October 2023 | Volume 21 | Number 10
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DOWNSTREAM PROCESSING

- Imaging Packed Chromatography Beds and Beads for Process Improvement
- Proteomics and Host Cell Proteins
- The Importance of Proactive Maintenance
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- Defining Permissible Daily Exposure Levels for Parenteral Products
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- INSERT: Gene Therapies

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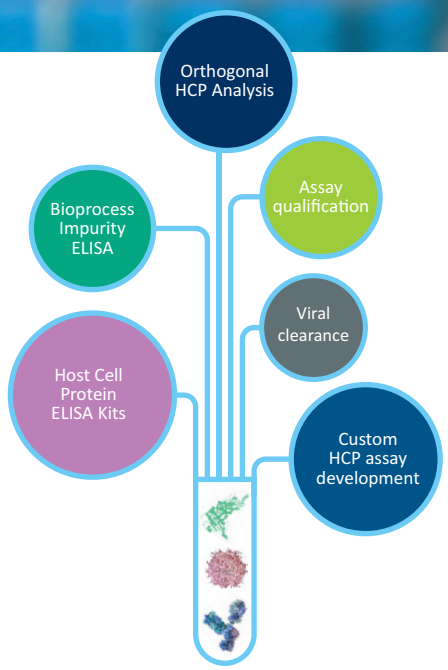

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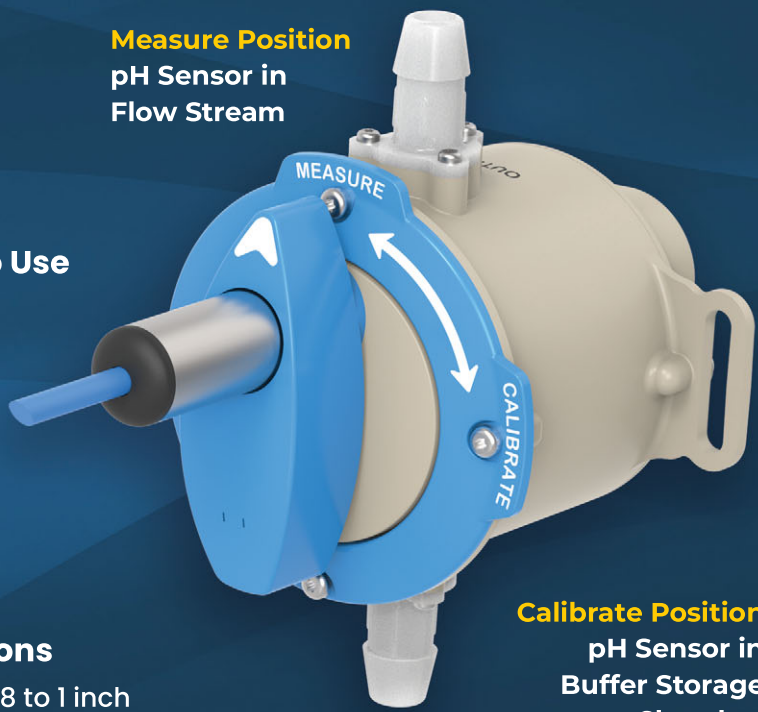
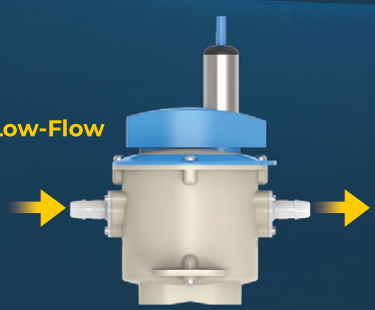


Measure Position
pH Sensor in Flow Stream

Calibrate Position
pH Sensor in Buffer Storage Chamber



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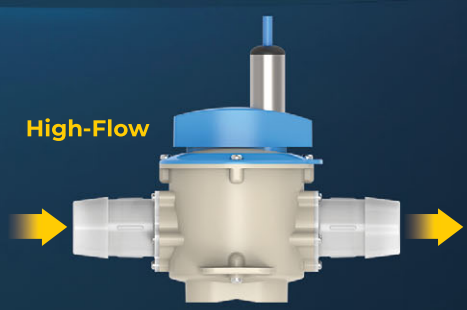

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BioProcess International

OCTOBER 2023
VOLUME 21 NUMBER 10

On the Cover
Chromatography and filtration systems dominate biopharmaceutical downstream processing, as illustrated by this image from Adobe Stock (HTTPS://STOCK.ADOBE.COM). *Learn more about this month's BPI contents — both in print and online — on page 4. And find us online at https://www.bioprocessintl.com.*

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BioProcess International (USPS 0022-044, ISSN 1542-6319) is published monthly except for combined issues in January, July, and November by Informa Connect at 605 Third Avenue, 22nd Floor, New York, NY 10158; phone 1-212-520-2700, fax 1-212-202-4567, https://www.bioprocessintl.com. Periodicals postage paid in New York, NY, and additional mailing offices. **POSTMASTER:** Send address changes to *BioProcess International,* 22701 West 68th Terrace, Suite 100, Shawnee, KS 66226-3583. Electronic subscriptions are available online.

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BPI's 2023 eBooks address many topics. This month, look for our feature on assay development.

Featured Report: Gene Therapies

As gene-therapy developers move their products into clinical trials and commercialization, associated manufacturing platforms continue to evolve. This month's **insert** highlights a number of technologies for producing DNA and viral vectors — and for characterizing and analyzing the results with an eye toward clinical testing.

Focus on Business

Opportunities abound for biotechnology entrepreneurs. Industry, scientific, and technical expertise isn't all they need, however, to turn their ideas into successful businesses. Running a company takes a distinct set of knowledge and skills. In beginning his occasional series on **page 10,** Martin Eckler introduces key elements of starting a biotechnology company.

Focus on Manufacturing

In biomanufacturing, minor production issues can lead to dire consequences, including compromised product quality and regulatory noncompliance. That makes proactive equipment maintenance indispensable to bioprocess operations. On **page 16,** Eric Whitley shows how proactive maintenance schemes can help companies secure regulatory compliance, reduce downtime, improve product quality, and manage risks.

Focus on Outsourcing

Outsourcing biomanufacturing and development processes is increasingly part of balancing budgets while ensuring reliable, high-quality production. As Anthony Newcombe writes on **page 14,** biopharmaceutical companies should choose contract manufacturing organizations (CMOs) that can offer the necessary expertise, regulatory support, and technological capabilities. Careful CMO selection prevents manufacturing delays, supply-chain disruptions, and setbacks. Companies successfully navigating the complex outsourcing landscape can mitigate risks and ensure manufacturing success through strategic decision-making and effective partnership development.

Looking Inside Chromatography Columns

Packed-bed chromatography is a vital downstream operation for purifying valuable biologics. Highly porous microspheres packed into cylindrical columns enable complex feed streams to be purified through characteristics such as size, charge, and hydrophobicity. On **page 30,** Thomas Johnson and Daniel Bracewell demonstrate high-resolution imaging techniques that can be used to visualize and characterize complex geometries, helping process engineers to understand how the detailed, internal structures of chromatographic materials relate to function and performance.

Patient Exposure to Inactivated Proteins

In multiproduct facilities, cross-contamination of pharmacologically active proteins must be controlled. Guidance on control strategies for solvents and small molecules does not apply directly to inactivated therapeutic proteins (TPs) occurring as impurities in subsequently manufactured drug products. TPs denature and degrade when exposed to cleaning processes. Permitted daily exposure levels (PDEs) specify the amount of residual TP that poses no risk to patient safety as an impurity in another drug. On **page 20,** Graham et al. examine available data to support a protective default parenteral PDE for denatured/degraded TPs.

Current Insights on Host-Cell Proteins

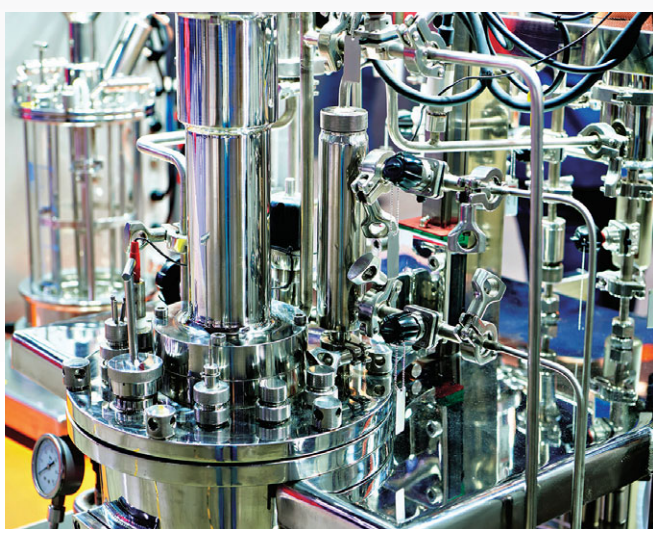
Host cell proteins (HCPs) are bioprocess-related impurities. Insights provided by proteomic analysis are shedding light on the related biophysics of downstream processes. On **page 34,** Abraham Lenhoff and Chase Herman provide an overview of this rapidly-evolving field, focusing on the role of Chinese hamster ovary (CHO) cell proteins in monoclonal antibody (MAb) bioprocessing.

On the Supplier Side

This month's supplier contributions offer solutions for downstream processing. On **page 44,** Sartorius defines the levels of process intensification and shows their positive effects on productivity and efficiency in chromatographic operations. And on **page 42,** Trelleborg highlights the sustainability and flexibility benefits that come with polypropylene-reinforced single-use chromatography columns.

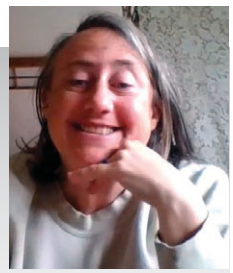
Dressing for the Occasion

Ensuring compliance with industry regulations is essential for keeping both workers and patients safe. Professional attire for working in laboratories and cleanrooms is integral to international standards. As Steven Cumper points out on **page 48,** compliance also helps companies look after their valuable teams.



DOWNSTREAM PROCESSING

FROM THE EDITOR



This issue goes to production as we editors board our flights from Eugene, OR, to the east coast for Biotech Week Boston. As you can imagine, it's our biggest show of the year — and it will be my first time back since the pandemic disrupted everything three years ago. I have missed greatly our yearly get-together with far-flung staff members from Europe and across the United States. It's almost like a family reunion with all the associated logistical complications and not-enough-time laments. We're very grateful for Informa's Streamly platform these days — as are most conference-goers, I'm sure — because it's hard to catch every interesting talk between meetings and parties and casual encounters with favorite authors and advisors in the corridors and exhibit hall. If you see us anywhere, don't hesitate to stop and chat because that's one of our favorite parts of business travel.

That's partly because we're always looking ahead to the next issue, next featured report, next eBook, and so on. Already our 2024 schedule is mostly planned out, but we expect to refine and adjust that based on what we hear from friends, colleagues, authors, readers, and advisors in Boston. It is the nature of publishing, unfortunately, despite the wise counsel of Yoda, who admonished Luke Skywalker in *Star Wars* for looking always "to the future, to the horizon. Never his mind on where he was. Hmm? What he was doing."

Consider the mental gymnastics required for copyediting — focusing intently on every word in every sentence of a manuscript, how they fit together, and all the many variations they may take to get from start to finish — while simultaneously juggling the main message of that article, how it will look on the page, the associated graphics, other articles and departments in a given issue, how they fit the overall theme . . . and keeping in mind the hard deadlines of print publishing, the articles in process at various stages for upcoming issues, custom-publishing projects that never seem to follow a set schedule . . . and add writing projects and freelancer management and public-relations questions and proofreading other editors' work and answering dozens of emails about all these things and more. And wait, where was I? Oh yes: writing an editorial.

Details matter. We can't lose sight of them as we lift our heads and look at the bigger picture. Yoda was right in redirecting Luke's attention to the here and now. Such advice applies far beyond an editor's world; it's vitally important in biomanufacturing, where ultimately lives are at stake. This month's theme of downstream processing is all about details. Our technical and supplier-side authors dig down into the nature and management of host cell proteins, the physics and chemistry of chromatography, and the allowable residuals from cleaning processes. The nontechnical pieces highlight the finer points of equipment maintenance, outsourcing, and entrepreneurship. So please take a moment to breathe, give them your attention, and reap the rewards of a few minutes spent in the now.

—Cheryl Scott

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BioProcess Insider

The BioProcess Insider *portal delivers financial and business news online alongside expert views about the commercialization of biopharmaceuticals. Here are a few recent stories edited for print. Visit* https://bioprocessintl.com/category/bioprocess-insider *to find in-depth discussion and sign up for the thrice-weekly newsletter.*

EU Clinical Trial Numbers Worrisome for Future CGT Approvals

by Millie Nelson

Although optimism surrounds the future of cell/gene therapies (CGTs) — in part because of the 10 regulatory approvals between the United States and Europe for nine different CGT products in 2022 — challenges surrounding advanced therapies remain a hot topic in the life-sciences space. Conversations address calls for innovative payment models, patient-access complications because of different healthcare systems, and difficult regulatory pathways to approval.

A panel discussion at Phacilitate's September 2023 Advanced Therapies Europe (ATE) event in Lisbon, Portugal, discussed the European landscape for CGTs, highlighting a low number of clinical trials taking place when compared with the number of studies in United States and Asia. "Europe has around half the number of clinical trials compared to the Asia–Pacific (APAC) region," said Elisabetta Zanon, director of EU public affairs and advocacy at the Alliance for Regenerative Medicine (ARM). "In phase 1 clinical trials, the European Union has around 80, whereas there are more than 300 in North America and APAC." Although Zanon acknowledged that it might be too early to say what will happen in 2024, "it is predicted that three times more regulatory approvals will happen in the United States compared [with] approvals [made] under the European Medicines Agency (EMA)." She described that forecasting as "really worrisome" and asked, "Does this mean in the future we will have [fewer] therapies being approved in Europe?" Because clinical trials must happen before commercializing a given product, she noted, evaluating "the whole ecosystem" and spurring action at both "an EU level and a national level" are both important for improving approval rates.

Miguel Forte, chief executive officer (CEO) of Kiji Therapeutics, concurred with Zanon's comments. He added, "We need lots of clinical trials. You need to have them to have future approval."

Chicken and Egg: Anthony Davies, CEO of Dark Horse Consulting, described the mutual importance of clinical trials and commercialization as a "chicken-and-egg situation" because "you do not get commercial approvals without clinical trials." Despite the lower numbers for clinical trials in Europe compared with other regions, he remained positive about the European CGT landscape and closed the panel by saying that he believes "the field is thriving, but it could thrive more."

HHS Award To Advance COVID-19 Vaccines and Therapeutics

by Millie Nelson

The US Department of Health and Human Services (HHS) awarded over US$1.4 billion through the Administration for Strategic Preparedness and Response's (ASPR's) Project NextGen, which aims to enhance preparedness for future COVID-19 strains and variants. "Project NextGen is a key part of the Biden–Harris Administration's commitment to keeping people safe from COVID-19 variants," said HHS secretary Xavier Becerra. "These awards are a catalyst for the program, kickstarting efforts to more quickly develop vaccines and continue to ensure availability of effective treatments." Here is a full breakdown of the funding allocation:

- $1 billion will go to four Biomedical Advanced Research and Development Authority (BARDA) clinical-trial partners to support vaccine phase 2b studies: ICON Government and Public Health Solutions in Hinckley, OH; Pharm-Olam, LLC, in Houston, TX; Technical Resources International (TRI) in Bethesda, MD; and Rho Federal Systems in Durham, NC.
- $326 million will go to Regeneron to support the development of a next-generation monoclonal antibody (MAb) for COVID-19 prevention.
- $100 million will go to Global Health Investment Corp (GHIC), a nonprofit organization that manages the BARDA Ventures investment portfolio. The funds will be invested in new technologies that will accelerate future pandemic responses.
- $10 million will go to Johnson & Johnson Innovation (JLABS) for a competition through Blue Knight, a BARDA–JLABS partnership.

According to HHS, funding clinical studies will advance the development of new vaccine candidates. The organization also pledged to provide a network of trials with the flexibility to use the most promising candidates as they become more established. The Regeneron partnership will speed efforts to prevent COVID-19 infections by producing a MAb therapeutic for patients who cannot be inoculated with available vaccines. Clinical trials for that candidate are anticipated to begin in fall 2023. The remaining funds will be used to

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explore technologies that can expedite development and production strategies. In turn, the funding will accelerate time lines and strengthen vaccine and therapeutic accessibility.

"As the virus continues to evolve, we need new tools that keep pace with those changes," said Dawn O'Connell, assistant secretary at ASPR. "Project NextGen combines the research and development expertise at HHS with the lessons we have learned about the virus throughout the pandemic, strengthening our preparedness for whatever comes next."

