

# Recovery and Purification



## SCIENTIFIC ADVISORY BOARD

**Joanne Beck, PhD**, director of biologics manufacturing, Abbott Research Center

**Uwe Gottschalk, PhD**, vice president, purification technology, Sartorius AG, Germany

**David W. Kahn, PhD**, director, late-stage purification development, Human Genome Sciences, Inc.

**Duncan Low, PhD**, scientific director, process development, Amgen Inc.

**Jill A Meyers, PhD**, principal consultant, BioPro Consulting

**Charles Schmelzer, PhD**, senior scientist, late-stage purification, Genentech, Inc.

**Peter Wojciechowski**, director, purification technology, global biologics supply chain, Johnson and Johnson

## Overcoming the Downstream Bottleneck: Sharing Experience

by David Kahn

The Recovery and Purification Track focuses on some of the most pressing issues faced by downstream process scientists.

A variety of recent improvements in mammalian cell culture processes and process development have led to significant increases in product titers. These large increases in product mass delivered from the upstream process pose a large challenge for downstream processing, at both the harvest and subsequent purification steps. On Thursday, the "Recovery and Purification from High Titer Feedstocks" session will focus on this issue with speakers from Amgen, Medarex, Merck, Lonza, Johnson and Johnson, and GE Healthcare.

Vendors of media used in conventional chromatography steps are being pressed to produce resins with higher capacities and higher throughput, while maintaining high selectivity. This challenge also provides additional impetus for process scientists to further consider implementing new and alternative technologies or methods that can be adapted from related processing industries. Technologies that will be discussed include new resin matrices, expanded bed chromatography, precipitation, crystallization, and aqueous two-phase extraction. The session "Applying Novel Process Technologies" on Tuesday will include speakers from Genzyme, Amgen, Genentech, Upfront Chromatography, and Lund University.



A continuous challenge for the biotechnology industry is the characterization, validation, and monitoring of downstream processes. On Wednesday, the session "Strategies for Process Validation and Characterization" will feature case studies from Amgen and Johnson and Johnson. Improvements in the speed and sensitivity of product and impurity assays have made more extensive real-time process monitoring feasible (process analytical technology, or PAT). Still, the industry is challenged in understanding the appropriate way in which to implement this technology and how it interfaces with conventional in-process and bulk release testing. The use of PAT also may affect characterization and validation of downstream processes.

Finally, cost-of-goods pressures are also routinely faced by process developers. Use of disposal components in downstream processes may help lower

costs by eliminating the time and expense needed for cleaning validation. Speakers from Sartorius, Micromet AG, BioSystem Development LLC, and RMC Pharmaceutical Solutions will talk in the "Use of Disposables in Bioprocessing: Plastics and Elastomers" session on Wednesday afternoon.

With the growth in the biotech industry, more companies are facing the task of tech transfer to multiple facilities either within the company or to contract manufacturers. Speakers from Biogen Idec, Genentech, and Wyeth will present their experience in this area on Wednesday morning. These experiences lead to suggestions for best practices that may be implemented going forward.

## JOSEPH P. KUTZKO



**Principal Scientist, Therapeutic Protein Development, Genzyme Corporation**

*The Use of Hydrophobic Interaction*

*Chromatography (HIC) in Commercial*

**Who will be most interested in the subject matter of your talk?** Those who seek to understand how HIC is used in

commercial processes today. Process designers, such as I, will either seek validation of their own ideas or learn different applications for HIC.

**What do you expect them to “take away” with them?** HIC is a powerful purification tool that can be used for a wide range of applications depending upon the properties of the protein being purified.

**Have there been recent advances in HIC that made it more useful in bioprocessing — and if so, what?** The manufacturers of HIC chromatography media continue to expand their product lines, making HIC more useful for a wider range of products. Companies such as GE Healthcare and Tosoh Bioscience are very expert in the manufacture of HIC chromatography media. They are more than happy to supply off-the-shelf samples for evaluation and will work with you for custom designed media if you so desire.

**Is there promising work on the horizon?** The body of information is always expanding, and there is a HIC Bioseparation Conference every other year that explores applications and innovations in depth ([www.hic-rpc.org](http://www.hic-rpc.org)).

**Which presentation(s) are you most looking forward to attending?** I prefer not to mention names but I have my favorites. I just look forward to networking and meeting new friends in the sciences.

**How long have you been in the industry?** Over 20 years

**When/why did you get involved in the industry? What interested you the most?** I've always enjoyed the biochemistry side of the business. No two proteins are the same, and every new product is both a challenge and fun to work on.

