbitzky, Ulf Altenhoener, Francesca Quattrini**

Lonza AG, R&D Peptides and Oligonucleotides CH-3930 Visp, Switzerland

## ABSTRACT

Downstream processing in custom manufacturing of APIs has to deal with numerous challenges like short time for process development and qualification, complex impurity profiles, which can vary throughout the different phase of process development, regulatory constrains and changes in specifications for the final API.

Today many peptidic APIs are obtained by solid phase synthesis (chain elongation on a polymeric support). Solid phase synthetic processes generate impurities with very similar structures to the target peptides (isomers, double insertion or deletion of one or more aminoacids), which show similar affinities to chromatographic supports.

This presentation will describe the different stages of developing downstream manufacturing process for peptidic APIs, from the early phase lab screening of chromatographic conditions through pilot scale production of the first kilogram-scale-GMP-batches for toxicity and clinical trials up to FDA approval and commercial scale production. A good understanding of process robustness is particularly crucial for scale-up and validation of API manufacturing processes.

Potential improvements of model based optimization of chromatographic processes can ease several challenges in the development and scale-up of DSP of peptidic APIs: one example will be shown where purification yield and costs could be significantly improved with a low number of lab experiments .by means of model based optimization.

**0142**

# Affinity-based Separations using Imprinted Polymers

**B. Sellergren,**

INFU, Technische Universität Dortmund, Otto-Hahn-Strasse 6, 44221 Dortmund, Germany,

## ABSTRACT

The demand for robust and economic alternatives to perform affinity based separations at a large scale is growing in several branches of chemistry. For instance environmental and health protection requires techniques for selectively purifying water from persistant organic pollutants showing adverse health effects. The same criteria apply to the food industry with respect to their raw materials whereas food processing in general may benefit from techniques allowing selective removal of spoilage agents or specific flavors.

It is in such applications where stationary phases based on robust and affordable molecularly imprinted polymers (MIPs) show distinct advantages. The recognition properties displayed by MIPs can be on a par with their biological counterparts although they commonly display poor water compatibility, lower affinity for the target, binding site heterogeneity and failure to recognize larger more complex targets. Based on a set of tools such as high throughput combinatorial imprinting[1], designed host monomers[2] and thin polymer film formats[3], we have developed polymers exhibiting high selectivity for some key targets related to the above mentioned applications. These efforts and future perspectives will be discussed in the talk.

(1) B. Dirion, Z. Cobb, E. Schillinger, L. I. Andersson and B. Sellergren Water compatible molecularly imprinted polymers via high throughput synthesis and experimental design. (2003) *J. Am. Chem. Soc. 125*, 15101

(2) J.L. Urraca, AJ Hall, M.C. Moreno-Bondi and B Sellergren. A Stoichiometric Molecularly Imprinted Polymer for the Class-Selective Recognition of Antibiotics in Aqueous Media, *Angew.Chem.Int.Ed.,* 2006, 45,1-5.

(3) B. Sellergren, B. Rückert, A. J. Hall. Layer by layer grafting of molecularly imprinted polymers via iniferter modified supports. (2002) *Adv. Mat.* 14, 1204

**0143**

# The combination of SMB Chromatography, Slurry wash and Batch Chromatography for the resolution and purification of a racemate.

**Kerstin Larson**

AstraZeneca, Södertalje, Sweden

## ABSTRACT

Smaller batches of the racemate had previously been resolved by Chiral Batch Chromatography.When 13 kg of the same racemate was delivered it contained > 30% of an impurity that was not present in the earlier batches. By combining SMB Chromatography and a following slurry dissolution, it was possible to reach both the wanted enantiomeric purity and to increase the chemical purity. The favourable differences in solubilities, of the pure enantiomer and the impurity, was used in this unconventional simple step.The final purification to 98 % area was performed by Reversed Phase Chromatography.

**0144**

# New Perspectives in the Downstream Processing of Large Molecules

**Wolfgang Kuhne, PhD[1]**

[1]Roche Diagnostics GmbH, Penzberg, Germany

## ABSTRACT

Monoclonal antibodies (mAbs) have captured the pharmaceutical market during the last 15 years significantly. Today, mAbs like MabThera®, Avastin® or Humira® are successfully used to treat oncological, autoimmune and other severe diseases. Due to the remarkably promising clinical results achieved, in particular during the last 10 years, many companies continue to focus on mAb (process) development. Consequently, mAbs account for 70-90% of the product development pipeline in many pharmaceutical companies today. However, companies have to face increasing drug demands for clinical and, primarily, market supply. For some mAbs, several hundreds of kilograms, if not tons per year have to be provided. In order to meet increasing demands also in the future, innovative procedures and concepts in process development and manufacturing have to be evolved and implemented. Ten years ago, cell culture titers for mAbs were typically only about 0.5 g/L. Due to advances in cell culture technology, mAb-concentrations of 3-5 g/L cell culture harvest, which corresponds to 30-50 kg per 10,000 L fermentation batch, can nowadays be achieved. In order to adequately and timely process these huge amounts by downstream processing (DSP), potential bottlenecks have to be eliminated or at least mitigated. Improvements regarding chromatography and nanofiltration efficiencies appear to be most important to streamline the purification process. Optimized ultrafiltration methods are required as well in order to avoid an undesired increase of aggregate levels during the “high-titer” downstream process. A need for alternative procedures regarding storage and shipment of API bulk from high-titer processes seems obvious, too. Platform approaches including improved process steps are increasingly realized in order to streamline the processes. However, “novel techniques” like aqueous two-phase extraction and crystallization, which are not part of the platform of mAb processes today, might complement or even replace certain “conventional” purification steps. Rapid process development needs implementation of high-throughput down-scale systems. In-depth process characterization and validation based on risk analysis and Design of Experiment (DoE) studies are required for implementation of robust downstream manufacturing processes which reproducibly deliver the desired product yields and the required product quality.

**0145**

# Present and Future Technology Trends in API Development

**Jan Bernd Lenfers and Michael Lögers**

Bayer Schering Pharma, Wuppertal, Germany

**ABSTRACT:**

Pharmaceutical industry is facing a challenging and rapidly changing environment characterized by declining numbers of new approvals, increasing R&D spending and an increasing importance of emerging countries, not only for low cost sourcing but also for development purposes.

A consequence for chemical process development functions is permanently increasing cost pressure demanding for lean development strategies and economic production processes. Besides regulatory requirements for new compounds are increasing and a portfolio shift to new substance classes is taking place.

This leads to more focus on development functions and increased openness for new technologies. The meaning of new technologies for Bayer-Schering compounds will be presented taking examples from the current pharma portfolio.

Currently production of active pharmaceutical ingredients is dominated by batch processes for the chemical reaction and the subsequent isolation and purification by crystallisation. With respect to the reaction continuous process technologies have the power to change API development and production in the future.

The meaning of preparative chromatography for purification purposes as an alternative to the batch crystallisation -which is currently the technology of choice in most cases- will be discussed. For some compounds like steroids chromatography is already an import purification method. Whether chromatography will become more and more important for new compounds is still open.

The view of BSP on these technologies will be presented.

**0146**

# "Synthetic Membrane Filtration: Breakthroughs and Needs"

**Georges Belfort**

Howard Isermann Department of Chemical and Biological Engineering
and Center for Biotechnology and Interdisciplinary Sciences
Rensselaer Polytechnic Institute, Troy, NY 12180

## ABSTRACT

In this retrospective, we will review some key theoretical and experimental discoveries and developments that form the foundation of pressure-driven synthetic membrane processes. We will also mention in closing several limitations that need to be addressed. Specifically, we will discuss the early work of describing fluid flow in porous ducts using regular perturbation methods (Berman and Yuan and Finkelstein in the 1950s), transport using the thermodynamics of irreversible processes (Spiegler, Kedem and Katchalsky in the 1960s), concentration polarization (Blatt, Dravid, Nelson and Michaels in the 1970s) and fouling through mass transfer modeling (Thomas, Howell and Field in the 1980s and 1990s) two-phase flow of suspended particles (fouling) (Altena and Belfort in the 1990s). Important experimental milestones were the formation of the asymmetric membrane (Loeb and Sourirajan in the 1960s and Frommer and Strathmann in the 1970s), high membrane surface density modules such as hollow fiber and spiral wrap (Hoene, Wesmorland, Bray, and Mahon in the 1960s), formation of the composite membrane (Cadotte, Petersen and Riley in the 1980s), membrane surface modification (Steuk, Ulbricht and Belfort in the 1990s) and moving membrane modules (Liebherr, Lopez Leiva and Belfort in the 1980s and 1990s). Recent developments have involved process methodology, membrane design with selectivity improvements (Van Reis, Zydney, Nystrom, Bacchin and Aimar). Future needs will also be addressed.

**0147**

# Process Optimization for Peptide Purification

**Francis Vix[1], Guido Ströhlein[2,3], Lars Aumann[2,3], Massimo Morbidelli[2]**

[1]F. Hoffmann - La Roche AG, Pharma Technical Development Actives, CH-4070 Basel
[2]Institute for Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zürich, CH-8093 Zürich, Switzerland
[3]ChromaCon AG, Technoparkstr. 1, CH-8005 Zürich, www.chromacon.ch

## ABSTRACT

The purification of peptides by RP-HPLC is a challenging task. The phase system optimization (organic modifier type, buffer type, buffer concentration and pH, etc.) allows achieving appropriate selectivity. The chromatographic properties of the developed p-HPLC system are characterized by a strongly nonlinear adsorption behavior.
Beside the conventional optimization approach, modeling has proven to be able to give crucial support in definition and description of optimal process conditions.
In this presentation, the power of modeling is demonstrated for different steps along the process development of peptide purification. After phase system optimization, the model parameters for a lumped-kinetic model with a Bi-Moreau isotherm were determined. Taking into account the adsorption of key impurities, a complete model of the chromatographic purification was established. The optimal process conditions (gradient profile and loading) have been calculated by using genetic optimization algorithms and the trade-off curve between yield and productivity has been obtained. Furthermore, the established model will be used for demonstrating the limits of the purification process and thus support a quality by design approach.
In practice the developed process has successfully been scaled up to supply scale and proved to be robust in operation with high yields.

**0148**

# Present and Future of Chromatography Purification: Peptides and Macrolides

**Berthold Schenkel**

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## ABSTRACT

Purification of Peptides and Macrolides to their final specified quality in the API is a demanding task in manufacturing drugs. A short overview will be given on selected approaches which are currently used in development and production of Novartis:

- Methodologies to develop chromatographic processes in lab scale
- Combination of chromatographic processes with work up steps and other purification technologies resulting in optimized overall purification processes
- Scale up of the lab processes into production
- Current and future manufacturing processes and technologies

**0149**

# How to Work in Industry

**Gerald Terfloth**

GlaxoSmithKline, King of Prussia, USA

## ABSTRACT

This presentation will be the third in a series started at Spica 2004. It will provide a view of working in the pharmaceutical industry. Topics to be covered are the organization of a pharma company, motivation for developing drugs, internal and external business drivers, opportunities and challenges, what employers look for in successful candidates and what to expect when moving from an academic to an industry environment.

## Oral Lectures

**1003**

# Development of a preparative ion exchange process for the separation of individual fractions of caseinomacropeptide using membrane adsorption chromatography.

**Markus Kreuß, Ulrich Kulozik**

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## ABSTRACT

An anionic membrane adsorption chromatography (MAC) process for the pilot plant scale fractionation of the biologically and technologically active whey peptide caseinomacropeptide (CMP) into its glycosylated (gCMP) and non-glycosylated (aCMP) fractions, which are supposed to differ in functionality, was developed. The fractionation was started from CMP material as raw material and then adopted for direct capture of CMP or gCMP out of fresh whey such that complex upstream operations can be minimized. The best separation was achieved using a food compatible sodium acetate buffer (0.02 M) with a pH of 4.1. The loading of the sample is conducted in a fast flow recirculation mode. After a washing step to clear out unbound molecules from the module, the elution of the bound gCMP was induced by increasing the NaCl level up to 0.7 M. It was further shown that both the loading and the elution of the sample could be operated at highest flow rates (10 times the MAC module volume) without any loss of purity and yield. With a 1000 ml module (membrane surface: 36,000 cm²) 10.2 g of gCMP (0.28 mg/cm²) could be separated in one step within 12 min overall process time. The results showed that only the gCMP is bound to the MAC module. The aCMP and all other whey proteins remain unbound in solution.

**1004**

# Modeling-based rational protein purification process synthesis methodology

**B.K. Nfor[1], T. Ahamed[1], G.W.K. van Dedem[1], L.A.M. van der Wielen[1], E.J.A.X. van de Sandt[2], M.H.M. Eppink[3], M. Ottens[1]**

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## ABSTRACT

The key to successful and efficient protein purification is the selection of the most appropriate purification techniques and their combination in a logical way to obtain the desired purification in the minimum number of steps [1]. However, the time required for a thorough process development is often a great practical barrier since there is always extreme pressure to get new drugs into the clinic as soon as possible. Consequently, protein purification process synthesis is mostly carried out qualitatively, based on substantial experience and heuristics, often resulting in overall suboptimal downstream processing. Rationalization of protein purification process synthesis is therefore necessary if a safe and economical process must be found quickly somewhere in the often extremely large design space.

Here, a rational protein purification process synthesis methodology is presented. At the heart of this methodology is the use of process modeling and optimization for rapidly but systematically screening several feasible process options to select and design the best one. In this way, product quality, economics, scalability and robustness issues are simultaneously addressed without the need for numerous additional experiments. The necessary model inputs are acquired by means of a micro-scale platform for the fractionation and characterization of crude protein mixtures. The use of the developed methodology is demonstrated with a case study in which an industrially relevant protein is purified from the crude protein mixture by means of chromatography.

## ACKNOWLEDGEMENT

This project is financially supported by the Netherlands Ministry of Economic Affairs and the B-Basic partner organizations (www.b-basic.nl) through B-Basic, a public-

private NWO-ACTS programme (ACTS = Advanced Chemical Technologies for Sustainability).

## REFERENCE

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**1012**

# Synthesis of Monolithic Columns Equipped with a Hierarchical Pore System of Micro/Mesopores and Macropores Using the Ice Template Method

**Shin R. Mukai, Shinya Murata, Kazufusa Onodera, Aiko Eguchi, Yasuhiko Yao and Takanobu Masuda**

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## ABSTRACT

Various porous materials can be obtained through sol-gel synthesis. Recently, we found that such materials can be molded into the form of monolithic microhoneycombs by freezing their parent hydrogel unidirectionally. As ice crystals which are formed within the hydrogel during freezing act as the template, we named this method the Ice Template Method. The sizes of the channels of such monoliths are in the micrometer range, therefore can be considered as macropores. The walls which form the channels have thicknesses around 1 micrometer, and have developed micro/mesopores within them. Therefore, such monoliths are equipped with a unique hierarchical pore system which makes them applicable to various purposes. Due to this unique structure, such monoliths do not cause severe pressure drops when fluids are passed through them, even though the lengths of the diffusion paths within them are extremely short. Such monoliths can be synthesized in vessels of various sizes, from capillaries with extremely narrow passages, to inch sized tubes. This presentation will show the details about this new method, and the characteristics of the materials obtained through it. Unique usages of this material will also be introduced.

**1016**

# A Versatile Solubility Screening Platform for Preparative Chromatography: Methodology and Applications

**Jan Priess, Eric Francotte**

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## ABSTRACT

Solubility of organic compounds plays a major role in many pharmaceutical areas. It is also an important parameter in many synthetic and chromatographic processes. In particular in chromatography, solubility is a critical factor as it is often the limiting feature for preparative throughput. Indeed, throughput in preparative chromatography is to a great extent directly related to the solubility of the compounds to be purified or separated in the mobile phase used for elution[1].

In this context, a systematic screening to determine thermodynamic solubility of the solutes to be purified or separated in water or organic solvents and their mixtures is of great importance in order to improve throughput in preparative chromatographic processes. As no commercial platform was available on the market to fulfill our needs, a dedicated solubility screening platform was setup in-house and is now routinely applied to determine solubility.

The presentation will focus on the approach, concept and design of the developed solubility screening platform. Combination of the solubility data gained from this screening with selectivity □ from chiral method development for a given solute have permitted to increase considerably the throughput of preparative chiral resolutions. Practical examples of optimized preparative chromatographic resolutions of chiral drug substances performed by simulated moving bed (SMB) chromatography as well as HPLC and SFC will be presented.

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**1018**

# Altering efficiency of hydrophobic interaction chromatography by combined salt and temperature effects

**Renata Kramarz , Wojciech Piątkowski, Dorota Antos**

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## ABSTRACT

Hydrophobic interaction chromatography HIC is an established and powerful bioseparation technique in laboratory-scale, as well as in industrial-scale purification of proteins. The protein retention and adsorption selectivity on hydrophobic stationary phases is strongly affected by salt concentration and temperature in the process. In HIC, the use of high salt concentration in the equilibration buffer and sample solution promotes the ligand–protein interactions and consequently the protein retention. Increasing the temperature enhances protein retention and lowering the temperature generally promotes the protein elution. Manipulation of salt concentration and temperature as operation variables requires proper design if process is performed on an industrial scale.

In this work the pulse propagation in a HIC column of the solute whose retention depends on salt concentration and temperature is studied in the context of the equilibrium theory and a dynamic model. In particular, combination of the effects of both the temperature as well as the salt profile on the protein retention in the HIC columns is analyzed. It is shown that proper altering velocities of the salt and the temperature wave allows improving the separation efficiency by reducing the band broadening and the sample dilution.

The origin of band splitting and band broadening due to propagation of salt and temperature profiles is shown. The effects of ineffective thermal equilibration on protein retention is discussed and illustrated on the basis of experimental band profiles.

Additionally, possibility to alter the structure of hydrophobic stationary phase by temperature is discussed.

**1022**

# Sample solubility - important parameter for successful preparative chiral chromatography

**Ulrich Emde, Melanie Dietz, Helmut Reubold, Herbert Pflug**

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## ABSTRACT

An overview about the preparative HPLC/SFC activities applied for the separation of stereo isomeric mixtures on chiral and achiral phases of the medicinal chemistry department at Merck Serono, Darmstadt will be presented. The general chiral column screening concept plus several examples of interesting preparative chromatographic separations of pharmaceutical compounds and/or synthesis intermediates are shown. Underlying theme is the importance of sample solubility in HPLC/SFC mobile phases and its impact on choosing the best chiral chromatographic column for a certain separation problem.

**1025**

# Robust design and process validation of preparative chromatography

**Marcus Degerman**[1], **Karin Westerberg**[1], **Bernt Nilsson**[1]

[1]Lund University, Lund, Sweden

## ABSTRACT

A model-based methodology for robust design and process validation of preparative chromatography is presented in this work. Based on a calibrated mathematical model, the process can be optimized using a suitable objective and the process quality will be assured by the design. The novel approach is to add descriptions of process disturbances into the optimization procedure. The process parameters vary within their normal operating range and the process quality has to stay within the specifications for all normal variations. The methodology ranks the important process variations, points out worst-case scenarios and suggests an optimal operating point that is robust with regard to all process disturbances.

The model-based robustness analysis is performed by generating batch scenarios with varying process disturbances using Latin Hypercube sampling. The batch scenarios are simulated and the variation of process performance and quality can be quantified. The process robustness and the probability of batch failure can be calculated from the variation in process quality. The variation in process quality will also determine the critical process parameters. Process robustness is defined as the distance from the normal operating point to the edge of failure. The method for robust design is a combination of the robustness analysis with an optimization routine to find an operating point with optimal performance and quality built in by design.

1036

# Material Design Strategy for Chromatographic Separation Steps in Bio-recovery Downstream Processing

**Eugene Ndocko[1], Reinhard Ditz[2], Jochen Strube[3]**

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[2]Merck KGaA, Darmstadt, Germany
[3]Clausthal University of Technology, Clausthal-Zellerfeld, Germany

## ABSTRACT

Manufacturing of therapeutic recombinant proteins, monoclonal antibodies or peptides involves complex separation tasks to purify the target compound from side components with minimal losses after fermentation. For the urgent reduction of manufacturing costs a faster and more efficient process development strategy is required, which exploits all accessible options of process intensification, to fulfil the economic needs of manufacturers and society.
As becomes more and more evident, material properties can play a significant role in process design and optimization. However, structural or functional optimizations of separation media are lengthy processes which do not fit into the tight schedule of a downstream process development. Therefore, it could be beneficial if an a priori material design strategy was conceivable which would allow to predict desired material properties. This would simplify selection and screening of suitable separation media, but especially it would enable to design and make in time new materials with better process performance characteristics. This cannot be covered by conventional screening concepts which are limited by what is available, which may not be what is really best for a specific process.
The approach pursued in this study incorporates an a priori material design strategy for these selective materials in combination with process development in order to achieve the full optimization potential of all parts involved.
In order to determine which aspects of material design parameters like pore size, pore structure, ligand type and density contribute most to a process optimization, variations of some of those parameters were assessed in a theoretical study presented here. As especially for the handling of large molecules transport and surface accessibility in porous structures is of special importance, first attention was given to what can be described as “irregularities” in the porous system. For example, radial gradients in pore size and ligand density distribution which sometimes cannot

be avoided during synthesis can negatively impact dynamic capacity. However, when selectivity is introduced for typical separation tasks based on differences of molecules in size and/or affinity, the situation chances. Here, radial gradients may improve separation performance significantly. The future challenge will be to define and manufacture them a priori. To that end a rigorous material design (RMD) model is proposed.

The theoretical results of the first study so far indicate that the potential improvements are worth to be followed up with the corresponding experimental studies. This degree of improvement, when combined with throughput enhancement of similar dimension could lead to process improvement by combined material design and process optimization in the range required for substantial economic enhancement.

**1039**

# Optimized Preparative Supercritical Fluid Chromatography Enantioseparation of Flurbiprofen

**Chen Wenda, Arvind Rajendran, Mohammad Aman Ullah**

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## ABSTRACT

Preparative supercritical fluid chromatography (SFC) has been recognized as a robust method for rapid enantioseparations. Compared to high performance liquid chromatography (HPLC), owing to higher diffusivity of the solute and lower viscosity of the mobile phase, SFC offers increased efficiency with low pressure drops. In addition, SFC is considered a “green technology” as it replaces bulk of the organic solvent by an environmentally benign solvent such as carbon dioxide.

In this study, the chiral separation of flurbiprofen - a key non-steroidal anti-inflammatory drug (NSAID) on chiral stationary phase based on polysaccharide derivatives (Chiralpak AD-H) is studied. Elution profiles of overloaded injections showed that the adsorption isotherm was favourable and hence a Langmuir model was used to describe the adsorption thermodynamics. The isotherm parameters were obtained by fitting simulated profiles to experimental ones. The effects of pressure and mobile phase composition on the adsorption isotherm parameters were studied.

An evolution based algorithm (non-sorting generic algorithm) has been used for the optimizing the process. The objective was to maximize the productivity while parameters such as operating pressure, modifier concentration and injection volumes were chosen as decision variables. Based on these results, the separation was scaled-up to a preparative SFC unit and the comparison of the experimental results with the optimization studies is discussed.

**1041**

# Understanding ligand-protein interactions in affinity membrane chromatography for antibody purification.

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## ABSTRACT

Affinity chromatography with Protein A beads has become the conventional unit operation for the primary capture of monoclonal antibodies. However, Protein A activated supports are expensive and ligand leakage is an issue to be considered. In addition the limited production capabilities of the chromatographic process drives the research towards feasible alternatives. The use of synthetic ligands as Protein A substitutes has been considered in this work. Synthetic ligands, that mimic the interaction between Protein A and the Fc portion of immunoglobulins, have been immobilized on cellulosic membrane supports. The resulting affinity membranes have been experimentally characterized with pure IgG solutions and a cell culture supernatant containing $IgG_1$. The effects of the membrane support and of the spacer arm on the ligand-ligate interaction have been studied in detail.
Experimental data have been compared with molecular dynamic simulations with the aim of better understanding the interaction mechanisms. MD simulations were performed in explicit water, modeling the membrane as a matrix of overlapped glucopyranose units. Electrostatic charges of the considered ligands and spacers were calculated through *ab initio* methods to complete the force field used to model the membrane. The simulations enabled to elucidate how the conjunct interactions of surface, spacer, and ligand with IgG contribute to the formation of the bond between protein and affinity membrane.

## ACKNOWLEDGEMENT

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**1047**

# “Mixed-mode” chromatography materials in downstream process development

**Felix Oehme, Klaus Kaiser, Jürgen Lenz, Jörg Peters**

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## ABSTRACT

“Mixed-mode” chromatography materials (Capto MMC, MEP /HEA/ PPA Hypercel etc.) contain ligands of multimodal functionality which allow protein adsorption by ionic interactions, hydrogen bonding and hydrophobic interactions. Complex mixtures like fermentation broth or cell lysates can be applied at relatively high conductivity while elution is usually achieved by electrostatic charge repulsion. We used “mixed-mode” materials for capturing and intermediate purification of several recombinant therapeutic proteins from different expression systems like yeast, E. coli, CHO cells and tobacco plants. Major contaminants, endotoxins and coloured impurities from fermentation broth were efficiently removed while the desired product was bound with high selectivity. The purity of the final product was usually > 80% after a single purification step which implies that “mixed-mode” materials act as a “pseudo-affinity” matrix. As the purification protocol can be easily scaled up to a pilot plant production scale, “mixed-mode” materials are being considered as potential elements of a general purification platform for recombinant therapeutic proteins from microbial, plant-based and mammalian expression systems.