Thermo Fisher's New Magnetic Particles Offer Cost-Reduction Option

by Dan Stanton

Through its Gibco division, life-sciences services company Thermo Fisher Scientific has launched a next-generation platform of Dynabeads superparamagnetic-polymer particles. They can be used by cell-therapy developers to adsorb bioreactive molecules and cells. According to company representatives, the CTS Detachable Dynabeads platform contains an active-release mechanism that uses a release buffer to help detach the product from target cells during the manufacturing process, offering "process flexibility, compatibility with automation, and scalability." The beads are designed to help users achieve greater control of their cell-therapy processes.

As part of Gibco's portfolio of buffers, cell-culture media, and reagents, Dynabeads particles contribute to Thermo Fisher's efforts to address the high costs of cell-therapy development and manufacturing. "We support the research work," said Thermo Fisher's CEO Marc Casper, who emphasized that such tools are key to enabling such work. Speaking at the Handelsbanken Third Annual Life Science Innovation Day in August 2023, he added, "The big challenge here is cost." Thus, industry suppliers have incentive to "drive the cost down so more patients can benefit from these medicines."

Along with providing media and reagents, Casper said that Thermo Fisher Scientific's goal is to help decrease cell-therapy costs. Beyond equipment and tools, the company has a contract manufacturing network that includes significant CGT capabilities. "Our goal is to drive the cost down meaningfully so that more . . . patients can benefit. It's going to be a journey. It took 20 years or so on the MAb side. And we're going to try to do it as fast as we can because it's worth it. The cures that are being brought out are huge, and if we can make [them] affordable, [they] will get adopted more significantly."

Rentschler, CGT Catapult, and Refeyn Team Up on AAV Processes

by Dan Stanton

Contract development and manufacturing organization (CDMO) Rentschler Biopharma is combining its expertise with those of CGT Catapult — a UK-based advanced-therapy incubator and collaborator — and analytical-instruments company Refeyn to address difficulties and inefficiencies in manufacturing adenoassociated viruses (AAVs). The goal of the two-year project is to develop a digitized and automated manufacturing platform for AAVs, which are used to produce over 65% of the gene therapies currently in development.

According to Rentschler, the collaboration "will improve understanding and control of the AAV-manufacturing process and help to increase process yield, robustness, and scale-up while keeping product quality attributes under control." The spokesperson added that if the project is successful, the resulting process will be applied across the company's full biomanufacturing workflow to pass benefits on to customers. "The project focuses on [process analytical technology] PAT during the upstream process. Learnings from that will translate directly into the Rentschler upstream-manufacturing platform process and help optimize productivity for production on behalf of our clients." The representative added, "The learnings will also help to implement PAT technologies into our downstream process. We also will explore the applicability of some key aspects for other viral vectors."

The project will be carried out at the CGT Catapult's location in Stevenage, UK, where Rentschler set up shop in 2021. The project has been funded through a Digitalisation and Automation of Medicines R&D and Manufacture grant from Innovate UK.

TherageniX and UK University Team To Develop Powdered Gene Therapy

by Millie Nelson

TherageniX, a spin-out from the University of Nottingham in the United Kingdom, is developing a dry-powder gene-therapy formulation for bone-graft augmentation. Innovate UK has provided TherageniX and the University of Nottingham with a £995,000 (US$1.2 million) grant. That funding will support the development of a gene-delivery system intended to

improve outcomes for patients who undergo bone-grafting procedures.

Initially, the grant will focus on orthopedic applications. Anandkumar Nandakumar, CEO of TherageniX, told *BioProcess Insider* that the funding will enable his company "to test different manufacturing methods, and we will select the optimal method based on factors such as cost [and] stability." Although TherageniX acknowledged that transplantation of autologous bone tissue "is the gold standard bone-repair strategy," the company cited drawbacks, stating that many bone implants risk failing because of infection or poor integration. When problems arise, patients can experience delayed recovery, reoperation, and higher treatment costs.

TherageniX's nonviral gene-delivery system transforms a liquid formulation into a dry-powder gene therapy. It works by combining autologous bone-marrow cells from a patient with the company's platform technology and advances the production of genes to aid the regenerative capacity of bone, skin, muscle, and cartilage postsurgery. Transfection of a patient's cells requires no additional time in the operating theater.

The grant will run for over two years, with the two beneficiaries hiring a team to work on the project. No specific worker count has been disclosed. The work will take place at the University of Nottingham's laboratories with help from a number of partner organizations that are based in the United Kingdom.

Benefits of Dry-Powder Formulations: The handful of gene therapies currently available are formulated as single-dose injections or infusions, but the US Food and Drug Administration (FDA) approved the first "redosable" gene therapy in May 2023: in the form of Krystal Biotech's Vyjuvek (beremagene geperpavec-svdt) gel. Regarding recent grants and approvals for nontraditional gene-therapy formulations, Nandakumar said that "different ways of administration are one of the facets of how the field is evolving." He added that the benefits of a dry-powdered gene therapy include "simpler storage so that highly specialized equipment may not be needed, which also means that we can deploy it in areas that do not have access to such equipment. A longer shelf life allows the end user to stockpile and [enables] us, as manufacturers, to have larger batch sizes to reduce costs. It is also easier to handle a powder compared with liquids during transportation."

GSK Invests $268 Million in Belgium Plant To Support Vaccine Sales

by Millie Nelson

GSK's Shingrix vaccine for shingles earned sales of £2.96 billion (US$3 billion) in 2022, up 75% from the previous year. Then in 2023, the FDA approved the company's Arexvy vaccine for respiratory syncytial virus (RSV), making it the first such product available for inoculation against that virus. Those events led the company to invest €250 million ($268 million) in manufacturing operations at a site in Wavre, Belgium, which the company describes as the largest vaccine-production site in the world.

"This major investment is once again recognition of the expertise and know-how of our employees in Belgium," said Emmanuel Amory, managing director at GSK Belgium. "Our business continues to evolve, and we adapt to future needs. Together we are developing new skills . . . and technologies . . . to stay at the forefront of the industry."

The new vaccine freeze-drying unit at GSK's Wavre facility is designed to improve product quality, increase the efficiency of manufacturing processes, and minimize environmental impact. The facility also includes suites for vaccine formulation and filling and freeze-drying. According to GSK, the lyophilization step is important to working with vaccines that are unstable at high temperatures or in need of a long shelf life. Freeze-drying supports the transport and distribution of vaccines in areas where a cold-chain supply may be difficult to maintain. The new center will be split into two wings, with one dedicated to "live" vaccines and the other to "nonlive" vaccines. GSK intends to manufacture tens of millions of doses annually and begin freeze-drying operations in 2027.

Shingrix Sales: GSK launched the Shingrix product in October 2017, and demand rapidly outstripped supply. Thus, the company expanded its network with a facility in France, then invested $100 million in its Hamilton, MT, site. In 2020, it invested $564 million in its facility in Belgium. The company reported a 47% decline in Shingrix sales in May 2021 because of market changes brought on by the COVID-19 pandemic. But under a year later, vaccine sales recovered with the Shingrix vaccine pulling in sales of $866 million during the first quarter of 2022, and $3 billion for the full year.

Dan Stanton *is founding editor, and* ***Millie Nelson*** *is editor at* BioProcess Insider*; dan.stanton@informa.com.*

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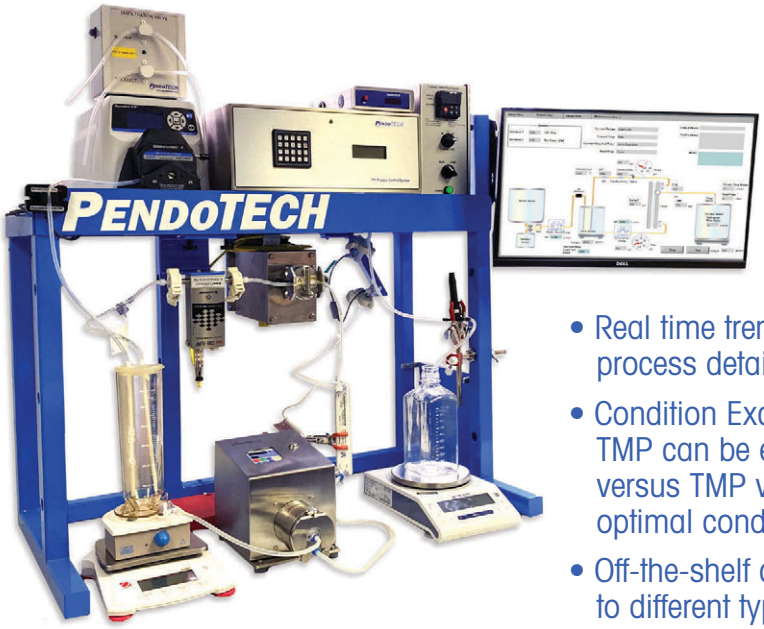

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Ideation and Conceptualization

Strong ideation is the first step to building a new business. The best business ideas solve problems, even if those problems are unrecognized by the people who have them. "Ideas for innovative biomanufacturing technologies are inspired by observing and pinpointing problems that have yet to be solved. Although you can be successful by "building a better mousetrap" and improving upon an existing solution, the biggest opportunities lie in inventing innovative solutions. You can use your knowledge, skill, and experience as an industry professional to observe industry needs and then reevaluate them from a business-building perspective.

Market Research and Validation

Before you develop a solution, market research can help you validate the existence of a market for your envisioned solution. It is best to begin by researching the market from a high-level perspective, studying products/services that are similar to your own idea and assessing their market size and growth trends. You'll need to confirm that there is a market gap that can be filled by your product/service.

You'll also want to determine the size of your target market — how many companies have the problem that you're preparing to solve? For your business to thrive, the market needs to be robust enough to support your idea.

Next, it is important to gauge the interest of your potential customers. You need to know how they feel about your concept and whether they are willing to pay money to address the problem you are preparing to solve. If you know people who have that problem, then speak with them directly about it. You also can assess market demand by using online surveys and establishing in-person or virtual focus groups.

Your next step should be to test the market by developing a *minimum viable product* (MVP), which is a basic version of your product that will function as a solution, but without any "bells and whistles." You can use minimal resources to build an MVP and then test the market by introducing it to customers. That saves you from spending excessive time and money building a product that people don't want.

Once you've developed your MVP, you'll test it among a group of *early adopters*, customers who agree to be among the first people to sample new innovations. These customers will provide you with feedback about your product, detailing how and why they use it and describing additional features they would like to have implemented. That feedback will help you to improve the design and development for the next version of your product.

Business Plan Development

You'll need to develop a business plan before you build the next version of your product. Doing so will guide you to think through each operational aspect of your business. As you develop a plan, you will need to research and develop strategies for all functions of your business.

Analyzing the budgetary aspects of your plan will help you to determine its financial viability. You'll need to understand your startup costs to determine whether you can fund your own launch, or whether you'll need to seek financial backing elsewhere. During this phase you also will calculate whether your business can turn a profit based on production costs and the amount that the market will pay. That information will help to inform business-growth projections as you gain new customers. Finally, a business plan is necessary for obtaining funding later. Even if you don't need startup money, you may need funding as you seek to grow your business in the future. A business plan has several components, including the following:

- a company overview describing your history thus far (even if you've only done ideation and market research), as well as your company mission, vision, and business structure
- a description of the problem that you're solving, your solution, and your proposed pricing model
- a market analysis that includes both the results of your market research and a competitive analysis
- a sales and marketing strategy for building awareness of your product and enticing people to buy it
- a technology strategy that details how your product will be developed and maintained from a technical perspective
- an operations plan for managing your day-to-day business
- a management and personnel summary identifying necessary roles and how will you fill them
- a financial analysis that addresses startup costs, revenue, and cost projections for at least three years
- an executive summary that provides highlights of your business plan. You'll write that section last, after you've developed your plan.

You may want to hire a professional to help you develop your plan. You often can find business-planning resources at local small-business development centers and business incubators.

Investors **SHARE YOUR GOAL** of growing your company quickly. They want it to reach a level at which they can exercise a successful exit, whether through the sale of the company, a merger or acquisition, or a public stock offering.

Funding and Investment

Many founders start businesses using their personal funds and then survive by "bootstrapping" until the company becomes profitable. Doing so is advantageous because you won't pay interest or give up equity to investors. However, growing a company significantly often requires a large amount of capital. Life-science industries have many people and companies that like investing and lending to support new innovations.

If you need to secure funding to start or support your business, banks offer several loan options. Many banks facilitate US Small Business Administration (SBA) loans, which are backed by government-supported SBAs and come with favorable interest rates. However, bank and SBA loans require repayment with interest that will reduce the cash flow to your business. Banks also offer little support for business management and strategic development.

Alternately, you can seek professional investors to provide capital in exchange for equity in your company, eliminating your need to make payments or pay interest. Investors often provide significant support in terms of resources and strategic advice, sometimes taking on managerial roles that can provide founders who lack business-management backgrounds with tremendous support. In such cases, founders may need to sacrifice significant equity in their companies and even some measure of control. But investors share your goal of growing your company quickly. They want it to reach a level at which they can exercise a successful exit, whether through the sale of the company, a merger or acquisition, or a public stock offering.

Usually investors seek a return of five to 10 times their original investment upon departing a company. For example, if they invest US$1 million for 30% equity in your business and seek 10× return on investment, they'd be looking for an exit price of $33.3 million with a personal share of $10 million. In such an example, you could walk away with $23.3 million. Not bad!

Your local business incubator is a good place to start identifying investors. Such services often provide access to seed funding programs, sometimes through government support. They can introduce you to "angel" or venture capital investors who specialize in life-science industries. Be prepared with a "pitch deck" of slides for presenting your business plan and capturing investor interest.

Product Development and Team Building

You are now ready to build the next version of your product. When designing, prototyping, and testing, it is important to incorporate learnings from your MVP to ensure that you are meeting the needs of your target market. You also may consider protecting your intellectual property with a patent if you think your work could be duplicated. Investors can help you assess the patentability of your product and sometimes can refer you to a local patent attorney.

Next, it's time to implement the management and personnel aspects of your business plan. At this stage, you may need to fill only some positions. Determine what roles are necessary to get your product to market and to maintain your business in the short term.

*Continued on **page 19***

Selecting a Contract Manufacturing Organization

Key Considerations for Successful Biomanufacturing

Anthony Newcombe

In our current financial climate, biotechnology companies are facing significant funding difficulties that necessitate careful decision-making when it comes to outsourcing biomanufacturing processes and balancing budgets. Reliable, high-quality bioproduction is paramount to success. Considering the complex nature of biomanufacturing and the intricate requirements involved, biotechnology companies should choose contract manufacturing organizations (CMOs) that operate within current financial constraints and that possess the expertise, regulatory compliance, and technological capabilities necessary to ensure seamless technology transfer and high product quality. Therefore, CMO selection is important to preventing manufacturing delays, supply-chain disruptions, and setbacks with clinical programs, development timelines, and critical milestones. By exploring the complex landscape of CMO selection, process sponsors can make strategic decisions and develop effective partnerships, helping to mitigate risks and increase the likelihood of manufacturing success.

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Manufacturing Expertise

A CMO's **track record** is important, especially regarding whether a manufacturer has worked with products that are similar to what a sponsor needs to produce. An experienced CMO can offer valuable insights, specifically with process analytics and troubleshooting. Equally important is whether a CMO has a history of successful technology transfers. A CMO with a team of experienced scientists, engineers, and manufacturing specialists can help sponsors to mitigate risks associated with scale-up to commercial production.

The Transition from Process Development to Commercial Production: Typically, CMOs performing good manufacturing practice (GMP) production do not need to use bioprocess equipment from the same vendor that sponsors used during small-scale development activities. Many equipment suppliers even provide predictive models for evaluating scale-dependent parameters, such as conditions for production-scale bioreactors. However, it is critical for sponsors to investigate potential equipment-related scale-up issues, which could result in extended timelines and increased costs. Moreover, equipment suppliers are likely to provide scale-up support for programs that use their technologies for both small-scale development and commercial manufacturing. Although downstream processes generally are considered to be less scale-dependent than are cell-culture processes, a sponsor still should determine whether a given CMO has successfully transferred small-scale downstream processes developed using specific equipment. Doing so can minimize risks of equipment issues.

A sponsor also should consider its long-term manufacturing strategy and how that aligns with available production scales at a prospective service provider. Although many CMOs for clinical activities offer batch sizes up to 2000 L using disposable bioreactors, such capacities may be unsuitable for future commercial manufacturing needs. Moreover, small batch sizes might be required for GMP clinical campaigns, potentially necessitating use of smaller bioprocess equipment or partly filled bioreactors. Although most bioreactors have a specified minimum fill volume, sponsors should evaluate a CMO's experience with manufacturing at different scales based on specific needs for clinical and production scales.

Technology-transfer experience and success are key aspects to consider when evaluating contract manufacturers. An experienced CMO will assign to each sponsor a dedicated project manager who possesses the necessary expertise to ensure a smooth technology-transfer process. An effective contract partner also will have

a well-established transfer process. That typically entails comprehensive assessment of process and facility compatibility, evaluation of equipment requirements, and identification and mitigation of potential risks. By gaining technology-transfer experience, CMO personnel learn how to integrate a client's technology seamlessly into their operations, ultimately contributing to the success of future projects.