#### NAVEEN PATHAK

*Senior Engineer, Manufacturing Science and Technology, Amgen Inc.*

*Computational Fluid Dynamics (CFD) Modeling of Commercial-Scale Chromatography Columns*

**Who will be most interested in the subject matter of your talk?** The subject matter will be most interesting to attendees who scale up chromatography operations to commercial scale; or for those who are involved in process improvement efforts for chromatography operations at large scale, or design large scale chromatography equipment.

**What do you expect them to “take away” with them?** They will take away an approach and a tool to assess the performance of the column using fundamental principles that can complement the testing that is usually required to assess such performance.

**What are some of the software programs available for CFD that could be used for work like you describe?** Fluent Inc. is the one we are using. There a couple of others

**You mention its use in column design — can it help with packing, etc. as well?** We have not explored this application until recently.

**How long have you been in the industry?** 12 years.

**When/why did you get involved in the industry? What interested you the most?** I like the complexity of biotechnology processes. Chromatography interests me the most.

#### JOSEFINE PERSSON

*Scientist, Early Stage Purification, Genentech*

**Case Study:** *Evaluation of Different Primary Recovery Methods for E. coli Derived Recombinant Human Growth Hormone and the Compatibility with Further Downstream Purification*

**Who will be most interested in the subject matter of your talk?** Industry representatives working with downstream purification.

**What do you expect them to “take away” with them?** Extractions in aqueous two-phase systems can be very efficient as a primary recovery step.

**What are the “further downstream” methods mentioned in your abstract?** Chromatography.

**What are the scale and throughput of your project?** This study was performed only at small scale (lab scale) at that point — we are starting to look into it again and will scale up to 10,000-L harvests.

**Which presentation(s) are you most looking forward to attending?** Case studies from the industry.

**How long have you been in the industry?** Five years.

**When/why did you get involved in the industry? What interested you the most?** I like to solve problems and I like the practical aspects of the work. The technical aspects and scale-up issues are some of my major interests.

**If visitors to the Bay Area could see/do only one thing, what would you recommend?** A drive on Highway 1.

**What is the best part of working in the Bay Area?** Perfect weather, not too warm and not too cold. A nice liberal environment.

#### WILLIAM K. WANG

*Senior Manager, Manufacturing Sciences, Biogen Idec*

**Critical Evaluation:** *Technology Transfer Considerations for the Relocation of a Commercial Manufacturing Process to a Different Facility*

**Who will be most interested in the subject matter of your talk?** Process development and manufacturing scientists, and tech transfer managers.

**What do you expect them to “take away” with them?** Decisions can be project- and situationally dependent, but the decision-making considerations should be consistent and comprehensive between projects.

**What's the general timescale of the project you'll be describing?** 12 months.

**Was the transfer complicated by long distances?** Yes, California to Massachusetts

**Were there comparability issues — and if so, did they require postmarket clinical studies?** No

**Which presentation(s) are you most looking forward to attending?** The new technologies session and the use of disposables session.

**How long have you been in the industry?** 13 years.

**When/why did you get involved in the industry? What interested you the most?** Interest in protein chemistry and process engineering during graduate studies.

**Senior Scientist, Purification Process Development, Amgen, Inc.**

**Case Study:** *Characterization of an Ion-Exchange Chromatography Step of a Recombinant Protein Purification Process*

**Who will be most interested in the subject matter of your talk?** People interested in process characterization. My talk will be centered on how the purification process development department at Amgen Colorado has been working on how to gain better understanding of the behavior of a given purification unit operation (in this case, an ion-exchange chromatography step) within the constraints of the operating parameters we impose upon it.

**What do you expect them to “take away” with them?** I hope to be able to give the audience our take on how we were able to incorporate performance data from bench, pilot, and clinical scales into a manageable series of steps that led to a defensible package of operating and performance parameters with associated classifications (critical/key/non-key), operating ranges, and acceptance criteria, that can act as a prelude to process validation.

**Have there been recent advances in IEC that made it more useful in bioprocessing — and if so, what?** Because IEC is so widely used, it was well suited for risk assessment tools such as FMEA that we used in the early steps of the characterization work. It was relatively easy to construct an exhaustive list of parameters from which to construct our initial list from which to work.

**Is there promising work on the horizon?** The use of PAT in IEC operations is gaining more attention; things such as transition analysis to examine column packing efficiency will, I'm sure, rapidly succeed over methods like HETP in the near future.