**1049**

# Automated iterative refinement of adsorption isotherm models for simulated moving-bed operation based on cyclic steady state data

**João M. M. Araújo, Rui R. C. R. Rodrigues, José P. B. Mota**

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## ABSTRACT

Enantiomeric separations by simulated moving-bed (SMB) chromatography are usually carried out under strongly nonlinear conditions. The accurate determination of the competitive adsorption equilibrium of the enantiomeric species is thus of fundamental importance to allow computer-assisted optimization or process scale-up. This is especially true for the SMB, because its operating conditions cannot be designed conveniently without knowledge of the adsorption isotherms of the feed components.

The inverse method of isotherm determination is currently becoming very popular as a quick procedure for estimating the adsorption equilibrium data necessary for designing SMB separations. It derives the isotherm from overloaded band profiles of individual solutes or of their mixture. In recent studies, reporting comparisons of frontal analysis and inverse methods, it is concluded that the inverse method gives accurate estimates of the competitive isotherm parameters up to the maximum elution concentration of the overloaded bands. However, it is only moderately accurate from the maximum elution concentration up to the injected concentration.

In this work we describe and validate experimentally a procedure in which the inverse method is applied directly to the cyclic steady-state (CSS) concentration profiles of the running SMB process to update the parameters of the prescribed adsorption isotherm model. The operating conditions are then optimized for the newly determined isotherm parameters and applied to the running SMB process. This process is iterated and automated in our monitoring and control software.

As a proof of concept of the proposed methodology, it has been successfully applied to the chiral separation of Reboxetine enantiomers on Chiralpak AD, using a mixture of Hexane-Ethanol-DEA as solvent. The system was operated near the solubility limit of the the racemic mixture. Reboxetine is an antidepressive NRI drug. Only the (R,R)- and (S,S)-pair is present as a racemic mixture in the active principle and commercial formulations. Recent studies support the hypothesis that the (S,S)-enantiomer is a more potent inhibitor than the (R,R)- and that it is responsible for the vasomotor and cardiac side effects of Reboxetine.

**1050**

# Comparison of Performance between Improved and Conventional Simulated Moving Bed (SMB) Process

**Shigeharu Katsuo**[1], **Marco Mazzotti**[1]

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## ABSTRACT

The use of Simulated Moving Bed (SMB) technology for the separation of enantiomers of chiral compounds is a success story because of superior performance compared to batch column chromatography [1]. In general chiral stationary phases have a high pressure drop due to the small particle size and compared to other stationary phases they can be relatively expensive. There has been a continuous effort to find modified SMB schemes to overcome these limitations, yet maintaining high purity of the products and high productivity.
The Improved SMB (ISMB) process is one of such modifications being widely applied in the sugar industry [2]. In the ISMB process the switch interval is divided into two sub-intervals; in the first step, the unit is operated as a conventional SMB, with two inlets (feed and desorbent) and two outlets (extract and raffinate), and no flow in section four; in the second step inlet and outlet ports are closed and the internal flow through the four sections allows to move the concentration profiles along the columns and to adjust their relative position with respect to the outlet ports [3]. Typically ISMB units use only four chromatographic columns, and the proponents claim to achieve higher productivity compared to a conventional SMB process.
This work analyzes the ISMB process in order to explore the potential of this technique in other application fields. The optimum operating conditions leading to maximum productivity and minimum solvent consumption are estimated for both ISMB and conventional SMB units using the process simulation tools together with a multi-objective optimization routine based on the evolutionary algorithm. The optimum solution for each system is a curve on the productivity-solvent consumption plane, the so-called Pareto set. The comparison of both systems is carried out based on the relative position of these curves.

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**1054**

# Theoretical Analysis of Continuous Chromatography with Adsorbing Additives

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## ABSTRACT

Additives are frequently used in chromatography, for example, to influence retention times or to serve as buffer components. In preprative applications adsorption is generally nonlinear and competitive. Therefore, an *adsorbing* additive can strongly influence elution behaviour of all components and competitive effects can give rise to phenomena like “additional” peaks and peak deformations. Recently, this was demonstrated in single-column batch chromatography for different enantioseparations. The additive as a third component caused peak shapes that resembled different combinations of Langmuir and anti-Langmuir adsorption isotherms [1].

An interesting question is under which conditions the effects of an additive can be purposefully utilised in continuous preparative chromatography. In order to facilitate an answer, a theoretical analysis is presented for continuous chromatography with the additive considered as a third adsorbing component. Besides numerical studies of the True Moving Bed (TMB) process, in particular the equilibrium theory (i.e., the hodograph method) will be applied in order to elaborate the different possible scenarios that arise depending on the order of adsorptivities. In a first step, systems with Langmuir adsorption isotherms are investigated for which a fully developed mathematical theory is available. Afterwards, the analysis is extended to systems with bi-Langmuir isotherms (as are typically found in enantioseparations). Based on the results, an attempt is made to derive design criteria for simulated moving bed (SMB) processes.

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**1055**

# EFFICIENT COMBINATION OF HPLC AND SFC AT PREPARATIVE SCALE FOR THE SEPARATION OF ENANTIOMERS: TIMINGS AND PRODUCTIVITY

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## ABSTRACT

The separation of enantiomers has been a flourishing field of research for over two decades which continues to grow [1-3]. The assessment of enantiomeric purity in drugs and other substances, as well as the interest of isolating pure enantiomers at preparative and even industrial level is undeniably important.
At analytical level, chiral chromatography is an essential tool to analyse racemic or enriched mixtures of enantiomers and to determine their enantiomeric excess (ee). But chromatography is also a cost effective methodology at preparative level. In the early stages of development of a new drug, chromatography is a fast and economic way to isolate the individual enantiomers to be used in the clinical and/or toxicological studies. In contrast to what happens in asymmetric synthesis, chromatography is able to render both enantiomers in a single step and the process is easily upscaled from the bench to the pilot plant.
Two main types of chromatographic techniques are today gaining general acceptance for the preparative separation of enantiomers at early stages: the classical HPLC and SFC (Supercritical Fluid Chromatography). The rational combination of both modes, together with the new chiral stationary phase (CSP) developments [3-6], leads to an efficient strategy for the production of enantiomerically pure compounds in pharmaceutical companies.
This presentation will review the different parameters needed to make preparative chromatography a time and cost effective technology. Examples of recent approaches and strategies to identify new chromatographic methods meeting the feasibility and economic requirements will be overviewed. Perspectives on future trends will also be considered.

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**1057**

# Optimisation of Affinity-enhanced Purification of Antibodies using Aqueous Two-Phase Extraction

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## ABSTRACT

More than 25 monoclonal antibody-based therapeutics have already been approved by the US Food and Drug Administration (FDA) for the treatment of several infectious diseases, cancers and autoimmune disorders [1-2]. The large demand and required high doses or chronic administration of these biotherapeutics led industry to enrich important improvements in the upstream productivity [3]. Innovative downstream processing technologies which have the potential to accommodate these improvements are then desperately needed since the downstream processing costs account for up to 80% of the overall production costs [4]. Extraction in aqueous two phase systems has attracted interest for many years and has been developed as a primary stage unit operation in downstream processing [5-7]. In order to make this unit operation more predictable and selective, affinity ligands can be introduced in one of the phase–forming components and consequently the target biomolecule can be partitioned to the phase containing the ligand [8].

In this work, the purification of human Immunoglobulin G (IgG) from an artificial mixture of proteins was first studied using polyethylene glycol 3350 (PEG 3350)/dextran aqueous two-phase systems in the presence of diglutaric acid functionalised PEG 3350 (PEG 3350-COOH) or triethyleneglycol (TEG-COOH). According to these results, the purification of IgG from a Chinese hamster ovary cells supernatant was also investigated and the best purification was achieved in the presence of TEG–COOH with a recovery yield of 93% and a selectivity to IgG of 11. A two level central composite design was then performed in order to optimise the PEG 3350, dextran and TEG-COOH concentrations for the purification of IgG. Statistically valid models were obtained and the optimal conditions for the isolation of IgG were observed for low concentrations of dextran and PEG 3350 and high concentrations of TEG-COOH. All IgG could be recovered with a final purity of 91%.

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**1058**

# Sequential Multi-Column Chromatography: New Disruptive Continuous Purification Technology for Downstream Processing

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## ABSTRACT

Continuous purification processes are well established in petrol, food, small molecule and antibiotic applications where cost of goods is critical. Technology principles and process know-how have been successfully extrapolated from these industries to create powerful new tools for the downstream processing of biopharmaceuticals.

One example is Sequential Multi-Column Chromatography (SMCC), a continuous purification technology, which can significantly increase purification productivity and reduce significantly buffer consumption and cost of goods compared to traditional approaches. This technology also offers the possibility to streamline biopharmaceutical-manufacturing processes. A detailed explanation of SMCC will be given along with evaluation of impact on cost of goods.

Two case studies were chosen to illustrate the potential of SMCC. The first example concerns the capture of human antibodies on a protein A matrix. Data from a second case study demonstrating the capability of the SMCC technology with a different molecule will also be presented.

Conclusions: SMCC enables faster downstream processing showing improved performance in an affinity capture step at elevated flow rates of: Working Capacity increased up to almost 3 fold, productivity almost doubled and buffer Consumption reduced by a factor of 3

In separate COGs studies performed by Biopharm Services a comparison of a continuous downstream manufacturing processes with a batch operation showed a reduction of cost of goods for downstream processing of 70% applying continuous processes. The study showed that the cost reduction mainly driven by savings of capital expenses, materials and labour.

**1060**

# Making Multi Columns Chromatography Simpler and Faster

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## ABSTRACT

One of the most challenging problems encountered during the development of a new pharmaceutical compound is linked to purification. The synthesis schemes are more and more complex and the request for rapid and efficient methods to isolate the target molecule from mixtures is crucial to ensure the success of the future drug. Preparative chromatography is widely used at the development stages. The technology offers a perfect answer to the main challenge of the developers : “go fast and be efficient”. The development of a chromatographic separation is easy at this stage; it is often possible to modify an analytical HPLC method to quickly isolate the purified product on a larger column. More efficient processes had been developed such as Cyclojet, SMB or Varicol

In the 60’s, Klesper proposed to use supercritical carbon dioxide for eluting a chromatographic column and developed the first supercritical Fluid Chromatography (SFC) equipment. The technology dramatically improved since 2000. SFC systems now incorporate efficient columns and devices to recycle carbon dioxide. As for liquid chromatography an SF-SMB was developed in 1994. Although this process is very attractive it has never been implemented at large scale due to its intrinsic complexity

A new process has been developed using only two columns with a simple design. Particularly simple and efficient, this process is applied to Supercritical Fluid Chromatography with benefits such as : high purities and yields even in low resolution cases, short columns, high flow rates.

Experimental results and methodologies to operate this process will be presented as well as the implantation of an automatic control for this two-column SFC process.

**1061**

# Molecular Dynamic investigation of the interaction of supported affinity ligands with Monoclonal Antibodies

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## ABSTRACT

Monoclonal antibodies are gaining an increasing importance in diagnostic and therapeutic treatment of acute diseases such as cancer. However, the production and purification of these pharmaceuticals is still extremely expensive. Among the different methods for MAB purification, affinity chromatography is one of the most employed. In this work we present our computational investigation aimed at obtaining some guidelines for the rational design of affinity ligands, through the study of their interactions with both the antibody (IgG) and two model support materials: agarose and cellulose. This analysis was carried out performing MD simulations of the support-spacer-ligand-IgG system in explicit water. Binding energies between IgG and two supported ligands, a di-substituted derivative of tri-chloro triazine and a tretameric peptide, were determined with the LIE and MM-GBSA approaches. A detailed study of the possible binding sites of the considered ligands was performed exploiting docking protocols and MD simulations. The energetic analysis revealed that van der Waals and electrostatic energies of interaction of the triazine ligand with the support are significant and comparable to those with the protein, thus decreasing the ligand affinity for the protein. This effect is less evident for the tetrameric peptide, since for steric reasons only two of its arms can interact at the same time with the agarose support, thus leaving the remaining available to bind the protein. These results indicate that the interaction between ligand and support is an important parameter, which should be considered in the computational and experimental design of ligands for affinity chromatography.

**1065**

# Design Method for Closed-Loop and Steady-State Recycling Chromatography

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## ABSTRACT

Seeking for a fast implementation of chromatographic processes very often classical batch chromatography is applied. With rising production scales and separation cost becoming more important a SMB unit might be considered, instead. However, smaller companies are often reluctant to invest in such complex equipment. In this case advanced batch process concepts like closed-loop (CLRC) and steady-state (SSRC) recycling chromatography are promising alternatives, because they consist of flexible and inexpensive equipment and they can lead to higher yields and lower eluent consumption. Although some publications (e.g. [1, 2]) deal with these concepts, a general design strategy does not exist.

In this talk we propose a method to determine optimal design and operating parameters for closed-loop (CLRC) and steady-state (SSRC) recycling processes. Both recycle the not completely purified fraction directly into the column, in case of SSRC inserting a portion of fresh feed into the recycle fraction. First we illustrate the influence of important optimisation parameters like plant volume (CLRC, SSRC) and point of re-injection (SSRC) on the process performance. Then we introduce a strategy for the optimal choice of all process parameters (design and operation parameters). The fundamental idea of this strategy is to apply detailed and experimentally validated process models and to reduce the number of influencing parameters by introducing and optimising dimensionless parameters. Jupke et al. followed this approach successfully for SMB and batch chromatography [3]. By introducing the dimensionless recycle time we prove experimentally that this concept is extendable to recycling chromatography processes.

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**1066**

# IEX membrane adsorbers for capturing of proteins: Model development, model validation and scale up

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## ABSTRACT

Membrane adsorbers have been introduced as an alternative for conventional chromatography approximately 20 years ago. During this period many advances have been made in manufacturing technologies and in functionalization chemistry with the goal to increase capacity, selectivity and reusability. Nowadays membrane adsorbers, functionalized with a broad spectrum of ligands, are commercially available from laboratory scale to process scale. No adequate simulation tools for evaluation of the performance of membrane adsorbers at process scale are available, which hinders the implementation of membrane adsorbers into commercial downstream processes. Therefore such a tool is developed in this work.
Dynamic experiments have been performed with bovine serum albumin and Sartobind ion exchange membrane adsorbers. The observed experimental breakthrough, washing and elution curves are hardly dependent on the applied flow rate. This behaviour was described in previous investigations for both affinity [1] and ion exchange [2] membrane adsorbers by using lumped parameter models. However the predictability of these lumped parameter models is restricted, since the relevant transport phenomena underlying membrane adsorption are not separately addressed. Therefore a better description and understanding of the transport mechanisms is required. This is achieved by the development of a more detailled modelling framework and an extensive experimental investigation on Sartobind S and Q membranes, in which the impact of molecular properties of the adsorbate (like molecular size) and of operating conditions (for example feed concentration and elution gradients) on process performance has been systematically analyzed. The resulting simulation tool is able to describe the enhanced performance of membrane adsorber configurations at process scale, as observed experimentally in literature [3].

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1071

# Utilisation of Peak Shape Tuning to Optimize Preparative Batch Chromatography

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## ABSTRACT

In SPICA 2006 we demonstrated that remarkable band shapes can occur in modern systems aimed at chiral preparative separations of enantiomers when an additive is present and that these effects depend on the relative adsorption strength of the additive and the solutes. We further showed that we could efficiently tune the peak shapes from Langmuirian to anti-Langmurian by changing the mobile phase composition. For a binary system we could tune the peak shapes such that they are (i) anti-Langmuir/Langmuir (the two sharp sides of the peaks points to each other), (ii) anti-Langmuir/anti-Langmuir, or (iii) traditional Langmuir/Langmuir.

In the present work we first made a simulation study, using the equilibrium dispersive model, to determine which peak shapes are most favourable in preparative batch chromatography when we have enantiomer solutes and a strongly adsorbed additive. Here both the additive concentration and the injection volume were varied systematically. We found that much can be gained, in terms of yield and productivity, if the additive concentration is tuned properly. Thereafter, we used an advanced global optimization computer algorithm to optimize the performance of an experimental system consisting of a teicoplanin stationary phase and a polar organic mobile phase with acetic acid/triethyl amine as additives and beta-blockers as solutes. The input data to the algorithm were the adsorption isotherm parameters of the solutes and additive as well as the van Deemter functions.

**1073**

# Advances and Case Studies in the Use of Chiral Chromatography in Pharmaceutical Process Research

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## ABSTRACT

Chromatography is playing an increasingly important role in early pharmaceutical development owing to its ability to rapidly solve purification problems with a minimun amount of labor. We will present some new advances and case studies in the use of chiral HPLC and SFC in pharmaceutical process research. Particular attention will be paid to newly developed loading study methodology that minimizes the required amount of compound to evaluate its feasibility for preparative chromatographic resolution. Using this methodology, synthetic route investigations can be carried out on milligram scale as only minimum amounts of intermediates are needed for chromatographic loading studies.

**1075**

# Facilitated extractions with selective resins – the use of MIPs in separations and analysis

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## ABSTRACT

Among the varieties of selective resins, molecularly imprinted polymers (MIPs) have gained wide acceptance as high performance, stable affinity separation materials and have achieved unsurpassed results when applied to demanding analytical tasks. MIPs typically consist of organic acrylic or styrenic polymers and contain designed binding sites in the polymer.

Compared to generic separation materials or to classical bioaffinity resins these MIPs offer a multitude of advantages, conferred by their high selectivity and unrivalled chemical stability. The enhanced stability of MIP resins leads to long life-times allowing repetitive and even harsh regeneration protocols. The selectivity enables fewer purification steps, higher productivities and better overall purification economics for the process or, for analytical uses, shorter times to analytical results.

We have demonstrated in a number of examplesthat the selectivity of MIPs is especially suited for the extraction of undesired impurities, toxic contaminants, or valuable low-level compounds present at trace concentrations in complex matrices.

In this lecture, we would like to present examples of the performance and benefits of selective MIPs both for analytical applications and for preparative/process purifications.

**1076**

# Continuous downstream processing of monoclonal antibodies using MCSGP with cation-exchange resins

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## ABSTRACT

The increasing number of therapeutic proteins, which are developed for the treatment of important diseases e.g. monoclonal antibodies against cancer, continuously require larger production capacities. The downstream part of the production process, and particularly the chromatographic purification step constitutes a process bottleneck. In order to overcome this bottleneck, it is indispensible to optimize the existing downstream processes and to implement innovative, more effective purification technologies. One of these new innovative technologies is the MCSGP process, which has been applied to the capture of a monoclonal IgG from a clarified cell culture supernatant (cCCS).

In fact, it has been found, that the MCSGP process can capture the mAb at high yield and purity for productivities being superior to the ones achieved by protein A affinity chromatography, in particular for very high titers. In contrast to the current capture processes, which are based on protein A affinity resins, the MCSGP process uses commercial cation exchange stationary phases, which decrease the resin costs by ca. 80%. For large scale productions these savings can reach several million US$ per year.

The lecture explains the principle of the MCSGP technology and covers experimentally the complete chromatographic downstreaming, including the countercurrent MCSGP capture and different polishing techniques.

**1081**

# Adsorption properties of DNA on charged surfaces

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## ABSTRACT

Anion exchange chromatography (AEC) is a useful and effective tool for DNA purification, but due to average pore sizes between 40-100 nm most AEC resins lack truly useful binding capacities for pDNA. Equilibrium binding capacities and uptake kinetics of anion exchange chromatography media including conventional media (Source-30 Q, Q Sepharose-HP), a polymer grafted medium (Fractogel EMD DEAE (M)), media with large pores (Celbeads DEAE, PL SAX 4000 Å 30 µm) and a monolithic medium (CIM-DEAE) were investigated by batch uptake or shallow bed experiments at two salt concentrations. Theoretical and experimental binding capacities suggests that the shape of the pDNA molecule can be described by a rod with a length to diameter ratio of 20:1 and that the molecule binds in upright position. The adsorption is clearly different to an enthalpy driven ion exchange interaction. The arrangement of DNA like a brush at the surface of the anion exchanger can be considered as a kind of self-assembly process which is inherent to highly and uniformly charged DNA molecules. This hypothesis has been also verified by thermodynamical analysis. With a random packing of DNA at the surface the electrostatic repulsion is not taken into account. The initial phase of adsorption is very fast and levels off, associated with a change in mass transfer mechanism. Feed concentrations higher than 0.1 mg/mL pDNA pronounce this effect. Monolithic media showed the fastest adsorption rate and highest binding capacity with 13 mg plasmid DNA per mL.

**1087**

# Bilayered SEC-IEC adsorbents for improved separation of bioparticulate nanoplex products

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## ABSTRACT

The emergence of larger and ever more complex 'bioparticulate nanoplex' products for the biopharma, diagnostics and nanobiotech sectors, presents a new set of challenges to bioproduct assembly, separation and formulation. Their properties dictate that efficient large-scale manufacture must follow a very different 'general' path to that which has been established for therapeutic human proteins of much smaller dimensions. Given the current trend to increase titres during fermentative production of bioproducts, big or small, combined with the difficulty in producing adsorbent materials with sufficiently high capacities (especially so for very large target species), the incentive to capture a product early on becomes less and less attractive. Conversely, 'negative' modes of adsorption chromatography (i.e. where the product runs through the column and contaminants are bound) are likely to find increasing favour. For subtractive adsorption chromatography to be successful in separating large nanoplex products from smaller macromolecular components sharing similar surface chemistry, new breeds of matrices should possess 'non-stick' exteriors or barriers that are freely accessible to smaller macromolecular species, but not to larger entities (i.e. nanoplexes), and in order not to compromise mass transport and sorption properties, they must also be very thin. In this presentation we describe our recent research on the development and evaluation of new multifunctional chromatography materials that enable efficient separation of nanoplex bioproducts from smaller, but chemically very similar 'problem' contaminants in a 'one column-one bead' process that combines size exclusion with ion exchange principles.

This work is supported by the Bioprocess Research Industry Club (BRIC) and BBSRC (grant number BB/F004982/1).

**1089**

# Tracking the movement of individual adsorbent particles in expanded beds

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## ABSTRACT

Based largely on indirect evidence derived from liquid dispersion studies it is often claimed that at stable fluidization individual adsorbent particles within an expanded bed orbit very close to their ideal position in the column – a position determined by size and density gradients, and the sedimentation velocity of individual adsorbent particles. In this study we illustrate the gross inaccuracy of this fundamental and widely-held assumption. Using the technique known as 'Positron Emission Particle Tracking (PEPT)' we have directly visualised the motion of individual adsorbent particles within expanded beds. PEPT relies on rapid detection of a tracer particle labelled with a positron-emitting radioisotope. Single long-lived $^{61}Cu^{2+}$ and $^{66}Ga^{3+}$ loaded positron-emitting tracer adsorbent particles of varying size, density and material were inserted into expanded beds composed of three different commercial media, and their movement tracked in pseudo-real time with a 'positron camera'. We have studied the influence of fluid flow rate on the motion of 'foreign' and 'native' tracer particles within expanded beds over extended times, and have also examined the motion of single 'native' adsorbent tracer particles in expanded beds of Q HyperZ during all phases of protein and DNA chromatography.

We wish to thank the BBSRC, Pall Life Sciences and Avecia Biotechnology for supporting this work.

**1096**

# Optimization of 8-zone simulated moving bed chromatography for ternary separation with recycle stream enrichment

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## ABSTRACT

The 8-zone simulated moving bed (SMB) chromatographic process, which has an internal recycle stream to create eight different velocity zones, allows realization of continuous ternary separation. However, for this modified SMB process, it can be proved by the equilibrium theory that „complete separation“ is not possible even if the nonidealities are ignored[1,2]. We first show using a rigorous dynamic SMB model that some optimization problems with a sufficiently high purity requirement are indeed found infeasible, but lowering the purity requirement can create a feasible optimal solution.

In order to overcome this theoretical limit, we introduce an enrichment unit for the internal recycle stream[3], typically realized by evaporation or membrane filtration. To demonstrate the advantage of this process, several case studies using the rigorous dynamic SMB model are investigated including the following cases:

the solvent is partially removed instantaneously with negligible dead volume

the recycle stream is fed into a vessel for solvent removal which has a significant mixing effect

An efficient deterministic optimization approach is employed to compare the performance[4]. We show that introducing an efficient recycle enrichment unit also creates a feasible optimal solution even for a high purity requirement.

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**1107**

# Continuous recovery of proteins using Large-scale Multicolumn EBA chromatography technology

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## ABSTRACT

The production of potato starch involves release of large amounts of potato fruit juice (PFJ). This aqueous solution contains large amounts of valuable proteins, some of which can be used for pharmaceutical application.

Solanic is a Dutch-based company that produces proteins derived from natural sources, including potato fruit juice. Solanic has developed a large scale process for recovering two classes of proteins from PFJ. The work-horse in the process is a continuous counter current multicolumn chromatography system. This system combines elements of Simulated Moving Bed (SMB) and Expanded Bed Adsorption (EBA).

In this presentation, we will outline the combination of SMB and EBA technologies. The impact of the configuration on the resolution and specific productivity will be discussed and compared to batch chromatography.

The presentation will cover the design and operation of a small pilot unit with (10 cm ID columns) and a large pilot production system (10 – 20 $m^3$/hr capacity), including operating experience on both scales.

One of the products that are purified in the Multicolumn EBA chromatography system is meant for pharmaceutical use. The validation of the large-scale unit will be briefly addressed in the presentation.

**1108**

# BioSMB™, Continuous Chromatographic purification with disposable columns and membrane adsorbers

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## ABSTRACT

Protein A Chromatography is one of the most powerful working horses in the purification of monoclonal antibodies. While expression levels in animal cell culture have increased dramatically, the antibody production capacity is approaching tonne-scale production rapidly. The production bottleneck is now shifting from the upstream process to the downstream process and to the Protein A chromatography step in particular.