Many CMOs specialize in drug-substance production and can make final bulk drug substance efficiently. However, some such companies do not have the facilities needed to undertake drug-product manufacturing, specifically capabilities for automated fill–finish activities. In such cases, it becomes necessary to engage another service provider that specializes in fill–finish operations. It could be advantageous to work with a CMO that offers only drug-substance services if a sponsor has an existing partnership with an established drug-product manufacturer. Leveraging such connections can reduce time and minimize potential delays.

The success rate of batches manufactured is a metric that holds particular importance. Typically, CMOs maintain a batch-failure or -rejection rate below 10%. However, that figure can vary depending on the complexity of the manufacturing processes involved. Batch failures can stem from process-related issues encountered with early stage projects. Thus, a lower-than-average success rate is not necessarily indicative of CMO performance.

New and Small CMOs: Working with a recently established or less-experienced contract manufacturer should not be ruled out because such partnerships can lead to long-term opportunities that result in mutual growth and success. A new CMO might be more flexible and open to tailoring its services to meet specific requirements than an established manufacturer might be. New contract partners sometimes offer competitive pricing to attract clients, and they can be more willing to negotiate pricing terms and offer cost-saving solutions. Because a new CMO is building a reputation and client base, it also might be able to give a project more attention than would a larger contract manufacturer. That said, drug developers should conduct thorough due diligence when evaluating a new service provider's capabilities and experience and when assessing risks for delays and problems.

Analytical Experience

One crucial criterion is whether a CMO has an established **quality control (QC) group** with expertise in verifying standardized analytical methods and procedures that have been established by recognized pharmacopoeial organizations, such as the United States Pharmacopeia and the European Pharmacopoeia. Experience with in-house method qualification and validation is valuable, too. Although it is common for some specific analytical tests to be outsourced, sponsors must determine whether a CMO's QC group can provide necessary support for in-process testing and product release.

A related consideration is a CMO's ability to perform **stability studies,** which involve long-term, GMP-compliant experiments that assess a product's stability over time under different conditions. Such studies provide crucial information about product shelf life, storage requirements, and recommended storage durations. Sponsors should determine the number of ongoing stability programs that a prospective CMO is performing on behalf of customers. That information can indicate a contract partner's experience with and capacity for coordinating and executing complex studies effectively, helping the sponsor to ensure accurate performance of analytical testing and reliable generation of data.

Although a CMO's **analytical instrumentation** need not be identical to what a sponsor has used during small-scale process development, there are certain situations in which using the same instrumentation is preferable. Simple laboratory instruments (e.g., for measurement of pH, conductivity, and absorbance levels) are unlikely to result in significant differences despite being from different suppliers. However, when measuring specific quality attributes (aggregate and particle levels) or product characteristics in a final drug substance or product, it can be advantageous to use the same vendor's equipment during process development and subsequent manufacturing activities. During contract negotiations, CMOs sometimes offer to provide specific analytical systems to support a project.

Evaluating a contract manufacturing organization's history of REGULATORY INSPECTIONS represents a key component of a sponsor's vendor assessment.

Regulatory and GMP Compliance

When evaluating CMOs, ensuring compliance with regulatory standards is of the utmost importance. A sponsor should determine whether a prospective partner consistently adheres to relevant regulations, such as GMPs and specific requirements set by health authorities such as the US Food and Drug Administration (FDA) and European Medicines Agency (EMA). Reputable CMOs maintain comprehensive quality management systems to support ongoing adherence to standards.

Evaluating a CMO's history of regulatory inspections represents a key component of a sponsor's vendor assessment. Results from routine GMP inspections provide insights into a manufacturer's compliance and identify areas for improvement. Audits conducted by other customers serve as an additional layer of scrutiny, offering valuable information about a contract organization's manufacturing processes, QC systems, and overall compliance. Requesting information about a CMO's audit schedule and the outcomes of regulatory inspections can provide a sense of the company's track record in meeting customer and regulatory expectations. International Organization for Standardization (ISO) certifications such as ISO 9001 (quality management) and 13485 (medical devices) further validate a CMO's

commitment to quality management principles.

During vendor selection and auditing, customers should communicate openly about their specific needs and expectations regarding regulatory compliance. Detailed audit observations and responses typically are treated as confidential information, but CMOs should be able to provide high-level summaries and discuss actions taken to address findings without breaching confidentiality. Willingness to provide high-level information demonstrates a CMO's transparency and dedication to addressing regulatory concerns while respecting the privacy of clients. Open communication and alignment of expectations are key to a successful partnership with a compliant and reliable CMO.

Location

Process sponsors would do well to consider location when shortlisting potential CMOs. Sponsor-company representatives should conduct site visits as part of contract discussions so that they can meet CMO project teams in person. Frequently, a member from the drug developer's quality assurance (QA) team also participates in a vendor audit as part of a supplier-qualification program. Therefore, sponsors should account for travel distance and logistics.

As a drug program progresses, someone from the client company might need to be present at the manufacturing facility to observe operations and provide input on manufacturing events and deviations. Time-zone differences across locations can pose challenges, especially when client input is urgently needed but representatives are unavailable because of differing time zones. As part of an overall CMO-selection process, sponsors should consider location, resource availability, needs for travel visas (if applicable), and local restrictions, such as those imposed during the COVID-19 pandemic.

Supply-Chain Management

Sponsors should ensure that their manufacturing partners can demonstrate efficient **inventory management**. Delays in obtaining raw materials and consumables, such as virus filters and chromatography resins, can influence project timelines significantly. It is an advantage if a prospective CMO has established relationships with key vendors to maintain a stock of chemicals and consumables that are identical to or comparable with the materials that a sponsor used during process development; such arrangements help to ensure smooth transfers between customer and CMO processes. Evaluating a CMO's inventory-control practices — e.g., for forecasting, demand planning, and inventory management — also helps to maintain a seamless supply chain. Large CMOs leverage economies of scale and often hold preferred-customer status with vendors, providing advantages such as reduced risk of material delays when ordering supplies.

Logistics and distribution capabilities are equally important to assess because such factors have bearing on timely delivery of finished products. Timing is especially important when bulk drug substance needs to be shipped from one contract manufacturer to another. Related considerations include a CMO's experience with cold-chain logistics, temperature-controlled storage, and complex export and import requirements during international distribution.

Relying on cost as the single factor when choosing a CMO can **INCREASE RISKS** for manufacturing delays and supply-chain issues, potentially causing setbacks in clinical programs, development timelines, and other milestones.

Cost Considerations

Cost is a crucial CMO-selection factor for most biotechnology and pharmaceutical developers. Sponsor companies need to adhere to budget constraints, particularly in the current financial climate. Therefore, selecting a CMO that offers competitive pricing within a sponsor's available budget is essential. But sponsors must strike a balance among cost, quality, and reliability. Relying on cost as the single factor when choosing a CMO can increase risks for manufacturing delays and supply-chain issues, potentially causing setbacks in clinical programs, development timelines, and other milestones.

Several **local factors** influence the overall cost of CMO services. For instance, employment costs and taxes in different regions can affect a CMO's overall cost structure significantly.

It is worth considering CMOs that offer **additional services** such as research and development support. Even if such services come at a high cost, they can bring significant value to sponsor organizations.

Sponsors should give serious consideration to the **availability of manufacturing slots**. Some CMOs have limited capacity or high demand for services, both of which can increase costs and timelines. Such manufacturers might offer favorable payment terms or negotiate upfront payments. Flexibility in financial arrangements might make a CMO seem more attractive, especially for preclinical-stage biotechnology companies with budget and cash-flow constraints. However, cost should be weighed against other considerations such as quality, reliability, and potential for long-term partnership.

Responsiveness to Questions

Sponsors should not underestimate a CMO's **responsiveness** (or lack thereof) to questions posed throughout the selection process. The time taken for legal review of a confidentiality agreement (CDA), the speed at which a proposal is provided, and the promptness with which technical questions are answered all serve as indicators of a CMO's commitment to effective communication during technology transfer.

The same goes for **attention to detail**. If a CMO representative includes

previous client names in a proposal, then that should raise concerns about the manufacturer's review process and overall attentiveness. Such errors can undermine confidence in a CMO's ability to handle sensitive information accurately and securely.

After receiving a proposal, a CMO is expected to respond promptly to client technical questions and to be proactive in scheduling a call with its technical team. Lengthy delays in issuing replies do not make good impressions, nor do discussions that are led primarily by sales representatives, who might have limited technical expertise. At such early stages, meaningful **technical input** from a CMO's team not only provides valuable insights, but also fosters effective collaboration.

Based on responsiveness to questions and attention to detail during the selection process, biotechnology companies can gauge a CMO's commitment to open communication, ability to meet project timelines, and professionalism. Prompt and thorough responses, along with meaningful technical engagement, contribute to building successful partnerships and technology transfers.

Partnership Development

Biotechnology companies are facing funding challenges that require careful decision-making when outsourcing manufacturing processes. Balancing budgets with the need for reliable, high-quality production is paramount. CMO selection plays a significant role in preventing manufacturing delays, supply-chain disruptions, and setbacks in clinical programs and development timelines. Key considerations include manufacturing expertise, analytical experience, regulatory compliance, location, supply-chain capabilities, cost, and responsiveness to questions.

Choosing a CMO that aligns with current cost constraints, possesses the necessary expertise, and demonstrates consistent regulatory compliance helps to ensure seamless production and high product quality. By considering such factors, biotechnology companies can navigate the complex landscape of CMO selection, mitigate risks, and ultimately achieve manufacturing success through strategic decision-making and effective partnership development.

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Proactive Maintenance

Five Ways It's Important in Biomanufacturing

Eric Whitley

Biopharmaceutical manufacturing companies create life-saving medications and treatments that are crucial to global healthcare. It is an industry in which minor production issues can lead to dire consequences, including compromised product quality and regulatory noncompliance, not to mention danger to patients. Thus, proactive equipment maintenance is indispensable. Below, I investigate why proactive maintenance is vital to pharmaceutical manufacturing operations and highlight its role in securing regulatory compliance, reducing facility downtime, improving product quality, and managing risks.

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Understanding Proactive Maintenance

Throughout its many complex processes, biomanufacturing demands strict quality adherence. To meet the highest standards consistently, the biopharmaceutical industry is turning to a strategy of proactive maintenance.

Definition and Explanation: *Proactive maintenance* is a way to anticipate and resolve potential problems before they crop up in manufacturing equipment. It involves regular checks, systematic inspections, and timely system upgrades to prevent unexpected downtime, enhance efficiency, and prolong the life of valuable equipment.

Proactive maintenance differs from the traditional "run-to-failure" model in which action is taken only after problems arise. Such reactive maintenance may seem cost-effective over the short term, but it always increases overall costs in the long run through unexpected production halts, hefty repair or replacement bills, and potential quality issues.

In contrast, proactive maintenance prioritizes continual improvement and risk reduction. This approach takes into account not only the current condition of equipment and instrumentation, but also their future performance. By identifying and addressing potential problems in advance, proactive maintenance aims to reduce downtime and maintain consistent process performance and product quality, safety, and efficacy in pharmaceutical manufacturing.

Relevance to the Biopharmaceutical Industry: The value of proactive maintenance to biomanufacturing cannot be denied. This sector's intricate, highly regulated production systems can turn minor inconsistencies into major quality issues, even posing risks to patient safety. A proactive-maintenance approach facilitates efficiency in production scheduling and capacity use, providing cost savings and improving a development company's profitability. Preventing instrument and equipment failure also reduces emergency repair costs and lengthens the useful lifetime of expensive systems, further contributing to long-term cost savings.

Five Key Benefits

The following five cornerstones illuminate the crucial role that proactive maintenance can play in manufacturing operations. It acts as a driving force for efficiency, regulatory compliance, risk mitigation, and most important, the delivery of safe, high-quality biologics to patients who need them.

Ensuring Compliance with Regulatory Requirements: Pharmaceutical

A proactive approach helps pharmaceutical manufacturers maintain comprehensive documentation that provides evidence of regulatory compliance and helps companies spot trends, monitor equipment performance, and make **DATA-DRIVEN DECISIONS.**

Proactive and Predictive Maintenance

Proactive Maintenance

Proactive maintenance focuses on preventing the root causes of equipment failure before it occurs. This is not just about fixing what's broken, it is also about understanding why things break in the first place and taking steps to prevent the occurrence.

Key Features:

- Root-cause analysis identifies and eliminates the root causes of failure.
- Regular inspections and routine checks identify potential issues.
- Preventive measures and actions are based on the findings from inspections and analyses.
- Organizational culture must shift to focusing on long-term asset health.

Benefits:

- By elimination of root causes, the likelihood of unexpected failures is minimized and downtime is reduced.
- Preventing a failure is often less expensive than fixing one — saving money in the long run.
- Emphasizing long-term reliability focuses on the long-term health of assets.

Predictive Maintenance

Predictive maintenance relies on data-driven insights to predict when equipment failure might occur. This uses a number of monitoring tools to track the condition of machinery and equipment over time.

Key Features:

- Condition monitoring uses sensors and data analytics tools to monitor the state of equipment.
- Data analysis uses advanced algorithms to predict when a machine is likely to fail.
- Timely interventions allows for scheduling of maintenance activities at the most opportune times.
- This data-driven approach relies heavily on technology such as internet of things (IoT) devices, machine-learning algorithms, and data analytics.

Benefits:

- Optimized minimizes impacts on production.
- Resource efficiency, using only what is needed when it is needed.
- Immediate return on investment (RoI) from reduced downtime and optimized resource allocation.

manufacturing falls under the purview of several regulatory bodies such as the US Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the World Health Organization (WHO) (**1**). Those and other organizations impose a host of regulations and standards to ensure the safety, efficacy, and quality of pharmaceutical products for populations around the globe.

Regulations such as good manufacturing practice (GMP) guidelines demand that companies maintain strict control over their manufacturing processes and environments (**2**). Biomanufacturers must validate their processes regularly, oversee their production settings and facilities, and keep all equipment in optimal working condition. Deviations from those requirements can lead to noncompliance, potentially triggering regulatory actions such as fines, warning letters, product recalls, and even total shutdowns.

In this heavily regulated environment, proactive maintenance is crucial for maintaining GMP compliance. Regular inspections and preventive equipment maintenance help to ensure that all systems operate within necessary parameters and are in control — key aspects of such compliance around the world. Additionally, a proactive approach helps pharmaceutical manufacturers maintain comprehensive documentation of all related activities. That provides evidence of regulatory compliance for reviewers and helps companies spot trends, monitor equipment performance over time, and make data-driven decisions to improve operations.

Detecting and Preventing Equipment Issues: Proactive maintenance in biopharmaceutical manufacturing relies heavily on continuous monitoring and regular inspection of key equipment, using advanced condition-based techniques. Those can include vibration analysis, infrared imaging, oil analysis, and ultrasonic inspection — each method targeted to uncover early signs of wear or performance decline before equipment failure occurs.

Vibration analysis can detect early problems in rotating equipment such as pumps, preventing them from wearing prematurely. Infrared imaging can identify potential electrical failures, which must be prevented in an industry where unexpected downtime can be catastrophic. Oil analysis maintains high-performance machinery by detecting contamination or abnormal wear early on to prevent friction and machine failure. Ultrasonic inspection detects leaks in pressure systems, which is crucial for maintaining sterility in upstream production processes, for example.

Minimizing Downtime and Maximizing Productivity: Equipment downtime can disrupt pharmaceutical manufacturing severely. Not only does it halt production, causing delayed orders and lost revenue, but it also can compromise the quality and integrity of products made. In certain cases, equipment failure might lead to the loss of an entire product batch, incurring substantial financial loss and possible damage to a company's reputation.

Proactive maintenance greatly mitigates the risk of unplanned downtime. Spotting potential problems early and planning maintenance during scheduled production breaks helps biomanufacturers prevent unexpected equipment failures. That helps to ensure uninterrupted production and boosts the efficiency and productivity of manufacturing operations.

Connected-worker technology is revolutionizing proactive maintenance (**3**). It uses predictive analytics,

A Step-by-Step Guide To Implementing Proactive Maintenance

Implementing a proactive maintenance strategy can reduce downtime and increase efficiency. Here's a step-by-step guide to help you get started in transforming your operations.

Step 1
Assess Current Maintenance Practices: Evaluate your current maintenance procedures. Identify areas of inefficiency and recurring problems.

Step 2
Secure Management Buy-In: Present the benefits of proactive maintenance to upper management. Secure budget and resources for the transition to a preventative scheme.

Step 3
Assemble a Dedicated Team: Form a team of experts focused solely on proactive maintenance. Ensure that the team has the necessary skills and training.

Step 4
Conduct a Risk and Resource Assessment: Identify critical assets and their failure modes. Assess the risk and impact of each failure mode.

Step 5
Develop a Proactive Maintenance Plan: Outline preventive and predictive maintenance tasks. Schedule regular inspections and condition monitoring.