**What's the nature of the product in your case study?** The actual nature of the product is of no consequence; it is the methods of the characterization itself that are the topic of the talk. I have not cleared the contents of my talk through our legal department, so I honestly cannot say whether I'm allowed to disclose the nature of the product at this time.

**Which presentation(s) are you most looking forward to attending?** I will be interested in hearing about how other companies are approaching process characterization, as well as platform purification technologies, PAT, postapproval process improvements, and tech transfer.

**How long have you been in the industry?** I've been in the biotech industry for about 23 years.

**When/why did you get involved in the industry?**

**What interested you the most?** In the early 1980s, just about anything we did was brand new and advanced the cause, so it was a very heady time. I was involved in protein solubilization and refolding from inclusion bodies and hold some of the early patents involving detergent solubilization. I was interested in how detergent molecules interacted with protein secondary structure and how they influenced refolding dynamics.



**Senior Process Scientist, Global Technical Service, Global Biological Supply Chain, Johnson and Johnson**

**Case Study:** *Revisiting and Updating a Process Validation Package*

**Who will be most interested in the subject matter of your talk?**

Management and professionals in the area of quality assurance, regulatory affairs, process validation, and manufacturing in companies with old commercial products.

**What do you expect them to “take away” with them?** Strategies to conduct the process validation gap analysis, address the gaps, and risk management.

**What's the nature of the product/process in your case study?** Biological commercial products approved years ago with outdated validation status.

**Can you provide quick definitions of “critical quality attributes” and “critical process parameters?”** *Critical quality attribute:* a quality attribute of the drug product/substance that must meet associated test specifications or is the subject of a validation claim related to product quality.

*Critical process parameter:* a process parameter that must be controlled within predetermined criteria to ensure that all related critical quality attributes are met.

**Which presentation(s) are you most looking forward to attending?** Several presentations in the Recovery and Purification track.

**How long have you been in the industry?** More than 12 years.

**When/why did you get involved in the industry?**

**What interested you the most?** I started my career in biotech and the biopharmaceutical industry in 1994. I was attracted by the biotech-biopharma industry for its technology and its ability to produce life-saving drugs for millions of patients.







UWE GOTTSCHALK



**Vice President,  
Purification  
Technology, Sartorius,  
Germany**

*Chairperson's Remarks  
and Overview:  
Economic Process  
Models for the Use of  
Disposable Technology  
in Polishing*

**Who will be most interested in the subject matter of your talk?** Development and Manufacturing specialists in the area of downstream processing. Also heads of operation and engineering.

**What do you expect them to "take away" with them?** Downstream processing is in a bottleneck situation and requires more resources. To keep the pace with fermentation and cope with process economy with require technological breakthroughs. Disposable technology is one solution and a very hot topic.

**What are the products/processes that will be discussed in your case studies?** Monoclonal antibodies — recombinant proteins

**We've seen cartridge filters, but what do disposable IEC and affinity technologies look like?** Good question! From the outside, exactly the same. A disposable membrane chromatography device looks like a filter, is operated like a filter (installation, handling, flow rates, etc.). Users even call it a filter (and install it as part of the filtration train) although it has the full capabilities of a chromatography column. Because of the much higher flow rates, it typically has only 1% of the volume. The reason why it looks like a filter is that we are working on platforms where the outer cartridge itself is the same.

**Which presentation(s) are you most looking forward to attending?** New strategies in manufacturing (platform approaches, generic processes, disposable manufacturing, integration of USP and DSP).

**How long have you been in the industry?** 13 years with Bayer HealthCare in PD and manufacturing, three years with Sartorius in purification technologies.

**When/why did you get involved in the industry?**

**What interested you the most?** I came with a background in drug targeting with monoclonal antibodies and followed their roller coaster ride to success within the past 20 years very closely.

SCOTT FULTON



**Chief Executive  
Officer, Tarpon  
Biosystems**

*Use of Disposable  
Technology for  
Very Large-Scale  
Chromatographic  
Purification*

**Who will be most interested in the subject matter of your talk?** We believe anyone involved in the development of downstream processing would be interested. Also, anyone involved in the design of new large-scale plants for MAb production.

**What do you expect them to "take away" with them?** The major "take away" message is that there is an interesting link between disposable technology and simulated moving bed (SMB) chromatography that both makes disposability practical for chromatography operations and enables processes to run at a much larger scale than previously contemplated.

