

Recovery and Purification



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The New Bottleneck

by Cheryl Scott

Thanks to improved cell line engineering and culture practices, the biggest challenge in downstream processing comes from increasing production titers. Just since the turn of this century, the industry has gone from considering 1 g/L an impressive showing in an animal cell culture process to that being the baseline. High-capacity resins are needed to keep column and tank sizes low, to minimize solvent consumption and disposal, and to make the best use of facility space. New technologies such as membrane chromatography, simulated moving bed chromatography, precipitation, and crystallization may help reduce utility use and overall cost of goods.

One idea that is gaining momentum is the “platform approach,” which begins with a boilerplate purification process that is adjusted and optimized for a given project. This is possible only because of growing experience and increased understanding of proteins and process streams and may not be an option for other types of products any time soon. High-throughput analytical methods can be used to screen chromatography resins, buffers, and operating conditions to save time and effort at this stage just as in upstream process development.

Material harvested from a fermentation (broth) or cell culture (supernatant) process usually presents as a liquid suspension of cells or cell fragments, various products including the protein of interest, and leftover media components and metabolites. Viral vaccines must be attenuated, inactivated, detoxified, or killed — in addition to the usual separation and purification required for recombinant products. Purification is a stepwise process that may begin with cell breakage (for intracellular proteins) or separation (for proteins secreted into the medium) followed by concentration and purification steps of increasing specificity or resolution. Depending on the product, centrifugation,

filtration, extraction, precipitation, and preparative column chromatography are used, often in combination.

Several different chromatographic methods form the basis of most purification processes, and usually more than one type is used. Less expensive methods typically occur “upstream” (with larger process volumes) of more expensive steps (for more refined process streams). But column chromatography may not dominate in the future. “New charged membranes in filtration will probably replace a lot of column chromatographic systems,” suggests one industry consultant. Purification process optimization does not mean perfecting each step separately and expecting the final results to add up. Unit operations must be examined in concert with one another. The ultimate goal is to make as much purified product as possible by the cheapest, best reproduced, and most robust and efficient route.

Adapted from “Chapter Three: Process Development — Turning Science into Technology,” in BioProcess International’s Supplement Series March 2006 installment The Bio Process — A Primer for Biotech Manufacturing

ALAHARI ARUNAKUMARI



Director of Process Development at Medarex Inc. (USA)

15+ years in the industry

Downstream Design Considerations for Efficient Batch Processing of High-Titer Cell Culture Processes for the Production of Human Monoclonal Antibodies

An alternative approach to economical downstream processes for antibodies will be presented. The affinity capture step is replaced with equally efficient and higher-binding cation-exchange resins. This simplified nonaffinity purification

process for human MAbs consists of cation capture and disposable anion membrane polishing chromatography steps. These ion-exchange purification technologies have been successfully scaled up to produce clinical material for multiproduct campaigns. The impact of this nonaffinity two-step purification scheme on facility output in terms of batch processing time and cost will be presented and compared with traditional schemes.

Who will be most interested in the subject matter of your talk? Biotech process development and manufacturing groups

What do you expect them to “take away” with them? An alternative and economical purification process technology platform for antibodies that positively affects cost of goods for manufacturing

Which presentation(s) are you most looking forward to attending? Strategies and advancements in process technologies and manufacturing efficiencies

What are the titer levels and volumes currently facing purification process developers — and what do you see in terms of numbers for the near future? On an average, 2 to 4 g/L and in exceptional cases above 5 g/L at the 20,000-L to 100,000-L scales — and in the future 5 to 10 g/L may be an average figure and batch volumes may be up to 100,000 L.

When/why did you get involved in the industry? What interested you the most? Genetic engineering and recombinant protein production

WOLFGANG BERTHOLD



Chief Technology Officer in SVP Technical Development at Biogen Idec Inc. (USA)

25+ years in the industry

Increasing Output: Interdependency and Constraints of Titer, Process, and Facility

This talk will address the series of technical bottlenecks that we encounter in our current facility design when the titers get very high. All these thoughts have to do with cost considerations. The message is that there could be a number of different strategies to either “save” the facilities (by adjusting the manufacturing processes) or establish new operations and designs.

Who will be most interested in the subject matter of your talk? Senior management of entrepreneurial process development and manufacturing plant managers

What do you expect them to “take away” with them? Increasing output will create a number of subsequent challenges that can be advanced in different ways for each plant and process.

Do you see “disposables” (single-use systems) as figuring prominently in the facility issues you’ll be addressing in your talk? Disposables will be mentioned but not prominently.