Step 6
Invest in Technology and Training: Invest in predictive-maintenance tools such as those for vibration analysis, infrared thermography, and ultrasound monitoring. Train your team on how to use these tools effectively.

Step 7
Implement the Plan: Roll out the proactive maintenance tasks according to the plan. Use a computerized maintenance management system (CMMS) for tracking.

Step 8
Monitor and Adjust: Continuously monitor the effectiveness of the maintenance tasks. Make adjustments to the plan as needed.

Step 9
Conduct Regular Reviews: Conduct quarterly or biannual reviews to assess the impact of the strategy. Update the plan based on key performance indicators (KPIs).

industrial internet of things (IIoT) devices, and machine-learning algorithms to collect and analyze vast amounts of real-time data from manufacturing equipment. Such tools enable users to detect minor changes in equipment performance that could indicate impending problems. Connected-worker technology also enhances real-time communication and information exchange among maintenance staff, improving the efficiency of their activities. Integration of advanced information technologies into proactive maintenance enables biopharmaceutical manufacturers to minimize downtime, increase productivity, and uphold stringent quality standards.

Enhancing Overall Product Quality: Keeping equipment in peak operating condition ensures that manufacturing processes function consistently as intended. That limits drug-substance and drug-product variation, improving product quality and consistency. Proactive maintenance also can thwart cross-contamination. Detecting and resolving issues such as leaks or equipment wear can prevent contamination that would jeopardize product quality or even prompt product recalls.

Without regular proactive maintenance, equipment will degrade over time, resulting in process deviations that can compromise product quality. For instance, a minor fluctuation in the temperature or pressure of a unit operation due to equipment malfunction could cause significant variations in final products, potentially making them ineffective or unsafe.

Reducing Risks: Biopharmaceutical manufacturing incurs numerous risks, including equipment failure, production delays, compliance breaches, and product recalls. The potential for cross-contamination, deterioration of active pharmaceutical ingredients (APIs) from equipment malfunction, or loss of entire batches to unexpected breakdowns poses significant financial and reputational risks.

A proactive maintenance strategy can alleviate those risks significantly. Regular inspections and condition monitoring lead to early detection and resolution of potential issues before they can escalate into severe problems. That lowers the risk of equipment failure and related production delays and helps companies to maintain process and product integrity, reducing the risk of costly recalls or compliance violations.

Insurance providers acknowledged the value of proactive maintenance. Biopharmaceutical manufacturers that can demonstrate a robust program can negotiate lower insurance premiums, bringing an additional financial incentive to adopting this approach.

A Real-World Example

Proactive maintenance is shaping how the biopharmaceutical industry addresses the concerns described above (**4**). AstraZeneca (AZ) showcases the power of proactive maintenance at its manufacturing facility in Mt. Vernon, IN. The critical first step of implementation in that location involved separating the reliability team from everyday operations, underlining a significant distinction between maintenance and reliability.

Facilities engineer Andrew Carpenter has highlighted the importance of this difference, with maintenance attending to daily tasks and reliability focusing on understanding and mitigating underlying equipment problems. This shift required a significant cultural change and backing from top management. The company propelled that transformation by investing in specialized training in advanced predictive technologies such as vibration analysis, infrared thermography, and ultrasound for the reliability team. Alongside those, other new technologies play a crucial role in predicting and addressing potential issues before they can spiral into significant problems.

AZ prioritizes quality, which is evident in its meticulously designed cleanrooms housing crucial equipment, airlocks preventing potential contamination, and even storerooms and warehousing. Chris Nolan (senior building and reliability manager) underscores the storeroom's importance as a mirror of such a facility's health: "What goes out of your storeroom is a huge check and balance of your maintenance process."

Another pillar of the company's proactive-maintenance approach is root-

cause analysis. Instead of just addressing an immediate issue, the team digs into its underlying cause to prevent future occurrences. Carpenter says that applying such a long-term focus on solutions — rather than quick fixes — has transformed operations.

The proactive-maintenance strategy initially found application in maintenance of utilities and purified water production; GMP maintenance; and heating, ventilation, and air conditioning (HVAC) systems. That allowed for persistent monitoring of critical aspects for ensuring product quality. The insights gained thereby have been crucial to the company's pursuit of process refinement and failure prevention — particularly by demonstrating the value of recognizing and analyzing problems early on.

AZ's Mount Vernon site primarily manufactures oral-solids medicines for type 2 diabetes treatment. AZ received early assistance from a consultant group (Life Cycle Engineering) to identify tools for showing overall criticality in terms of business cost, quality, and mean time between failures.

Means to an End

From guaranteeing compliance with stringent regulatory standards to enabling early identification of equipment issues, proactive maintenance's crucial role in biopharmaceutical manufacturing facilities is evident. Not only does this strategy minimize downtime and optimize productivity, but it also ensures the consistent production of top-quality drug and biologic products. Outstanding performance at AZ is a strong testament to these benefits.

More than a mere operational strategy, proactive maintenance serves as a tool for quality assurance, a risk management measure, and a compliance enabler (**5**). The approach is indispensable for continued smooth functionality of biopharmaceutical manufacturing processes, which supports the industry's duty to produce safe and effective drugs.

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Be selective in your hiring; you want qualified people who share your vision.

Going to Market

Now you're ready to start making sales. You'll need to implement your marketing and sales strategies, which should be tailored to your target market. Artificial intelligence (AI) provides powerful tools not only for scientific research, but also for marketing. In fact, AI is projected to have a stronger impact on marketing than on any other aspect of business. AI tools can help you to personalize messaging based on your target market's needs. They also enable the creation and placement of targeted ads based on the online habits of potential customers (**1**).

Be sure to measure the effectiveness of your marketing over time so that you can focus on strategies that work. You'll also need to stay apprised of market and industry trends. You may need to adapt your product and strategy and find opportunities to expand into new markets. Continuous innovation is necessary to remain competitive in the technology and science sectors.

By putting your skills and knowledge to work in the business world, you can build something of value that can last for generations. Take a thoughtful approach to starting and developing your business, just like you do in your field of expertise, and you can achieve amazing things.

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A Parenteral Permissible Daily Exposure for Inactivated Therapeutic Proteins

An Approach Based on Literature Review

Jessica Graham, Selene Araya, Kamila Blum, Janet Gould, and Thomas Pfister

In multiproduct biopharmaceutical manufacturing facilities, cross-contamination with pharmacologically active proteins must be controlled in a good manufacturing practice (GMP) environment (**1, 2**). Although guidance on control strategies exists for solvents and small-molecule pharmaceutical impurities, that is not directly applicable to inactivated (e.g., denatured and/or degraded) therapeutic proteins (TPs) occurring as impurities in a drug substance (DS) and/or drug product (DP). Small-molecule drugs and TPs differ in their molecular structures, pharmacological mechanisms of action, hazards, and potential impurities, so their cross-contamination control strategies also should be considered differently. Unlike small molecules, TPs are known to denature and degrade when exposed to pH extremes and/or heat and thus are expected to become pharmacologically inactive during the cleaning process (**2**).

PRODUCT FOCUS: PROTEINS

PROCESS FOCUS: DOWNSTREAM PROCESSING

AUDIENCE: MANUFACTURING, PROCESS DEVELOPMENT, QA/QC

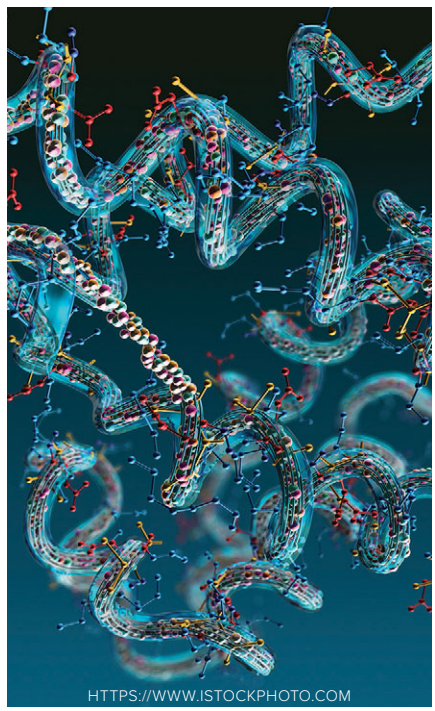
KEYWORDS: PROTEIN IMPURITIES, CLEANING VALIDATION, DRUG SUBSTANCE, DRUG PRODUCT, RISK MANAGEMENT, PRODUCT- AND PROCESS-RELATED IMPURITIES

LEVEL: ADVANCED

In cleaning activities, permitted daily exposure levels (PDEs) support the amount of residual DS that poses negligible risk to patient safety if it is present as an impurity in another drug. The PDE is a daily dose of a compound that is not expected to cause adverse effects (pharmacological or toxicological). Note that PDEs are established based on the activity of TPs as intact, pharmacologically active molecules (**2–4**). However, given the inactivation of proteins during cleaning, using PDEs that were established based on the pharmacological activity of a DS is not applicable (**1**).

Our aim herein is to examine available data to derive and support a protective default PDE for denatured and/or degraded TPs that present in parenteral (intravenous, intramuscular, or subcutaneous) DS and DP. We refer to such denatured and/or degraded TPs as *inactivated TPs*.

Characteristics of Therapeutic Proteins: Numerous TP modalities are in development and on the market: monoclonal antibodies (MAbs), antibody fragments, fusion proteins, and other biopharmaceuticals such as therapeutic enzymes. TPs exert their pharmacodynamic effects by binding to receptors or targeting particular antigens involved in the pathophysiology of disease. TPs can be fully human, humanized (e.g., with protein sequences modified to increase their similarity to human antibodies), and/or chimeric (consisting of human and nonhuman proteins). Although TPs have varying arrangements of large peptide and/or



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protein constituents, they are composed of amino acid (AA) chains — which are the building blocks of all proteins.

Proteins have four different levels of structure: primary, secondary, tertiary, and quaternary (Figure 1) (**5**). Quaternary structure is critical for a TP's ability to interact with molecules in the body, and the relationship between conformation and function is crucial to ensuring pharmacological activity (**6, 7**).

Impurities in Therapeutic Proteins: TPs generally consist of three-dimensionally (3D) arranged AA chains produced through expression in biological organisms — e.g., Chinese hamster ovary (CHO) cells or *Escherichia coli* bacteria (**8**). During TP manufacturing, a number of

Figure 1: Protein structure, degradation, and denaturation; proper folding and conformation of a therapeutic protein (TP) are critical for its ability to exert pharmacological activity. Once disrupted by denaturation and/or degradation, a TP is not anticipated to have pharmacological activity and thus is regarded as being "inactivated."

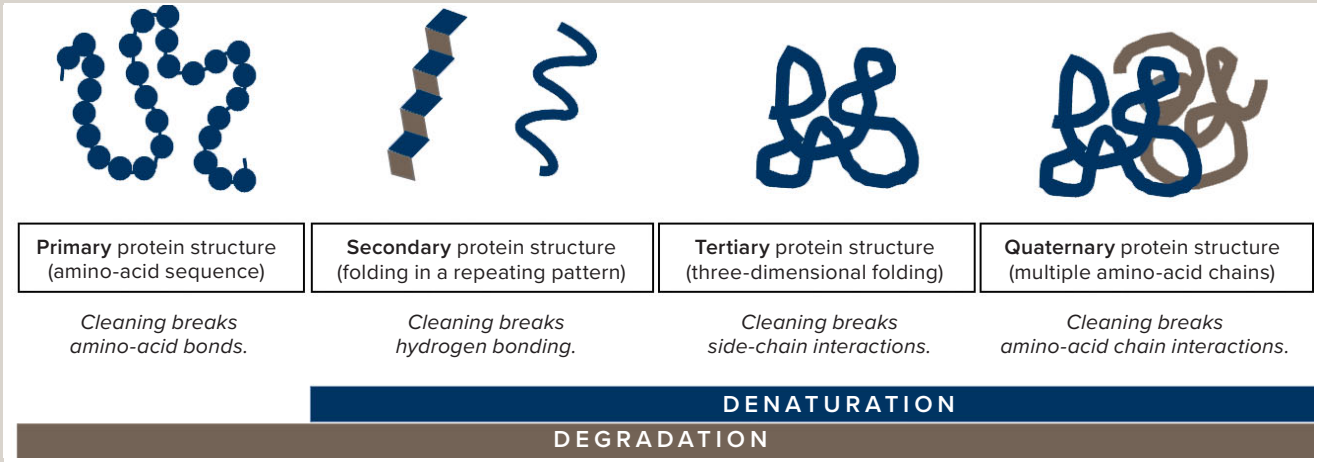
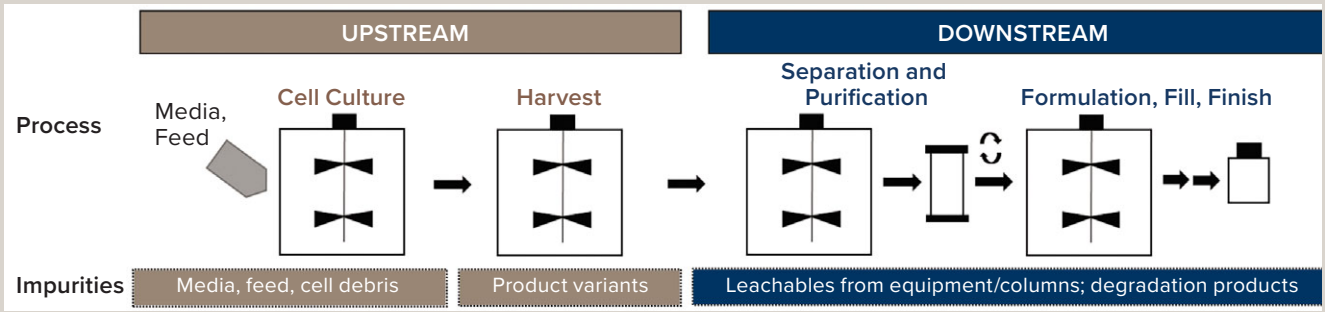


Figure 2: Overview of TP manufacturing and potential process-related impurities, which may be derived at any step of a process and be detected in quality control (QC) samples after cleaning activities; such impurities (as defined by ICH Q6B) include cell-culture media components, host-cell proteins, DNA, leachables from equipment and purification columns, and TP fragments/aggregates. This list of impurities is not extensive; other impurities can be formed and/or present in some processes.



impurity types can make their way into a DS: e.g., host-cell proteins (HCPs), cell debris, leachables from equipment, and active/variant TP products (Figure 2). Inactivated TP (after cleaning activities) from previously manufactured TP can carry over into a new batch of TP (whether the same or a different product).

Of all the impurities mentioned, an important carryover risk is DS from a previously manufactured product, which has been concentrated in downstream steps and was intended to have pharmacological activity. Upon completion of cleaning activities, residual TPs are expected to be inactivated. This assessment addresses the acceptability of a level of inactivated TP that is present as an impurity in a DS and/or DP from a previously manufactured batch of the same or a different product.

TP Inactivation

TPs generally are unstable under normal environmental conditions (e.g., exposure to light and ambient temperatures) and sensitive to physical and chemical degradation (**9**). Therefore, they require strict practices for their handling, administration, and storage — often at specific temperatures in solution with buffers and/or stabilizers. Specific formulations and modifications often are critical to improving their stability and preserving their pharmacological activity in DP development (**9–12**).

TPs lose their specific pharmacological activity when the molecular structure necessary for their pharmacological effect(s) is altered or destroyed (**13**). That loss of activity is known as *protein inactivation*. In principle, it can occur through two distinct processes: denaturation or degradation.

Denaturation of Therapeutic Proteins: *Protein denaturation* is the disruption and destruction of a protein's secondary, tertiary, and quaternary structures. Such uncoiling and disruption of higher-order structures typically comes as a consequence of chemical processes or physical stress (Figure 1). Examples of denaturing agents include alcohol, which disrupts hydrogen bonds in secondary and tertiary structures; acids, bases, and heavy-metal salts, which can disrupt salt bridges in tertiary structures; heat, which can disrupt hydrogen bonds and nonpolar hydrophobic interactions; and reducing agents, which can disrupt disulfide bonds (**10, 14, 15**).

Degradation of Therapeutic Proteins: Degradation occurs when the primary structure of a TP — its AA chain — is fragmented, usually through hydrolysis, but degradation may be spontaneous on occasion. The process can be catalyzed by compounds such as enzymes, metal salts, acids, and bases (e.g., sodium and potassium hydroxides) and can be accelerated through heating (**16, 17**). Once its primary structure has been degraded, then a protein's secondary, tertiary, and quaternary structures are also disrupted (**18**).

Denaturation and Degradation During Equipment Cleaning: In practice, a

number of denaturation and degradation methods are applied to clean multiuse equipment in biopharmaceutical production facilities. Biopharmaceutical cleaning cycles generally are designed to expose product-contact equipment to extremes of pH (<2 and >13) and temperature (60–80 °C) for several minutes, yielding undefinable proteinaceous material or peptide fragments that lack specific biological activity (**19**). Upon completion of such cleaning activities, no recognizable TP structures should remain in the equipment. To demonstrate the effectiveness of cleaning procedures, quality control (QC) samples demonstrating control of carryover risk often are taken from the worst-case location(s) in equipment and tested for the presence of residues after final rinsing of cleaning agents (**20**).