**With the high cost of certain chromatography adsorbents (e.g., affinity resins), can disposable technology really be economically viable in this type of unit operation?** It is very clear that the key barrier to making chromatography a disposable process is the high cost of the resin per gram of product produced (the unit resin cost). This unit cost is dependent upon both the effective capacity of the resin and the practical number of cycles that the resin can be run (the effective cycle life). SMB technology affects both capacity and the effective cycle life in a very positive way and, we believe, may make disposable chromatography at last practical. SMB also can be used to dramatically increase the practical scale for chromatography, which is its primary application in the chemical process industries. Very interestingly, we believe that the technologies of disposable processing are critical to making SMB a viable approach for biopharmaceuticals.

**Is membrane chromatography the answer?** The capacity of membrane systems has to date been too low for anything except polishing applications and unusually high MW products such as plasmid DNA or viral vaccines. Although new membrane adsorbents may improve upon this situation, we believe that the chemistry of the resin material is critical to achieving the purification needed, and that forcing users to change media to obtain other process benefits (such as disposability) will be difficult. We think a much better approach will be to bring these benefits to the industry with the resins currently in use.

**What are the typical “very high volumes” mentioned in your abstract?** A major focus for us has been the downstream purification challenges posed by the standard 15–20,000 L bioreactors with expression levels at 5 g/L and above (i.e. 75 – 100 kg product per batch), which is rapidly becoming the state of the art for MABs.

**Which presentation(s) are you most looking forward to attending?** I am most interested in the Economics and Recovery and Purification sessions.

**How long have you been in the industry?** I have been in the industry for 29 years.

**When/why did you get involved in the industry? What interested you the most?** I have mostly worked for “tools” companies (Amicon and PerSeptive Biosystems) on development of chromatography and filtration systems, but more recently have worked in biopharmaceutical development, particularly with transgenic systems. The most interesting thing for me about bioprocess is the fascinating interplay between very sophisticated engineering and biology.

HANS J. JOHANSSON



**Senior Scientist, GE Healthcare, Sweden**

*Current and Future Advances in Development of Downstream Processes for Purification of Monoclonal Antibodies*

**Who will be most interested in the subject matter of your talk?** Anyone interested in downstream process development or production.

**What do you expect them to “take away” with them?** Recent developments in design of chromatography media.

**Do you see disposables as figuring heavily into solving the downstream “bottleneck?”** I think they will be increasingly used, not so much in full scale production of high volume products (such as MABs), but definitely in preclinical and clinical production.

**What about membrane chromatography?** As a scavenger for trace contaminants, yes.

**What’s on the horizon that we should be looking out for?** New expression systems giving rise to new challenges in DSP.

**Which current products will you be assessing in your talk?** Agarose-based chromatography media with affinity (Protein A) or ion-exchange function.

**Which presentation(s) are you most looking forward to attending?** Anything related to industrial purification of MABs.

**How long have you been in the industry?** Over 25 years.

**When/why did you get involved in the industry?**

**What interested you the most?** Being born in Uppsala where modern chromatography evolved at the University of Uppsala and Pharmacia, it was pretty natural to get into separation science.

## POSTER PRESENTATION

### The Use of Chitosan to Improve the Throughput of Fed-Batch Mammalian Cell Culture Harvest During Clarification Unit Operations

*Julie Belliveau (Genzyme, 45 New York Avenue, Framingham, MA 01701)*

Cell harvest clarification is a critical but often challenging unit operation in the large-scale purification of recombinant proteins. The use of flocculants to improve the clarification of mammalian cell culture harvest has not been extensively studied. In this paper we examine the utility of adding chitosan to NS0 cell culture harvest to improve material throughput while maintaining high product (monoclonal antibody) recovery. Optimal chitosan concentration and flocculation conditions were selected based on scale-down studies. Chitosan was then added to harvests, which was subsequently clarified using a pilot-scale continuous flow disk-stack centrifuge followed by filtration on depth and absolute filters. Chitosan treatment improved filter throughput six to seven fold with no negative effect on monoclonal antibody recovery and purity.

Chitosan is inexpensive, free of mammalian derived components, and the procedure for its use is facile and applicable to large-scale production.