Recently, the benefits of Simulated Moving Bed Technology (SMB) -or continuous multicolumn processing- for Protein A Chromatography have been demonstrated. There is no doubt that this allows higher productivities and simultaneously a lower buffer consumption, and thus the technology contributes well to process intensification.

Tarpon Biosystems is developing a multicolumn chromatography system that comprises an entirely disposable fluid path, hence dealing with cleaning and validation issues. BioSMB relies on disposable format modules for the valve system as well as for the columns.

In this presentation, we will present technological solutions to overcome hurdles related to multicolumn chromatography processes. In addition to that, we will present the benefits of this technology, supported by experimental results obtained during the isolation of a monoclonal antibody from a CHO cell supernatant, both for disposable columns and for membrane adsorber cartridges.

**1109**

# Industrial Crystallization of Recombinant Biopharmaceutical Proteins

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## ABSTRACT

Preparative crystallization may be an alternative to costly unit operations such as liquid chromatography and lyophilization. Limited binding capacity and scalability of chromatographic media and continuously increasing fermentation titers represent a bottleneck in large scale downstream processes and cause time consuming and expensive purifications. Bulk crystallization provides high yields and purities, considerable volume reduction, short process times, nearly unlimited scalability and low cost of goods due to inexpensive chemicals.
General approaches to implement protein crystallization into a purification process were proposed theoretically, but reports about successful implementation of such programs are rare.

Here we present a systematically and practically approved approach to develop and establish a crystallization step into a downstream process for recombinant therapeutic proteins. Crystallization yields above 90 %, significant reduction of impurities and reduced process times of several hours could be achieved. In addition, the generation of phase diagrams and re-crystallization principles will be discussed. Seeding is shown to be an option to crystallize at conditions without nucleation. Also the influence of temperature and pH as well as crystal shape and size will be depicted. Furthermore accompanied problems like co-precipitation of impurities will be discussed.

As a conclusion, it could be clearly shown that industrial protein crystallization can be a powerful tool with economic significance for purification, concentration and formulation of therapeutic proteins. Finally we will describe general guidelines how to proceed during development and implementation of crystallization into downstream processes.

1117

# A new generation of powerful chiral stationary phases for HPLC, SFC, and SMB.

**Eric Francotte*, P. Richert, G. Diehl, D. Huynh, J. Priess**

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## ABSTRACT

The usefulness and broad applicability of chromatography on chiral stationary phases (CSPs) as a powerful approach for the preparative separation and isolation of pure stereoisomers from the milligram scale up to the production scale is now indisputably recognized. The technique is now considered as a complementary approach to the more conventional methodologies such as enantioselective synthesis and enzymatically catalyzed transformations. This successful development would not have been possible without the design and preparation of efficient CSPs, exhibiting the specific characteristics required for preparative chromatographic separations regarding performance, loadability, physical and chemical robustness. Although the stereoisomers of most chiral compounds can be separated using a limited number of chiral stationary phases, the poor solubility of the enantiomeric or diastereomeric mixtures to be separated in the applied mobile phase still remains an obstacle to a more extensive and productive exploitation of the chromatographic technique. Indeed, solubility of the stereoisomeric mixture is often a critical issue and considerably affects throughput and productivity.

However, the recent developments made in the field of CSPs have opened fully new perspectives. Over the last few years, we elaborated processes to immobilize polysaccharide-based phases, making the CSPs resistant to any kind of solvents and thus considerably extending the possibility to improve the solubility of the solute by varying the mobile phase [1, 2]. Moreover, we found in many instances that the enantioselectivity could also be significantly increased when using ‘unconventional’ mobile phases. In order to fully exploit the potential of the new CSPs, we set up a fully automated screening system, capable of testing up to several hundreds of different combinations of CSPs/mobile phases over 24 hours [3].

By using deliberately the mobile phase as a key optimization parameter of enantioselectivity and solubility (of the solute), we were able to enhance remarkably the throughput of preparative chiral separations. Applications demonstrating the advantages of the novel immobilized CSPs will be shown for the three major

preparative chromatographic modes which are currently applied in the pharmaceutical industry, high performance liquid chromatography (batch), supercritical fluid chromatography (SFC), and simulated moving-bed (SMB) chromatography.

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1119

# ADVANCES IN CHROMATOFOCUSING WITH UNRETAINED BUFFERS FOR CHALLENGING PREPARATIVE PROTEIN SEPARATIONS,

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## ABSTRACT

Complex pH transitions occur in exchange columns containing weak acid or weak base during salt steps or gradients if the mobile phase is buffered and the buffering species do not interact with the stationary phase. If not anticipated and planned for within a separation step, they can result in large equilibration volumes and potentially negative effects on pH-sensitive proteins. On the other hand, such pH variations, if controlled and directed, can be beneficial to the separation of proteins. The behavior of chromatographic columns packed with resins containing both weak cation exchange and weak anion exchange groups is investigated in order to obtain protein separations by means of internally generated pH gradients in response to step changes in buffer composition. A local equilibrium model is developed to predict pH transitions using non-adsorbed buffers, i.e. containing neutral or anionic buffering species with cation exchangers and neutral or cationic buffering species with anion exchangers. In agreement with experimental results, the model, based exclusively on the resins' titration curves, predicts practical, fairly linear gradients, which are formed using suitable mixtures of acidic buffers for cation exchange and basic buffers for anion exchange. The separation of mixtures of ovalbumin, albumin, and transferrin is used as a model system with cation exchange columns while the separations of transferrin and of mAb charge variants are used as model systems with anion exchange columns. The peaks obtained with this approach are much sharper than could be obtained isocratically or using externally generated, unretained gradients as a result of the peak compression caused by the axial pH gradient formed along the column. Moreover, separation is obtained at very low ionic strengths (2-3 mS/cm). The effects of flow velocity, mobile phase composition, time of injection, and protein load on retention and elution pH are investigated systematically demonstrating a range of ways in which the separation can be controlled and optimized.

**Keywords:** pH gradients, chromatofocusing, local equilibrium theory, protein chromatography, resolution of protein variants

**1125**

# A new amino acid-based affinity chromatography approach to supercoiled plasmid DNA purification on arginine-agarose

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## ABSTRACT

Supercoiled (sc) plasmids are an important component of gene-based delivery vehicles, applied in new therapeutic strategies like gene therapy or DNA vaccination. However, the general distribution of plasmid DNA (pDNA) therapeutics requires a procedure to efficiently purify the sc plasmid isoform. Taking advantage of affinity interactions between amino acids and nucleic acids it was possible the implementation of new and interesting chromatographic strategies for the purification of pDNA [1, 2]. Arginine-agarose matrix was first used to fully separate supercoiled (sc) and open circular (oc) pDNA isoforms [2]. The characterization of sc retention was also achieved by performing several binding/elution experiments, which lead to the establishment of two possible elution strategies, either using NaCl or arginine increased gradients [3]. These results showed that the arginine matrix promotes multiple interactions with pDNA, including not only electrostatic and hydrophobic but also biorecognition of nucleotide bases by the arginine ligand [2]. For the reason that pDNA production is mainly achieved by autonomous replication in recombinant *E. coli*, the main challenge following is the sc recovery devoid of contaminating host biomolecules. In this way, the present work also describes a strategy which uses arginine-chromatography to purify sc pDNA from other isoforms and *E. coli* impurities present in a clarified lysate. Control analysis already showed that the isolated sc pDNA conforms to specifications in terms of pDNA homogeneity (≈100% sc) and no RNA and proteins are detectable. Furthermore, the transfection efficiency of COS-7 cells (62%) was significantly higher when compared with the efficiency (25%) of a pDNA control.

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1127

# An 18 Litre Process Scale High Performance Counter-Current Chromatography Centrifuge

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## ABSTRACT

The universal acceptance by industry of counter-current chromatography will depend on whether it can be seen as a competitive/complementary separation system in its tool chest of processes. Predictable scale up has been demonstrated [1,2], but comparative studies at the process scale where 30kgms of crude are handled in a few days for toxicology trials are required before industry will be convinced of the competitiveness of the process.

Dynamic Extractions Ltd has developed a range of high performance CCC centrifuges from analytical to process scale. Dedicated research has been performed at Brunel University into the scale up of countercurrent chromatography and new facilities like the Advanced Bioprocessing Centre have been invested in to house both pilot and process scale facilities in its hazards laboratory.

***Figure 1:*** Prototype 4.6 litre DE-Maxi centrifuge on the left and the newly installed 18 litre DE-MAXI centrifuge on the right. In the centre and on the screen in the control room in the foreground are the liquid handling system and control software

The 4.6 litre capacity DE-Maxi Centrifuge (R=300mm; d=10mm) has been running routinely and reliably now for 3 years. A number of manufacturing contracts have been performed, hence little has been published. Sample loadings on the 4.6 litre centrifuge have typically been 5% of the coil volume (230ml) and have varied from

100-500mg/ml giving throughputs of up to 15 kg a day when working with the bobbins in parallel and 7.5 kg/day in series working a 2 shift 16 hour day.

The latest 18 litre Maxi-DE centrifuge (Figure 1) has now been installed. The two centrifuges are mounted side by side with a common liquid handling control system which can operate each centrifuge separately in parallel or series depending on the required resolution.
Column capacities of 2.3, 4.6, 9 and 18 litres are now available to cope with separations requiring different resolution. Liquid handling flow rates up to 3,000 ml/min for parallel operation and up to 1,500 ml/min for series operation. Interestingly, while separations on the 18 litre Maxi will take 4 times longer than on the 4.6 litre Maxi, the throughput will be the same as the sample size can be increased in proportion to the capacity. Results will be presented demonstrating the performance of the new Maxi-DE centrifuge.

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Keywords: Counter-current Chromatography, CCC, Dynamic Extraction, Scale-up, Process Scale

**1131**

# Retention of amino-acids in ion exchange/exclusion chromatography

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## ABSTRACT

Ion exchangers uptake counterions from the solution through ion exchange on the resin's charged sites, whereas they exclude coions because of electrostatic repulsion. At the same time ion exchangers uptake neutral species through sorption thanks to van der Waals interactions on their polymer backbone in the case of resins. Strong electrolytes such as metal ions or halogen ions are either exchanged or excluded; species that cannot dissociate, e.g. sugars, are only sorbed. Weak acids and bases, particularly aminoacids, e.g. aspartic acid, are present in solution in undissociated form (typically as zwitterions), or in protonated form (at very low pH), or as dissociated acid (at higher pH). The different forms interact with the ion exchanger differently, i.e. one is retained whereas the other is excluded. In an ion exchange column the pH may vary along the column due to the development of the elution profile for all the species involved. Accordingly, the net valence of a given amino-acid may vary along the column, and so may its effective retention behavior. Such feature has to be carefully accounted for when modeling the behavior of systems involving the species mentioned above. In fact, this is of key importance in determining the elution behavior of the amino-acids, and can be also exploited for separation purposes, e.g. in multicolumn chromatography.
In this work, we develop a model of an ion exchange column in the presence of amino-acids.

**1133**

# ‘Cycle to Cycle’ optimizing control of simulated moving beds – experimental implementation for a nonlinear chiral separation

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## ABSTRACT

Different approaches for the control of SMB processes have been proposed in recent years [1, 2]. Independently of how complex the control algorithm might be, they all require accurate feedback information to achieve good control performance. In the case of chiral separations the choice of monitoring techniques is rather limited. In a previous work our group has made use of online optical detectors, i.e. UV and polarimeter, whose accuracy limited the performance of the controller [3]. To overcome these difficulties a monitoring system based on HPLC measurements has been developed, implemented and validated.

In this monitoring system the HPLC measurements are carried out less frequently, i.e. once per cycle and the analysis time is in the range of the cycle time, which introduces a significant time delay in the measurements. As a consequence, the controller actions are based on more accurate and straightforward but less frequent and time delayed feedback information of the SMB plant.

This work presents new experimental results for the separation of guaifenesin enantiomers in Ethanol on Chiracel OD using our ‘cycle to cycle’ control concept together with the automated HPLC monitoring system. Various case studies have been chosen to assess the performance of the controller under common situations encountered during SMB operations: separation of racemic mixtures with different overall concentrations, in a range from 0.1g/L (linear) to 15g/L (nonlinear) regime; rejection of disturbances due to pump malfunctioning; setpoint tracking in the case of varying purity specifications or if the feed concentration changes in the course of the operation.

The results demonstrate the effectiveness of the SMB controller in combination with the newly developed monitoring system for chiral compounds to guarantee the fulfillment of process and product specifications.

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**1134**

# Direct capture of antibodies from fermentation broth using a new multi-modal high salt binding ion exchange material

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## ABSTRACT

With increasing production needs of especially monoclonal antibodies there is significant interest in developing efficient ion exchange processes for capturing proteins directly from high conductivity fermentation broths. Even though electrostatic interactions between proteins and oppositely charged immobilized ligands is the primary mode of interaction, additional interactions such as hydrophobic, van der Waals interactions and hydrogen bonding may also have a significant impact on selectivity in ion exchange systems used for protein capture. Protein properties, mobile phase composition and resin chemistry have an influence on the strength of these interactions.

A novel cation exchange multi-modal chromatographic material for the capture of antibodies from cell culture supernatant has been was evaluated with regard to its selectivity and performance. The combined effect of charge and other secondary interactions with this multi-modal chromatographic material enables binding of proteins under high salt conditions. In addition the secondary interactions contribute to the unique selectivity for the recovery of antibodies. Since the cation exchange material exhibited high binding at elevated salt concentrations the work with this material focused on a study of elution strategies to remove host cell proteins as well as multimeric forms of the antibody. After evaluating various elution protocols, a combined strategy of pH change and salt elution were shown to be an effective for the selective elution of antibodies.

**1135**

# Preparative Extraction by Chromatography of Organic Pollutants from Water Streams

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## ABSTRACT

A procedure designed to selectively extract traces of organic pollutants from a large amount of water is described and modeled. The procedure uses a continuous belt made of a thin canvas enclosing a mass of fine fibers of a nonpolar adsorbent. The belt moves through the body of polluted water, then through a tank filled with a suitable organic solvent, e.g., methanol. The organic pollutants are selectively adsorbed on the fibers contained in the belt, then desorbed by the organic solvent in which they are progressively concentrated. The system permits the progressive extraction of large quantities of trace organic pollutants from the water and the concentration of these pollutants into an organic solvent. This extraction procedure could be used to purify water streams from trace organic pollutants, either in the case of local, accidental pollution or in the case of systemic pollution of rivers by waste water. It seems particularly convenient to extract pharmaceuticals and pharmaceutical metabolites from waste waters or from drinking water.

## Poster Presentations

**2002**

# Resin optimization for affinity chromatography media

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## ABSTRACT

Protein A affinity chromatography has been the workhorse in downstream processing of monoclonal antibody. With the advance in expression technology, fermentation with higher titer is becoming common practice. This demands increased capacity and productivity in the affinity chromatography step. One of the common base matrices for affinity chromatography media is controlled pore glass (CPG). As a relatively mature material, controlled pore glass provides rigidity, uniform pore size, and superior flow property. In this study, we examined parameters of CPG base matrix and identified key factors that influence the dynamic and static binding capacity of polyclonal and monoclonal antibodies. Pressure-flow property of the optimized media was also studied to ensure optimum productivity for mAb processing. This work provides guidance for base matrix optimization of Protein A affinity media. The result is a new media with ultra high capacity and productivity.

**2005**

# TREATMENT OF WASTEWATER CONTAINING TOXIC (Chromium) AND HEAVY (Cadmium) METALS EXTRACTION WITH OXYDE TRI-N-OCTYL PHOSPHINE

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## ABSTRACT

Humanity has been for a few years in front of an alarming growth of the water pollution by heavy metals, one can quote primarily chromium, cadmium, lead, mercury, nickel, the zinc and copper contained in the galvanic rejections they have an impact on the plants and the products for current human consumption, even with low contents.

The reactions for the extraction of Cd(II) from orthophoric acid are quite complicate because various of Cd(II) species may be present: $CdH_2PO_4^+$, $CdPO_4^-$, $HPO_4^{2-}$ , $H_2PO_4^-$ and $PO_4^{3-}$ in aqueous phase were taken into account in the analysis of extractions. In this study, the mechanisms extraction of Cd (II) from orthophosphoric acid solutions with the oxyde tri- n- octyl phohoric were investigated.The determination of the distribution coefficients permitted to identify the mechanisms of extraction and transfer

The reactions for the extraction of Cr(VI) from sulphuric acid are quite complicate because various of Cr(VI) species may be present. In this study, the mechanisms extraction of Cr(VI) from sulphuric acid solutions with the kerosene solution of tri-n-octylphosphine were

Investigated. Various form of Cr(VI) and sulphuric acid, $H_2CrO_4$, $HCrO_4^-$, $Cr_2O_7^{2-}$ $CrSO_4^{2-}$, $HSO_4^-$ and $SO_4^{2-}$, in aqueous phase were taken into account in the analysis of extractions.

A chemical modelization has allowed to identify the extraction mechanism.

The process purification by membrane.would be particularly judicious in the case of the treatments of the industrial wastes containing heavy metals, it is very sparing out of water and raw materials (recycling of the reagents and extractants).

**Key words**: chromium, cadmium, purification, extraction, ,treatment, effluent.

**2008**

# Chiral chromatographic separation of o,p' dichlorodifenyldiclhoroetane (mitotane) in semi-preparative columns of o,o'bis[4-terc-butylbenzoyl]-n,n'-diallyl-l-tartardiamide and separation regions for a simulated moving bed

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## ABSTRACT:

Mitotane (o, p'-dichorodiphenyldichloroetane) is a drug used in the treatment of adrenocortical carcinoma. It is marketed in the racemic form of their R and S enantiomers. This study concerns the separation of mitotane enantiomers using HPLC columns with the chiral stationary phase O,O'-bis[4-*terc*-butyl-benzoyl]-N,N'-diallyl-L-tartardiamide. Pulses experiments with diluted solutions of a inert compound and the enantiomers were accomplished at different flow rates and temperatures. The system porosities, chromatographic parameters, equilibrium constants, axial dispersion and mass transfer parameters were obtained. The results showed satisfactory separation with number of plates overcoming 9000 and separation factors in the order of 1.13. The overall mass transfer coefficient achieved values higher than 300 $cm^{-1}$, demonstrating low mass transfer rate effect and consequently a prevalence of the thermodynamic effects. Experiments in overload conditions were realized in order to determine the isotherms using the method of frontal analysis as well as the chromatograms under these conditions. For the mixture concentration smaller than 16 g/L the isotherms showed a good adjustment to the Langmuir model. Based on the experimental data for one column, the separations regions for a simulated moving bed system were determined with different feed concentrations, which permit the comparison of the performance parameters using the continuous system and the batch ones.

**2010**

# Engineering of a scFv affinity column for separation of cytokines

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**ABSTRACT:**

There is a paradigm shift in use of small peptide and protein ligands for biopharmaceutical production. With the aid of a highly selective affinity chromatography as capture step a multistep procedure can be reduced to two steps. Recently GE Healthcare has launched a two step purification procedure for production of recombinant antibodies.
Single chain fragment variable antibodies (scFv's) against Interferon $\gamma$ are used as model system to explore the efficiency of single chains for cytokine purification. ScFv's generated from human single fold scFv library have been immobilized on Sepharose FF. After demonstrating the high selectivity by purification of *E.coli* derived recombinant human Interferon $\gamma$, the engineering parameters to characterize process affinity chromatography have been elucidated. Uptake kinetics in an infinit bath, breakthrough curves and adsorption isotherms were determined. Also the affinity of the ligand was measured by surface plasmon resonance. Applying a pore diffusion model effective diffusivities have been calculated. A scale up scenario will be discussed.

**2011**

# A Potential Paradigm Shift in Downstream Chromatographic Purification of Monoclonal Antibodies

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## ABSTRACT

Chromatographic purification of monoclonal antibodies appears to be safely sheltered in platforms, currently well-established in the leading bio-pharmaceutical companies by means of multiple large scale processes. However, natural upstream process evolution compels us to peer into the future to prepare ourselves before downstream chromatographic purification process becomes a severe bottle-neck. This is heightened not only by a projected increase in the number of monoclonal antibody therapies, but also by concomitant improvements in upstream productivity, and the drive to improve process economics at scale. As a contribution to ongoing efforts to address this potential downstream bottle-neck issue, more recent generation of chromatography resins have been engineered to have improved resistance to pressure (ex. rigid or semi-compressible resins), affording increased flexibility in scaling up processes. This case-study presentation will discuss overall productivity gains using semi-compressible resins in a high-performance-liquid-chromatography (HPLC) column to purify a monoclonal antibody with various modalities (affinity and IEX). Key benefits and challenges pertaining to the use of the semi-compressible resins in HPLC mode will be highlighted.

**2013**

# Important Aspects when Designing Chiral Preparative Separations using Coated Polysaccharide Stationary Phases

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## ABSTRACT

Acidic and basic additives are frequently used in chiral chromatographic separations of acidic and basic compounds in order to improve peak shape by suppressing achiral interactions. In preparative chromatography, conditions without additives are desirable in order to achieve an uncomplicated solvent recycling process. It is also an important point that the resolved compounds should be easily isolated, and that additives can lead to degradation as they are accumulated during work-up. Data demonstrating the interesting phenomenon that overloaded studies are needed early in method development in order to choose CSP and decide if mobile phase additive is needed will be presented together with examples where Kromasil® CelluCoat™ show very unique characteristics compared to what is expected.

Another important aspect especially in batch chromatography is to achieve a highly efficient and stable packed bed without mechanically destroying the silica. Data illustrating the influence of packing pressure on plate-count for Kromasil® CelluCoat™ and Kromasil® AmyCoat™ will be presented. The data clearly demonstrates the benefit of mechanically strong silica that can withstand a high packing pressure.

**2014**

# The chromatographic isolation of reference standards – a case study

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## ABSTRACT

The production of active pharmaceutical ingredients requires the development of precise and efficient analytical methods able to identify minor impurities unambiguously. For the development of these analytical methods and the characterization of impurities it is often required to obtain the by-products and intermediates of a synthesis sequence in g-amounts. Various approaches can be considered to isolate the compounds, ranging from re-work of mother-liquors, crystallization, and chemical synthesis to chromatographic purification of the impurities.

A case study will be used to explain strategies how to isolate minor impurities via chromatography as a reference standard, giving special attention to the best choice of stationary and mobile phase and the conditions for isolation of the compound of interest. The structure elucidation of the isolated compound using 2-dimensional NMR techniques and LC-MS will be described.

**2017**

# Systematic Study of Production Processes Integrating Chromatographic Separation and Isomerisation Reactions

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## ABSTRACT

A systematic study is performed of integrated processes combining chromatographic separation and isomerisation reactions like, for example, racemisation reactions. In the first part of the study, different true moving bed (TMB) processes with different degrees of integration are investigated and optimized. In particular, the concept of spatially distributing the “reaction functionality” within the unit is investigated. The influence on process performance of parameters such as the equilibrium constant or the separation coefficient is demonstrated. Furthermore, beneficial effects of the “distributed” schemes that were deduced from the optimization results will be discussed. Their origin will be analysed on the basis of equilibrium theory.
A second part of the study investigates the possibilities to transfer the results to the simulated moving bed (SMB) process. Further, conventional integrated schemes (like the Hashimoto process which uses side reactors) are compared to fully integrated SMB processes with distributed functionalities (i.e., process that employ chromatographic reactors). It is shown that -for a low number of columns- the latter can outperform the classical Hashimoto concept. This is a motivation for further investigations to develop and design improved integrated chromatographic processes.

**2019**

# Adsorption behavior of sugars versus their activity in single and multicomponent liquid solutions

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## ABSTRACT

The importance of carbohydrate solutions in many industrial processes is well recognized nowadays. Carbohydrates are produced as mixtures of different types of sugars; therefore further separation is required for most applications. The successful prediction of separation process performance rests on the ability to describe the relevant thermodynamic and rate parameters. The accurate prediction of activity coefficients in solutions is essential for the process design.
In this work the activity of carbohydrates: sucrose, glucose and fructose in high-concentrated aqueous solutions was studied for both single and binary mixtures. The solubility properties of sugars for different temperatures were correlated basing on the Schröder-van-Laar-equation with the NRTL (Non-Random-Two-Liquid) model of activity coefficient formulation. The activity of individual sugars was quantified from the solubility properties and incorporated into the isotherm model, which reproduced accurately convex shape of the isotherm courses obtained in a previous work [1]. Based on the phase equilibria for binary systems of sugars with water, the sugar activity in mixtures was established. The determined activity along with the calculated single component isotherms was used to predict competitive adsorption isotherms by applying the Real Adsorbed Solution theory [2]. The isotherms were compared to the data of competitive adsorption from the former study [1]; the experimental results were found to be fully predictable.
The results obtained will be presented and discussed.

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**2020**

# Binding of Water-Soluble, Globular Proteins to Anionic Model Membranes

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## ABSTRACT

The role of electrostatics in the adsorption process of proteins to preformed negatively-charged (phosphatidylcholine/phosphatidylglycerol, PC/PG) and neutral (PC) small unilamellar vesicles (SUVs) is studied. The interaction is monitored, at low ionic strength, for a set of model proteins as a function of p*H*. The adsorption behaviour of lysozyme, myoglobin and bovine serum albumin (BSA) with p*I*s = 10.7, 6.9 and 5.5, respectively, with preformed SUVs is investigated, along with changes in the fluorescence emission spectrum of charged proteins, *via* the adsorption on SUVs. Significant adsorption of the proteins to negatively-charged SUVs is only found at p*H* values, where the number of positive charge moieties exceeds the number of negative charge moieties on the protein by at least 3 e.u. Negligible adsorption to SUVs composed of zwitterionic lipids is observed in the tested p*H* range (4–9), except for formally dianionic cardiolipin (CL). The fluorescence emission of positively-charged proteins increases after adsorption on negatively-charged SUVs. With increasing protein to phospholipid ratio, the increase in the fluorescence emission levels off and reaches a plateau; protein adsorption profiles show a similar shape. Analysis of the data demonstrates that neutralization of the SUV charge is the controlling factor in their adsorption.