Potential Hazards of Inactivated TPs

Inactivated TPs are an unspecified mixture of undefinable proteinaceous material, essentially endogenous substances (AAs). Studies of such compounds would be unsuitable for setting health-based exposure limits. Additionally, determining a definitive quantitative PDE for variable, inactive proteinaceous material is infeasible. Consequently, a pragmatic exposure limit for inactivated TPs must be based on reasonably conservative assumptions that consider the basic properties of the human immune system.

Biological Activity: For TPs such as antibodies, the intact molecules (sequence, structure, and posttranslational modifications) are necessary to impart full potency (ability to bind to cellular receptors or intended targets) and stability in a person's bloodstream (**21–23**). The probability for a component of a denatured and/or degraded TP to refold or present the proper structure and modifications necessary to effect a biological function is considered to be minimal. Therefore, a degraded protein fragment or peptide is not anticipated to undergo specific changes that result in pharmacological activity at another (unintentional) target receptor, so they are expected to be pharmacologically inactive (inactivated).

When comparing general hazards associated with inactivated and intact TPs, the former are considered to be less hazardous for several reasons. Note that proteins and their metabolic products (AAs) are endogenous to all living organisms. In living cells, proteins are constantly synthesized and degraded (e.g., into smaller peptides and individual AAs). That makes classical biotransformation studies such as those performed for small-molecule pharmaceuticals unnecessary for TPs (**24, 25**). The regulatory position reflects a general belief that degradation products of TPs, unlike those of small-molecule drugs, have limited potential to cause unexpected off-target activity (**26, 27**). Additionally, biotechnology-derived therapeutics are not tested for genotoxicity or carcinogenicity because they are not expected to interact directly with DNA or other chromosomal material (**25**). That expectation also could apply to degraded proteins. Additionally, from a safety standpoint, the potential toxicity (e.g., pharmacological activity) of an inactivated TP is expected to be negligible in comparison with the respective active TP.

Absorption, Distribution, Metabolism, and Elimination (ADME): Absorption: Upon parenteral administration, even a small quantity of inactivated TP present as an impurity in a DP can disperse immediately throughout the 3.5–5 L of blood volume (IV) in an average human adult — or slowly from muscle or other tissue (IM, SC) to enter circulation (a lower C_{max} value). Upon tissue uptake, metabolism/catabolism of inactivated TPs takes place before the remains are excreted as smaller peptides and AA degradants — or are recycled for synthesis into other proteins.

Distribution: Because of their size and hydrophilic nature, circulating inactivated TPs that lack secondary, tertiary, and quaternary structures are expected to have a low ability to bind to cellular receptors and be internalized by cells (**28**). Tissue distribution is limited for large, inactivated TPs not only because of their size, but also their charge and binding properties. Therefore, inactivated TPs in a parenterally administered drug can be expected to remain in circulation, where specific proteases can degrade them further into smaller protein fragments.

Metabolism: Although proteolysis occurs widely in humans and animals, its kinetics and mechanistic details are poorly understood, especially for large TPs such as MAbs (**27, 29**). Products of degradation from cellular proteins are transferred from tissue into systemic circulation by the lymphatic system through a highly regulated process that protects endogenous proteins from uncontrolled degradation (**27, 30**).

Elimination: TPs are cleared through the same catabolic pathways used to eliminate endogenous and dietary proteins, and the same is expected for their inactivated counterparts (**30**). Immunoglobulin G (IgG) clearance occurs mainly through intracellular catabolism by lysosomal degradation into AAs upon cellular uptake, with a small amount cleared through biliary excretion (**30, 31**). Renal excretion plays a major role in elimination of protein degradation products smaller in molecular weight (MW) than the glomerular filtration threshold (~55 kDa). Proteins and peptides <30 kDa are filtered most efficiently by our kidneys and have a short half-life in circulation, usually between two and 30 minutes, because of proteolytic degradation and the fact that they are not reabsorbed in the renal tubules (**32**).

Immunogenicity is a general concern with the administration of biological materials. Risk factors for potential immunogenicity hazards include the proportion of foreign protein present, the stability of the proteins, and their tendency to aggregate.

Foreign Proteins: The immunogenic potential of a biologic increases with the proportion of foreign protein present. Thus, humanized proteins are less likely to cause a systemic immune response than are chimeric (e.g., murine) antibodies (**33, 34**).

Protein Stability and Aggregation: Sensitization to a protein allergen generally is anticipated to be more likely when such proteins preserve their native, 3D structure after chemical, physical, or enzymatic interactions (**35**). Such properties are extremely rare, but they have been reported for some major food allergens.

Aggregates of intact proteins generally have reduced activity and — more important — greater immunogenicity potential because of their multiplicity of epitopes and/or conformational changes. Concentration-dependent antibody aggregation is a great challenge during development of highly concentrated TP formulations. The recommended allowable aggregate level in commercial intravenous immunoglobulin products is limited to <5% (**36**).

Several features give inactivated TPs lower potential for immunogenicity than that of their intact, active counterparts. Proteins and their metabolic products (AAs) are endogenous to all living things; thus, so are the AAs and proteins resulting from denaturation/degradation of TPs. Note, however, that chimeric proteins have some nonhuman sequences that could be immunogenic. Structural integrity is important for allergens, as demonstrated in studies showing that active and denatured allergens — beta lactoglobulin (BLG), alpha lipoic acid (ALA), and beta casein — had reduced antibody-binding capacity from their loss of conformational epitopes (**35**). Known allergenic proteins contain certain motifs and conformations that are critical for allergenicity, whereas inactivated TPs do not have tertiary or quaternary structures and thus would not retain such activity. That expectation is consistent with the properties of residues after postprocessing techniques used in the food industry to reduce oral allergies (e.g., enzymatic hydrolysis and heat treatment). For example, heat-treated protein hydrolysates often are described as "hypoallergenic" formulas (**37**).

In allergies, IgE antibodies are produced against specific epitopes from foreign proteins or glycoproteins. Repeated exposure to the same epitope is required for type 1 hypersensitivity responses. Under the harsh conditions of cleaning methods used in biomanufacturing, the resulting fragmenting and degradation should not produce consistently similar protein epitopes at sufficient concentrations to induce type I hypersensitivity at low exposures (e.g., 100 µg/day). In conjunction with the unlikelihood of de novo epitopes being generated during inactivation, it is reasonable to consider that the allergenicity of degraded TPs is considerably lower than that of common environmental allergens or the parent TP.

Considering Available Limits

It is important to remember that what is in question is the safety of an additional amount of inactivated TP added to a given DP formulation. Essentially, what amount of inactivated TP is not anticipated to pose a safety risk to patients if it is present in a DP? For residual inactivated TPs, the goal is to determine not necessarily the highest level possible, but rather an acceptable level that could be justified using available scientific information that leverages historical safety data.

Risk Assessment Process (RAP) maps published by Jolly et al. in 2022 present a framework to facilitate the establishment of health-based exposure limits (HBELs) for endogenous compounds (**38**). Because of the general lack of formal toxicological studies and exposure information on endogenous substances, the RAPMAP framework includes evaluating whether an existing limit can be used or adapted to establish an HBEL. What follows is an overview of relevant available limits along with an evaluation as to how each limit could be used and/or adapted to accommodate the anticipated hazards and nature of inactivated TPs. Upon evaluating these limits (Table 1), a protective PDE for inactivated TPs can be established at 100 µg/dose.

Applying Limits for Intact/Active TPs to Residual Inactivated TPs: In 2017, Pfister et al. proposed a default PDE of 10 µg/day for a parenterally administered MAb (Table 1) based on historical evaluation of PDEs for other MAbs (**39**). To extrapolate from a pharmacologically active dose to a "no observed adverse effect level" (NOAEL), ICH Q3C proposes a factor of 10 (**40**). Using that 10-µg/day exposure limit for pharmacologically active and intact TPs as a benchmark — and with the conservative assumption that inactivated TPs will be 10-fold less active/potent — gives a default PDE of 100 µg/day. Exposure at or below that limit is expected to pose negligible safety concern for inactivated TPs.

From a toxicological perspective, material derived from degradation and/or denaturation of proteinaceous or peptide TPs other than MAbs (those without a globulin structure) can be regarded to have properties similar to those of inactivated MAbs — provided that those other molecules are completely inactivated. The PDE for inactivated protein residues is independent of the potency or modality of intact TPs.

Applying Limits for Intact/Active Host Cell Proteins to Residual Inactivated TPs: Besides contamination from carryover, proteinaceous impurities in TPs also can derive from biomanufacturing processes (Figure 2). Because intact HCPs sometimes trigger unpredictable immunogenic responses, regulatory guidelines stipulate that such proteins need to be identified and quantified to protect patient safety (**41**).

For example, production of TPs in CHO cells yields low levels of CHO proteins (CHOPs, considered to be process-related impurities) in resulting DPs. Specifications placed on final DPs thus include HCP levels of <100 ppm (**36**). A recent report indicates that the most likely range of HCPs in biologic products reviewed by the US Food and Drug Administration (FDA) is 1–100 ppm (**42–44**). If the dose of a TP is 1,000 mg (1 g), then the acceptable tolerance limit of 100 ppm is consistent with the PDE of 100 µg/dose (**42, 45**).

A limit of 0.1 mg/dose (100 µg/dose) has been proposed for residual HCPs based on the NOAEL from a keyhole limpet hemocyanin (KLH) antigen study in monkeys with CHOPs (**46**). The proposed PDE of 100 µg/dose should be protective for inactivated TPs given that the same limit has been proposed to be safe for residual, intact HCPs.

Applying Limits for Protein Fragments to Residual Inactivated TPs: Low–molecular-weight (LMW) species (e.g., truncated protein-backbone fragments) and high–molecular-weight (HMW) species (e.g., antibody dimers) are both examples of common TP-related impurities. Aggregation-formed HMW species within a DP can compromise both drug efficacy and safety. Additionally, LMW species often have low or substantially reduced activity relative to a TP's monomeric form. Thus, both types of impurities are considered to be critical quality attributes (CQAs)

that must be monitored routinely during drug development and as part of release testing for purified DPs (**47**). A level of ≤5–10% of soluble protein aggregates in a TP DP has been recommended (**48**); a level of <5% of HMW immunogenic aggregates is recommended (**36**). For a TP administered at 1 mg/kg (IV) to a subject weighing 50 kg, with the assumption that ≤5% of the dose consists of product-related impurities, the resulting 2.5-mg/dose mixture of protein impurities would be more immunogenic than residual inactivated TPs. The proposed PDE of 100 µg/dose is 25-fold lower than the recommended level for aggregate impurities present in the TP dose.

In an attempt to develop an acceptable limit for pharmacologically inactive fragments of human TPs, Sharnez et al. reported on their studies with gelatin in 2013 (**49**). They chose gelatin because it is a complex protein with fragments (15–400 kDa) and is of animal origin (which should be more immunogenic than degraded human TPs would be). Also, given that gelatin is derived by exposing collagen to pH and temperature extremes, its protein fragments are considered to be chemically comparable with the TP fragments in bioprocess residues after cleaning and sterilization (**49**). Gelatin also is used in blood infusions and a number of vaccines. Based on clinical experience, the safe and acceptable limit for inactive gelatin fragments was ascertained to be 650 µg/dose. Given the nonhuman nature of the protein, that provides greater confidence that a PDE of 100 µg/dose is a protective and acceptable exposure limit for inactivated residual TPs.

Applying Threshold of Toxicological Concern (TTC) Approaches to Residual Inactivated TPs: The TTC approach presented by Dolan et al. in 2005 proposed and supported exposure limits of 1, 10, and 100 µg/day respectively for compounds that are likely to be carcinogenic, those expected to be potent or highly toxic, and those that are neither (**50**). Originally established for pharmacologically active, small-molecule APIs, the approach also is commonly used in setting PDEs for other data-poor substances (**51**). Inactivated TPs are unlikely to be potent, highly toxic, or carcinogenic — giving them an acceptable daily intake (ADI) of 100 µg/day. That limit is consistent with the PDE of 100 µg/dose proposed herein for inactivated TPs (assuming daily administration).

In 1998, Munro and Kroes proposed a similar approach based on the Cramer structural classification scheme and evaluation of NOAELs for >600 substances tested in repeat-dose toxicity studies (**52–54**). Briefly, Cramer class I substances have simple chemical structures, known metabolic pathways, and low potential toxicity. Normal biological constituents (aside from hormones) thus are included in that class (**52, 53**). Cramer class II substances have less-innocuous structures than those in class I but no positive indication of toxicity. Cramer class III substances contain structural features that suggest the potential for significant toxicity.

The TTC values established were 90, 540, and 1,800 µg/person/day for Cramer class III, II, and I substances based on a recipient's body weight of 60 kg (**54**). Consistent with Cramer class I compounds (1,800 µg/person/day), AAs and inactivated TPs are not expected to pose a risk of significant toxicity. As a protective measure, if there is uncertainty regarding immunogenicity potential, denatured and degraded TPs also can be regarded as Cramer class II (540 µg/person/day) with the proposed PDE over fivefold lower. Even considering the most stringent class (Cramer class III, 90 µg/day), which is associated with a clearly positive indication of toxicity and data-poor substances, the PDE proposed herein is conservative and within an order of magnitude.

Applying ICH Guidance for Impurities in Small-Molecule Therapeutics to Residual Inactivated TPs: Small molecules generally are considered to be those with a molecular weight of <900 Da. In the case of degraded proteins, fragments can consist of single to multiple AAs, which range 57–186 Da in MW. Thus, an AA or a peptide fragment could be thought of as a small molecule.

Although not directly applicable to impurities in biologics, ICH Q3A recommends qualification of impurities present at a concentration threshold of 0.15% in a DS dosed at ≤2 g/day for nonmutagenic small-molecule impurities or to an impurity limit (per impurity) of 1 mg/day, whichever is lower (**55**). Note that these impurities can include pharmacologically active molecules. Notably, for a TP administered once daily, the level of 100 µg/dose is 10-fold below the 1-mg/day threshold. Additionally, if a DS is dosed at 2 g, then 0.15% would be 3 mg, which is 30-fold higher than the proposed PDE of 100 µg/dose of inactivated TP.

ICH Q3B recommends qualification of impurities present at a concentration threshold of 1.0% for nonmutagenic small-molecule impurities present in drug products dosed at <10 mg/day or to a threshold (per impurity) of 50 µg/day, whichever is lower (**56**). Such limits apply to each impurity, not to the total amount of all nonmutagenic small-molecule impurities present. Additionally, those impurities can be pharmacologically active. With that in mind — and the expectation that inactivated, denatured, and degraded TPs are mixtures of many proteinaceous compounds — the level of 100 µg/dose would be conservative. Note that the thresholds presented in ICH Q3A and Q3B are related more to product quality than patient safety.

Additional Considerations

Residual Circulating Inactive TPs: An important goal is protecting patients from potential acute effects driven by endogenous substances at peak systemic exposures (e.g., upon TP administration). Circulating endogenous IgG levels vary ~9.5–12.5 mg/mL (**57**). Considering a plasma volume of 3.5 L for a 70-kg adult, a residual amount of 100 µg degraded protein in circulation would be at 0.029-µg/mL concentration (29 ng/mL). A nanogram-level change is anticipated to be marginal compared with the total amount of degraded proteins present from physiological processes in our bodies. Therefore, an additional parenteral exposure of 100 µg inactivated TPs would be at 0.0002–0.0003% of circulating IgG levels, which is not anticipated to have a significant impact.