### Optimization of Mammalian Cell Culture Centrifugation Using the Partial Discharge Method

*Prince Bhebe (Amgen, Inc., One Amgen Center Drive, Thousand Oaks, CA 91320)*

The ability to optimize and control the centrifuge performance when processing increasingly higher cell densities in large-scale mammalian cultures is a key for successful, cost-effective recovery of product. In this work, I examine work that was performed at two different pilot scales to characterize the effects of critical centrifuge operating parameters on separation (clarification and dewatering), as measured by turbidity, volumetric yield, and filterability. Two Westfalia continuous disk stack centrifuge models, CSA-1 and CSA-8, were used to characterize the effects of feed flow rate, bowl speed, bowl opening time, percent bowl fill, and back-pressure on concentrate clarity using a partial discharge



method. Feed flow rate was found to be negatively correlated to clarification efficiency within the tested range. Maximum cell shear in the centrifuge bowl was observed at the initiation of the feed cycle. Cell lines that form aggregates during cell culture exhibit characteristic variability in percent solids content, which may affect accuracy in the determination of maximum feed volume before discharge. In addition, this work presents a pilot-scale comparison of separation efficiency between partial discharge and full discharge operations.

### **An Improved Method for Virus Filter Qualification and Implementation: Using Flow Decay to Determine Processing Endpoint**

*Jennifer Campbell (Millipore Corporation, 290 Concord Road, Billerica, MA 01821)*

Current practices for carrying out virus filter validation studies mimic a scale-down of "anticipated" manufacturing scale conditions, using equivalent hydraulic conditions (delta P or flux), volumetric throughput (L/m<sup>2</sup>), feed concentration, etc. The throughput (L/m<sup>2</sup>) at which virus reduction is demonstrated in the qualification study becomes the maximum throughput the manufacturer can process. However, qualification based upon volumetric throughput limits manufacturers to processing within the protein concentrations tested in the qualification. Furthermore, the addition of virus stock solutions during spiking significantly limits the volumetric throughput obtainable across the virus filter. We propose an alternative method, based on flow decay, as a more pertinent measure to determine the endpoint during filter qualification and during manufacturing, particularly for some parvovirus filters. Flow decay correlates to LRV more strongly than volumetric or mass loading.

Validation studies conducted with flow decay monitoring produce the required data to safely support full-scale manufacturing processing at a range of product concentrations.

### **Comparison of a CaptureSelect® VHH Antibody Fragment to a Conventional Monoclonal Antibody for Purification of Recombinant Factor VIII Protein**

*Anthony Chikere (Bayer, 800 Dwight Way, Berkeley, CA 94710)*

Heavy chain antibodies (VHH) commonly found in *Camelidae* are unique in that they lack the CH1 domain and the complete light chain. These antibodies can be isolated from llamas, which have been immunized against a particular target protein of interest. The VHH antibody fragment gene can then be cloned into microbial expression systems and screened for desired binding and elution conditions for the target protein. Unlike conventional antibody fragments (VH/VL), these fragments are highly soluble. Like conventional monoclonal antibodies, these fragments show high specificity and affinity for target proteins. These fragments, however, can be expressed inexpensively in a yeast expression system.

Recombinant FVIII, used for the treatment of Hemophilia A, is expressed in very low levels in mammalian cell culture. Typically immunoaffinity chromatography is required to capture and purify the protein to homogeneity. In this study a VHH ligand coupled to Sepharose® media was evaluated against a conventional monoclonal antibody. FVIII recovery, purity, and integrity were measured using optimized binding and elution conditions for both chromatography media. The VHH ligand coupled media was shown to give comparable or higher recovery and purity, while maintaining the integrity of the FVIII protein during the chromatography.

This new affinity medium offers the potential of substantial cost savings over conventional monoclonal antibody coupled media while giving equal or superior performance.

### **Applications of CaptureSelect® Affinity Ligands in Biological Product Purification**

*Bruce Dawson (BAC, Huizerstraatweg 28, Naarden 1411 GP, The Netherlands)*

Affinity chromatography is a well-established technology for purifying biological molecules from complex source materials. The most commonly used application of large-scale affinity chromatography is monoclonal antibody purification by bacterial coat protein (Protein A) resins. The widespread use of affinity purification in large-scale downstream processing has been hampered by the absence of safe affinity ligands that fulfill the needs of large-scale affinity chromatography including chemical stability, "tunable" affinity, high selectivity, short development times, and cost in use.

We have developed a technology that fulfils these basic demands by making use of *Camelidae* heavy chain antibody fragments. BAC's CaptureSelect® ligand technology is based on the rapid identification of highly stable and specific affinity ligands using immune antibody libraries efficiently expressing these heavy chain antibody fragments in the yeast *S. cerevisiae*. These ligands can be used for generic purification solutions such as human monoclonal and polyclonal IgG. CaptureSelect ligands can be custom made to solve virtually any biotherapeutic purification challenge.