**2021**

# Sub-/Supercritical fluid chromatography with propane

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## ABSTRACT

During the last 20 years the interest in use of supercritical fluids for preparative chromatography has been increased permanently [1]. Compared to chromatography with conventional liquid solvents, the use of supercritical fluids as mobile phase offers many advantages, e.g. the low viscosity makes possible high flow rates at a moderate pressure drop. As a consequence of the high diffusion coefficients of supercritical fluids a high number of theoretical plates can be reached. Both lead to high productivity. The most common used supercritical fluid is carbon dioxide. This study deals with the sub-/supercritical fluid chromatography using propane as exceptional mobile phase.

Carotenoids, in particular β-carotene, have long been known for their provitamin A activity, as they can be transformed into vitamin A in vivo. The separation of carotenoids can be achieved using supercritical fluid chromatography with supercritical $CO_2$ as mobile phase [2]. However, due to the low solubility of carotenoids in supercritical $CO_2$, cosolvents such as methanol often are necessary. While the solubility of carotenoids in propane is much higher [3], cosolvents aren't needed. Solubilities and adsorption isotherms in propane were determined. The influences of temperature and pressure were investigated and the differences of SFC with propane and SC $CO_2$ in retention behavior of carotenoids were compared.

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**2023**

# A new silica gel from Zeochem AG for preparative HPLC: ZEOsphere

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## ABSTRACT

Zeochem AG produces on large scale silica gels for liquid chromatography since 25 years.

ZEOprep, which is an irregular, broken material, is mainly used in batch adsorption processes, in flash chromatography or in SPE.

The new spherical silica gel of Zeochem AG – **ZEOsphere** – is made for preparative HPLC.

Physical properties (pore characteristic parameters, particle and pore size distributions, purity, particle shape and mechanical stability) of this new media will be presented and compared to competitor's products.

The comparison will be enlarged involving the chromatographic properties (hydrophobicity, silanol activity, steric selectivity, hydrolytic stability and separation of basic compounds) of C18 modified ZEOsphere.

**2024**

# Determining optimal operating conditions for preparative purifications with varying feed composition

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## ABSTRACT

Today, changes in the feed quality are considered a part of the control space of preparative purification processes. This means that a process will purify feeds with varying amounts of impurities in the same way. We propose to analyse the feed composition and use this information to at-line set the optimal process performance for each batch to be purified. This extension of the design space will ensure a higher performance at all times in the purification processes.

The methodology uses a calibrated model of the process to optimize the process for different amounts of target product and impurities. This will give optimal operating conditions for a number of feed scenarios. For example, easier purifications with lower amounts of impurities might give the opportunity to increase the loading factor and thus increase the productivity. Lowering the loading factor for batches with higher amounts of impurities can give higher yields. Elution gradients and cut point settings can also be changed for the different feed scenarios, ensuring an optimal process at all times. For easy use in the process, the operating conditions can be tabled for different feed compositions.

A case study on the purification of an insulin analogue will show the method's validity and usefulness.

**2026**

# Determination of robust pooling in preparative chromatography

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## ABSTRACT

In preparative chromatography the pooling of the eluted peaks is based on the UV-signal, which does not differentiate between proteins. Process variations may cause the eluted peaks to overlap; therefore the choice of UV-gate for the target protein becomes a critical process parameter. In this poster we present a model-based method for robust determination of UV-gate. Separation of Immunoglobulin G (IgG) from Myoglobin and Bovine Serum Albumin (BSA) on an ion-exchange column was modeled. Using Latin Hypercube sampling, process variations within the normal operating range was simulated and the optimal UV-gate with respect to productivity was found for each scenario. Robustness, expressed as probability of batch failure, was related to the expected yield for the UV-gate choices. The worst-case UV-gate was completely robust but reduced the yield significantly. The process knowledge embedded in the model could be used to refine the UV-gate further; the modeled outcomes were used to correlate the UV-gates to variations in other peak characteristics such as time of elution, conductivity in the buffer and time of elution of a peak preceding the product peak. This allowed for varying the UV-gate between batches and thereby increased the yield while keeping the risk of batch failure low.

**2027**

# Cation-exchange adsorption of the two major whey proteins

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## ABSTRACT

This paper describes the ion-exchange adsorption of the two major whey proteins, alpha-lactalbumin (ALA) and beta-lactoglobulin (BLG) with the purpose of optimizing a process for isolating them from whey. Adsorption of pure BLG and ALA was studied onto the cation-exchanger SP Sepharose FF, at 20$^{o}$C using 0.1M acetate buffer. Batch experiments were carried out at pH 3.7, 4.0, 4.5 and 5.0 for BLG; and pH 3.7 for ALA (due to its limited solubility at the other pH values), and the relevant Langmuir isotherm parameters, dissociation constant, $K_d$, and maximum binding capacity, $q_m$, were determined. For BLG, the most favourable pH range for adsorption when considering the values of $q_m$ was found to be 4-4.5. However when considering the values of $K_d$, the lowest value, corresponding to the strongest binding, was achieved at pH 3.7. At pH 3.7, both $K_d$ and $q_m$ pertaining to ALA were found to have higher numerical values than those of BLG, implying different characteristics of adsorption of the two proteins on this adsorbent. The $K_d$ for the former protein was almost four times larger than the latter, while $q_m$ was 1.3 times higher. Packed-bed column adsorption was performed on Akta Explorer 100 using a 1-ml column at 20$^{o}$C, pH 3.7, flowrate 1ml/min and initial concentration ($C_o$) of 3mg/ml for BLG and 1.5mg/ml for ALA both in 0.1M sodium acetate buffer. The $t_{1/2}$ for the resulting ALA breakthrough was 75% longer than its BLG counterpart. The above results suggest the possibility of the occurrence of competitive adsorption between the proteins when adsorbed simultaneously.The packed-bed adsorption and elution of a mixture of ALA and BLG were then investigated under the above conditions but using a 5-ml column. BLG breakthrough occurred first, and its concentration in the outlet exceeded its feed value by 1.6 fold before declining to the feed value, followed by the breakthrough of ALA. ALA displaced and eluted all the BLG from the column in a pure form. Pure ALA could then be eluted with good recovery. The evidence of the competitive nature of adsorption observed in binary mixtures can be used to develop a facile separation procedure for the two proteins from whey.

**2028**

# Simulation and optimization of two and three components SMB separations

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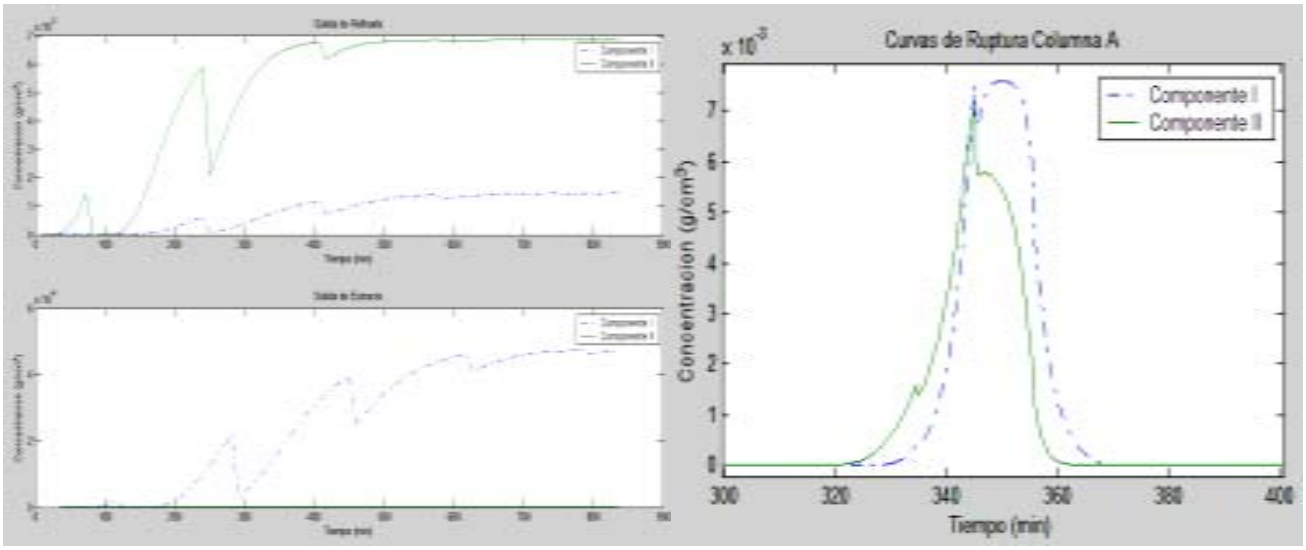
## ABSTRACT

The software capable to simulate the behaviour of a continuous separation SMB system has been developed. The system can model two or three component separation. It also can be used in the determination of single components breakthrough curves. The parameters that must be included in the program are the geometrical characteristics of the column (length, diameter and system dead volume), the porosity of the packing substance and the constants that define the adsorption isotherm which can be linear or Langmuirian. The optimal flows that circulate inside each one of the four areas of the SMB and the switch time are determined using the “Triangle Diagram”. The program allows choosing between different column conformations in order to reach the optimum of operation. In two component separation it is possible to work with an SMB with 4, 6 or 8 columns. In three components separation, it is done in two linked SMBs with different number of columns: 4+4, 6+6, 6+4, 8+8, 8+6 or 8+4 taking into account that each zone has not necessarily the same number of columns. The optimal conformation depends on the purity of the obtained products and the amount of time needed to reach the steady state. The results that present the program are graphics concentration *vs.* time both in Raffinate and Extract and purity of the products in the same currents; the Euclidean graphic (number of switches to reach the steady state) and finally the chromatogram of each column at each switch time. The comparison of these graphics for each conformation allows choosing the best one for each process.


Curvas de Ruptura Columna A
x 10^-3
Componente I
Componente II
Concentracion (g/cm^3)
Tiempo (min)
300
320
340
360
380
400
0
1
2
3
4
5
6
7

**2029**

# Characterization and evaluation of Amberlite XAD7HP for use in the expanded bed adsorption of flavonoids

**Jing Li and Howard A. Chase**

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## ABSTRACT

The expansion and hydrodynamic properties of expanded beds of the macroporous adsorbent Amberlite XAD7HP were investigated and analyzed. The bed expansion as a function of operational fluid velocity was measured and correlated with the Richardson-Zaki equation. Theoretical predictions of the correlation parameters (the terminal settling velocity $u_t$ and expansion ratio $n$) were improved by modifying equations in the literature. The residence time distribution (RTD) was studied using acetone as a tracer. Three evaluation factors (the height equivalent of theoretical plate, Bodenstein number and axial distribution coefficient) were investigated and compared to commercial EBA adsorbents developed for protein purification. A suitable bed expansion ratio was found to be 1.25, with a corresponding flow velocity of 183 cm/h and the mean residence time of liquid in the expanded bed was around 28 min for an initial settled bed height of 40 cm. Under these flow conditions, the axial mixing coefficient $D_{ax}$ was $7.54\times10^{-6}$ $m^2$ $s^{-1}$ and the Bodenstein number was 28; the number of theoretical plates ($N$) was 19 and the height equivalent of theoretical plate ($HETP$) was 2.77 cm.

Rutin trihydrate was used as a model flavonid for characterization of the adsorption properties of Amberlite XAD7HP. Adsorption was observed to reach equilibrium within 3 hours with 70% of the adsorption capacity being achieved within 30 min. The maximum equilibrium adsorption capacity for rutin was estimated to be 43.0 mg/(g resin) and 46.4 mg/(g resin) when the results were fitted to Langmuir and Freundlich isotherms respectively. The adsorption performance was not seriously impaired by the physical presence of *Ginkgo biloba* leaf powder. The maximum adsorption capacity (Freundlich isotherm) was only reduced by 3.8% with a 5.5% increase in the dissociation constant, even when the concentration of *Ginkgo biloba* leaf powder was as high as 50 mg dry mass/mL. Assessment of adsorption kinetics revealed that the rate constant of for adsorption was only reduced by 15 % in the presence of leaf powder at 50 mg/mL. The results demonstrated that Amberlite XAD7HP should be suitable for expanded bed adsorption of flavonoids from crude extraction solutions.

**2030**

# The use of Expanded Bed Adsorption (EBA) to purify flavonoids from Ginkgo biloba L.

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## ABSTRACT

Three techniques (liquid-liquid extraction, packed bed adsorption and expanded bed adsorption) have been compared for the purification of flavonoids from the leaves of *Ginkgo biloba L.*. A crude Ginkgo extract was obtained by refluxing with ethanol for 3 hours. The yield of flavonoids for crude extraction was about 19 wt% and the purity of flavonoids in the concentrated extract was from 1.9 to 2.3 wt%. The crude extract was then dissolved in MilliQ water and centrifuged where necessary to prepare the feedstock for further purification. For the method using liquid-liquid extraction with ethyl acetate, the purity, concentration ratio and yield of flavonoids were 25.4-31.0 %, 16-18 and >98%. For the method using packed bed adsorption, Amberlite XAD7HP was selected as the adsorbent and clarified extract was used as the feedstock. The dynamic adsorption and elution curves were measured. For a feedstock solution containing flavonoids at a concentration of 0.25 mg/mL, the appropriate loading volume to reach a 5% breakthrough point during the adsorption stage was estimated to be 550-600 mL. The results from the elution stage indicated that the majority of impurities were eluted by ethanol concentration of 40% ethanol or below and efficient separation of flavonoids from the impurities could be achieved by elution with 50-80 % ethanol reaching an average purity of ~25 %. For the method using expanded bed adsorption with Amberlite XAD7HP as the adsorbent, the optimal operation conditions scouted during the packed bed experiments were used but unclarified feedstock could be loaded directly into the column. The overall yield of flavonoids from expanded bed adsorption reached 22.5 % and the productivity increased by about 33.4 % over that achieved using the expanded bed process. The overall process time and the volume of solvent used were decreased in the EBA process. The results suggest that the adsorption of EBA can greatly simplified the process flow sheet and in addition reduced the cost and time to purify flavonoids from *Ginkgo biloba L.* These results clearly demonstrate the potential for the use of EBA to purify pharmaceuticals from plant sources.

**2031**

# Rapid development of monoclonal antibody downstream processes for production of clinical Phase I/II material. A case study.

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## ABSTRACT

This presentation describes the development of a monoclonal antibody (MAb) purification process. Screening of chromatography resins and operation conditions are done in a High Throughput Process Development (HTPD) format. This is followed by optimization and robustness testing done in a small column format. The purification process is then transferred to a scale suitable for clinical Phase I/II. Clinical material for Phase I/II is produced in only a limited number of batches after which the used chromatography resins as well as other consumables are being discarded. At this stage pre-packed, pre-qualified and sanitized columns can with great benefits be used. A comparison between a process that utilizes ready to process (pre-packed) columns and a conventional process will be given.

**2032**

# Lab-scale optimization and scale-up of two purification steps using a mixed-mode and a novel ion exchange sorbent

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## ABSTRACT

The purification of β-lactoglobulin and of α-lactalbumin from microfiltrated whey were optimized, using pre-packed 1 mL-columns, on a mixed-mode sorbent (HEA HyperCel™) and a novel ion exchange sorbent, respectively.
After the direct loading of whey with no pH or conductivity adjustment, β-lactoglobulin was fully retained on HEA HyperCel equilibrated in PBS, pH 7.4 and α-lactalbumin, fully retained on the novel ion exchanger in a phosphate buffer, pH 6.8. β-lactoglobulin was recovered at pH 4.0 from HEA HyperCel while most of the proteins (including α-lactalbumin) were eluted at pH 3.0. α-lactalbumin was recovered in a phosphate buffer containing 80 mM NaCl from the novel ion exchanger, while most of the proteins were recovered in the flow through (IgG) or in a buffer containing 200 mM NaCl (β-lactoglobulin). After these steps, the purity of β-lactoglobulin and α-lactalbumin was over 75%. The transfer of these steps to pilot-scales, using a 1 Litre- and 4 Litre-column, and to large-scale, using a 21 Litre-column, was successful for the separation of α-lactalbumin on the novel ion exchanger. It required some optimization for the separation of β-lactoglobulin on HEA HyperCel.
The main benefits of these purification steps were that no pre-treatment of the microfiltrated whey was required before loading on the 2 sorbents and both proteins of interest were partially-purified over 75% using scalable single steps.

**2034**

# New Process Scale Stationary Phase for Peptide Purification

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## ABSTRACT

Here we report about DAISOGEL MP, a novel type of chemically modified stationary phase aimed to push the silica frontiers further. A very important aspect of the chemical modification is to shield the base silica from the aggressive mobile phase. The previously introduced DAISOGEL BIO types can be used at a wider pH range by means of a higher surface bonding density and special endcapping, while the DAISOGEL MP is a polymerically modified type: the abundance of bonded phase is serving as the protective layer. DAISOGEL BIO has a wide application range, while MP is focused on molecules around 6000 Dalton molecular weight.
On the poster the results of alkaline (basic) and acidic resistance tests will be provided and discussed.

The most important outcome of our research is a whole new group of extended pH durability featuring silica based process scale stationary phases. It is clear, that each separation problem needs a custom made stationary phase. While it is difficult to actually design one perfectly matching phase for each and every process, a group of phases with definitely different SEPARATION properties to choose from is the so far missing tool for the large scale RP chromatography improvements.

**2035**

# Equilibrium theory based design of simulated moving bed processes under reduced purity requirements. Linear isotherms

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## ABSTRACT

SMB schemes operated under reduced purity requirements can be interesting where complete separation of the binary feed mixture is not essential. Such requirements, which are likely to grow in the coming years, exist in the agrochemical industries, where only an enantiomerically enriched product is often required. This is also useful for hybrid separations such as the SMB-crystallization process where a partial enrichment of the products is performed by the SMB.

The design of simulated moving bed processes under reduced purity requirements for systems whose isotherm is linear is considered. Based on the equilibrium theory of chromatography, explicit equations to uniquely identify the separation region that will ensure specified extract and raffinate purities are derived. In the frame of the "triangle theory", this results in a pentagonal region in the ($m_2$, $m_3$) plane, operating within which will ensure an extract and raffinate purity better than the specified one. The identification of the vertices of this region requires only the knowledge of Henry constants of the solutes, the concentration of the solutes in the feed and the purity specifications. The derivation of the design equations is illustrated and these results are validated using numerical simulations.

**2037**

# ROBUST DESIGN AND OPERATION OF TWO- and THREE-COLUMN COMPACT SMB PROCESS FOR BINARY AND TERNARY SEPARATIONS

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## ABSTRACT

Over the last years there has been intensive research on simulated moving-bed (SMB) chromatography. As a result, several new operating procedures and schemes have been developed to improve the separation efficiency, thus allowing for increased productivity, unit downsizing, or simplification of the process flowsheet.
In this work we report a class of simple SMB schemes, using only two and three columns, to achieve both binary and ternary separations. These schemes have the advantage of employing fewer columns, and requiring only two or three pumps, which renders the system more economic; the set-up is also simpler to build. However, these compact SMB schemes expand the degrees of freedom for operating the cycle, which renders them more sensitive to disturbances, namely flow-rate stability and parameter uncertainty. Therefore, a robust design of the system is needed.
A robust design of the proposed two- and three-column processes has been developed for protection from flow-rate, dead-volume, and isotherm uncertainties. The optimal cycle parameters are chosen only among candidate solutions that are robust feasible. This gives rise to a robust approach to optimal design in which the nominal problem is replaced by a worst case problem. The nominal optimization problem and its robust counterpart are formulated using a full-discretization approach for steady periodic dynamics. The resulting nonlinear programming problems are solved by an efficient interior-point solver.
The procedure is successfully employed to find robust operating conditions for the linear separation of nucleosides mixtures by reversed-phase. The robust schemes are validated experimentally. Emphasis is given to the pros and cons of running the processes under nominal and robust operating conditions.

**2038**

# Development of a 'cycle to cycle' control for the MCSGP-process for a monoclonal antibody variant separation

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[2] ChromaCon AG, Technoparkstr. 1, CH-8005 Zürich, www.chromacon.ch
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## ABSTRACT

The MCSGP process is a new continuous chromatographic process specifically designed for the purification of proteins and peptides. Due to its counter-current purification principle, the performance increase with respect to the batch process can be up to 10x in productivity and more at even higher yields[1]. Various successful applications of the MCSGP-technology have been reported, e.g. the purification of monoclonal antibodies from high-titer supernatants with ion-exchange resins and the purification of polypeptides.

However, optimum MCSGP operation is a challenge and the current practice is to operate the MCSGP units at sub-optimal conditions to guarantee robustness. As a result, control and automation of MCSGP is of great interest in order to exploit the full economic potential of this process. An automatic control algorithm for MCSGP units that guarantees an optimal, robust operation with product purities in specification is a challenging problem because of the complex dynamics involved in this process, i.e. its cyclic and hybrid nature due to the inlet/outlet port switching with strong nonlinearities and delays in the feedback information.

In this work, a control algorithm based on previous work[2] is developed for the MCSGP-technology. The flow rates as well as the salt gradients have been chosen as manipulated variables. The suitability of the controller is proven using the separation of three monoclonal antibody variants as model system for the simulations.

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[1] http://www.chromacon.biz/PressRelease_MCSGPpeptides_Novartis.pdf
[2] Grossmann, C., Erdem, G., Morari, M., Amanullah, M., Mazzotti, M., Morbidelli, M., 'Cycle to cycle' optimizing control of simulated moving beds. AICHE JOURNAL, Volume: 54, Pages: 194-208, Published: 2008

**2040**

# Supercritical Fluid Chromatography Enantioseparation of Flurbiprofen: Determination of Non-Linear isotherms

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## ABSTRACT

Supercritical fluid chromatography, owing to its reduced solvent consumption and the use of benign solvents is being increasingly adopted as an essential chiral separation tool in the food and the pharmaceutical industry. Further, since SFC offers, much higher efficiencies at larger flow rates, analysis and separation times can be considerably reduced directly leading to reduction in batch processing times.

In this study, the chiral separation of flurbiprofen - a key non-steroidal anti-inflammatory drug (NSAID) on chiral stationary phase based on Chiralpak AD-H was studied. Carbon dioxide modified with methanol was used as a mobile phase. The elution profiles of the pulse injections indicated that the isotherm was Langmuirian. The isotherm parameters were estimated using two methods. The first method involved the determination of the isotherm parameters by fitting the simulated elution profile to the experimentally measured one. The second method involved an inverse method which uses the Henry constant measured from dilute injections and the elution time of the shock front from the overloaded injection of a pure enantiomer. The effect of operating conditions, particularly operating pressure (130 to 180 bar) and modifier compositions (13 to 23 %) on the adsorption isotherm parameters was studied. At high injected concentrations, the elution profiles showed marked deviation from the theoretical predictions. These deviations were characterized by increased elution times of the tails of the heavier component with increasing injected concentrations. It is believed that these deviations are caused due to poor solubility of the solute at the column inlet. A mathematical model to account for this was constructed and the deviations from the theoretical predictions were accounted for.

**2042**

# Influence of different spacer arms on A2P affinity membranes for human IgG capture

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[2] Department of Analytical Chemistry and Food Chemistry, University of Vienna, Waehringerstr. 38, A1090 Vienna, Austria

## ABSTRACT

In the course of an ongoing research program to investigate the biomimetic synthetic affinity ligand A2P for capturing of monoclonal antibodies, A2P was immobilized onto a cellulose based membrane (Sartobind Epoxy) via different spacer chemistry. In the first attempt A2P was bound via a standard spacer by Prometic Biosciences (A2P-Sartoepoxy). These membranes are endowed with a good binding capacity for IgG, although an incomplete protein recovery had been observed together with high sensitivity towards Pluronic F68. This led to the development of two new, alternative immobilization strategies for A2P: i) via a thiophilic spacer and ii) via a triazole spacer as a result of a Click Chemistry concept.
Both reaction types are well controllable and reproducible, resulting in membranes with higher binding capacities. These new affinity membranes have been fully characterized in batch and dynamic experiments obtaining improved binding capacities for IgG and performances when compared to the A2P-Sartoepoxy affinity membrane used as a benchmark.

Acknowledgement:
This work has been performed as part of the “Advanced Interactive Materials by Design” (AIMs) project, supported by the Sixth Research Framework Programme of the European Union (NMP3-CT-2004-500160).

**2043**

# Theoretical investigation of separation of a ternary mixture by Simulated Moving Bed Chromatography

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[4]Chair of Chemical Process Engineering, Otto-von-Guericke-University, Magdeburg, Germany

## ABSTRACT

Simulated Moving Bed (SMB) chromatography is a well-established process that can be applied at widely different scales for adsorption based separations. There is an increasing interest in isolating a target component out of a multicomponent feed mixture. Since the conventional 4-zone SMB system is unable to produce a pure product stream if the desired component is neither the strongest nor the weakest adsorbable component, various modifications of SMB systems have been proposed [e.g. 1, 2].

In this work a separation of a ternary mixture based on simulated moving bed technology and the assumption of linear adsorption isotherms is investigated.

Two coupled 4-zone SMB units connected in series by extract or raffinate port operated in semi-continuous and continuous mode are analyzed and subsequently compared to an integrated 8-zone SMB unit with internal recycle. As the major aspect of this research, the influence of the separation factors and the excess of the weaker/stronger adsorbed component in the feed mixture on the separation performance are studied. Separation under reduced purity requirements for the target component is also analyzed. Finally, the potential of modulating the solvent strength during the process (gradient operation) is investigated.