Dose, Frequency, and Duration: TP administration schedules vary, and low-level chronic exposures to protein

Table 1: Published limits and their proposed applicability to inactivated therapeutic proteins (TPs) relative to the proposed permissible daily exposure (PDE) of 100 µg/dose; DP = drug product, DS = drug substance, HCP = host-cell protein, HMW = high molecular weight, IV = intravenous

Published Limit	Applicability	Relevance to Inactivated TPs	Adjusted/Adapted Limit for Inactivated TPs	Compared with the Proposed PDE	References
10 µg/day	Parenteral PDE for data-poor therapeutic monoclonal antibodies (MAbs)	Limit is for pharmacologically active TPs (established based on the hazard of pharmacological activity); inactivated TPs consist of protein fragments that lack pharmacological activity.	After applying a factor of 10 to adjust conservatively for the lack of pharmacological activity, the resulting limit is 100 µg/day.	Justifiably similar	39
<100 ppm/dose	FDA tolerance for HCP impurities in a TP	Limit is for active/intact proteins, but inactivated TPs lack activity.	<100 µg/dose if the TP dose is <1,000 mg (<1 g)	Justifiably similar	36, 42–44
0.1 mg/dose	Limit for residual HCPs in a TP	Limit is for active/intact proteins, but inactivated TPs lack activity.	100 µg/dose	Justifiably similar	46
100 µg/day	Limit for a compound not likely to be potent, highly toxic, or carcinogenic	Inactivated TPs are unlikely to be potent, highly toxic, or carcinogenic.	100 µg/day	Justifiably similar if the TP is administered once daily, every day	50
650 µg/dose	Limit for inactive protein fragments in TPs	Inactivated TPs are anticipated to consist of inactive protein fragments.	650 µg/dose	6.5-fold higher than the proposed PDE	49
1,800 µg/day	Limit for a compound not expected to pose a risk of significant toxicity	Inactivated TPs are not expected to pose a risk of significant toxicity.	1,800 µg/day	18-fold higher than the proposed PDE if the TP is administered once daily, every day	54
<5% per dose	Limit for HMW immunogenic aggregates in a TP	Limit is for aggregates of intact TPs, but inactivated TPs lack pharmacological activity.	2.5 mg/dose if considering a dose of 1 mg/kg (IV) and a body weight of 50 kg	25-fold higher than the proposed PDE	36
≤5–10% per dose	Limit for soluble protein aggregates in a TP	Limit is for aggregates of intact TPs, but inactivated TPs lack pharmacological activity.	5 mg/dose if considering a dose of 1 mg/kg (IV) and a body weight of 50 kg	50-fold higher than the proposed PDE	48
<0.15% in a DS dosed at ≤2 g/day; limit per impurity of 1 mg/day	Quality-based limit for an impurity in a small-molecule DS	Limit applies to each individual impurity in a DS, including impurities that are pharmacologically active; inactivated TPs are anticipated to be a mixture of proteinaceous material and not pharmacologically active.	1 mg/day for each type or variant of proteinaceous fragment and inactivated TP impurity	For a DS dosed at 2 g, 0.15% would equate to 3 mg (30-fold higher than the proposed PDE); 1 mg/day per impurity is >10-fold higher than the proposed PDE	55
1.0% in a DP dosed at <10 mg/day; limit per impurity of 50 µg/day	Quality-based limit for impurities in a small-molecule DP	Limit applies to each individual impurity in a DP, pharmacologically active molecules; inactivated TPs are anticipated to be a mixture of proteinaceous material that are not pharmacologically active.	50 µg/day for each type or variant of proteinaceous fragment and inactivated TP impurity (anticipated to be >>2)	For a DP dosed at <10 mg, 1.0% would equate to 0.1 mg, which is similar to the proposed PDE (50 µg/day per impurity is within an order of magnitude of the proposed PDE)	56

degradants (as impurities in TPs) are unlikely to occur daily because most TPs are administered weekly or less frequently. From this standpoint (as elsewhere herein), the proposed PDE of 100 µg/dose to inactivated TP impurities is conservative.

Some individuals receive multiple drugs per day. Our evaluation focuses on additional risk to patients posed by a level of 100 µg of inactivated TP if present in an administered TP. It is important to note that the PDE for inactivated TPs is a conservative estimate. Exposure to such a level of inactivated TPs in more than one DP administered at roughly the same time should be acceptable for each TP administered with negligible safety concern. Similarly, impurity assessments for small-molecule drugs focus on the DS or DP at hand and not the possible agglomerate of impurities if multiple drugs are administered on the same day (**40, 50, 55, 56**).

Analytical Considerations: Applying a PDE in cleaning validation requires consideration of analytical feasibility. Inactivation studies usually are based on bioassays — e.g., enzyme-linked immunosorbent assays (ELISAs) — which measure the relative amount of biologically active product by investigating binding sites that are functionally intact (**49**). Measuring inactivated TPs does not require such specific analytical methods. Commonly used analytical methodologies detect all proteinaceous material and generally cannot tease apart whether a detected degraded protein comes from a TP or other sources (e.g., HCP). The standard method is a combination of total organic carbon (TOC) analysis for impurity quantification and a sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) assay for analyzing fractions of proteinaceous material (**58, 59**). TOC has a limit of quantification (LoQ) of ~0.2 ppm, which is sufficient for most cleaning validation applications.

Limitations and Applicability

In certain circumstances, application of the PDE proposed herein could benefit from a risk assessment. For example,

that could be helpful for evaluating chimeric TPs that include foreign sequences and thus might present increased risk of immunogenicity. Another scenario that calls for additional risk assessment is when a TP is administered by a less common route such as intravitreal injection (**60, 61**). Highly stable TPs that can be administered orally also could benefit from in-depth assessment because the digestion processes (stomach acids and enzymatic activity) to which they are subject can be similar to harsh conditions of some cleaning processes.

Our proposed PDE of 100 µg/dose is not applicable to inactivated proteins from antibody–drug conjugates, protease inhibitors, enzymes, or plasma-derived TPs. For TPs dosed below the PDE threshold level, product-quality concerns come into question. The proposed PDE is based on safety, with product quality considerations aside.

After reviewing data and limits from available literature, we anticipate that a parenteral PDE for inactivated (denatured and/or degraded) TPs in the range of 100–3,000 µg/dose is generally acceptable within the above constraints. Available information supports that an exposure limit of 100 µg/dose is protective for inactivated TPs.

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Visualization and Characterization of Chromatography Structures

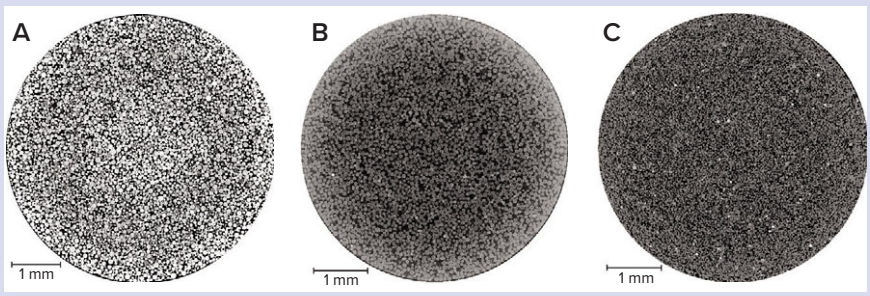
Imaging at Packed-Bed and Individual-Bead Scales

Thomas Johnson and Daniel Bracewell

Packed-bed chromatography is a vital downstream operation for purifying valuable biological products, including monoclonal antibodies (MAbs) and emergent therapeutic modalities such as viral vectors. Conventional chromatography unit operations in bioprocessing use highly porous microspheres packed into cylindrical columns, purifying complex feed streams through characteristics such as size, charge, and hydrophobicity. The porosity of both a packed bed and its constituent beads relates directly to the intended function and optimal performance in terms of both chemical and physical separation (**1**).

High-resolution imaging techniques have developed sufficiently to the point at which they can be used to visualize and characterize complex geometries such as packed columns. These methods help us understand the detailed, internal structure of many different materials (**2**). X-ray computed tomography is an effective method for imaging at nanoscale resolutions in three dimensions (3D) while negating the need to section samples physically. We used that technology to image several commercially available chromatographic resins and packed beds to improve our understanding of how their structures relate to function and performance.

Figure 1: Horizontal slice through 1-mL prepacked beds of chromatography media imaged at a 3-µm pixel size; (A) agarose resin, (B) cellulose resin, (C) ceramic resin — adapted with permission (**3**)



Product Focus: Biologics

Process Focus: Downstream processing

Audience: Manufacturing and process development

Keywords: Resins, media, image-based analysis, column, X-ray computed tomography

Level: Intermediate

3D Imaging Setup

We imaged three resins consisting of agarose, cellulose, and ceramic base matrices using X-ray computed tomography using two instruments: a Zeiss Xradia 810 Ultra X-ray microscope for individual beads and a Nikon XTH 225 system for packed beds. After critical-point drying, individual beads were adhered to the top of a pinhead for scanning. We captured 1,601 images while rotating each sample, taking 20 hours for each single scan, using two pixel sizes: 64 nm to image entire beads and 32 nm for higher resolution at the expense of limiting the field of view. We also imaged 1-mL prepacked columns at 3-µm pixel size, taking 3,142 images over five hours for each scan. Then we loaded reconstructed volumes into Avizo software (Thermo Fisher Scientific) for digital processing. Samples were binarized into material and void phases for analysis of porosity (expressed as a percentage) and *tortuosity* (the effective path length through a complex structure, expressed as a ratio, with a value of 1 given to an uninterrupted path).

Packed-Bed Imaging: The ability to visualize the internal structure of a packed bed in the unchanged environment of an unused column improves our understanding of the detailed geometry of real bioprocessing materials. Because X-ray computed tomography is nondestructive and requires no sample preparation, the imaging can capture details of such samples in their nascent state. Optimizing image quality is essential to obtaining the most representative quantitative information. In this study, we achieved that through empirically determining the number of individual

Figure 2: Structural analysis of three resins, with edge measurements defined as the volume within 250 µm of the column wall; (A) porosity, (B) tortuosity — adapted with permission (**3**)

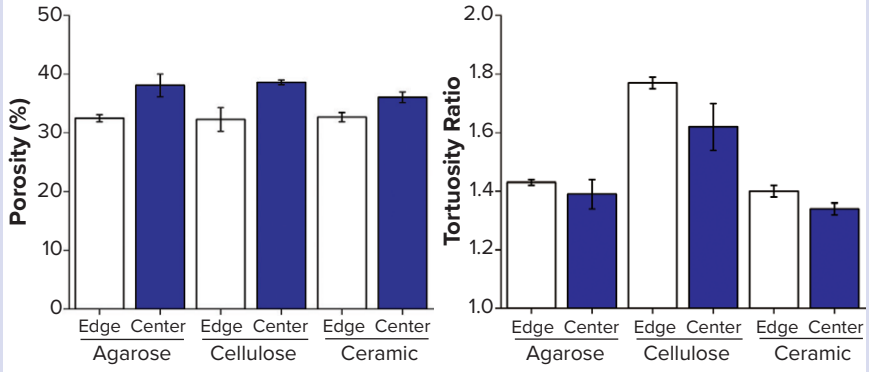
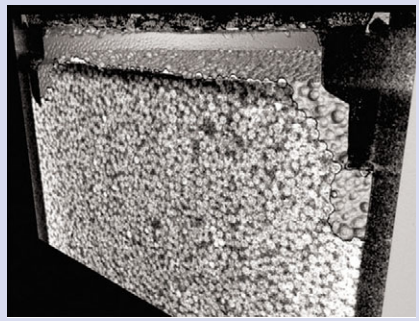


Figure 3: 1-mL cellulose packed bed following compression from excessive flow rates — adapted with permission (**6**)

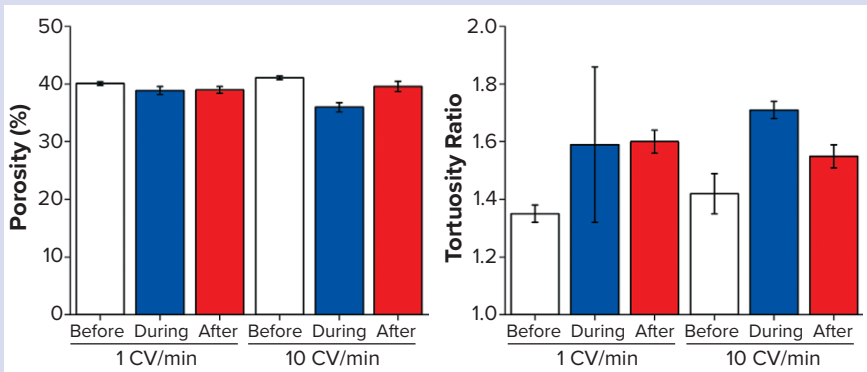


radiographs at the best signal/noise ratio achievable while fine-tuning our equipment settings. Figure 1 displays two-dimensional (2D) slices through each type of column, showing clear physical differences among the three base matrices. For example, agarose and cellulose beads are spherical, whereas ceramic particles are less so. Note that in all cases, the beads are neither uniform in size nor arranged into distinct plates.

Generating 3D digital representations of packed beds in chromatography columns enables structural analysis of their key characteristics. Although structural features of columns can be measured using conventional approaches — e.g., a blue dextran pulse for determining interbead porosity — imaging techniques enable positionally based analyses (**3**). Shalliker et al. imaged iodine pulses in a time series to visualize chromatography wall effects, in which a radial packing disparity arises from geometrical and frictional interactions close to column walls (**4**). X-ray computed tomography reconstructions of the three column types enabled us to measure interbead porosity and tortuosity at the edge and center of each column. Figure 2 provides results from our analysis.

We observed that interbead porosity was lower at the edge than the center for all three resins, which demonstrates further their radial packing disparities. Indicating the effective path length that the mobile phase must travel in a chromatographic process, tortuosity was another factor of critical interest in this study because of the importance of transport phenomena between and within chromatography beads. Historically, tortuosity has been measured indirectly using a relationship to overall sample porosity, as described by Tjaden et al. (**5**). The advent of digital volumes from 3D imaging has enabled use of the complex geometries of these materials to simulate tortuosity. In all cases, the tortuosity measured in this study was lower at the edge of the columns than at their centers.

Figure 4: Structural analysis of three resins before, during, and after flow-induced compression; (A) porosity, (B) tortuosity — adapted with permission (**6**)



Packed-Bed Compression: We applied X-ray computed tomography to investigate how a packed bed of chromatographic media changes during and after compression. Frictional support through wall effects is diminished as columns are scaled up to larger diameters, which can lead to compression when combined with relatively soft resin materials such as cellulose and mobile phases containing foulants. To deliberately compress columns in this study, we passed 20% ethanol through a 1-mL prepacked bed at a reasonable flow rate of 1 column volume (CV) per minute and an excessive flow rate of 10 CV/min (**6**). Figure 3 shows an irreversibly compressed packed bed, with an obvious gap between the new bed height and the surrounding plastic molding.

X-ray computed **TOMOGRAPHY** reconstructions of three column types enabled us to measure interbead porosity and tortuosity at the edge and center of each column.

As above, we collected 3D data sets to evaluate interbead porosity and tortuosity. Columns were imaged before, during, and after flow at both 1 and 10 CV/min. To enable capture of high-quality images during flow, a steady state had to be reached at which beads were no longer moving; even so, we

Achieving suitable resolution is **ESSENTIAL** for representative visualization and characterization, but that often comes with drawbacks, including a decreased field of view and increased scan times.

detected a noticeable reduction in signal/noise ratio while imaging the live flow. As Figure 4 indicates, the porosity measured for the top 3 mm of the bed is reasonably consistent at 1 CV/min, but a clear reduction in porosity is apparent during 10-CV/min flow (reverting after flow ceased).

Individual Bead Imaging: The internal structure of chromatography beads is essential for separation during size-exclusion chromatography as well as for providing a substantial ratio of surface area to volume to bind products and impurities (**7**). Applying X-ray computed tomography at the nanoscale enabled us to view the internal structure of three types of chromatography beads across their entire diameters (Figure 5). Pores are obvious throughout in each case, with each sample having a distinct geometry.

For acquisition of the most representative images and the best results from their analysis, the finest features must be distinguishable within each chromatography bead. That requires an appropriate resolution, so for this study we improved the pixel size from 64 nm (Figure 5) to 32 nm (Figure 6). Far more detail could be seen on the chromatography beads at the improved resolution, which was noticeable particularly for the cellulose sample. Note that only the largest spherical pores can be seen in Figure 5B, whereas those larger pores are surrounded by more detailed structures in Figure 6B. Imaging at improved resolution requires sacrifice in other aspects, however, most noticeably the substantially reduced field of view that images only a small region of each bead.

We compared the two pixel sizes for both porosity and pore size (Figure 7). Porosity measurements were reasonably consistent at both pixel sizes; however, the difference in average pore size is clear. Thus, imaging focused on capturing an entire sphere within the field of view is insufficient for detecting the finest features within each chromatography bead. Therefore, achieving suitable resolution of a given sample is essential for representative visualization and characterization, but that often comes with drawbacks, including a decreased field of view and increased scan times.