What about modular construction? Plants are already modular, a plug and play mode could be a consideration for special problems.

How long have you been in the industry? More than 25 years in biotech and process development

When/why did you get involved in the industry? What interested you the most? I was working on interferon and wanted to see it benefit patients.

THOMAS R. KREIL



Director of Global Pathogen Safety for Baxter Bioscience (Austria)

15 years in the industry

Process Development and Virus Reduction Studies: The Link

During product development, limits for process parameters critical for virus reduction are defined by the interaction of process validation and virus reduction studies. Later, in routine production, process excursions are evaluated on the basis of those earlier generated results. Potential pitfalls and practical solutions for coordinating process development, operations, and virus validation are presented.

Who will be most interested in the subject matter of your talk? Late-stage R&D scientists, process validation engineers, and pathogen safety scientists

What do you expect them to “take away” with them? Only if communication flows openly and the above-mentioned groups work together in an inclusive fashion will the process of bringing a development product into the manufacturing scale be effective.

How long have you been in the industry? 15 years in different R&D and operations functions

When/why did you get involved in the industry? What interested you the most? I was already doing my PhD thesis there. Beyond the science, it’s about making a difference in patients’ lives!



JOHN LIDDELL



*Head of Process Science at
Avecia Biotechnology
(UK)*

10+ years in the industry

New Directions in Downstream Processing: Nonantibody Proteins

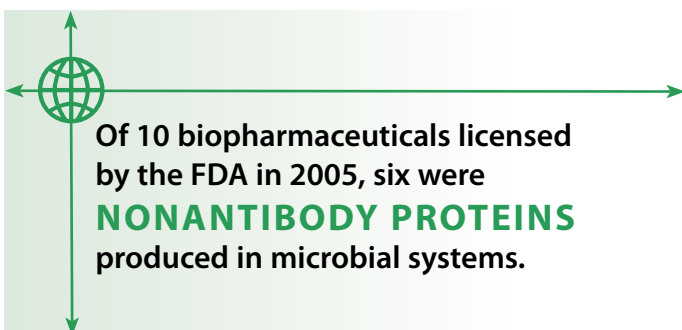
Nonantibody protein therapeutics require a case-by-case approach to downstream process development. New approaches to overcome development bottlenecks have been emerging: e.g., high-throughput process development techniques, reassessment of alternative techniques to chromatography, novel chromatography methods, improved predictive approaches, and data mining to inform new process development. The potential for new approaches to affect development timelines for biotherapeutics will be reviewed, with discussion of the regulatory situation and learning from previous innovations in protein purification.

Who will be most interested in the subject matter of your talk? People most interested in the subject matter of the talk will be those involved in purification of nonantibody therapeutic proteins. Presentations at this type of conference in recent years have been dominated by discussion of antibody production issues. Given the number of antibody therapeutics in clinical development that is understandable. However, of 10 biopharmaceuticals licensed by the FDA in 2005, six were nonantibody proteins produced in microbial systems.

What do you expect attendees to “take away” with them? An up-to-date overview of developments in purification development, some of which may be less familiar such as rational approaches and data mining techniques

Which presentation(s) are you most looking forward to attending? The presentations by Arne Staby and Dr. Christian Echemann will be the two I'm most looking forward to attending. They should provide interesting perspectives to new methods in process development.

What current technological advances do you think have the most potential for nonantibody recombinant proteins? In the nonantibody recombinant protein area, technical advances



having the most potential come from application of high-throughput screening techniques combined with advanced analytical methods.

Can single-use systems revolutionize the industry? To an extent that revolution is already here. Certainly at Avecia we make extensive use of disposables (e.g., buffer bags and filters) across all scales of operation.

How long have you been in the industry? I have been involved in biopharmaceutical protein production for over 10 years.

When/why did you get involved in the industry? What interested you the most? My PhD was in protein structure determination using X-ray diffraction, and things just evolved from there. What interests me in this industry is the ability to make a real difference to people's health through purification of therapeutically active proteins.

DUNCAN LOW



*Scientific Director of
Process Development
at Amgen Inc.
(USA)*

~30 years in the industry

The Future of Antibody Purification

The larger quantities of antibody emerging from upstream processing are putting considerable pressure on current processes and facilities. Consequently, manufacturers are exploring multiple ways of streamlining product recovery and purification. These include methods for decreasing the number of steps and alternative formats such as expanded and moving beds, membrane chromatography, and nonchromatographic purification methods (e.g., precipitation, crystallography, and aqueous two-phase systems). Such nonplatform technologies will be examined in terms of speed to clinic and cost regarding economies of scale and capital avoidance.