In this poster we present several case studies of applied research and development using CaptureSelect ligands to purify a variety of antibodies, antibody fragments, recombinant proteins, and viral gene therapy vectors.

### **Determination of Optimal Packing Conditions for a Polymeric Reversed Phase Resin**

*Jon Fisher (Rohm and Haas Company, 727 Norristown Road, Spring House, PA 19477)*

Polishing chromatography processes require highly efficient chromatographic columns. Smaller, mono-sized resins can deliver high column efficiencies, but only when they are packed in their optimal configuration. Additionally, protocols used for the packing of reversed-phase silica do not provide satisfactory results when used with reversed-phase polymeric resins. Therefore, a factorial design study was conducted with a new mono-sized, polymeric reversed-phase resin, Amberchrom™ HPR10, to determine what conditions provided the highest efficiencies. The results demonstrate that proper packing conditions will provide the high column efficiency required for difficult separations.

Large-scale packing results also demonstrate the stability of this polymeric resin in preparative- and process-scale columns.

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

*Jon Fisher (Rohm and Haas Company, 727 Norristown Road, Spring House, PA 19477)*

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



detergent removal protocol using short chromatographic columns has been developed.

Polystyrene divinylbenzene polymers, packed in chromatography columns, were used for removal of nonionic, zwitterionic, and anionic detergents from protein solutions. The results demonstrate that this resin exhibited high capacity and was effective in removing a wide range of detergents at high linear velocities (>600cm/hr) with minimal loss in protein yield.

### **Tangential-Flow Microfiltration (TFF) Is Improved Significantly with the Use of Chitosan, a Polycationic Flocculating Agent**

*Garo Mikaelian (Genzyme Corporation, 76 New York Avenue, Framingham, MA 01701)*

In this poster we detail how TFF of a monoclonal antibody harvest with the addition of chitosan simplifies clarification, improves recovery, and increases product passage. Chitosan is a polycationic flocculating agent that improves product passage and flux through a TFF membrane to a point where TFF as a stand-alone filtration method is now feasible. The advantage of using TFF as a stand-alone system is that, it is a single unit operation rather than multiple unit operations, higher product yields are obtained, and it is reusable. These factors may contribute to lower operating costs than technologies currently used. Today, the most commonly used clarification technique is centrifugation together with either dead-end or tangential-flow filtration. Centrifugation/filtration of these "fed batch culture harvests" is difficult because of the high amounts of host cell components and cellular debris. Tangential flow filtration (TFF), as a stand-alone clarification method, has not been commonly used because of low flux, membrane fouling, and poor product yield. We have shown that these challenges can be overcome by using chitosan in conjunction with TFF. Chitosan improved TFF filtrate flux and product passage, making TFF as a stand-alone filtration unit operation more feasible.

### **Removal of Caseins from Transgenic Pig Milk Using Calcium Phosphate Microparticles**

*Tulin Morcol (BioSante Pharmaceuticals, Inc., 4600 A&B Highlands Parkway, Smyrna, GA 30082)*

We previously described a novel process for selective removal of caseins from normal and transgenic animal milk. During the initial stages of process development, we used normal cow milk supplemented or spiked with various human proteins as our "simulated transgenic milk" model.

In the present study, we tested our model process with milk from transgenic pigs producing high levels (3–4 g/L) of human coagulation factor IX (FIX). As the first step in the process, we "deconstructed" casein micelles in transgenic milk by destroying their natural calcium phosphate cores with chelating agents, such as a mixture of sodium citrate and EDTA. We speculated that any recombinant FIX associated with or entrapped within the large micellar structures would thus be freed in the EDTA/citrate-clarified milk. The next step involved the "reconstruction" or "reformation" of casein micelles away from natural whey proteins and the recombinant FIX using our proprietary calcium phosphate (CAP) particles.