Figure 5: Two-dimensional (2D) slice through individual chromatography beads imaged at 64-nm pixel size; (A) agarose resin, (B) cellulose resin, (C) ceramic resin — adapted with permission (**8**)

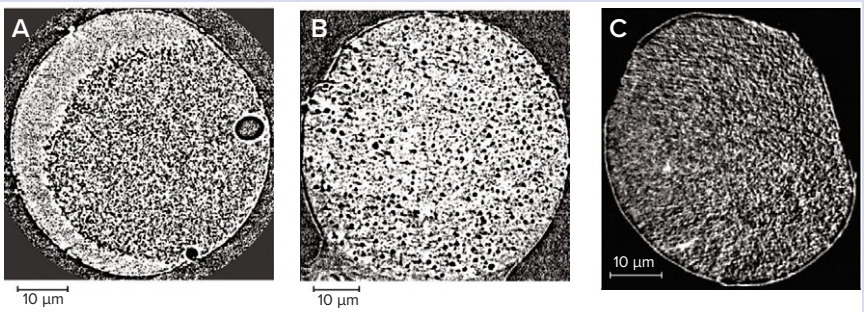


Figure 6: Vertical slice through a subsection of chromatography beads imaged at 32-nm pixel size; (A) agarose resin, (B) cellulose resin, (C) ceramic resin — adapted with permission (**8**)

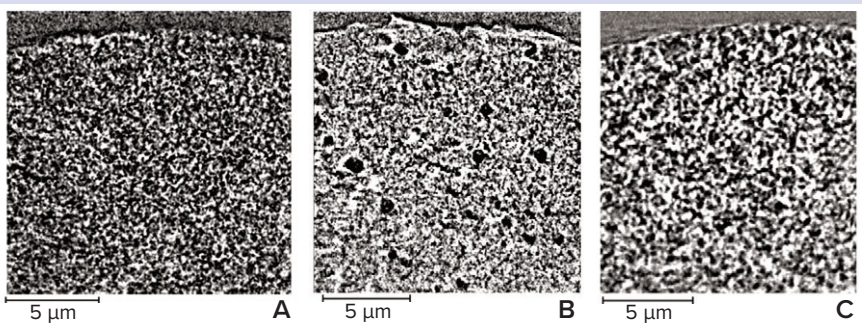
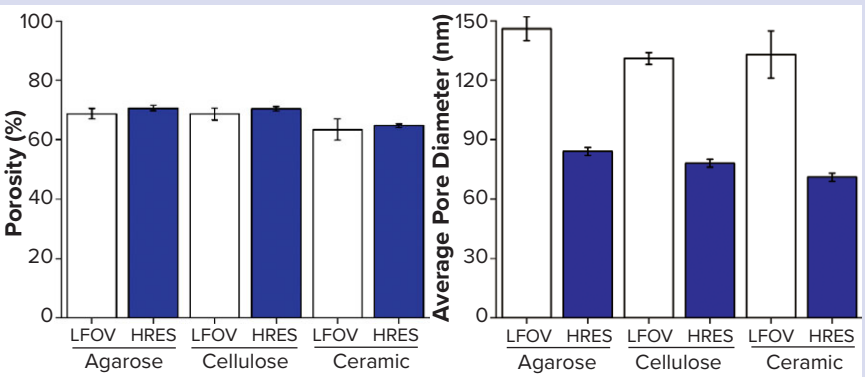


Figure 7: Structural analysis of three individual beads; (A) porosity, (B) average pore size; LFOV = large field of view at 64-nm pixel size (Figure 5), HRES = imaging at higher resolution of 32-nm pixel size (Figure 6) — adapted with permission (**8**)



Outlook

High-resolution X-ray computed tomography allows us to visualize and characterize the detailed, internal structures of chromatographic packed beds and individual beads for a range of industrially relevant resins. Representing 3D geometries and associated flow paths of real materials enables analysis of complex transport properties through porous media using state-of-the-art simulation software.

In this study, we focused on tortuosity. Improving the biopharmaceutical industry's understanding of how structure relates to function and performance will influence design and fabrication of bioprocessing and biopurification materials across multiple scales. That is of critical and timely importance as multiple emerging therapeutic modalities provide exciting opportunities in the clinic while presenting substantial challenges to manufacturing at high process yields with acceptable product quality.

Acknowledgments

This research was funded by the UK Engineering and Physical Sciences Research Council (EPSRC) grant EP/L01520X/1 and UK Research and Innovation (UKRI) grant MR/W004399/1 through an Innovation Scholarship. We're grateful to Nigel Jackson and Dave Hayden at Cytiva in addition to Francesco Iacoviello and Paul Shearing at University College London's (ECL's) Electrochemical Innovation Laboratory. This research was undertaken as part of the UCL-Pall Centre of Excellence, established in 2018.

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1. Publication Title: BioProcess International
2. Publication Number: 15426319
3. Filing Date: 10/1/2023
4. Issue of Frequency: Monthly except for combined issues in January, July & November
5. Number of Issues Published Annually: 9
6. Annual Subscription Price: Free to Qualified
7. Complete Mailing Address of Known Office of Publication (Not Printer): Informa Business Media, Inc., Informa Connect 605 Third Ave., 22nd Floor, New York, New York County, NY 10158 — Contact Person: Lauren Mena, Telephone: 813-428-4357
8. Complete Mailing Address of Headquarters or General Business Office of Publisher (Not Printer): Informa Business Media, Inc.,605 Third Ave., New York, NY 10158
9. Full Names and Complete Mailing Addresses of Publisher, Editor, and Managing Editor - Publisher: Christopher Johnson, Informa, 605 Third Ave., New York, NY 10158; Editor: S. Anne Montgomery, Informa, 605 Third Ave., New York, NY 10158; Managing Editor: Brian Gazaille, Informa, 605 Third Ave., New York, NY 10158
10. Owner - Full name and complete mailing address: Informa Media, Inc., 605 Third Ave, New York, NY 10158; Informa Operating Holdings, Inc. (owns 100% of the stock of Informa Media, Inc.), 605 Third Ave, New York, NY 10158
11. Known Bondholders, Mortgagees, and Other Security Holders Owning or Holding 1 Percent or More of Total Amount of Bonds, Mortgages or Other Securities: None
12. Tax Status (For completion by nonprofit organizations authorized to mail at nonprofit rates) (Check one)
The purpose, function, and nonprofit status of this organization and the exempt status for federal income tax purposes: N/A
13. Publication Title: BioProcess International
14. Issue Date for Circulation Data: August 2022
15. Extent and Nature of Circulation

	Average No. Copies Each Issue During Preceding 12 Months	No. Copies of Single Issue Published Nearest to Filing Date
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Control of Host Cell Proteins in Monoclonal-Antibody Bioprocessing

Using Proteomic Analysis To Understand Impurity Clearance and Persistence During Purification

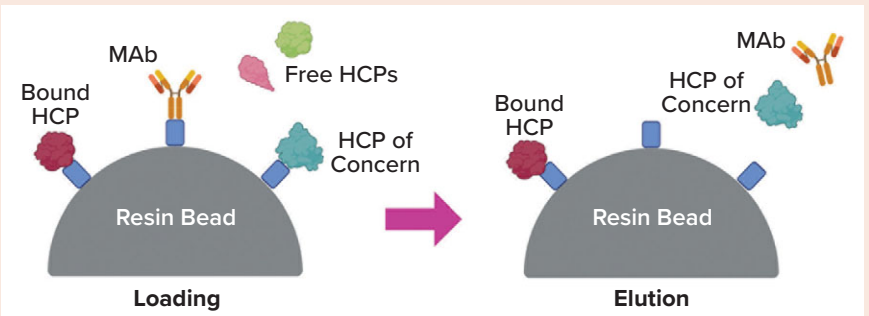
Abraham M. Lenhoff and Chase E. Herman

Downstream process development can proceed like a detective novel, starting with evidence of something seriously wrong and rapidly evolving into a "whodunit." The evidence often comes as precipitate particles in what is supposed to be a stable formulation. The whodunit takes the form of root-cause analysis into the degradation mechanism of a biopharmaceutical product or of a key ingredient in its formulation. And the culprit often turns out to be an enzyme present in such small quantities as to be almost undetectable. The rash of such cases during manufacturing of monoclonal antibodies (MAbs) and other biopharmaceuticals has changed the field of impurity clearance, including associated assays and control strategies.

Issues with impurity clearance stem almost invariably from host cell proteins (HCPs). Along with host-cell DNA (hcDNA), cell debris, lipids, and viruses, HCPs are categorized as *process-related impurities*, distinguishing them from *product-related impurities* such as product aggregates (often referred to as *species of high molecular weight* (HMW)) and fragments. Historically, the extremely large and heterogeneous class of HCPs has been considered as a unit, both for specific analyses (e.g., as a set of impurities to be detected using enzyme-linked immunosorbent assays (ELISAs)) and for assessment of downstream clearance, with overall levels in a MAb drug substance needing to fall below the target of 100 ppm (ng HCP/mg MAb) and with products typically having ~10 ppm. Cases in which low concentrations of individual HCPs were found to have deleterious effects on drug substances, formulations, and recipients have since led to classification of dozens of specific species as "difficult-to-remove" or "high-risk" (**1**). The latter category includes various proteases such as cathepsins, lipases, and hydrolases.

Identification of a single problematic HCP can prompt downstream scientists to give it specific analytical attention and make focused efforts to ensure its removal to sufficiently low levels, preferably below a method's limit of detection. Cases of such adaptation have directly influenced current approaches to process development, including considerations for associated analytical support. For instance, although platform processes for MAb purification have been well established for about two decades (**2, 3**), and despite the emergence of liquid chromatography with mass spectrometry (LC-MS) as an enabling technology for HCP analysis during process development and (increasingly) manufacturing, proteomic analyses of MAb process streams are shedding light on new biophysical aspects of downstream processes. Here, we overview that rapidly evolving field, specifically for proteomic assessments of product proteins and impurities generated by Chinese hamster ovary (CHO) cells. Our presentation is necessarily brief, and although we address implications for analytics and specific assays, we focus primarily on application of proteomic methods to biomanufacturing processes.

Figure 1: Coelution is a possible mechanism of host-cell protein (HCP) persistence through chromatography of a monoclonal-antibody (MAb) product (e.g., a protein A step), shown below from loading through elution (image created using BioRender software).



PRODUCT FOCUS: MABS

PROCESS FOCUS: PURIFICATION

AUDIENCE: PROCESS DEVELOPMENT, MANUFACTURING, QA/QC

KEYWORDS: HOST CELL PROTEIN CLEARANCE AND PERSISTENCE, PROTEOMICS, COELUTION, PRODUCT ASSOCIATION, AGGREGATION

LEVEL: INTERMEDIATE

Proteomics of CHO HCPs

LC-MS technology has matured rapidly over the past decade and has displaced two-dimensional gel electrophoresis to assume a dominant role in proteomics. Today, analysts often can identify and quantify hundreds to thousands of discrete protein species in a single sample, a capability that should serve outstandingly well in HCP analysis (**4, 5**). For some HCPs, methods even have been developed to detect levels as low as 0.1 ppm (**6**). However, access to seemingly comprehensive data sets can be highly misleading without adequate perspective on uncertainties that are inherent to proteomic analysis.

Converting the exquisite sensitivity of modern mass spectrometers into large data sets involves multiple steps both upstream and downstream of an LC-MS instrument (**7**). A sample must be exchanged into an appropriate buffer environment and digested by a protease before the resulting peptides are separated by one or two LC steps, with the eluting peaks then analyzed by MS. Some analyte components might be lost during sample preparation, whereas others could be obscured by coeluting LC peaks. And after LC-MS data have been acquired, they are interpreted using software packages in which selected parameter values can influence final outcomes.

Although all assays have limitations, LC-MS analysis of HCPs is especially susceptible to "false negatives," in which HCP species are present in a sample but remain undetected. Amid substantial quantities of a product protein, peaks from MAb-derived peptides can obscure those from HCP-derived peptides such that the number of HCP species detected is reduced by more than an order of magnitude (**8**). To overcome such issues, researchers have developed new workflows that leverage depletion of MAbs in a sample to yield one or more low-MAb fractions in which individual HCPs can be detected more easily (**6, 9, 10**). However, complete elimination of false negatives is difficult to envisage.

HCP Clearance in Downstream Processing

That a MAb product is secreted into the cell-culture fluid is one of the strengths of CHO and related mammalian cell lines. However, many HCPs that must be removed during downstream processing are likewise secreted (**11**). Moreover, cell death and lysis during culture can release intracellular HCPs and more complex biophysical structures such as organelles. In principle, the most direct way of dealing with HCPs would be at their source, and proteomic analysis is indeed performed within the cell-culture context (**12, 13**). However, our emphasis below is on HCP clearance during purification of MAbs from harvested cell-culture fluid (HCCF) under typical cell-culture conditions (e.g., with titers of ~10 mg/mL).

A downstream process can achieve robust clearance of all impurity classes through multiple unit operations performed in series with orthogonal patterns of separation. For MAb biomanufacturing, the well-established platform process involves a capture step using protein A affinity chromatography, which is highly specific and typically achieves HCP log reduction values (LRVs) of ~3 $\log_{10}$ (**2, 3**). Remaining impurities, usually already at low concentrations, are then reduced to acceptable levels by additional polishing steps. Selection of unit operations (mainly of different chromatography modes) often is guided by heuristics regarding their effectiveness at removing different impurities (**3**). For instance, flow-through anion-exchange (AEX) chromatography is applied widely to reduce HCPs and hcDNA, but it is not considered to be effective at removing MAb aggregates, for which cation-exchange (CEX) chromatography and hydrophobic-interaction chromatography (HIC) are used more frequently.

Platform processes structured in such ways can reliably reduce overall HCP concentrations to low levels. This fact might seem inconsistent with the observation that damaging levels of individual HCPs sometimes persist in drug substances and drug products. One factor to bear in mind is the extremely low concentrations at which some enzymes can have deleterious effects on therapeutic proteins. A related consideration is the fairly long shelf-life expected for most biopharmaceuticals.

Often, multiple individual HCPs are detected in drug substances. In the absence of harmful effects on a product or patient, such persistence generally is considered to be benign. However, recurring issues arising from high-risk HCPs have prompted the biopharmaceutical community to investigate mechanisms that might contribute to persistence of individual HCPs through, for instance, a chromatography step. Three mechanisms have received the most attention.

Coelution: The most straightforward explanation for poor separation is that a given product protein and HCP behave nearly identically in the separation train, specifically in having similar binding and elution properties during chromatography (Figure 1). Although coelution is plausible within a single unit operation (**14**), its likelihood is much lower when considering all of the multiple, nominally orthogonal operations in a usual downstream train.

Product Association: A potential driver of persistence is noncovalent association between an HCP and product, with the complex having similar separation properties to those of the product (**15**) (Figure 2). Researchers have investigated the possibility of such "hitch-hiking" in various ways — e.g., by placing material from a null HCCF process (no MAb present) in direct contact with a MAb immobilized onto chromatographic

Figure 2: Product association as a potential mechanism for host-cell protein (HCP) persistence (image created with BioRender software)

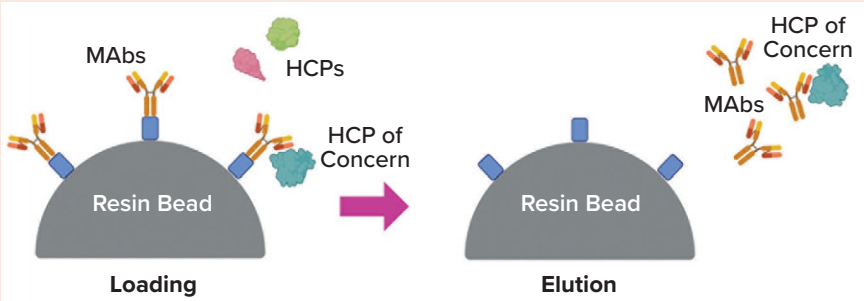
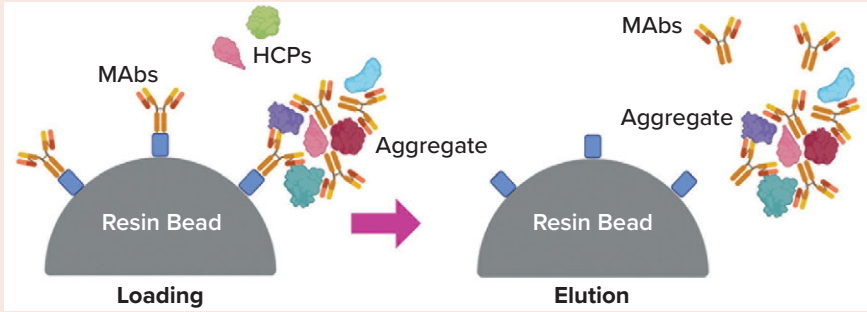


Figure 3: Aggregation as a potential mechanism for host-cell protein (HCP) persistence (image created with BioRender software)



particles (**16, 17**). Such studies have helped to identify dozens of HCPs that appear to bind with multiple MAbs — sometimes with most or all of those tested. Such observations could help explain not only the repeated finding of certain persisting HCPs, but also less predictable cases in which a certain high-risk HCP is found to be problematic for a particular MAb process.

Implicit in much of the product-association discussion is the notion that such binding is strong and specific — it is the high-affinity binding that reflects a (fortuitously) high degree of molecular complementarity between the proteins concerned. However, in a few reported cases, direct measurement of the MAb dissociation constants (K_D values) indicated that the affinities are not particularly high (K_D ~ 1 µM) (**18, 19**). In addition, most of the HCPs that appear to exhibit product association are among the most abundant ones in HCCF (**19**). Thus, the association seems to be driven less by high affinity than by mass action coupled with moderate affinity, possibly in multiple binding configurations (hence more accurately characterized as avidity).

Such observations suggest a situation in which product association indeed contributes to persistence of an HCP, with repeated reequilibrations leading gradually to its depletion. Reequilibration could occur, for instance, during a wash step in a chromatography process, and the conditions of the wash (e.g., pH or the presence of an excipient) might further modulate the dissociation constant of the complex.