To describe the performance of considered arrangements a standard dynamic model was applied. Different approaches for optimization will be compared.

[1] G. Paredes, S. Abel, M. Mazzotti, M. Morbidelli, J. Stadler, Ind.Eng.Chem.Res. 43 (2004) 6157

[2] B.J. Hritzko, Y. Xie, R.J. Wooley, N.L. Wang, AIChE J. 48 (2002) 2769

**2044**

# An economical approach to upstream decolorization

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## ABSTRACT

Within the pharmaceutical and biopharmaceutical industry, there are many process streams which require purification by some means or another. Typically, this requires that a specific impurity is removed or that a range of impurities are eliminated. In the latter case, these impurities are never fully characterized and are generally given the name “color species”. In all cases, the “color” is regarded as an impurity which must be removed in order to avoid any adverse quality problems downstream with the final pharmaceutical product.

Color in a process stream can be derived from a variety of different sources like fermentation from which the product is derived or degradation products. The molecular weight of these degradation products vary greatly but tend to have a carboxylic functionality.

These “color species” can foul and discolor resins used in the upstream portion of purification processes. Fouling and discoloration of expensive ion exchange resins can be avoided by the use of inexpensive ion exchange resins that can be used econmically in a disposable mode. We demonstrate protocols for effective decolorization of fermented product process streams.

**2045**

# A STREAMLINED TWO-COLUMN SMB PROCESS FOR CHIRAL SEPARATION

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## ABSTRACT

Simulated moving bed (SMB) chromatography is a powerful tool for chiral separation, because of its many advantages with respect to discontinuous batch chromatography, namely higher product purity, less solvent consumption, and higher productivity per unit stationary phase. Newly developed cyclic operating schemes and column configurations are pushing the trend toward the use of units with fewer columns and more economical set-ups.

Despite the lower productivity and higher solvent consumption of batch processes, they are advantageous in other respects, such as in their simplicity (less design effort is necessary and the scale-up is easier), flexibility (changes in conditions can be easily handled), and versatility (multi- and center-cut separations from multi-component mixtures are easy to implement).

Somewhere in between, and in an attempt to work with the best of “two worlds”, we have developed a compact, two-column open-loop SMB process for enantiomeric separation, which is particularly effective when the resolution is limited.

On the other hand, product quality control is mandatory for chiral separations, which requires robust on-line monitoring of the individual enantiomer concentrations. We have successfully implemented an on-line monitoring system, comprising an HPLC set-up with two UV detectors, to measure the individual enantiomeric concentration profiles in the SMB unit. Our monitoring scheme does not use a polarimeter.

The chiral separation considered in this work is the separation of Reboxetine enantiomers on Chiralpak AD, using a mixture of Hexane-Ethanol-DEA as solvent. This system is one in which there is clearly a lack of resolution between the two components. It is thus difficult to obtain both products with high purity and yield by HPLC for the chosen chromatographic conditions. Reboxetine, (RS)-2-[(RS)-□-(2-ethoxyphenoxy)benzyl]-morpholine is an antidepressive NRI drug. Only the (R,R)-, (S,S)-pair is present as a racemic mixture in the active principle and the commercial formulations. Recent studies support the hypothesis that the (S,S)-enantiomer is a more potent inhibitor than the (R,R)- and that it is responsible for the vasomotor and cardiac side effects of Reboxetine.

**2046**

# Rapid Removal of Detergents from Protein Solutions using Polymeric Reversed Phase Resins

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## ABSTRACT

Detergents are commonly used in hydrophobic cell membrane protein purification and viral removal/inactivation. These detergents must eventually be removed during the purification process, and specifically in vaccines production.

A rapid detergent removal protocol using short chromatographic columns has been developed. A number of different polymeric resins were screened for detergent removal. A polystyrene divinylbenzene polymer, Amberchrom ™ CG161, and an acrylic polymer, Amberchrom ™ CG71C, were employed for the removal of non ionic detergents from protein solutions. The results demonstrate that these resins exhibited high capacity and were effective in removing a wide range of detergents at high linear velocities with minimal loss in protein yield.

**2048**

# Implementation of an automated online HPLC monitoring system for SMB processes

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## ABSTRACT

In continuous chromatography, Simulated Moving Bed (SMB) is a firmly established powerful technique for the separation of fine chemicals and enantiomers [1]. Nowadays, SMB units are most often operated sub optimally to guarantee robustness of the operation and to account for system and operation uncertainties. The use of a controller can optimize the operation conditions and increase the productivity of an SMB unit.
In our group, a 'cycle to cycle' optimizing control concept for SMB units was validated experimentally for the separation of achiral and chiral components [2, 3]. The feedback information for the controller was determined with online optical detectors, i.e. UV-detector and polarimeter, positioned at the extract and raffinate ports. The accuracy of such devices is highly sensitive to experimental factors like impurities in the mixture to be separated or pressure fluctuation in the measuring cell; and has a direct impact on the performance of the controller. To overcome the limitations imposed by these devices an automated online HPLC monitoring system was developed and installed to measure the product composition at the outlet streams. This measurement technique leads to more accurate results, can handle multicomponent systems, and is less affected by possible impurities in the products compared to the optical detectors. However, the HPLC measurements can be carried out less frequently leading to an inherent time delay of the feedback information. This work presents the design and implementation of an automated online HPLC monitoring system that determines the average concentrations of the raffinate and extract stream over one cycle. The monitoring system was validated by comparing experiments with offline analysis and simulations.

[1] Mazzotti M. et al., *Journal of Chromatography A* ***769*** (1997) 3-24.
[2] Amanullah M. et al., *Journal of Chromatography A* ***1165*** (2007) 100-108.
[3] Grossmann C. et al., *AIChE Journal* **54** (2008) 194-208.

**2051**

# Measurement of Adsorption Isotherms of Tröger's Base Enantiomers for Chromatography-Crystallization Combined Process Design

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## ABSTRACT

The development of processes for the separation of enantiomers of chiral compounds has become more important over the last decade. Crystallization and chromatography are key technologies in this field and it is well known that Simulated Moving Bed (SMB) chromatography is widely applied. More recently, a combination of SMB chromatography, used for initial enrichment of the racemic mixture, followed by crystallization, for final purification of the enantiomers, has been proposed and discussed as a possibility for improving the process performance [1,2]. For the whole design of this process a thorough knowledge of the system is essential, i.e. competitive adsorption isotherms and solid-liquid ternary equilibrium among the two enantiomers and the solvent.

In this work, the adsorption isotherm of Tröger's base is determined on Chiralpak®AD with ethanol as mobile phase, which is supposed to be used as the model system for the combined SMB-crystallization process. Overloaded pulse injection and breakthrough experiments are performed to obtain the isotherm. The elution profiles revealed Langmuirian-like behavior. Additionally, taking advantage of a detailed SMB simulation model and a thermodynamic model for the solid-liquid equilibrium of Tröger's base enantiomers in ethanol [3], the design of the combined process is discussed and its performance (productivity and solvent consumption) is analyzed for different cases.

[1] H. Lorenz, P. Sheehan, A. Seidel-Morgenstern, Coupling of simulated moving bed chromatography and fractional crystallisation for efficient enantioseparation, J. Chromatogr. A, 908 (2001) 201.

[2] M. Amanullah, M. Mazzotti, Optimization of a hybrid chromatography-crystallization process for the separation of Tröger's base enantiomers, J. Chromatogr. A, 1107 (2006) 36.

[3] J. Worlitschek, M. Bosco, M. Huber, V. Gramlich, M. Mazzotti, Solid-liquid equilibrium of Tröger's base enantiomers in ethanol: Experiments and Modelling, Helvetica Chimica Acta, 87 (2004) 279.

**2052**

# Equilibrium and kinetics of IgG adsorption on a strong cation-exchanger.

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## ABSTRACT

Chromatographic techniques belong to the most important unit operations in the purification of monoclonal antibodies for therapeutic use. Multiple models of protein binding such as affinity, ion exchange or hydrophobic interactions are employed. The cation-exchange chromatography was the main focus of this study. This type of chromatography is most typically applied as a polishing step in downstream purification after Protein A affinity chromatography although it has been also reported to be an effective capture step. A wide use of ion-exchangers is due to their low cost and mild conditions of purification.

Adsorption on cation-exchangers is highly dependent on the ionic strength. For this reason, the adsorption behaviour on FractoGel EMD SE Hicap (M), a strong cation-exchanger often used in monoclonal antibodies purification, was tested at several salt concentrations. The adsorption isotherms for IgG were determined using the static method at pH 4.5 in a 50 mM citrate-phosphate buffer. Individual isotherms were fitted by means of the Langmuir model. The steric mass action model was successfully applied for a simultaneous fit of all equilibrium data. An investigation of adsorption kinetics was carried out in batch stirred vessel with a recycle loop through a flow spectrophotometer. The kinetic data were evaluated using a pore-diffusion model.

Acknowledgement: This work was supported by VEGA project, Slovak Grant Agency for Science, No. 1/3565/06

**2053**

# Investigation of Bimodal Pore Structure of Sartobind Q Membrane Adsorbent Using Different Experimental Techniques

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## ABSTRACT

Sartobind Q membrane is a strong basic anion exchanger used in chromatographic separations that is formed by a macroporous cellulose support HydroSart and a grafted, swelling gel layer at the macropore walls. The quantitative characterization of pore structure was based on several experimental techniques: batch size exclusion, inverse size-exclusion chromatography, mercury porosimetry and permeability. The materials were used either in commercial, laboratory membrane modules, flat membrane chunks or packed columns. The size-exclusion experiments were carried using redistilled water, phosphate buffer and Tris-HCl buffer as mobile phases. Different ionic strengths of the buffers were set by the means of three different sodium chloride concentrations: 0 mM, 150 mM, 1000 mM, were conducted. Dextrans with varying molecular weights were used as molecular probes.

Evident differences in the bimodal pore structure of the adsorbent were observed depending on the composition of pore-filling liquid. Combining the results from all above-mentioned techniques for HydroSart, the mean specific volume and radius of macropores were determined. Permeability and batch size-exclusion measurements on Sartobind Q allowed a quantitative assessment of swelling/shrinkage of gel layer and consequently of the macropore pore radius and specific volume. Batch-size exclusion experiments provided the specific volumes of micro- and mesopores. The dependence of the partition coeffcient on hydrodynamic radius of solute probe obtained by size-exclusion chromatography could be then very well described by a simple bimodal model. The solute partitioning in the macropores were characterized by a simple cylindrical pore model for the mean macropore radius. The solute partitioning in the micro- and mesopores were characterized by the Ogston-Laurent-Killander model which two parameters were estimated from the fit of the data.

Acknowledgements: This study was supported by a grant of VEGA, Slovak Grant Agency for Science, No. 1/3565/06.

**2056**

# Dynamic Discrete Event Modelling and Mixed Integer Optimisation of Protein Downstream Processes

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## ABSTRACT

While in chemical and oil industry the use of computer aided process design tools based on detailed mathematical models is a state of the art method for the efficient design, scale-up and optimisation of integrated process, the application of models considering mass and heat transfer phenomena for process analysis and process optimization purposes, to design biological processes is not common yet. However, the potential benefits of computer-aided design methods lead to an increasing acceptance in the biopharmaceutical industry as the downstream process costs often exceed 50% of the overall production costs and the time-to-market has to decrease despite of limited budgets. The application of such tools helps reducing process development times by allowing different process sequences and operating conditions to be examined inexpensively by model based design. In this work a generic process model on the basis of detailed dynamic models for ion-exchange membrane adsorber, ion-exchange and size exclusion chromatography and ultra-/ diafiltration is presented. This approach allows for predicting the internal concentration profiles and performance parameters like yield or selectivity after each purification step. Moreover, transient states in the unit operations reflecting the influence of operation conditions on overall process performance can be taken into account to provide an improved accuracy for the detailed process design. The developed generic process model is linked to an evolutionary optimisation algorithm allowing for discrete continuous process optimisation with respect to the process structure and the process design variables on the basis of detailed dynamic models yielding a powerful simulation and optimisation tool.
A case study based on the chromatographic downstream process of human serum albumin demonstrates the applicability of the developed tool for process optimisation and process analysis. The required steric mass action model parameters for the ion-exchange membrane adsorbers present in the purification sequence have been experimentally determined on commercially available Sartobind Q and S membranes.

**2059**

# Preconceived ideas about Preparative Chromatography

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## ABSTRACT

Multicolumn continuous chromatography is more cost effective than HPLC for chiral separation - SFC is more productive than HPLC - Batch chromatography is a better process than continuous chromatography for the separation of complex mixtures – Doubling productivity will halve production cost - SFC is greener than liquid chromatography – etc.

For a number of years, the scientific community has developed, studied and compared preparative chromatography processes and defined some general statements which are now widely accepted. This has resulted in some interesting lectures and articles, but these general conceptions can be challenged by some “real life” examples.

A more general issue will be addressed: shouldn’t we spend more time convincing our fellow chemists to try chromatography a couple of steps earlier or later, or even to try it at all?

After all these years of efforts, do we think we have succeeded in bringing chromatography at the right level in the pharmaceutical industry? We will explore some market data and discuss the role that chromatography could play in tomorrow’s industry.

**2062**

# Integration of PAT (Process Analytical Technologies) in Continuous Chromatographic processes: The Advanced Control System

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## ABSTRACT

At SPICA 2006, we presented the experimental application of a novel Advanced Control System (ACS) for multi-column chromatography. Since that time intensive work has been performed and new results will be presented.

The ACS module make continuous chromatography fully PAT compliant and provide a better understanding and control of the process. This module will adjust operating conditions to improve the process robustness. These adjustments are possible thanks to two kinds of measurements: an “in-line” measurement (UV detector, polarimeter, densimeter…) and an “at-line” measurement (HPLC analysis). The combination of these measurements ensures a continuous control of the process operating conditions. This regulation is quick and simple with the “in-line” measurements, and accurate with the periodical “at-line” measurements.

The implementation of an ACS module greatly simplifies the use of SMB or Varicol® processes.

At production scale, the goal is to secure the process and optimize the productivity. Thus, the ACS detects technical problems, secures the process, automatically corrects deviations and maximizes the productivity while maintaining high purity and yield.

At laboratory scale, the goal is to simplify the use of such systems and to speed up process development. Thus, for each new separation, the user sets the specifications (purity, yield) and the ACS automatically brings the system to the optimal separation conditions.

**2063**

# Design and Optimization of a Chromatographic-Crystallization Hybrid Process for the Separation of Tröger's base Enantiomers

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## ABSTRACT

The hybrid chromatography-crystallization process, where the separation task is apportioned between Simulated Moving Bed (SMB) and crystallization processes to improve the overall process performance, has been proposed in the literature [1,2]. The key challenge for the design and optimization of such a process primarily come from availability of equilibrium data about the adsorption isotherm in the SMB process and the ternary solid-liquid equilibrium data in the crystallization process, in the same solvent and at the same temperature. Besides, a proper formulation of the optimization problem and an efficient computation tool are vital for a realistic evaluation of the hybrid process.

This work aims at design and optimization of the hybrid process for the separation of Trögers base enantiomers. The separation in the SMB part of the hybrid process is considered on Chiralpak AD stationary phase and in ethanol mobile phase. Adsorption isotherm for this system has recently been determined experimentally. The crystallization operation is accounted for through material balance assuming equilibrium between enantiopure crystals and mother liquor for which experimental data is available in the literature [3]. The multi-objective optimization problem is solved in terms of operating parameters and column length for the optimum SMB purity at which the performance of the hybrid process, in terms of maximization of the productivity and minimization of the solvent evaporation cost, is the maximum. Besides, the results are compared with a previous work where the SMB separation was investigated on microcrystalline cellulose triacetate stationary phase.

[1] H. Lorenz, P. Sheehan, A. Seidel-Morgenstern, Coupling of simulated moving bed chromatography and fractional crystallization for efficient enantiosepartion. J. Chromatogr. A. 908 (2001) 201.
[2] M. Amanullah, M. Mazzotti, Optimization of a hybrid chromatography-crystallization process for the separation of Tröger's base enantiomers. J. Chromatogr. A. 1107 (2006) 36.
[3] J. Worlitschek, M. Bosco, M. Huber, V. Gramlich, M. Mazzotti, Solid-liquid equilibrium of Tröger's base enantiomers in ethanol: Experiments and Modelling, Helvetica Chimica Acta, 87 (2004) 279.

**2064**

# Choice of Process Concept in Preparative Chromatography

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## ABSTRACT

In HPLC process design vast effort is made during method development. However, this essential step alone does never guarantee a separation operating at its economical optimum. The technical implementation of the separation as a batch, closed-loop or steady-state recycling or SMB process is the next crucial step on the way to a successful operation. Simple and therefore limited decision trees working with criteria like “scale”, “range of k'” and “number of fractions” are sometimes used to give a pre-selection [1]. Lacking more detailed methods classical batch chromatography is often the preferred concept, especially in early phases of process development.

Fast methods assisting the decision for a process concept promise a considerable improvement over the arbitrary application of batch chromatography. We aim at a method choosing and designing process concepts based on a small number of experiments and computer simulations. Our first step is to improve the above mentioned rough guidelines by means of further heuristic rules, i.e. a knowledge base generated by experimental as well as numerical methods. For the parameter studies performed we apply validated models of the respective process concepts. Utilisation of artificial adsorption isotherms helps us gathering information about various kinds of adsorption behaviours. Optimised process alternatives are evaluated by typical characteristics such as specific productivity, yield and eluent consumption with different emphases depending on the cost structure. Performing our studies with different feedstock compositions we account for the impact of displacement and tag-along effects. It becomes clear how the nature of the adsorption isotherm and the composition of the feedstock influence the suitability of continuous and discontinuous process concepts. The methods and guidelines derived will be presented.

1. Schulte M., Wekenborg K. and Wewers W., *Process Concepts*, in *Preparative chromatography of fine chemicals and pharmaceutical agents*, H. Schmidt-Traub, Editor. 2005, Wiley-VCH: Weinheim. p. 173-214.

**2067**

# Chromatographic purification of reference standards – a case study

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## ABSTRACT

The production of active pharmaceutical ingredients requires the development of precise and efficient analytical methods able to identify minor impurities unambiguously. For the development of these analytical methods and the characterization of impurities it is often required to obtain the by-products and intermediates of a synthesis sequence in g-amounts. Various approaches can be considered to isolate the compounds, ranging from re-work of mother-liquors, crystallization, and chemical synthesis to chromatographic purification of the impurities.

A case study will be used to explain strategies how to isolate minor impurities via chromatography as a reference standard, giving special attention to the best choice of stationary and mobile phase and the conditions for isolation of the compound of interest. The structure elucidation of the isolated compound using 2-dimensional NMR techniques and LC-MS will be described.

**2068**

# Implementation of a new Strategy for rapid Enantiomer Screening

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## ABSTRACT

When dealing with a rapidly increasing number of synthesized chiral compounds, the former way of enantiomer screening using analytical HPLC systems proved to be much to time-consuming. In order to increase our throughput in accordance with the number of chiral compounds synthesized at our site, we have adopted a new strategy for rapid enantiomer screening. Our goal was to substantially speed up the HPLC process as well as subsequent data analysis in order to find at least one condition per compound yielding sufficient enantiomer separation. A broad range of stationary and mobile phases was included into the essentially empirical screening process. Using a set of columns and eluents for the reverse phase chiral separation screening process, we increased throughput eightfold by using an HPLC system capable of running every sample on 8 different columns in parallel. This was possible due to the 8x FlowControl device of the 8 x Sepmatix System guaranteeing the same flow rate in all 8 channels. We present an example for quick enantiomer separation and subsequent data analysis that highlights how we have succeeded with the implementation of a rapid enantiomer screening strategy.

**2070**

# Design and Optimization of a Pilot Scale Simulated Moving Bed Unit for Citric Acid Separation from Fermentation Broth

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## ABSTRACT

The conventional citric acid (CA) fermentation broth down-stream process is based on a calcium salt precipitation technology what generates huge amounts of $CO_2$ and gypsum. Here, a novel CA recovery process is based on the Simulated Moving Bed (SMB) technology and a tailor-made CA- highly selective resin. No environmentally harmful wastes are produced, since deionized water (eluent) is the only substance added to the separation process.

This work presents the modeling, design and optimization of a pilot-scale SMB plant with 16 columns. For the sake of simplicity, CA and glucose are chosen as model components.

The SMB unit is modeled on the basis of the experimentally determined hydrodynamics, adsorption equilibrium and kinetics, using a semi-preparative chromatographic column. The glucose and CA equilibrium isotherms are of linear and modified Langmuir types, respectively.

Different mathematical models, comprising different degrees of complexity, are used and validated in prediction of the CA and glucose breakthrough and elution profiles. The Transport Dispersive Model (TDM) gives a satisfactory prediction. It has been selected for the SMB simulation, design and optimization due to shorter computation time.

The equivalent True Moving Bed TMB is used for the SMB pilot scale design based on the triangle methodology. The influence of the switching time and feed concentration on SMB performances was studied.

The SMB unit configuration and operating conditions leading to the maximum CA productivity with minimal eluent consumption, with minimal CA purity (99.5%) and reasonable recovery (85%) requirements have been calculated using the commercial available gOPT software. The optimized pilot scale SMB unit has been scaled up to an industrial plant of a production capacity of 5000 tons/year.

**2074**

# Integrated and Model-based Bioprocess Development from Upstream to Downstream - and Beyond

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## ABSTRACT

Downstream processing plays a crucial role in biotechnological production processes. Product concentration and purification require complex, multi step downstream processing, with a significant investment of chemicals and auxiliary material and energy, yet resulting so far in a product loss of 5-15% per process operation.
Therefore, while recovery of valuable materials from wastes and residues poses an ecological and economic challenge, at the same time new opportunities for better resource exploitation are created.

In the area of larger scale bulk chemicals, the proportion of processing costs in the total production costs run at about 30%, for small to medium sized products of the specialty chemicals, food and pharmaceutical industries they can be as high as 70-90%. Thus, improvements in processing efficiency directly influence production costs. A total process efficiency assessment, however needs to include the steps beyond the downstream steps, i.e. the formulation steps as well as the workup of the waste-containing process fluids.

The presentation focuses on the following methodological approach:

- The unit operations of the base cases of the process examples for each product range are first described by a short-cut method to allow process and production cost evaluations which quantify potential and work load for the recycling of auxiliaries from each process step.
- The aim is further to develop explicit solutions to the previously defined recycling tasks by comparison of various unit operations under the recycling strategy and to arrive at GMP-compliant solutions. Therefore, rigorous models

are applied to generate a quantitative understanding of the sensitive parameters.

The thereotical study, presented in the lecture, aims at the development of design methods for process development as well as optimization strategies to improve productivity especially with regard to total process improvement. To illustrate that, process examples from white biotech-based fine chemistry to red biotechnology pharmaceuticals are shown.
In order to integrate upstream and downstream process development and so exploit the full potential of total process optimization, first concepts are proposed for further discussion to optimize the upstream operation regarding side component and contaminant spectrum under which a cost optimized down-stream processing is possible.

[1] S. Sommerfeld, et.al. (2005), Challenges in biotechnology production – generic processes and process optimization for monoclonal antibodies, Chemical Engineering and Processing 44 (2005) 1123-1137

[2] J. Strube, et.al. (2007), Ed. G. Subramanian, Bioseparation and Processing, Volume 1, Chapter 3, Wiley-VCH (2007) 65-100

**2077**

# Systematic Design of Downstream Processes – Modeling and Simulation

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## ABSTRACT

Scale up of biotechnical downstream processes out of lab scale often results in ill-conceived industrial solutions leading to low product yields and large resource consumption. Systematic design and evaluation of alternative process concepts fails, because current simulations tools do not meet the requirements of downstream processes. Especially the simulation of batch processes with unconventional unit operations, unknown substances and missing property data is not possible with commercial software solutions.

For this reason we develop a new software tool fulfilling the requirements for the simulation of downstream processes. Existing software for event-driven simulation INOSIM Batch provides the basic framework to handle the scheduling of batch processes. It is extended by an adaptive model library and an innovative qualitative-quantitative material property model processing qualitative as well as quantitative material and process data. This setup enables the user to balance entire processes even in early project phases, when only little material properties are know precisely. Rough estimations based on molecular structure for example allow categorizing the size of molecules as small, medium or large without knowing its accurate figure. Using these qualitative categories separations based on molecule size (e.g. size exclusion chromatography or membrane processes) can be balanced with simple black-box models. As soon as more detailed material or process data is acquired during process design the model depth is adapted to the growing data base. Model depth can be varied from black-box models based on simple correlations or heuristic rules up to rigorous dynamic process models. This approach constantly enables biotechnical process design to base the economic evaluation upon closed mass and energy balances.

We will demonstrate the improvements of our tool on the systematic process design of Baccatin III, a precursor of the anti cancer drug Paclitaxel. The example presented will show the economic potential of the tool developed and how its application can support the workflow of biotechnical process design.

**2078**

# Adsorption Equilibria of Fructose, Glucose, Sucrose and Fructooligosacharides on a Cation-Exchange Resin

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## ABSTRACT

Biocatalytic transformation of sucrose by fructosyltransferase is one way of industrial production of fructooligosaccharides, important prebiotic compounds. Fructooligosaccharides (kestose, 1- nystose, $1^F$-fructofuranosyl nystose) are separated from fructose, glucose and sucrose on large scale by simulated moving bed chromatography. The knowledge of adsorption equilibria of these saccharides is thus important for the design and optimization of the chromatographic separation. Adsorption equilibria of single saccharides and their binary mixtures on the resin Amberlite (TM) CR1320 CA at the operation temperature of 60°C were obtained by frontal chromatography in the concentration range of 0–400 g $dm^{-3}$.