After we removed the reformed casein micelles using low speed centrifugation, recombinant FIX was recovered in a clear, casein-depleted supernatant fraction for further processing. Using protein

determination, SDS-polyacrylamide gel electrophoresis, Western blot analysis, and human FIX-specific ELISA methods, we demonstrated that 1) caseins from normal or transgenic animal milk can be eliminated at neutral pH conditions using CAP particles; 2) the degree of casein removal from milk is dependent on the amount of CAP used in the process; and 3) the process provides about 90% recovery of the recombinant protein of interest in a clear, casein-depleted supernatant fraction that can be used in the succeeding purification steps (e.g., chromatography) with zero or minimum manipulation. In conclusion, we believe that the process presented here has great potential to be a viable alternative to existing transgenic milk processes because it removes the caseins in a simple process efficiently, without the need for extreme pH conditions; reduces the product loss significantly during the initial milk processing steps, and thus improves overall yields at low cost by providing a product-rich, clear fraction for the targeted chromatography steps.

### **Light Exposure of the Cell Culture Supernatant Leads to the Charge Variant Shift and Aggregation of Therapeutic Monoclonal Antibodies**

*Guihang Zhang (Human Genome Sciences, 14200 Shady Grove Road, Rockville, MD 20850)*

This poster summarizes our observation of the light-induced charge variant shift and aggregation of therapeutic monoclonal antibodies (MAb) in the mammalian cell culture supernatant. The culture supernatants were stored under various conditions with or without room light exposure. The therapeutic MAbs in the supernatant were then captured by Protein A affinity column, and the elution pool was further analyzed with SEC-HPLC, AEC-HPLC, and SDS-PAGE. The results of two therapeutic MAbs were given here. In the first example, the MAb supernatant was stable in the dark for two weeks.

However, a time-related product aggregation and charge variant shift were observed in the supernatant samples exposed to room light. The light-induced aggregation of the antibody product could not be fully reduced to a normal heavy-chain and light-chain and gave an additional 75 kD band in the reduced SDS-PAGE gel. The charge variant shift was partially related to the aggregation. In the second example, 76% of the charge variant shifting was observed under the room light exposure for six days. The light-induced charge variants had significantly reduced bioactivity. The light-induced antibody charge variant shift was related to the cell culture components and type of antibodies. Further characterization of the charge variant is in progress.

Based on these observations, it is recommended that the light exposure of certain cell culture supernatants during and after recovery be minimized for downstream processing.

### **Separation of a Covalently Bound HSA-Fusion Protein Dimer from a Noncovalently Bound Dimer by Blue Sepharose FF Chromatography**

*Guihang Zhang (Human Genome Sciences, 14200 Shady Grove Road, Rockville, MD 20850)*

HSA-fusion protein technology is a proprietary technology of Human Genome Sciences, Inc. that combines the therapeutic activity of a known protein with the long half-life of human serum albumin (HSA). Aggregates of an HSA-fusion protein (including dimer, trimer, and multimer) are found in the yeast fermentation supernatant as well as purification pools. In purifying these protein aggregates for biological and biochemical

characterization, we observed that there were two forms of dimer, one covalently bound and the other noncovalently bound. The two dimers could not be separated by size-exclusion chromatography or ion-exchange chromatography. Incidentally, we found that the two forms of dimer were readily separated by Blue Sepharose FF chromatography using a step gradient for elution. The potency assay indicates that the noncovalently bound dimer is biologically active, whereas the covalently bound dimer is inactive.

This study demonstrates the different biological activity of the two HSA-fusion protein dimer variants and suggests a new and potential application of Blue Sepharose FF chromatography to separation of covalently bound and noncovalently bound protein dimers.

## Effective Cleaning and Sanitizing of Anion-Exchange Chromatography Resins

Ng Paul (Bio-Rad Laboratories, 6000 James Watson Drive, Hercules, CA 94547)

An anion-exchange column soiled by DNA and/or endotoxin was successfully cleaned in place. A cleaning procedure using common and readily available chemicals was tested. Spiking studies showed >99% clearance of DNA in fractions containing 0.5M NaCl. The clearance of endotoxin by the cleaning and sanitizing procedure was greater than 6 log. Selectivity of the resin using gradient separation of model proteins was not affected.

## Recovery and Purification

from our archives at [www.bioprocessintl.com](http://www.bioprocessintl.com)

Arnold TE. **Fluid Purification Using Charge-Modified Depth Filtration Media.** *BioProcess International* 2005, 3(10): 44–49.

Bloomington G, Gandhi P. **Engineering Design Considerations for Column Packing in Large-Scale Biotechnology Facilities.** *BioProcess International* 2005, 3(6): 44–51.

Capodici J, et al. **Large-Scale Beta Propriolactone Inactivation of HIV for Vaccines.** *BioProcess International* 2006, 4(2): 36–42.