Who will be most interested in the subject matter of your talk? Process development scientists and engineers and financial controllers

What do you expect them to “take away” with them? There is a balance between time to market and the most optimal process.

Which presentation(s) are you most looking forward to attending? There are many fine talks, so I wouldn't like to single out one in particular.

What current technological advances do you think have the most potential for “the future of antibody purification?” Ideally, any unit operation that can provide a high-yield, high-purity, and low-cost initial purification and recovery concentration step

Can single-use systems revolutionize the industry? There are multiple areas where they can be used to advantage, but *revolutionize* seems a bit strong.

How long have you been in the industry? Since the late 1970s

When/why did you get involved in the industry? What interested you the most? My early ambitions for working in industry were the typically craven ones of an impoverished PhD student. I also thought a job in Switzerland would be cool because I ski, and the taxes are low. I ended up in Sweden, where the countryside was flat but the taxes sky high — but hey, it begins with *Sw!* I'm glad to say that, as the biotechnology industry took root, I was fascinated by its potential to bring benefit and delighted that I had developed the skills to contribute.

FRED MANN



*Technical Marketing Manager
for Millipore Corporation
(United Kingdom)*

23 years in the industry

Debottlenecking Downstream Purification: Current Trends and Future Solutions for Production of Monoclonal Antibodies

Advances in cell culture development for monoclonal antibodies have created downstream processing challenges in how to process an increasing mass of protein both economically and in a time efficient manner. This presentation will focus on the clarification and initial purification of such feedstocks and will discuss current and new solutions for both secondary clarification and capture and how to use them.

Who will be most interested in the subject matter of your talk? Those interested in maximising throughput and increasing productivity in downstream processing for monoclonal antibodies; and those looking to increase flexibility in manufacturing through increased use of disposable technologies

What do you expect them to “take away” with them? Be aware of recent developments in clarification and capture technologies in downstream processing for monoclonal antibodies.

What presentations are you most looking forward to attending? There are a number of interesting-looking presentations: “Increasing Output: Interdependency and Constraints of Titer, Process, and Facility” by Wolfgang Berthold (Biogen Idec); “Scale-Down Model for High-Throughput Process Development” by Christian Echerhmann (Boehringer Ingelheim); “A Return from the Darkside” by Kevin Cox (Avecia); “The Future of Antibody Purification” by Duncan Low (Amgen); and “Continuous Counter Current Liquid-Liquid Chromatography: Potential for Future Large-Scale Purification” (speaker to be announced).

Will you discuss single-use technology as part of your talk? Yes, a key element of the talk is developments in integrated disposable solutions to clarification.

How long have you been in the industry? Too long — seriously, since 1983

When/why did you get involved in the industry? What interested you the most? I joined Amicon in 1983 working on the development and scale-up of process chromatography columns and systems. The opportunity to take my chromatography knowledge gained at the bench during postdoctoral studies and take it to larger scale for industrial use

ARNE STABY



*Head of Protein Separation at
Novo Nordisk (Denmark)*

12 years in the industry

Robot Technology, Mathematical Modeling, and Miniaturization to Speed Up Process Optimization for Ion-Exchange Chromatography

The increased demand of material for clinical trials and handling of a higher number of projects in the biopharmaceutical industry are calling for new ways of performing process development. This talk presents the implementation of high-throughput screening techniques as stand-alone and in combination with mathematical modelling for purification development, troubleshooting, and batch release for ion-exchange chromatography steps. These techniques will be discussed in relation to scale-up, general accuracy of results, and potential regulatory issues.

Who will be most interested in the subject matter of your talk? In general, people performing process development at laboratory scale

What do you expect them to “take away” with them? A new set-up for performing process development where traditional chemical engineering meets biotechnology approaches combined with HTS techniques

Which presentation(s) are you most looking forward to attending? Usually, I attend to meet collaborators and get new contacts — I have probably seen most of the presentations in various forms before.

Does the approach you'll be discussing apply equally to other types of chromatography processes (e.g., affinity, hydrophobic-interaction, etc.)? Yes, but so far we have mainly looked at IEC.

What are the relative timelines between your method and “traditional” process optimization? You can save up to 75% in process development time, with less need for material.

When/why did you get involved in the industry? What interested you the most? Options to stay in academia were very few, and the offer I got from Novo Nordisk was very close to a dream job (I did not apply for any other jobs).

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