All determined isotherms were linear. The distributions coefficients of the saccharides decreased with the increasing molecular weight, i.e. in the order fructose > glucose >
sucrose > kestose > 1-nystose > $1^F$-fructofuranosyl nystose. This order reflects the size-exclusion effect of the resin. The binary adsorption equilibria showed that the distribution coefficients of all saccharides besides fructose were affected by the presence of another saccharide. The high value of the distribution coefficient of fructose was a consequence of the formation of a complex of fructose with the $Ca^{2+}$ functional group of the resin. The decrease of the distribution coefficients of glucose, sucrose and kestose in the presence of a second saccharide was due to steric hindrance and pore blocking effects. The distribution coefficients of 1-nystose and $1^F$-fructofuranosyl nystose had very small values in all cases and hence they were only little affected by the presence of other saccharides.

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**2079**

# Analysis of the optimal shape of gradients for systems characterized by complex adsorption isotherms

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## ABSTRACT

In preparative chromatography gradient elution is increasingly applied for the purification of fine chemicals and /or pharmaceuticals products. The concept can outperform conventional isocratic operation due to the additional degree of freedom offered be the variation of the elution strength during the chromatographic operation [1].
Up to now mostly positive (upward) gradients have been considered, i.e. the exploitation of monotonously or step wise increasing strengths. In this work we investigated systematically optimal gradient shapes for the production of the middle component of ternary mixtures which are characterized by complex adsorption isotherms. Systems following the multi-Langmuir model, the multi-Freundlich model and more complex isotherm models (capable to describe inflection points within the course of the isotherms) were evaluated. Two types of problems were considered. In the first case the more difficult problem was the separation of the target component and the first eluting component. In the second case the main problem was to separate the target component and the last eluting component. Craig's cell model was used to quantify the chromatographic process. An artificial neural network based method was used to solve the optimization problem. Optimum gradient shapes, operating conditions and performance criteria were determined and compared for each case with the respective optimum isocratic operation. The results reveal that under certain circumstances, downward oriented or more complex gradient shapes should be used.

[1] P. Jandera, J. Churacek, Gradient Elution in Column Liquid Chromatography. Theory and Practice, Elsevier, Amsterdam, 1985.

**2083**

# Modified ligand exchange chromatography for amino acids separation

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## ABSTRACT

In the present work the separation of racemic mixtures of amino acids (phenylalanine, tyrosine, valine, methionine and leucine) applying ligand exchange chromatography was investigated. The mobile phase used contained the chiral selector N,N-dimethyl-L-phenylalanine as a copper complex dissolved in water. Achiral RP C8 columns was used in the experiments. In order to enable the recovery of the chiral selector nonionic surfactants were added to the mobile phase at concentrations higher than the critical micellar concentration. In these micelles the selector was immobilized and could be re-used. It was shown that no negative influence on the separation (due to surfactant adsorption on the achiral stationary phase) occurred and the retention factors could be controlled with changing the surfactant concentration.

Temperature and pH effect on separation efficiency was determined. Temperature had no influence on separation but lowered the retention times. Thermodynamic parameters were obtained with van't Hoff plots. The van Deemter plots showed a marginal influence on dispersion and high separation efficiency with low HETP values for all the investigated amino acids could be achieved.

**2084**

# Rapid, Efficient Packing methodologies for ™ Ceramic Hydroxyapatite

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## ABSTRACT

Standardized packing methodologies for CHT™ Ceramic Hydroxyapatite will be presented which allow for rapid process-scale packing/unpacking of this important purification resin. CHT Ceramic Hydroxyapatite can be very efficiently packed (>10,000 plates/m) in Bio-Rad InPlace™ columns, with no need for any excess media. Additionally, a novel procedure for slurrying in place has been developed for re-packing without removing the column top or the media from the column. The method is highly reproducible, and the column can be re-packed and ready for use in less than one hour. Both repacking and unpacking the InPlace column can be achieved using fewer than 2 column volumes of buffer.

**2085**

# Managing pH excursions in hydroxyapatite columns associated with changes in NaCl concentration mobile phase concentration

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## ABSTRACT

Sodium chloride gradients are often used with hydroxyapatite to purify proteins or nucleic acids even when using reasonable concentrations of phosphate buffer. For example when using 0.15 M NaCl, 20mM phosphate buffer, pH 7.2 as a running buffer and 2% CV amount of 1.2 M NaCl, 20 mM phosphate buffer, pH 7.2 as elution buffer a pH shift is observed in the column effluent at the moment the 1.2 M NaCl exits the column.

Many purification applications use much lower concentrations of phosphate buffer in order to promote protein binding and subsequent molecule purification by NaCl steps or linear gradients. Excursions in pH relative to the input buffer can be as high as 1.5 units. The consequence may be a prolonged excursion into the acid range especially when the initial buffer pH is 6.5 or less. These acidic pH excursions can chemically erode hydroxyapatite. Goods buffers are recommended to reduce the magnitude of the pH excursion, for example 2-(*N*-morpholino)ethanesulfonic acid or MES. MES is a good buffer for the acidic pH range of 6.2 to 6.5 but it, along with other Good's buffers, has properties that might affect their use because of environmental disposal issues. For example they have midrange pKa, maximum water solubility and minimum solubility in all other solvents, minimal salt effects, minimal change in pK with temperature, chemically and enzymatically stable, have minimal absorption in visible or UV spectral range and are reasonably easily synthesized (1). Never the less, to prolong the integrity of a packed column for an economical number of purification cycles, mobile phase enhancements may be necessary beyond those established at minimal purification recommendations.

Previously we demonstrated that a simple insertion of low phosphate or WFI rinses eliminates an unintentional agent that causes increased column pressure drop. In this presentation we discuss the results for a Good's buffer and alternative reagents to manage the pH shift.

(1) Good, Norman E. et al., Biochemistry, 5, 467-477 (1966)

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**2088**

# Chromatographic materials displaying 'smart' new functions

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## ABSTRACT

Today's chromatographic materials are infinitely superior to those of yesteryear, but in many ways their development has stood still. For example, the basic design and tasks expected of a chromatography support have changed very little over the last 50 years, and like their ancestors, most of today's materials still perform just a single function. Given the dominant role that chromatography has played in the past and is expected to play long into the future, shouldn't we now expect much more from 'next generation' chromatography matrices?

In this presentation we shall describe some of our recent research on the development of multifunctional chromatographic materials designed to address sub-optimal elution/cleaning performance and excessive buffer consumption/waste generation. Here, we specifically describe how modification of macroporous anion exchange adsorbents and SEC supports with thermoresponsive polymer brush pore linings can convey unique properties on the resulting materials, such as 'all or nothing protein binding' and 'self-elution' in AEC, tunable fractionation ranges in SEC, and 'self –cleaning' in both AEC and SEC.

This work was supported by the European Commission 6$^{th}$ Framework Programme (STREP grant 013469 'NanoBioMag').

**2091**

# Introduction of a fast and widely applicable analytical screening method for the design of new materials for antibody purification.

**Jeannie Horak and Wolfgang Lindner**

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**ABSTRACT:**

The major weakness in material design for antibody purification, which is often underestimated is the lack of a single, fast and accurate analytical method to classify the quality and applicability of the newly designed materials for different buffer, salt and pH-conditions or even for a mock-feed solution containing other proteins besides IgG in a serum-free cell medium (SFCM) or even a real feed solution.
The here presented method resembles an extension of the commonly used Protein A HPLC method for the quantification of IgG by additional determination of the overall protein content in the flow-though fraction with and without combination with size exclusion chromatography (SEC), which depends on the test solution of choice. This method enables the simultaneous quantification of two proteins e.g. IgG and BSA and thereby determining their cross-selectivity as well as the %-binding of impurities from SFCM during static binding studies (SBC) of protein capturing materials. In direct combination with SEC, the performance of a new material can be visualized for protein isolation from real feed solutions, for which their performance can be quite different compared to tests employing standard proteins in pure buffer systems. Overall the quantitative results for material screening of novel anion exchange materials using pure standard protein mixtures as well as real cell supernatant solutions will be presented and discussed in comparison to results obtained with the classical photometric detection at 280nm and the Bradford method. The major advantage of this method is however, the necessity to establish only one calibration curve for two proteins, binding and non-binding e.g. IgG and BSA, for solutions with different buffers, pH-values, salt content and even for SFCM at a LOD of 0.15 [µg abs] and a LOQ of 0.5 [µg abs], which is equivalent to the Bradford method plus a linear range of almost four orders of magnitude.

Acknowledgements: This study was supported by the 6$^{th}$ Framework Program of the EU, Project AIMs (Advanced Interactive Materials by Design), No. NMP3-CT-2004-500160

**2092**

# Novel Fluorous Phase for the Separation of Organic Compounds

**Hugo St-Laurent, Lynda Tremblay, Philippe Couture, Geneviève Gingras, François Béland**

SiliCycle® Inc, Quebec, Canada

## ABSTRACT

Successful methods for combinatorial and parallel synthesis must couple efficient chemical reactions with simple purification methods. Even in other types of synthesis, chemists are always eager for new tools and new technology that would allow rapid synthesis of multiple compounds.

Fluorous solid-phase extraction (FSPE) is an attractive technique for strategic separation of the desired fluorous-tagged molecule from the reaction mixture (reagents and/or by-products). In fact, fluorous techniques constitute a growing area in synthetic organic chemistry.

Silia*Bond*® Fluorochrom is a novel fluorinated sorbent with high level of fluorine. It is very useful for the reversed-phase separation of fluorine containing molecules from non-fluorous one and can successfully be used either as sorbent in SPE or Cartridges.

**2093**

# Direct estimation of competitive Langmuir isotherm parameters from overlapping bands of binary injections

**Arvind Rajendran**[1]

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## ABSTRACT

The estimation of adsorption isotherm parameters is vital to the design, scale-up and optimization of chromatographic separation processes. Based on the equilibrium theory of chromatography explicit algebraic equations are derived to estimate competitive Langmuir isotherm parameters from the elution band profiles of binary injections. The key requirement for using this method is that the elution bands of the two constituent solutes should not be completely separated, a condition often encountered in practical situations. For systems where the saturation capacities of the two solutes are unequal, a case typically encountered in enantiomer separations, the method requires the measurement of elution profiles of two overloaded binary injections with the same concentration of the binary mixture but with different injected amounts, e.g. injections loops with different volumes. For systems where the saturation capacities of the solutes are equal, a requirement for thermodynamic consistency, only one injection of the mixture is required. In both the cases, the only measured parameter is the elution time of the shock front of the more strongly adsorbed component. This method offers several advantages compared to the available techniques. First, only the retention time is required, and hence this method effectively eliminates the need for detector calibration. Secondly, by using binary injections, it eliminates the need for pure components. Finally, since the experiment involves a pulse injection it reduces the amount of solute needed for isotherm determination. The effectiveness of this method in rapid determination of isotherm parameters is highlighted, and its limitations with respect to aspects such as column efficiency are discussed.

**2094**

# Integrated operation of SMB, biocatalysis and cross-flow filtration for the production of fine chemicals

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## ABSTRACT

On-line coupling of simulated moving bed separation and enantioselective (bio)synthesis constitutes an attractive option to overcome a broad set of constraints frequently encountered in biotransformations such as thermodynamic limitations, compromised reaction yields by long reaction times, product inhibition and product instability (Fig. 1). Next, integration of a chiral SMB with a mild enzymatic racemisation enables –theoretically- 100% conversion of the racemate into the desired (single) enantiomer. Such an installation automatically gives access to both enantiomers, in contrast to enzymatic process routes that require three different enzymes for the same flexibility [1]. In this work we present the development, implementation and optimization of two hybrid processes for the production of enantiopure amino acid: (i) Asymmetric synthesis of L–*allo*-threonine from glycine and acetaldehyde catalyzed by an aldolase (reaction yield 40% at equimolar substrate conc.). (ii) Coupling of an amino acid racemase catalyzed racemisation with an SMB enantioseparation of

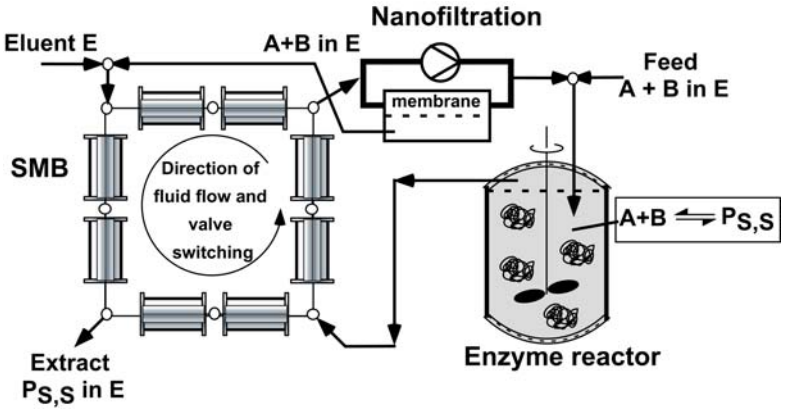


Figure 1 By coupling a continuously operated bioreactor to an SMB that provides efficient separation of the substrate/product mixture of the reactor effluent and subsequent recycling of the substrate –theoretically- 100% chemical yield can be achieved. The inherent dilution in SMB operation requires concentration of the substrate effluent by cross-flow filtration.

racemic amino acids.

Due to the complex operation and interoperability of the three unit operations a rational design of the process is indispensable. Consequently, each unit operation was characterized (adsorption isotherms, enzyme kinetics, enzyme stability, membrane rejection and stability) as a function of potentially interoperable medium compositions (organic solvent content, pH, buffers). Based on the obtained data numerical process optimization was performed and the results validated on a lab-scale system.

[1] M. Bechtold, S. Makart, M. Heinemann, S. Panke, J Biotechnol 124 (2006) 146.

**2095**

# SMB design for chiral stationary phases showing an analyte–related memory effect

**M. Bechtold, M. Füreder, S. Panke**

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## ABSTRACT

Teicoplanin-aglycone bonded chiral stationary phases (Chirobiotic TAG, Astec, USA) enable excellent enantioseparation of amino acids in aqueous solutions with economic loading capacities. Since amino acids show decreasing solubility with decreasing water content and prep-scale material is commercially available, the TAG column constitutes a promising option for large-scale amino acid enantioseparation. However, a strong memory effect was observed that could be related to amino acid overloading. Consecutive finite amino acid injection HPLC runs (employing a 90/10 v/v 50mM $NH_4Ac$ pH 5.8 / MeOH mobile phase) carried out directly after the column was treated with a constant amino acid feed (e.g. 90/10 v/v 33mM D,L-Met 50mM $NH_4Ac$ pH 5.8 / MeOH for 2 hours) revealed a slow dynamic change in the adsorption behavior (Fig. 1). The underlying mechanism of this effect could only partially be identified by a model-based experimental analysis [1]. Still, variations in the pH and organic modifier concentration could be excluded as potential source of the memory effect. Obviously, such a behaviour severely aggravates SMB design. Employing operating conditions calculated by adsorption isotherms - that were estimated by widely used model-based analysis of pulse injections (inverse method) or the perturbation method – did not yield the projected purities in SMB operation. By

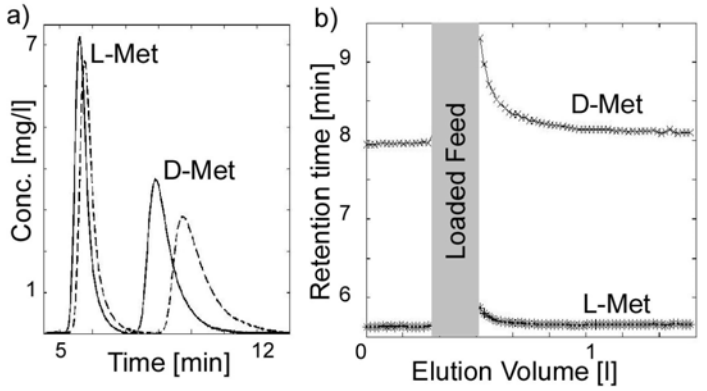


Figure 2 Chromatograms recorded before (solid line) and directly after (dashed line) harsh overloading of the column b) retention times in consecutive finite injection methionine HPLC separations before and directly after harsh overload conditions

applying a modified perturbation method that incorporates the projected SMB feed concentration as additional intermediate concentration plateau, the resulting isotherm description predicts SMB operation fairly accurate. The characterisation of the memory effect and the developed SMB design method will be presented in detail.

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2097

# Automated Fraction Trapping of Purified Compounds from Preparative Chromatography

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[2] Gilson, Inc., Middleton, USA

## ABSTRACT

Studies have shown that synthetic scale up can create impurities or additional bi-products that were not observed at an analytical scale compromising the purity of the active compound. Therefore preparative chromatography is employed to separate and isolate the active compound. Preparative purification (e.g. > 50 x 150 mm, column geometry, flow rate > 75 mL/min, compound mass 0.3 – 15 gm) creates a fraction volume in the 20-120 mL range. The purified fraction collected requires obviously dry down to remove the mobile phase. Since the common preparative technique employed for purification is RP-HPLC the purified fraction will contain an aqueous and organic component including a modifier (e.g. TFA, TEA, formic acid). Elimination of the solvent associated with the purified fraction requires vacuum/temperature increase. Concentration of the mobile phase modifier has been shown to cause hydrolysis of functional groups, possibly destroying the activity of the purified compound. In addition evaporation or dry-down is time consuming (e.g. 8-24 hrs) and requires manual intervention. Employing an activated media packed in a SPE cartridge, the automated system can concentrate the fraction containing the compound via the SPE cartridge. In the case of large preparative fractions a specially designed polymer resin column is brought in-line through a switching valve in order to concentrate the fraction. Both approaches eliminate all the possible caveats and limitations regarding preparative purification. These two approaches allow for over 30 purified fractions/compounds to be processed in less than 2 hours without manual intervention. General cost savings is approximately $5,000/week based on research/instrumentation reduction.

**2098**

# Effect of Multiple Acidic Buffers on the Retention of Ionogenic Molecules

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## ABSTRACT

Acidic buffers are used in the solvents of a variety of chromatographic bio-separation processes mainly for stabilising the solvent pH. There are other side benefits, like suppression of the effects of residual silanol group on the solid-phase. Apart from being a donor of the H+ ions in the solution, the anionic part of the buffer forms ion-pair with ionogenic analytes and can modulate their (analytes') retention behaviour substantially. The strength and modulation capability of these ion-pairs, on the other hand, depend strongly on the properties of the associated anionic group.

In the downstream process line multiple chromatographic steps are used. Each step, in turn, may employ different buffers according to respective suitability. Now, depending on relative affinity and process conditions, the buffers used at different stages can be carried over to the subsequent stages in varying quantity, leading to a mixed buffer environment. In most of the situations, the effect of having multiple buffers does not become prominent till one goes to sufficiently high overloading where unexpected phenomenon, like peak splitting, starts occurring.
The proposed presentation will discuss the outcome of a study carried out to understand and formulate the effect of mixed buffer on the retention behaviour of ionogenic molecules at highly overloaded conditions.

**2099**

# Newly developed high strength and chemically stable silica gel based preparative reversed phase packing materials (1)

**Britta Bloedorn[2], Masakatsu Omote[1], Kiyoshi Morishita[1], Noriko Shoji[1], and Naohiro Kuriyama[1]**

[1]YMC Co., Ltd., Ishikawa, Japan
[2]YMC Europe GmbH, Dinslaken, Germany

## ABSTRACT

A new high strength silica gel and a bonding technology based on preparative bulk packing materials for HPLC have been developed to provide improved recovery, selectivity, and longer life time for the preparative peptide separations. The novel preparative silica particle was successfully prepared by the new generation process, which allows the higher gel density than typical silica gel and the particle size distribution would be practically mono-dispersed character. For the effective reversed phase peptide separations, pore size and pore volume of these new particle were optimized depending on the molecular weight of peptides. To enhance chemical stability and selectivity under the typical peptide purification conditions, the combination of chemical bonding method and functional group density was optimized for maximum performance.

By repeated packing and unpacking of this synthesized gel with large dynamic axial compression column, it was demonstrated that no fine has appeared and no back pressure increasing has occurred comparing to commercially available packing materials. Also cost effective peptide purification with high loadability, productivity, and recovery was achieved with significant small and large peptides.

2101

# Dynamic Model for the Ultrasonic-assisted Extraction Process of Curcumin from Curcuma Longa L.

**Trung Kien Tran[1], Lan Huong Phung[1], Son Nghiem Xuan[1], Van Thiem Pham[2]**

[1]Department of Chemical Engineering, Hanoi University of Technology, Hanoi, Vietnam
[2]Center for Education and Development of Chromatography (EDC), Hanoi University of Technology, Hanoi, Vietnam

## ABSTRACT

Curcumin, the major yellow-orange pigment extracted from Curcuma Longa L., is a promising material for pharmaceutical industry and food industry, thanks to its bioactive effects: antioxidant, anti-inflammatory, anti-cancer etc. However, its application is limited due to difficulties met in the isolation procedure. One of these difficulties is the long time taken in the extraction process, which also lead to energy consumption and curcumin decomposition. In this paper, an extraction method using ultrasonic technique, that saving more than 90% of required extraction time in comparison with another method, is proposed. For the investigation of the process, a dynamic model is also developed and experimentally validated. High performance liquid chromatography (HPLC) is applied for the determination of curcumin composition in samples and in extractive phase.

**2102**

# New generation of semi prep column by YMC

**Britta Bloedorn[2], Masakatsu Omote[1], Kiyoshi Morishita[1], Noriko Shoji[1], and Naohiro Kuriyama[1]**

[1]YMC Co., Ltd., Ishikawa, Japan
[2]YMC Europe GmbH, Dinslaken, Germany

## ABSTRACT

An efficient and speed up development of variable compounds has been needed in the pharmaceutical area. To prepare standard molecules or impurity compounds, preparative column should have stable under any conditions, more effective productivity and reproducibility than conventional type.
Recently, new technology has been applied to semi prep column. Its column structure is quite different from conventional type, and the column shows longer life time than conventional prep column. These advantages are offered by mainly packing material density uniformly so every performance could be improved.
Now YMC introduces brand new product named 'YMC-Actus' (*Axial Compression Technology with Ultimate separation*) which combines improved uniformly packing technology and YMC's various packing material. Comparing our conventional product, competitors with Actus, as the result, our new comer shows competitive longer column life time with higher plate count and symmetrical peak shape.

**2104**

# COMPARISON Batch vs SMB/Varicol for a racemic mixture and for a complex mixture

**Jan Hoogendoorn, David Stadermann**

SOLVAY Pharmaceuticals B.V., Weesp, The Netherlands

## ABSTRACT

A comparison will be given for two processes performed by using PrepHPLC in batch mode and in SMB or Varicol-mode, all on Kg-scale. The first process is a standard racemic mixture, however, the second process is a complex mixture of diastereomers and achiral by-products.
For the complex mixture the purified product had to comply with a very high specification set. The comparison will show the effect on solvent consumption and production time and workload.

**2105**

# Separation and Purification of Polar, Basic Pharmaceutical Relevant Compounds by MS Directed Preparative Supercritical Fluid Chromatography (SFC)

**Rui Chen, Peter Ridgeway**

Thar Instruments, Inc., Pittsburgh, PA, USA

## ABSTRACT

In the past decade, supercritical fluid chromatography (SFC) has experienced a striking resurgence and exponential growth in acceptance, particularly in pharmaceutical and chemical laboratories. In SFC, a “supercritical” fluid, most commonly $CO_2$, in combination with one or more polar organic solvents, such as alcohols, are used as mobile phase. Due to the lower viscosity and high diffusivity of supercritical fluids, fast separation can be achieved without the loss of chromatographic efficiency by SFC. Other advantages, including cost-effectiveness, elimination of halogenated solvents, and significant reduction of post-purification endeavor, have been unambiguously manifested in preparative SFC and welcomed by industry. With the specificity and universality of mass spectrometer (MS), MS directed preparative SFC will further heighten the aforementioned benefits inherent to SFC.

The polarity of $CO_2$ is similar to that of hexane, and thereby making SFC a normal phase like chromatographic technique. As a result, SFC holds a unique advantage over reversed phase liquid chromatography (RPLC) in separating polar compounds that have little retention and/or selectivity. We present herein an example of using a MS directed preparative SFC system to separate and purify 8 polar, basic pharmaceutical relevant compounds. Important parameters, including the choice of mobile phase, temperature, and pressure, and their impact on separation will be discussed. Other relevant system attributes, such as purity and recovery, will also be presented.

**2106**

# Chiral Resolution for full spectrum of racemates

**Presenter: Niteen A. Vaidya, Ph.D.**

CTO, ChiroSolve Inc.

## ABSTRACT

Chiral resolution by Diastereomeric Crystallization technique is the oldest and the most widely used method for manufacturing optically pure products. It is the most well adopted and well accepted method in the industry. But this does not mean that it is the most straightforward method with simple solution for every type of racemate.

Today I would like to discuss some of the more complex examples of the Diastereomeric Crystallization applications that require pre-processing and/or post-processing as part of the separation process. These examples include Boronic acids, aldehydes, alcohols, etc.

We at ChiroSolve, have used ChiroSolv® kits for many such examples to achieve very fast screening that identifies the ideal separation process for the racemate. As part of our Chiral Enhancement Services, many times we get unknown racemates which do not posses either acidic or basic functionality. For example, amino acids or boronic acids; where internal salt/zwitter ionic nature of the molecule makes it necessary to break it by ion-exhchange chromatography.