Carpenter JF, Hesterberg LK, Randolph TW. **High-Pressure Disaggregation and Folding of Recombinant Proteins: Examples and Production Cost Comparison.** *BioProcess International* 2005, 3(5): 46–55.

Curling J. **Final Word: The Challenges of Biopharmaceutical Processing.** *BioProcess International* 2005, 3(9, supplement): 73–74.

Dosmar M. **Could Membrane Fouling Be a Thing of the Past?** *BioProcess International* 2005, 3(2): 62–66.

Dosmar M, Meyeroltmanns F, Gohs M. **Factors Influencing Ultrafiltration Scale-Up.** *BioProcess International* 2005, 3(8): 40–50.

Gagnon P, et al. **Vendor Voice: A Ceramic Hydroxyapatite Based Purification Platform — Simultaneous Removal of Leached Protein A, Aggregates, DNA, and Endotoxins from MAbs.** *BioProcess International* 2006, 4(2): 50–60.

Immelmann A, et al. **Validation and Quality Procedures for Virus and Prion Removal in Biopharmaceuticals.** *BioProcess International* 2005, 3(9, supplement): 38–44.

Jakobsen M, Sandstrom C. **An Approach to Optimizing Large-Scale Cell Harvest: Meeting Design Challenges to Maximize Productivity.** *BioProcess International* 2006, 4(2): 28–35.

Jin Z, et al. **Vendor Voice: A Method for Automated Multistep (Multidimensional) Purification Process for Protein Recovery.** *BioProcess International* 2005, 3(5): 56–66.

Lander R, Daniels C, Meacle F. **Efficient, Scalable Clarification of Diverse Bioprocess Streams: Using a Novel Pilot-Scale Tubular Bowl Centrifuge.** *BioProcess International* 2005, 3(10): 32–42.

Lentine KR, et al. **Preparation of Redundant, Disposable Filtration Systems: Wetting, Integrity Testing, and Drying.** *BioProcess International* 2006, 4(6, supplement): 44–47.

Liu J, Rouse D. **Using Liquid Nitrogen To Maximize Lyophilization Manufacturing Capacity.** *BioProcess International* 2005, 3(2): 56–60.

Luo R, et al. **High-Concentration UF/DF of a Monoclonal Antibody: Strategy for Optimization and Scale-Up.** *BioProcess International* 2006, 4(2): 44–48.

Mills GJ, Bardo B. **Biopharmaceutical Filtration Validation: A Perspective on Regulations and Business Risk.** *BioProcess International* 2006, 4(5): 30–38.

Mora J, et al. **Disposable Membrane Chromatography: Performance Analysis and Economic Cost Model.** *BioProcess International* 2006, 4(6, supplement): 38–43.

Sellick I. **Vendor Voice: Chromatography Advisor #3 — Economic Benefits of Protein A Alternatives.** *BioProcess International* 2005, 3(5): 68–70.

Sellick I. **Vendor Voice: Chromatography Advisor #4 — Capturing Very Large Biomolecules with Membrane Chromatography.** *BioProcess International* 2005, 3(10): 58–59.

Schmidt S, et al. **An Integrated Concept for Robust and Efficient Virus Clearance and Contaminant Removal in Biotech Processes.** *BioProcess International* 2005, 3(9, supplement): 26–31.

Schroeder HG. **Sterility Assurance with Filtration: Taking Bioburden, Membrane Integrity, and Process Conditions Into Account.** *BioProcess International* 2006, 4(6): 38–47.

Scott C. **Chapter Three: Process Development — Turning Science Into Technology.** *BioProcess International* 2006, 4(3, supplement): 24–41.

Scott C. **Chapter Four: Formulation Development — Making the Medicine.** *BioProcess International* 2006, 4(3, supplement): 42–57.

Shukla AA, et al. **Strategies to Address Aggregation During Protein A Chromatography.** *BioProcess International* 2005, 3(5): 36–45.

Tepper F, Kaledin L. **Vendor Voice: A High-Performance Electropositive Filter.** *BioProcess International* 2006, 4(6): 64–68.

Wang C, Wang L, Geng X. **Refolding rHGCSF Expressed by *E. coli*: A Case Study Using the Unit of Simultaneous Renaturation and Purification for Proteins.** *BioProcess International* 2006, 4(5): 48–54.

Zhou JX, Tressel T. **Membrane Chromatography as a Robust Purification System for Large-Scale Antibody Production.** *BioProcess International* 2005, 3(9, supplement): 32–37. 🌐