**2111**

# Rapid development of Cleaning-In-Place protocols for affinity media

**Hans J Johansson, Hanna Tengliden and Anna Grönberg**

GE Healthcare Bio-Sciences, Uppsala, Sweden

## ABSTRACT

Commonly used protein-based affinity ligands (like Protein A) are very stable and allow potential use for many hundreds of purification cycles. Instrumental to maximum functional resin life-time is the use of efficient and resin compatible cleaning-in-place (CIP) solutions.

One major challenge is that all biological materials are unique and will behave differently with respect to fouling of resins and development of suitable CIP protocols. Traditional small scale column studies are very labour intensive, time and sample consuming.
Therefore, we have developed a rational methodology for design of CIP protocols using Protein A affinity media and capture of mAb from harvested cell culture fluid from mAb expressing Chinese hamster ovary (CHO) cells as a model.

This paper describes the development of a methodology for screening of a large number of CIP solutions in 96-well format. Applications and final verification in parallel small scale column format are also described

**2112**

# Optimization of silica based RPLC materials for peptide purification

**Anders Törncrona1, Johan Ekeroth1, Britt Kofoed-Hansen1 and Sylvia Winkel-Pettersson[1]**

[1]Eka Chemicals AB/ Separation Products, Bohus, Sweden

## ABSTRACT

Reversed phase liquid chromatography (RPLC) has proven to be a very efficient tool to polish crude solutions of polypeptides.

In the present work, the main focus has been to study the effect of pore size and surface chemistry on the selectivity, purity and recovery of insulin. Both analytical and preparative injections were made on the studied materials and the results were compared. It was found that both surface chemistry and pore size affect the purification performance of the materials.

Frontal analysis was employed to determine the saturation capacity of the prepared materials. The results obtained by frontal analysis were compared with the peak width of the preparative insulin chromatogram for each material. It was found that a higher saturation capacity, as determined by frontal analysis, results in a narrower peak width in the preparative chromatogram.

In contrast to what has been reported elsewhere, the optimal pore size for insulin purification seems to be in the size range 100-150 Å.

However, the surface chemistry of a RPLC-material in combination with an appropriate RPLC-method, i.e. gradient and mobile phase, are the most important factors to obtain a high selectivity, purity and recovery of insulin.

**2113**

# Batch and Continuous Liquid-liquid Partition Chromatography of Biopolymers in Aqueous/Ionic Liquid two phase systems

**Christian Birkner[1], Ursula Haering[1], Joachim Kinkel[2], Tobias Gubbey[2]**

[1]Roche, Diagnostics Division, Penzberg, Germany
[2]Ohm-University of Applied Sciences, Nuremberg, Germany

## ABSTRACT

Work presented will give the results of the separation of biopolymers as DNA and proteins of diagnostic purpose by means of batch and continuous countercurrent chromatography.
For the first time, the successful separation of DNA in an aqueous/ ionic liquid two phase system has been successfully accomplished.
A discussion on the potential of this aproach based on the experimental data of preparative runs is included.

**2114**

# New polar phases for preparative HILLIC applications

**Joachim N. Kinkel[1], Andreas Zucker[2], Peter Koeppel[1],**

[1]Ohm-University of Applied Sciences, Nuremberg, Germany
[2]Biontis GmbH, Geesthacht, Germany

## ABSTRACT

The paper will give the design and chromatographic characterization of different HILLIC-type packings based on monomer- or polymer modified silica or crosslinked hydrophilic porous polymers. Polar neutral, charged and zwitterionic ligands have been bonded as silanes or acrylic monomers resulting in monomeric or tentacle-type polymeric packings.

Chromatographic performance of these packings has been tested with various polar test mixtures
From aqueous solutions and alcohols or acetonitril as modifier.

The preparative usefullness of such packings will be discussed.

**2115**

# LLPC /SMB: The continuous Separation of Biopolymers on Polyacrylamide silica

**Joachim N. Kinkel**

Ohm-University of Applied Sciences, Nuremberg, Germany

## ABSTRACT

The paper will give the experimental results of the application of Liquid/Liquid partition chromatography for the separation of DNA and proteins on tentacle Polyacrylamide-silica in combination of SMB technology.

Despite the merits of LLPC for the preparative separation of Biopolymers in Aqueous Polymer Two Phase systems demonstrated by W. Mueller as early as 1985, this approach has never been applied in SMB mode.

The results clearly demonstrate that the sucessful transfer of Mueller's experiments to continuous mode comprise a rare possibility to perform biopolymer separations under isocratic conditions.

**2116**

# ENANTIOSELECTIVE MEMBRANES CONTAINING L-PROLINE-DERIVED CHIRAL CARRIERS

**Biotza Gutiérrez[1,2], Cristina Minguillón[1,2]***

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[2]Laboratori de Química Farmacèutica, Faculty of Pharmacy, University of Barcelona, E-08028 Barcelona, Spain.

## ABSTRACT

The strict requirements established by health authorities for the commercialization and control of the enantiomeric composition of chiral drugs have promoted the development of highly efficient separative techniques for this purpose. Liquid chromatography is a suitable technique to carry out both analytical and preparative separations. Membrane technology has also been considered a possible tool for enantioseparation and is a promising alternative to classical methodologies and an interesting and challenging research field. Among diverse membrane configurations,[1] liquid membranes containing a selective carrier have already been used for the separation of enantiomers.[2,3] However, chiral solid membranes are a more reliable and attractive approach.[4,5]

Here we used liquid membranes as a tool to screen the enantiorecognition capacity of L-proline-derived chiral selectors (CS). The effects of solvent nature and CS concentration on enantioselectivity and transport were studied. Chosen CSs were covalently bonded to polysulfone or, alternatively, polymerized to obtain materials suitable for membrane preparation. Transport experiments through the solid membranes obtained were performed in aqueous and organic media. The effect of the density of chiral entities on enantioseparation and transport was examined. Structural and morphologic characterization of the polymeric materials and membranes are also presented.

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**2118**

# Orlistat – a successful approach to an efficient preparative liquid chromatography purification method

**Lucien Charles* and Thomas Kaiser**

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## ABSTRACT

Liquid Chromatography is one method of choice enabling efficient purification of a broad range of compounds like e.g. pharmaceutical compounds in high yields in short time. Using this powerful technique there are several stationary phases based on base materials like polysaccharides (sepharose, agarose, cellulose, dextrane etc.), synthetic organic polymers (polystyrene, acrylamid, methacrylate etc.) as well as mineral compounds (silica, silica-hybrid, ceramic, zircon oxide, titan oxide etc.) which the user can chose in order to find the best matrix for the separation conditions. Focusing pressure stable and high resolution matrices, silica materials are still first choice when using preparative liquid chromatography purifications for e.g. for organic compounds. We will show our results in establishing an appropriate silica-based liquid chromatography purification method for purifying the drug **Orlistat** by using a unique systematic “intelligent” matrix selection approach to adopt the best appropriate stationary phase on base of our broad silica lines and using our unique surface modification mode. **Orlistat**, a tetrahydro-Lipstatin, is a compound designed to treat obesity having a strong growing demand within the market. The anti-obesity drug primary function is preventing the absorption of fats from the human diet and it is intended for use in conjunction with a physician-supervised reduced-calorie diet. Showing these results we present users alternative ways in using/finding appropriate silica/modified silica for a given topic by focusing the specifications of the aim molecule in first instance and to adopt the best appropriate and economic matrix for the separation problem.

**2120**

# Optimize the performance of Denali RP media for preparative purification by using MODcol SpringColumn and MultiPacker technology

**Dr. Saar J[1]., Dr. Nguyen R. T[2], Denoulet B.[3]**

[1] Grace Davison Discovery Sciences, Worms, Germany
[2] Grace Davison Discovery Sciences, Hesperia, USA
[3] Grace Davison Discovery Sciences, Lokeren, Belgium

## ABSTRACT

RP-HPLC is the method of choice for the analysis and purification of peptides and proteins due to its unparalleled resolution. Especially in large scale LC purification processes not only the media properties, but also the type and quality of the column hardware has a major influence on the loadability, purity and yield of such a downstream processing step.

Hormones like insulin represent an important part of the current market for biochemical drugs. The use of the MODcol Spring Column with patented dynamic axial compression (DAC) technology can enhance the recovery of insulin when packed with Denali C18 silica based material. The MODcol Spring Columns are the only DAC columns on the market that can be handled independently from their packing unit while still maintaining their DAC functionality.

Packing of the Spring Column hardware is performed on the new MODcol 25mm and 50mm MultiPacker unit. This column packing system is equipped to pack both 25mm I.D. and 50mm I.D. MODcol Spring Columns of various and flexible bed lengths. The system is fully compliant with CE and ATEX regulations and features an integrated, TÜV-approved safety concept. This MODcol 25mm and 50mm MultiPacker is the most advanced packing system commercially available and sets the new standard for self-packing of Prep-LC column hardware from a perspective of safety, performance and user-friendliness.

**2121**

# The use of high quality silica media in pilot and process scale chromatography using custom designed high pressure process LC columns and systems.

**Dr. Saar J[1]., Vind K.[2], Denoulet B.[3]**

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[2] Peak Biotech, Kvistgaard, Denmark
[3] Grace Davison Discovery Sciences, Lokeren, Belgium

## ABSTRACT

In order to obtain high levels of purity together with a high recovery in pilot and production scale LC purifications both the packing media and the equipment used are important factors. From the media side both irregular (Davisil) and spherical (Vydac) media are available from Grace in scalable sizes to cover a very wide range of applications.

Davisil is available in a broad variety of pore sizes, ranging from 30A to 2500A. The high surface area of the small pore silica grades allows an increased loading capacity and maximized throughput per run. The use of extra wide pore medias allows the efficient purification of sterically challenging molecules like large proteins. In both cases the high purity of the silica reduces unwanted interactions and contamination. Vydac media have a pore size of 300A and are typically used for peptide and protein purifications.

Both media types are used in Peak Biotech LC columns which are designed for continuous or batch processing and which are in compliance with all relevant standards within the pharmaceutical and biotech industries. Performance chromatograms of different media types packed in a Peak Biotech LC column will be presented.

The Peak Biotech columns and also the LC systems are custom designed and are available in many different configurations with a large variety of possible options. Each column and system is tailor-made to the specific requirements of the end users and their purification needs. The different options for packing and unpacking the columns as well as their unique leak detection system are only some example of the unrivaled features Peak Biotech can offer for their process LC columns.

2122

# Vydac MS RP-HPLC Columns Provide Unique Selectivity and High Recovery for Peptide and Protein Separations

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## ABSTRACT

Based on specially treated large pore silica and enhanced with a proprietary bonding process, Vydac MS reversed-phase (RP) HPLC columns offer superior performance for peptides and proteins. The deamidation of human growth hormone (HGH) has been monitored for many years by RP using Vydac columns.

The Vydac MS C4 column provides the best overall performance characteristics (recovery, resolution, and peak symmetry) for the common important assay of HGH and desamido HGH. Although hydrophobic membrane proteins are particularly difficult to separate, the Vydac MS C4 column provides better separation and recovery (up to 86% higher vs. other leading columns) for a reptilian reovirus p14 protein and myristolyated form, a component of a potentially new vaccine delivery system.

Separation of the trypsin digest of fetuin, a glycoprotein, exhibits improved selectivity for peptide mapping on a Vydac MS C18 column compared to other C18 columns, revealing some peaks otherwise not seen. The improved selectivity for peptides on the Vydac MS columns results in better primary structure definition and easier identification of degradation products and other protein characteristics.

**2123**

# A rational approach to modelling of uptake rate of large proteins by porous chromatographic materials

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## ABSTRACT

A novel rational approach is employed to the modelling of the uptake rate of large proteins by chromatographic materials in batch systems. In contrast with common lumped pore models, which fail to describe experimental data at several concentrations, the proposed model takes into account factors such as progressive mass transport impairment inside porous structure due to adsorbed molecules and improved interpretation of adsorption isotherm data for a non-constant final concentration profile inside adsorbent particle. On the other hand, several phenomena are described by effective representation, e.g. mean pore radius instead of pore size distribution and local equilibrium of adsorption reaction instead of intrinsic kinetics. This increases a practical value of the model, since many complex models suffer from over-parameterization if multiple parameters are not available from independent measurements and must be fitted directly from an experimental dataset.

The applicability of this approach is demonstrated on several previously published experimental data of human IgG uptake by protein A adsorbents and other protein adsorption systems at a broad range of process conditions (protein concentrations in solution). A practical utilization of this approach is suggested for a systematic development of novel adsorbents when textural properties are coupled with mass transport performance.

**2124**

# Histidine affinity chromatography of homo-oligonucleotides: the role of multiple interactions on retention

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## ABSTRACT

Interest in producing large quantities of supercoiled (sc) plasmid DNA (pDNA) has recently increased as a result of the rapid evolution of gene therapy and DNA vaccines. Hence, large-scale manufacturing processes have been developed particularly to improve purification strategies. Several chromatographic methods have been reported, but not all strategies achieve efficient pDNA isoforms separation. The recent application of the histidine–agarose affinity support in plasmid purification takes advantage of the biorecognition of nucleic acid bases by histidine ligand [1]. This consideration prompted the need for better understanding the interactions involved in affinity chromatography with the histidine-agarose support. Histidine-nucleotide base interactions have been recognised at atomic level in several protein-DNA structures [2]. According to some atomic studies, the events that can occur between nucleosides and the aromatic side-chains of histidine are related to stacking interactions, hydrogen bonds, van der Waals contacts, electrostatic interactions or water mediated bonds. This work describes the affinity chromatography of homo-deoxyoligonucleotides with sizes up to 30 bases on a L-Histidine-agarose gel. The results suggested that multiple interactions between oligonucleotides and the histidine ligand play an important role on their selective retention in accordance with the molecular mass of the oligonucleotides, the hydrophobic character of the individual bases, the presence of secondary structures of the oligonucleotides and the concentration of ammonium sulphate in the eluent. Otherwise, the temperature manipulation has not showed a direct influence on oligonucleotides retention. In fact, the increase of temperature only induced conformational changes on oligonucleotides presenting secondary structures, as previously described for pDNA studies [3].

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**2130**

# NON-CLASSICAL COMPOSITION FRONTS IN NONLINEAR CHROMATOGRAPHY

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## ABSTRACT

The local equilibrium theory of chromatography is well established as a tool to analyze multicomponent nonlinear chromatography. Such approach is based on a simplified model of the chromatographic column consisting of first order partial differential mass balance equations (conservation laws), which account for convection and adsorption, but neglects dispersion and mass transfer resistances. In the case of two-component chromatography described by a general isotherm the theory allows to solve the model equations semi-analytically, whereas this is possible for multicomponent systems only in the case of a Langmuir isotherm. The equilibrium solution for a Riemann problem (constant initial state in the column, and constant feed composition) consists of constant composition states separated by transitions, i.e. concentration fronts. The latter can be continuous simple waves that fulfill the partial differential equations or discontinuous shocks that fulfill the so called Rankine-Hugoniot condition, where it is assumed that no mass accumulation on the shock front is possible.

In this work, we study the solution of Riemann problems in the case of two-solute chromatography, where adsorption of the species to be separated (species 1 and 2, i.e. the less and more retained component, respectively) is characterized by the following isotherm: $n_1=H_1 c_1/(1-K_1 c_1+K_2 c_2)$ and $n_2=H_2 c_2/(1-K_1 c_1+K_2 c_2)$, with $H_2 > H_1$. Note that contrary to the Langmuir isotherm the $K_1 c_1$ term in the denominator has a negative sign. In this case the model equations are of a mixed type, i.e. they are hyperbolic in a region of the composition space (hence the method of characteristics can be applied as in the Langmuir case) and elliptic in the remaining part of it (being parabolic on the boundary between the two regions). This isotherm fulfills necessary conditions of thermodynamic stability.

It will be shown that for some Riemann problems in the hyperbolic region the solution of the model equations is not constituted of constant states separated by simple waves and shocks only. As a matter of fact, in these cases the Rankine-Hugoniot condition, that implies the conservation of mass through the shock with no

accumulation on the shock itself, cannot be fulfilled. The solution can accommodate the excess mass only by admitting material accumulation on the shock, thus generating a spike that grows while traveling on top of the shock discontinuity along the column; mathematically, this is a Dirac-delta. The results will be demonstrated through the application of the method of characteristics and through numerical simulations. Contrary to the case of classical shock, the propagation velocity of the traveling spike cannot be predicted within Equilibrium Theory. On the contrary, it can be shown through simulations that this depends on the functional form chosen to describe axial dispersion along the column.

**Keywords:** nonlinear chromatography, equilibrium theory, delta-shock, singular shock, traveling spike

**2136**

# Optimization of Lovastatin production by *Monascus purpures* MTCC 369 using Plackett-Burman and central composite statistical experimental design

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## ABSTRACT

Lovastatin is a hypercholesterolemic potent drug for lowering blood cholesterol which acts by competitively inhibiting the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase, the first enzyme in cholesterol biosynthesis. In this present study, lovastatin producing various fungi were screened and *Monascus purpureus* MTCC 369 was selected for the production. The production was carried out in both the submerged and solid state fermentation. Twelve medium components were evaluated initially using Plackett-Burman two level factorial design. The significant variables affecting lovastatin production were found to be glucose, NaCl, peptone, $KH_2PO_4$, $MnSO_4.H_2O$. The effect of various carbon sources and various solid substrates (agro industrial wastes) in the production of lovastatin were tested in submerged and solid state fermentation respectively. A maximum concentration of 84 mg $l^{-1}$ and 179 mg $g^{-1}$ of lovastatin was obtained at the optimum combination of medium constituents and fermentation conditions in submerged and solid state fermentation respectively. Unstructured kinetic models, Logistic model and Luedeking-Piret model were used to describe the cell mass and lovastatin production respectively. Analysis of lovastatin was carried out in shimadzu HPLC (LC 20 AT prominence) at 238nm in Luna $C_{18}$ column of particle size 5µ and (250X4.6) mm I.D, UV detector (SPD 20 A) and the column oven (CTO-10 AS vp) at 45$^{o}$C. Binary gradient system was used and the samples were injected manually using Rheodyne Injector of 20 µl. The mobile phase used was 60:40 acetonitrile-phophoric acid (0.1%) at a flow rate of 1.5ml $min^{-1}$.

**2137**

# Evaluation of Chiral Stationary Phase packed AXIA HPLC Columns

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## ABSTRACT

A series of AXIA HPLC columns (Phenomenex, Torrence, USA) were packed with Chiralpak AD, 20 □m (CTE, Illkirch Cedex, France) chiral stationary phase. AXIA HPLC columns with different bed length (50, 75 and 100 x 21.2 mm) were packed at Phenomenex (Torrence, CA), according to their proprietary packing technique. The efficiency of this new packing technology was evaluated by determining the chromatographic performance of the columns using a number of commercially available racemates. Resolution ($R_s$), selectivity factor (□, plate number (N), peak width ($w_{1/2}$) and peak symmetry were considered for the evaluation of the performance. Additionally, a preparative loading study was performed.
The presentation will focus on the chromatographic performance obtained on these columns by comparison with those obtained on a corresponding conventional HPLC column. Finally, the usefulness of these AXIA columns will be demonstrated with selected examples of preparative chiral chromatographic separations of pharmaceutical stereoisomers using HPLC and SFC.

## Last Minute Poster Presentations

**4153**

# Modeling Study of On-Line Estimation of SMB Parameters in Mixture of Quercetin and Rutin

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## ABSTRACT

The simulated moving bed (SMB) is a continuous chromatographic separation which is becoming an important technique on the basis of the purification level. One of the key areas in the design of SMB is the determination of zone flow rates and the switch time of the SMB process. The determination of operating conditions for high purities depends on the accurate parameters in SMB models. In the operation of the process, the off-line determined parameters may no longer be a true reflection of the process and need to be re-determined. From the practical point of view, the most effective way to prevent the designated purity from being affected by process changes is to do parameter estimation on-line and carry out an on-line design. In this research, the parameters can be determined using the measured data based on the knowledge of the position and propagation of wave profiles. To verify the proposed method, chromatographic separation of Quercetin and Rutin is simulated. Simulation and parameter estimation are used simultaneously to develop a healthy SMB model for the current operation status.

**4154**

# Performance Monitoring of SMB Operation Using UV On-Line Systems

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## ABSTRACT

A new monitoring method of the SMB performance based on two multi-wavelength UV detectors is proposed. The UV detectors provide the required independent signals by measuring the UV absorbance at multiple wavelengths simultaneously. Although we can make use of UV signals for computing the concentration level in the extract and raffinate streams, the archived data can also be used to identify potential problems in an incipient fashion, like the malfunction of multi-position valves, failure of check valves, and anomaly in operation of HPLC pumps, for preventative maintenance. To this aim, different faults are implemented on a lab-scale, eight-column and four-section SMB plant for the high purity separation of Quercetin and Rutin. In this study, different fault patterns through UV on-line systems can be successfully visualized with the measured data. The visualized fault patterns allow operators to observe the current status of SMB operation. This noninvasive technique, without much complex knowledge, can efficiently diagnose the possible cause of performance degradation. It is a useful method that can enhance the stability of SMB in the continuous operation.

**4155**

# Novel Small-Molecule Additives to Refold Proteins – A Simultaneous and Systematic Evaluation

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## ABSTRACT

Production of foreign proteins in E. coli may yield inclusion bodies – dense, insoluble aggregates of mis-folded protein. Inclusion bodies, however, are easily purified, resistant to proteolysis and can be solubilized with chaotropes. Defining a condition that promotes refolding of a chemically solubilized target protein into its native conformation is both empirical and difficult. This difficulty can be lessened by simultaneously and systematically evaluating multiple refolding conditions. Previously, we described two 96-well plate-based fractional factorial refolding screens – the iFOLD™ Protein Refolding Systems 1 and 2. Here we introduce the third generation of the iFOLD Systems. Like its predecessors, the iFOLD Protein Refolding System 3 is a 96-well plate-based refolding screen but differs from Systems 1 and 2 by utilizing novel protein refolding additives, termed the FoldACE™ Reagents. The FoldACE Reagents comprise five low molecular weight organic salts that were identified during multiple refolding screens. Using the iFOLD System 3, we have found that the FoldACE Reagents support the refolding of a variety of proteins, including a GFP-fusion protein, proteases, and an engineered antibody. Furthermore, the FoldACE Reagents support refolding at levels significantly greater than those obtained when using more traditional refolding additives, such as glycerol and l-arginine. Finally, we have demonstrated that following protein refolding, the FoldACE Reagents are easily and efficiently removed using size exclusion chromatography.

**4156**

# Prep SFC Method Optimization

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## ABSTRACT

A COMPREHENSIVE APPROACH WAS APPLIED TO DEVELOP A CHIRAL PURIFICATION METHOD FOR AN ANALYTE THAT WAS FOUND TO BE UNUSUALLY DIFFICULT TO SCALE-UP USING PREP SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC). IN THE CASE STUDY MAJOR FACTORS SUCH AS THE SOLUBILITY OF AN ANALYTE IN SFC CO-SOLVENT, CHIRAL STATIONARY PHASES, PRESSURE DROP, AND INJECTION CYCLE TIME WERE ANALYZED. A SERIALLY AND PARALLEL COUPLED PREPARATIVE COLUMN SYSTEM OF THE SAME CSP WAS DEVELOPED BY CONNECTING TWO P-SFC COLUMNS TO INCREASE RESOLUTION AND SOLVE THE SCALE-UP PROBLEM. THE INFLUENCE OF THE COLUMN SEQUENCE, THE LOADING AMOUNT AND PRESSURE DROP ACROSS THE COLUMN WERE ALSO STUDIED.

**4157**

# Thin-Layer Chromatography (TLC) as a versatile tool for preparative liquid chromatography process development

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## ABSTRACT

Thin-Layer Chromatography (TLC) has been used for a long time as a versatile tool for in-process control in organic synthesis as well as natural product fractionation. It can also be used in a straightforward way for the optimisation of process conditions for preparative liquid chromatography.
TLC has several clear advantages in method development over the approach to use small diameter HPLC-columns:

- It can be easily operated in parallel
- Strongly adsorbed compounds can be detected, giving an idea about sorbent pollution by sticking compounds.
- By using group-specific detection methods compounds of interest can be identified

Some prerequisites with respect to the phase system have to be taken into account. The most important ones are the use of stable mobile phases and the constant selectivity between the sorbent used for TLC and for prep LC.
We will report examples for the purification of natural compounds as well as for the determination of the maximum load on pre-purification silica columns. In the second example we will present a new approach using the quantification of scanned TLC-plate for the determination of fraction purities.

**4158**

# Local equilibrium theory for the binary chromatography of species subject to a generalized Langmuir isotherm: Wave interactions

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## ABSTRACT

The generalized Langmuir isotherm model was recently developed to describe the competitive adsorption equilibria of a binary mixture [1]. This formalism captures the competitive effect of solutes that show either Langmuirian or anti-Langmuirian behaviour. This results in four isotherm types that show markedly different chromatographic behaviour. The equilibrium theory of chromatography has been developed in a previous work to study Riemann problems (saturation, elution and the chromatographic cycle) that are of practical relevance [1].
This work focuses on the development of the equilibrium theory to account for wave interactions, and is divided into two parts. In the first part, the theory of wave interactions is developed based on the method of characteristics. It is shown that the rules developed for competitive Langmuir isotherms can be extended to describe possible wave interactions in the case of the generalized Langmuir isotherm [2]. The second part of the study deals with the development of the chromatographic cycle for binary pulse injections. The general case of short pulses in which the waves from the adsorption and desorption front interact within the column is considered. The solution of this problem results in a set of algebraic equations that describe the movement of the solutes in the column. The solution of the problem is unique to the type of adsorption isotherm considered owing to the different sequence in which wave interactions occur in the column. Finally the elution profiles for the four isotherm types obtained from the equilibrium theory are shown to be fully consistent with those obtained through numerical simulations.

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[2] Rhee, H.-K; Aris, R.; Amundson, N. R. *First-order partial differential equations*; Prentice-Hall: Englewood Cliffs, NJ, 1989; Vol II.

4159

# Optimization of IgG Binding on a Strong Cation Exchanger using DOE

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## ABSTRACT

Downstream processes are challenged to improve throughput over their current status as the MAb titer approaches 5 g/L. In order to keep up with demands and advances in upstream processes due to higher efficiencies in cell cultures, 4-6 times of the current downstream capacity or throughput will be needed. There is, therefore, a need for selective and cost effective purification processes. Of all factors determining throughput in a chromatographic process, media capacity, linear velocity of the mobile phase, as well as selectivity of the chromatography media need to be closely examined. Variables such as flow rate, pH and conductivity play a major role in resulting binding capacities of proteins on selected chromatographic media. However, finding conditions for maximum dynamic binding capacity (DBC) by changing one parameter at a time in experiments is often time-consuming due to the interaction of several variables. The following study is an attempt to utilize a simple approach to understand different parameters affecting the chromatographic process and meet the challenges posed by complex separations, as well as high throughput requirements. This study demonstrates that statistical tools such as Design of Experiments (DOE) along with Regression Analysis are useful in determining the optimum conditions for loading a selected protein, using a minimum number of experiments. We have employed simple DOE methodology and studied a strong cation exchanger, PolyCSX, to show how several interacting factors are important in determining dynamic binding capacity of large proteins such as IgG.

**4160**

# High Volume Radial Flow System to separate proteins and polyphenoles from beer

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## ABSTRACT

The poster describes the Combined Stabilisation System CSS as an alternative to traditional stabilisation procedures of beer. Breweries are enabled to adsorb both haze forming proteins and tannins in a single step after pre-filtration in order to increase the shelf-life of beer.
Base of the CSS is the use of regenerable, high grade cross-linked insoluble agarose beads which are permanently retained in one or more High Volume Radial Flow Columns. Implemented in an existing beer filter line, un-stabilised beer is pumped through this chamber where both proteins and tannins are being adsorbed within seconds. Finally, the adsorbed substances are removed by regenerating the agarose by using rock salt and caustic.

High flow rates, low pressure drops and no oxygen uptake have been found especially in production scale systems. The beer flow speed though the RFC system ranges from 15.000 liter per hour to well over 60.000 liter per hour. The radial flow columns withstand 6 bar overpressure and can be sterilized with hot water.

Other applications for the High Volume radial Flow Column are currently looked into.

**4162**

# Macroporous Polymeric Monoliths by Reactive Gelation for Protein Purification

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## ABSTRACT

The use of macroporous polymer supports for the chromatographic separation of biomolecules is old. Such supports are typically synthesized by suspension polymerization in presence of porogenic agents. Though remarkably simple, this process is not conceptually fully understood, so that its control remains difficult. The reactive gelation approach presented here mimics the mechanism of pore formation of suspension polymerization by a sequence of independent steps. Namely, crosslinked particles are first produced by emulsion polymerization and then gelated in controlled way, which determines size and type of porosity of the final polymer. The obtained gel is then re-polymerized to impart enough mechanical resistance to the material, so to obtain a porous polymeric monolith. Monolithic supports prepared by this technique have some key advantages upon conventional polymeric macroporous supports. First, the formation of the monolith is involving the polymerization of a small fraction of the total polymer only and it carried out in water as continuous medium, so that heat removal is much more efficient and large monoliths for industrial applications can be obtained. Second, the monolith formation as a sequence of independent steps allows a precise control of the porosity. In particular, monoliths with large permeability and no size exclusion can be easily obtained. Finally, the use of emulsion particles as starting building block allows a precise and economic surface functionalization of the support. A number of experiments will be shown to exemplify the control capabilities of the reactive gelation technique. Finally, some experimental results are presented to show the chromatographic applications of these monoliths. Hydrophobic (non-functionalized) supports, made of crosslinked methyl methacrylate, have been synthesized. These are applied for the purification of proteins by hydrophobic interaction chromatography. A second set of functionalized supports is presented for the use as a strong cation exchange material.

**4163**

# Ion Exchange Centrifugal Partition Chromatography: A convenient method for bioactive compounds purification

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## ABSTRACT

Centrifugal Partition Chromatography (CPC) is a countercurrent chromatographic method that was developed in the 1960's by Ito. This support-free liquid-liquid technique relies on the partition of the analytes between the phases of an equilibrated biphasic solvent system.

CPC in elution mode solves a wide range of purification problems, due to the infinite number of possible biphasic systems. Charged or ionizable molecules can also be efficiently purified by displacement CPC, a development mode that includes pH-zone refining and ion-exchange chromatography. The latter has been intensively developed in our laboratory during the last ten years. It can be used to purify anionic and cationic species, the chromatographic process being driven by a strong or by a weak exchanger. The resulting four separation types were used for the purification of a wide range of bioactive compounds.

Glucosinolates are potentially cancer protective, anionic molecules that were obtained by strong ion-exchange CPC. An anionic exchanger was used to prepare anthocyanins at gram-scale. Low molecular-weight heparins were fractionnated by means of a weak cationic exchanger.

The most recent purification protocols combine a weak and a strong displacement process for the isolation of biologically active peptides from vegetal sources.

**4164**

# Dynamic binding capacity of a cyanine-affinity chromatographic support using lysozyme as a model protein

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## ABSTRACT

A constant development of dye-affinity chromatography to replace more traditional techniques is being verified, with the aim of increasing specificity in the purification of biomolecules. The establishment of new chromatographic supports imposes their complete characterization, namely with relation to the binding capacity for proteins, in order to evaluate its applicability on global purification processes. In this work, following previous studies [1] the adsorption of lysozyme onto a thiacarbocyanine dye immobilised on beaded cellulose was investigated. The effect of different parameters, like temperature, ionic strength, pH, protein concentration and flow rate, on the dynamic binding capacity of the support to retain lysozyme was also studied. In this study we have concluded that increasing the temperature and the concentration of lysozyme had a positive effect on the dynamic binding capacity (DBC), whereas increasing the ionic strength and the flow rate resulted in the opposite. It was also discovered that the pH used had an important impact on the lysozyme binding to the support. The maximum DBC value obtained for lysozyme in this investigation was 6.2 mg/mL, which was achieved at 30°C, pH 9 with a protein concentration of 0.5 mg/mL and a flow rate of 0.05 mL/min.
The dissociation constant ($K_d$) obtained was $2.61 \times 10^{-5}$M, proving the affinity interaction between the thiacarbocyanine dye ligand and the lysozyme.

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**4165**

# Polishing of a monoclonal antibody with a mixed-mode resin in bind and elute mode

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## ABSTRACT

Monoclonal antibodies are typically obtained in a cell culture supernatant. The downstream processing is usually done with two main steps: the antibody is captured in the first chromatographic step. This is done here with a cation-exchange resin with the novel MCSGP technology. Run in a continuous operation mode, a purity of the antibody of approximately 95% with a titer of 4.5g/l is reached. The polishing is then done with the mixed mode resin Capto adhere from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). The resin is a strong anion exchange resin. Furthermore the stationary phase exhibits properties of hydrophobic interaction resins under certain conditions. This resin was developed for polishing of monoclonal antibodies in flowthrough mode. However, it is shown here, that the resin can be operated in bind and elute mode with excellent results. Beside the monoclonal antibody, the feed of the polishing step consists mainly of aggregates and fragments of the antibody. It will be shown, that high loadings at high pH lead to very pure product fractions. Purities of the antibody higher than 99.5% are achieved. The product quality was checked with an analytical Protein A resin, as well as size exclusion chromatography and ELISA. Various conditions for the polishing step with Capto adhere were checked. Optimal conditions regarding pH, modifier concentration and resin capacity were explored.

**4166**

# Design of steady-state recycling chromatography processes under arbitrary yield and purity constraints

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## ABSTRACT

Besides SMB systems, several single-column techniques, such as closed-loop recycling and peak shaving, have been suggested to enhance the yield of chromatographic separation by recycling the chromatogram partially or as a whole. Steady state recycling (SSR) chromatography is a further development of these concepts. In an SSR process, in addition to collecting the leading and trailing parts of the elution profile, a constant amount of fresh feed is added to each recycle fraction, which causes the process to attain a periodic steady state.

Here we present a method (based on the equilibrium theory) for analysis and design of SSR chromatography with arbitrary purity or yield requirements. The approach applies to SSR processes in mixed-recycle operation and allows for the direct prediction of the steady state and the design parameters without performing dynamic simulations. Therefore, it simplifies optimal design of SSR processes and simultaneously enables evaluation of process performance.

We have shown that, when the purity and/or yield of the product fractions is specified, the total injection width is the only free operating parameter in mixed recycle SSR chromatography. Theoretical analysis of the process revealed that the productivity is necessarily lower than that of an optimized separation by batch chromatography. On the other hand, the SSR process always outperforms batch chromatography in terms of eluent consumption and product concentrations.

**4167**

# RAPPTor™ – a new technology for high throughput downstream development

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## ABSTRACT

Downstream processing is becoming the main cost driver in the production of biopharmaceuticals since upstream technologies nowadays achieve very high product titres whereas downstream processes have to deal with only moderate advances in throughput capacities. Together with the demand for short development timelines a major challenge arises to develop both not only safe and robust but also highly economic downstream processes. With RAPPTor™, Boehringer Ingelheim´s new technology platform for protein purification development, highly optimized chromatographic steps are accomplished at a fraction of time and effort compared to conventional techniques. The experimental space of the platform is tremendous. In early product development phases when little information is available about molecule or cell line properties multiple binding and elution conditions can be rapidly screened and evaluated in parallel. At later stages the robotic technology may assist probing limits of individual process steps. Verification at small scale and scale-up showed excellent agreement with the micro-scale screening platform. The RAPPTor™ platform also supports an integrated process development accelerating time to clinic.

**4168**

# L-Ribose Separation from L-Arabinose Using Competitive Langmuir Isotherms in a Four-Zone SMB Unit

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## ABSTRACT

L-ribose and its necleoside derivatives have high potential as a precusor of anti-viral drugs. L-ribose and its derivatives have to be synthesized from L-arabinose because ribose in nature exists as D-form sugar or necleosides. Conventional four-zone SMB (1-1-1-1) was adopted for the separation of two L-sugars; four chromatographic columns were 2.5 cm inner diameter and 19.0 cm bed hieght in dimensions. In order to design an accurate SMB operation, adsorption isotherms of L-ribose and L-arabinose are a critical parameter. The chromatographic columns were packed with Dow 50WX4 200-400mesh ($Ca^{2+}$ form). Single-step frontal analysis with single-component at different concentrations of L-ribose or L-arabinose were carried out to determine the retention behavior of those in the column. Single-step frontal analysis with mixture of L-sugars were also performed to confirm the competitive interaction among L-sugars and the packing material. Better agreement with the simulation was obtained using a lab-scale SMB unit with new pump placement in practical operation. The mixture of L-ribose and L-arabinose was successfully separated using lab-scale SMB with purity and yield above 99.9 and 99.8%, respectively. The enrichment was 53.3 and 88.3% for L-ribose and L-arabinose, respectively. The scale-up study using a pilot-scale SMB, 5 cm inner diameter and 50 cm length in dimesions of column, for the separation of L-ribose from L-arabinose is being performed at the present time.

**4169**

# Development of novel mixed-mode cation exchange materials for antibody purification

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## ABSTRACT

Cation exchangers are often used in the downstream processing of monoclonal antibodies for which the need of adjustment of the salt concentration and pH of the cell culture supernatant is still a challenge. In this context we investigated the impact of functional group increments of the ligand to trigger binding capacities of the polyclonal IgG Gammanorm® onto the novel sorption materials under various NaCl concentrations. As support material epoxy activated Fractogel® (Merck, Darmstadt) was chosen, and a related strong cation exchanger functioned as benchmark. First we investigated a sulphonic acid in combination with a "thiophilic" sulphide bridge ligand which did not improve binding capacities significantly. The replacement of the sulphonic with a carboxylic acid led to a significant pH shift and improvement of the IgG binding maximum in conjunction with salt tolerance. Further studies proved the importance for the insertion of aromatic moieties near to the carboxylic group realized e.g. in a N-benzoyl cysteine scaffold. The static binding capacities of all materials have been measured under different pH (5-7) and 4 NaCl concentrations. The thus optimised mixed mode cation exchanger materials showed a binding capacity of 26g/L at pH 6.5 and 150mM NaCl compared to less than 1g/L for Fractogel® EMD $SO_3^-$. The materials with the best performances were tested for their total dynamic binding capacities with IgG Beriglobin® at 75 and 150mM NaCl and pH of 5.5 and 6.5 with binding capacities for the latter conditions of 18.9g/L.

**4170**

# Synthetic Peptide Purification: Are isocratic elution and volume overload viable options to improve process economics?

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## ABSTRACT

Synthetic peptide production is one of the major growth areas in biopharmaceutical drug development. Although there have been major advances in synthesis methodology many of the peptides still require purification with reversed phase HPLC being the method of choice. However, as the complexity of the peptides increases so does the challenge of purification.
For a purification process to be viable it must provide the API at the required purity and recovery but it must also meet the economic criteria. Contributions to the process economics will come from the capital equipment outlay and the consumables running cost so both must be considered to achieve the optimum process.

Many of the processes used for synthetic peptide purification are based around small particle high perfomance RP-silica materials and use gradient elution and so have high capital and running costs. We have investigated the possiblilty of using isocratic elution conditions for the purification of model synthetic peptides of the size typically produced as API candidates, 8 to 15 amino acids. Solubility is always a challenge in any purification – mass overload strategies are preferred to volume overload. Some peptides are only soluble at high pH or in agressive solvents such as strong acids or DMSO. The solubility in solvents compatible with an RP-HPLC method may still be limited and so in such cases a volume overload strategy maybe the only option.
Data will be presented to show how a synthetic peptide can be purified using volume overload and the effectiveness of mass and volume overload strategies compared.

From the work presented in this poster methods for the purification of synthetic peptides will be proposed which may result in improvements in process economics.

**4171**

# The influence of silica pore size on column loadability in reversed phase HPLC

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## ABSTRACT

There are a number of factors which must be considered when choosing a stationary phase for a prep or process HPLC separation. Some relate specifically to the process such as availability, manufacturers support and cost while others refer to the target compound and impurity profile. At the process development stage the focus is on the target compound and achieving the required purity and recovery to make the production economically viable. There are two dominant factors, the first is the selectivity of the HPLC media, maximising the resolution between the target compound and the impurities and the second is the capacity of the media - both of these will contribute to efficiency of the purification and the throughput of the process.

The work presented in this poster will look at the importance of the HPLC media pore size on the loadability and selectivity of the purification. Two HPLC materials are used in this study, SepTech ST60 10-C18 and SepTech ST150 10-C18, which are manufactured using silica of different porosity but with the same bonding chemistry. As the selectivity will be similar a comparison of the influence of pore size on the loadability of RP-silica materials can be made. Pore size characterisation data will be shown for the two materials and loading studies on a series of model model compounds of different sizes carried out. Using this data recommendations for pore size selection will be made.

**4172**

# SFC method development for the purification of the RG-3399 steroid API

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## ABSTRACT

During the production of pharmaceutical APIs or intermediates there are cleaning steps which had traditionally been achieved by selective crystallization. But nowadays - thanks to the stricter purity requirements – different types of fluid chromatographic cleaning methods are becoming more and more important. Preparative HPLC has got several advantages and significant disadvantages too. One of these disadvantages that after a HPLC cleaning a considerable quantity of (mostly organic) solvent mixture remains, which must be treated by regeneration and/or incineration.

For the past few years limit values of residual organic solvents in the APIs has considerably been decreased and the most commonly used solvents of normal phase chromatography (dichloromethane, n-hexane) is gradually being pushed out from application. As a result of the above listed reasons, some alternative techniques have come into the front, one of them is Supercritical Fluid Chromatography (SFC) which was studied in our work.

In our poster we present the method development of SFC cleaning of RG-3399, a steroid API produced by Gedeon Richter Plc. Eight organic solvents (methanol, ethanol, isopropanol, acetonitrile tetrahydrofurane, methyl-tert. buthyl-ether, ethyl-acetate and methylene chloride) were used as co-solvents and selectivities of RG-3399 and two critical impurities were determined at different pressures and temperatures in analytical SFC experiments. Having chosen the most appropriate stationary phase and mobile phase pairs, preparative SFC separations were carry out to determine optimal operating parameters and to study regeneration of the stationary phase. Finally, newly developed SFC method was compared with the presently applied preparative HPLC method.

**4173**

# Hydrophilic Interaction Liquid Chromatograpy for Preparative Purposes

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## ABSTRACT

Despite that reversed phase liquid chromatography is the most used separation technique, and can be employed for a variety of applications in junction with all common detection principles, certain solutes, i.e. polar and hydrophilic compounds, are not retainable. Hydrophilic Interaction Liquid Chromatography (HILIC) is a feasible alternative for such situations, as it is “orthogonal” to reversed phase chromatography. Solutes that have little or no retention and that may be affected by wettability problems on a reversed phase column generally have strong retention on a HILIC column. The separation mode utilises an eluent containing a high content of water miscible organic solvent (e.g. acetonitrile) to promote hydrophilic interactions between the analyte and a water wetted hydrophilic stationary phase, and is thus as technique in principle comparable to traditional normal phase chromatography. However, with respect to analyte solubility in the mobile phase and matrix compatibility, HILIC is superior, as the mobile phase compositions used are comparable to reversed phase separations. Herein, examples will be presented which all in all will illustrate the potential for HILIC for preparative scale applications.

**4174**

# Effluentless and bufferless amino acids and peptides separation by means of ESIEX-Electrical Swing Ion Exchange

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## ABSTRACT

Feasibility of a 3 steps process, involving neither pH buffer nor generation of effluents, was investigated to purify amino acids and peptides. The experimental set up is an electrodialysis cell with anion exchange resin between the membranes, namely an electrodeionization cell.

In the first step, the mixture to be separated is percolated on the ion exchange bed. Conditions must be chosen in order that only one species is fixed on the resin. For example, dipeptide GLYGLY in water is fixed on an anion exchange resin in carbonate initial form.

The second step is elution of GLYGLY with a carbonic acid solution (obtained thanks to the third step of the previous cycle). This solution allows to recover pure GLYGLY in water, contrary to other classical methods in ion exchange where desorption occurs in a buffer or in a salt solution. During this step, resin converts to bicarbonate form.

The third step is to return the resin to carbonate form. This is done by passing an electrical current trough the cell. An anion exchange membrane is chosen so that HO- move toward the resin bed. Half of the bicarbonate anions move trough the opposite anion exchange membrane of the cell toward a compartment where they dissolve in water. In this compartment, protons are produced by a bipolar membrane: thus elution solution for the second step is regenerated. The second half of bicarbonate anions transform on the resin to carbonate anions. Thus initial state is recovered.

**4175**

# Comparison of Chromatography and Polymer/Salt Aqueous Two-Phase Processes for Downstream Processing of Recombinant Phenylalanine Dehydrogenase

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## ABSTRACT

Phenylalanine dehydrogenase (PheDH; EC 1.4.1.20) is an important enzyme of amino acid dehydrogenases family that increasingly used as a valuable biocatalyst in neonatal screening kits and synthesis of L-phenylalanine. According to our study goal, we directly compared a polymer/salt aqueous two-phase system (ATPS) with an established chromatography process for the downstream processing of recombinant phenylalanine dehydrogenase produced by a recombinant *Escherichia coli* BL21 (DE3) cell. Direct comparison of chromatography and ATPS procedures clearly revealed that the ATPS consisting of 9% (w/w) PEG-6000, 16% (w/w) $K_2HPO_4$-$KH_2PO_4$ and 16% (w/w) KCl with pH of 8.0, $V_R$=0.25, temperature of 25 °C and 40% (w/w) cell-lysate was the most desirable procedure for downstream processing of recombinant PheDH. It contained five operation steps produced an overall specific activity of 4231.42 U/mg, yield of 96.66% and purification factor of 29.43. In contrast, the chromatographic protocol involved ten series of separation steps produced an overall specific activity of 553.57 U/mg, yield of 25.12 % and purification factor of 40.52. ATPS protocol could be completed less than 4 h, while the chromatography process performed in 72 h. In addition, based on economic analysis, the proposed ATPS was preferred (4 € Eur /system) to the chromatography process (2000 € Eur /column). Altogether, ATPS was cost-effective, time-saving and important at the commercial viewpoint.

**4176**

# New column format for process development

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## ABSTRACT

Development of purification processes is preferably done in small scale due to sample consumption, convenience, time and cost. The aim is to obtain an efficient, robust and scalable process with highest possible throughput at lowest cost.
The new HiScreen™ columns are prepacked with 13 different BioProcess™ media. The volume 4.7 ml and bed height 10 cm, make HiScreen columns suitable to use in process development. Two columns can also easily be connected in series to give a bed height of 20 cm.
All media prepacked in the HiScreen are available in other different prepacked formats, as well as in bulk packs. This makes it possible to use the same medium for development work, pilot studies and routine production.
Purification on HiScreen MabSelect Sure™ was scaled up 4.3 times with the same bed height. The results were equivalent for both columns. The effect on the selectivity is shown for the HiScreen HIC column family.
The robust design of the new prepacked HiScreen columns, together with optimized media provide ideal prepacked columns for screening of parameters such as selectivity and method optimization. Equivalent results are obtained when scaling up using larger columns showing the usefulness of these columns as process development tools.

**4177**

# Development and optimization of a new Single Column SMB technology on the laboratory scale controlled by an ÄKTA Explorer

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## ABSTRACT

Simulated moving bed (SMB) chromatography is a quasi-continuous counter current process that combines high productivity and high purities with reduced buffer consumption. With its advantages this technique is of growing interest for the biopharmaceutical industry. Especially the high requirements in validation are unique for this industry, in such cases the feed material is often limited and a laboratory scale unit would be helpful. We have developed a laboratory scale single column SMB (SC-SMB) unit with all four separation zones in one column. Distributors embedded within the chromatographic medium allow introduction and withdrawal of liquid between the zones. As a big advantage this single column unit exhibits homogenous packing in all zones, reduced headspace, less complex tubing, fewer valves, and almost undisturbed plug flow between the separation zones. Furthermore, the SC-SMB unit is operated with a modified ÄKTA Explorer workstation, which has been specifically developed for the handling of biological fluids. For operating the SC-SMB unit the ÄKTA workstation has been modified with one additional pumping system. Successful operation has been demonstrated with a binary model solution.

**4178**

# Optimisation of Temperature Induced Aqueous Two-Phase Extraction of Human Antibodies

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## ABSTRACT

The global market for the therapeutics with monoclonal antibodies reached US$10.3 billion in 2004 [1] and is forecasted to reach up to US$30 billion in 2010[2]. Although monoclonal antibodies are a powerful technique for the treatment of various human disorders only a few have been commercialized. This relatively small number of bioproducts shows how challenging and expensive is to produce effective antibodies. The downstream processing of antibodies can represent up to 80% of the total production costs [3] mainly due to the utilization of the expensive affinity chromatography technique in the capture step. For that reason several alternative techniques such as aqueous two-phase systems are being evaluated. Temperature induced ATPS is a powerful technique for the purification of monoclonal antibodies [4] because they allow and provide integrated clarification, concentration and purification of the target bioproduct in one unit operation [5] at reasonable costs. Due to high purity requirements for diagnostic and therapeutic proteins, several ligands have been developed in order to increase the selectivity of this technique. In order to achieve its full potential the system can be optimised using for that statistical valid models [6].

In this research work, the purification of IgG from a CHO cell supernatant with UCON 2000/dextran/ TEG-COOH system was optimised using a central composite design. Using an ATPS composed of 8% UCON, 6% dextran and 20% TEG-COOH (triethyleneglycol), IgG was purified, in two steps, with a global yield of 85% and 88% purity. These results open promising perspectives for applying ATPS with back-extraction induced by temperature as a first step in the purification of monoclonal antibodies from cell culture supernatants.

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**4179**

# Development of a thermally responsive agarose based ion-exchange chromatographic resin

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## ABSTRACT

Resins with thermally responsive functional groups have the potential to reduce the cost and environmental impact of separation processes in the food and pharmaceutical industry. A temperature responsive cation exchange resin was developed by grafting poly(*N*-isopropylacrylamide-*co*-acrylic acid-*co*-*N*-*tert*-butylacrylamide) onto crosslinked agarose (ItBA) Carboxymethyl modified crosslinked agarose (CM) with a similar ion exchange capacity to that of ItBA was also prepared. Lactoferrin adsorption isotherms at 20ºC and 50ºC were obtained statically for both resins and the maximum adsorption capacities ($B_{max}$) were determined using a Langmuir isotherm model. Resins were packed into PEEK columns (100× 4.6mm i.d.) and the dynamic adsorption and desorption characteristics of lactoferrin were studied at 20ºC and 50ºC. For CM there was no significant difference between the adsorption isotherms and $B_{max}$ values obtained at 20ºC and 50ºC. For ItBA there was a significant difference between adsorption isotherms at 20ºC and 50ºC, and the $B_{max}$ value at 50ºC was approximately 3 times higher than the $B_{max}$ value at 20ºC. When adsorping and desorping dynamically, a significant proportion of the adsorped lactoferrin was desorped from ItBA when the temperature was reduced from 50ºC to 20ºC. However, under the same conditions there was no desorption of lactoferrin from the CM. In conclusion, the developed ItBA resin shows temperature responsive adsorption and desorption of lactoferrin and maybe employed to reduce salt usage and processing costs in the food and pharmaceutical industry.