Aggregates: Often called *HMW species*, aggregates long have been treated as a major class of product-related impurities to be cleared during downstream MAb processing (**20**) (Figure 3). Typically, they are regarded as MAb oligomers, and their properties during chromatography tend to be similar to those of a MAb monomer, including their capacity for persistence through protein A chromatography steps. About a decade ago, Gagnon et al. reported evidence that some aggregates can serve as carriers of HCPs and thereby might be responsible for HCP persistence in many cases (**21**). The writers hypothesized that such aggregates form around chromatin particles, in which histones are highly positively charged and DNA is highly negatively charged; other species, including HCPs and MAbs, would then bind promiscuously because of strong electrostatic attraction. Several subsequent studies have shown that pretreatment to remove aggregates can improve HCP clearance appreciably during protein A chromatography (**22–24**).

More recent evidence supports the likely role of aggregates in HCP persistence. Proteomic analysis shows that MAb aggregates, classified somewhat arbitrarily into larger and smaller fractions (with radii up to ~50 nm and ~10 nm, respectively), contain many hundreds of different HCPs (**8**). Those include cellular-defense proteins such as chaperones, suggesting a possible origin for aggregates other than histones (**25**). Size-exclusion chromatography (SEC) analysis of protein-A fractions reveals that although free HCPs almost entirely flow through during column loading, aggregates bind similarly to — and evidently competitively with — MAb monomers, and a significant proportion of those aggregates coelute with the monomers (**26**). That finding supports the conventional wisdom that aggregates contain MAb molecules, but the proteomic data show that both large and small aggregates contain HCPs as well.

SEC fractionation of material from flow-through AEX presents a complementary picture (**26**). For AEX processes, conditions are chosen such that MAb monomers do not bind to resin beads, but results from SEC analysis show that such conditions limit binding of small aggregates, too, explaining why flow-through AEX is disfavored for aggregate clearance. In contrast, large aggregates may bind and be removed, making flow-through AEX effective for HCP clearance as a polishing step. The small aggregates appear to be more MAb-rich than the large ones are, so small aggregates more closely resemble the conventional view of HMW species as MAb oligomers (**8**).

Making Sense of HCP Persistence

The picture that emerges from such findings is helping researchers to explain the mechanisms underlying the fate of HCPs during downstream processing. Some moderately abundant HCPs appear to persist by product association, but they also seem to be depleted during operations such as wash steps that enable dissociation of MAb–HCP complexes and removal of freed HCPs. This model of HCP persistence is consistent with the observation that product association strength differs across product species, resulting in differing amounts of residual HCPs.

Aggregates, regardless of their origins, provide another likely and perhaps more widespread mechanism for HCP persistence. Available data clearly demonstrate that aggregates can elude clearance during protein A chromatography but that polishing steps such as flow-through AEX can remove them effectively. That aggregates are probably the principal vectors of HCP persistence is supported by a broad correlation between HCP numbers/concentrations and HMW content in tested material (**26**) and by correlation of HCP persistence with MAb aggregation propensity (**27**).

Such a model of HCP persistence muddies the customary distinction between product- and process-related impurities, the two categories into which HMW and HCP species are normally classified, respectively. Aggregates that contain HCPs represent both product- and process-related impurities; consequently, they can confound heuristics regarding

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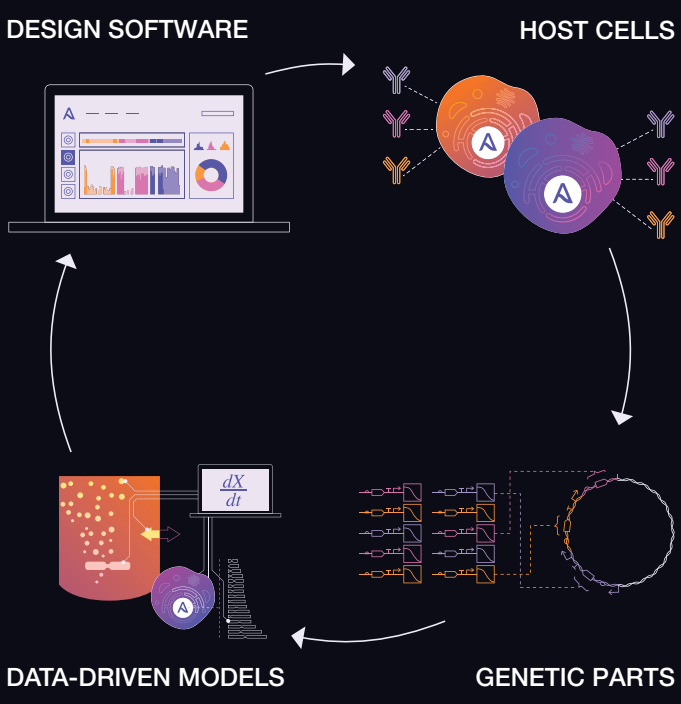

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the suitability of different unit operations for removing given types of impurities. For example, flow-through AEX typically is considered to be effective at removing HCPs but not HMW species. That notion seems to be inconsistent with the observation that large, HCP-containing aggregates are indeed cleared effectively. Resolution of that apparent conflict lies in the poor clearance of small aggregates, which might be biased heavily toward MAb oligomers even if they also include simple MAb–HCP complexes.

What do such findings tell us about the structure and operation of downstream processes to remove HCPs and other impurities? For MAbs, the established platform process generally is highly effective, but enhanced analytical support can help to identify areas of potential concern and improved control. Recent study results confirm what Gagnon et al. proposed a decade ago: that despite its effectiveness and dominance over other purification strategies, the Achilles heel of protein A chromatography is poor clearance of HCP-containing aggregates. Resins and complementary technologies that are modified to address such impurities could be beneficial. For modalities other than MAbs, questions remain about whether a given product and formulation are susceptible to damage from individual HCPs. Lessons learned from experiences with MAbs will provide both guidance and adaptable purification methods.

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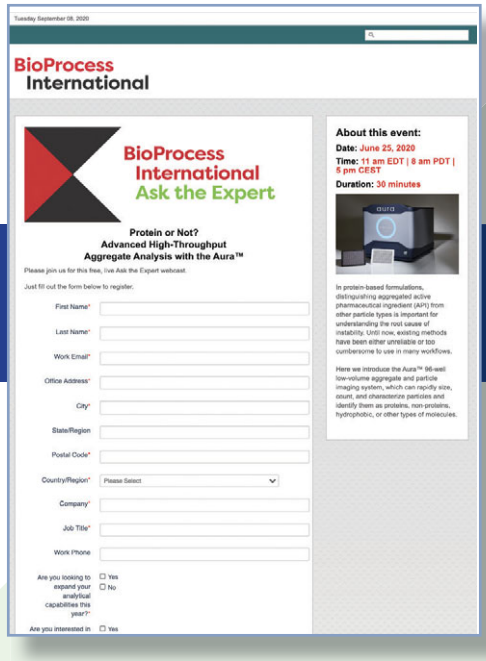

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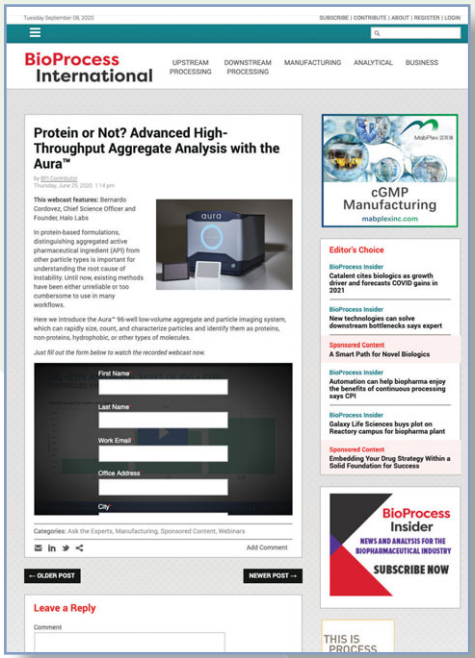

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An Innovative System for Advanced, High-Throughput Aggregate Analysis

with Bernardo Cordovez

Distinguishing aggregated active pharmaceutical ingredients (APIs) from other particle types is critical to evaluating a protein product's stability, but standard characterization tools struggle to discriminate proteins from nonproteins with similar sizes, shapes, and morphologies. In a 25 June 2020 "Ask the Expert" webcast, Bernardo Cordovez (founder and chief scientific officer of Halo Labs) introduced his company's Aura aggregate-analysis solution. He explained how the device combines an innovative microscopy technique with sophisticated imaging software to characterize subvisible particles more quickly and accurately than incumbent technologies can.

Cordovez's Presentation

Cordovez observed that flow imaging requires large sample volumes and cannot distinguish proteins from plastics or degraded polysorbates. Raman and Fourier-transform infrared (FTIR) spectroscopy can do so but require proteins and aqueous suspensions can have similar optical properties.

Unlike spectroscopy, the Aura system is high throughput. It requires little sample (~5 µL), evaluates 96 samples in under an hour, and integrates easily with automation tools. In minutes, Aura software collates measurements of particle shape, size, morphology, and intensity to generate particle-, sample-, and experimental-level assessments. The system also analyzes entire samples, such that all particles become visible. By contrast, a flow imager reveals ~20% of a sample's particles.

A fluorescent-labeling workflow enables the Aura instrument to identify particles. After performing the brightfield steps, an analyst can apply 40 µL of 5 mM thioflavin T (ThT) dye to a well plate, suction out residue, and return that plate to the system for imaging. Cordovez noted that ThT is useful because it binds to protein amyloid structures — but not to plastic, stain and remained dark. IgG and ETFE also showed distinctive side-angle scattering signatures. Based on measurements of such properties, Halo analysis determined that 15.5% of measured particles in the IgG–ETFE blend were nonproteinaceous, which was consistent with readings from the bright-field quantitation step.

Although IgG and ETFE appear to be identical using flow-image microscopy, the Aura system can distinguish between even highly similar particles. It can render data in several ways to offer high-level insights about samples, and it features a second channel that can apply dyes for identification of lipids and protein monomers. Now, Halo Labs is exploring multichannel fluorescence and side-scattering techniques to enhance particle identification further.

Questions and Answers

What dyes besides ThT can Aura systems accommodate? Defaults for the system's second channel include TMA-DPH and


ASK THE EXPERT

Translating Inhaled and Nasal Technologies for Biologics Delivery

with Mark Parry

Gastric delivery is unachievable for most biopharmaceuticals, so drug developers formulate biologics primarily for intravenous infusion or injection. However, inhaled and nasal delivery options are attracting considerable attention because they enable targeted delivery of a wide range of therapeutic proteins. On 23 June 2020, Mark Parry (technical director at Intertek) presented an "Ask the Expert" webinar that described critical considerations for inhaled and nasal delivery for biologics.

Parry's Presentation

Because biopharmaceuticals are complex products, developers need compelling reasons to choose inhaled and nasal formulations. Targeted drug delivery is one such reason. As researchers have observed with therapeutics for asthma/chronic obstructive pulmonary disease (COPD) and antibiotics, local administration facilitates delivery of therapeutic doses. NALT provides a large mucosal platform, which can help provoke systemic immune responses. The olfactory region offers a "back door" to the brain, enabling delivery of drugs that otherwise cannot pass the blood–brain barrier. That holds promise for treating anxiety, depression, migraines, Alzheimer's disease, and Parkinson's disease.

Biologics developers can select from several nasal devices. Solution- and suspension-based sprays are inexpensive and can facilitate biologics administration. But some devices do not tolerate freeze–thaw cycles. Reconstitution of drug product (DP) close to administration and careful device screening and design can circumvent that problem. Many biologics developers favor dry powder, which can increase drug-product stability and longevity.

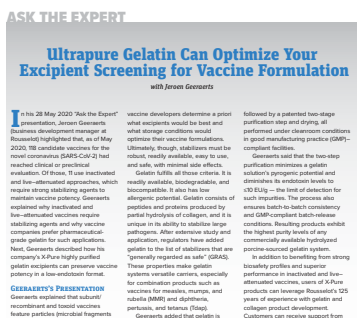
Pressurized metered-dose inhalers (pMDIs) present significant formulation challenges for biologics, so the focus is on aqueous and solid formulations for

Analytical assays are critical to translating biologics from injectable to inhaled or nasal products. Developers know how their products behave out of a vial, but formulations for the nose and lungs also require characterization of DP emission from devices. Successful translation requires aerodynamic particle-size distribution and emitted-dose/dose-uniformity assessments to establish product specifications.

Questions and Answers

Which is the better option for systemic delivery: inhaled or nasal formulation? Emerging research tends to focus on nasal products for systemic delivery. Inhaled products often perform well in terms of dosing immediacy, but they can raise formulation challenges.

Can inhaled and nasal formulations deliver small proteins such as antibody fragments and nanobodies? Inhaled and nasal delivery can accommodate a wide range of proteins from antibody


ASK THE EXPERT

Ultrapure Gelatin Can Optimize Your Excipient Screening for Vaccine Formulation

with Jeroen Geeraerts

In this 28 May 2020 "Ask the Expert" presentation, Jeroen Geeraerts (business development manager at Rousselot) highlighted that, as of May 2020, 118 candidate vaccines for the novel coronavirus (SARS-CoV-2) had reached clinical or preclinical evaluation. Of those, 11 use inactivated and live–attenuated approaches, which require strong stabilizing agents to maintain vaccine potency. Geeraerts explained why inactivated and live–attenuated vaccines require stabilizing agents and why vaccine companies prefer pharmaceutical-grade gelatin for such applications. Next, Geeraerts described how his company's X-Pure highly purified gelatin excipients can preserve vaccine potency in a low-endotoxin format.

Geeraerts's Presentation

Geeraerts explained that subunit/recombinant and toxoid vaccines feature particles (microbial fragments vaccine developers determine a priori what excipients would be best and what storage conditions would optimize their vaccine formulations. Ultimately, though, stabilizers must be robust, readily available, easy to use, and safe, with minimal side effects.

Gelatin fulfills all those criteria. It is readily available, biodegradable, and biocompatible. It also has low allergenic potential. Gelatin consists of peptides and proteins produced by partial hydrolysis of collagen, and it is unique in its ability to stabilize large pathogens. After extensive study and application, regulators have added gelatin to the list of stabilizers that are "generally regarded as safe" (GRAS). These properties make gelatin systems versatile carriers, especially for combination products such as vaccines for measles, mumps, and rubella (MMR) and diphtheria, pertussis, and tetanus (Tdap).

Geeraerts added that gelatin is followed by a patented two-stage purification step and drying, all performed under cleanroom conditions in good manufacturing practice (GMP)–compliant facilities.

Geeraerts said that the two-step purification minimizes a gelatin solution's pyrogenic potential and diminishes its endotoxin levels to ≤10 EU/g — the limit of detection for such impurities. The process also ensures batch-to-batch consistency and GMP-compliant batch-release conditions. Resulting products exhibit the highest purity levels of any commercially available hydrolyzed porcine-sourced gelatin system.

In addition to benefiting from strong biosafety profiles and superior performance in inactivated and live–attenuated vaccines, users of X-Pure products can leverage Rousselot's 125 years of experience with gelatin and collagen product development. Customers can receive support from

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We also prefer using first-person (*I, we, my, our*) and sometimes second-person (*you, your*) perspectives rather than overly formal and distancing third-person constructions (*one, it, there is*). This approach facilitates the exchange of practical information.

BPI pays strict attention to correct uses of demonstrative and relative pronouns: e.g., *this* and *that, these* and *those,* and *which* and *that.* Our readers are busy, so we don't want them to waste time stumbling over ambiguities. Thus, *since* (referring to time) is not used in place of *because* (causality), and *while* is not used to mean *although*.

BPI often defines and abbreviates key terms because acronyms can stand for different concepts across disciplines and might be unfamiliar to readers who are new to the industry. For example, we still use "CGMP" to abbreviate *current good manufacturing practice,* following the US Food and Drug Administration's early lead in capitalizing the "C." (Although often used in biopharmaceutical industry discourse, the similar abbreviation *cGMP* can be confused to mean *cyclic guanosine monophosphate* in some contexts.)

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Single-Use Systems

Providing Biopharmaceutical Manufacturers with Cutting-Edge Material and Assembly Solutions

Mike Urbanski

Recent years have witnessed biopharmaceutical manufacturers transition swiftly from traditional stainless-steel systems that require harsh sterilization between applications to single-use systems (SUS) that are less expensive, faster to produce, and — perhaps counterintuitively — more compatible with sustainability initiatives (**1**). Now that disposable systems have become industry standard, biopharmaceutical original equipment manufacturers (OEMs) are seeking full-service components partners that can offer further innovations in SUS.

Integrated Solutions

Some suppliers can provide biopharmaceutical OEMs with tailored assembly solutions in addition to finished components. Such work could involve integrating connectors or sensors to extruded tubing assemblies and providing complete system assemblies such as single-use chromatography columns (see the "Chroma — what?" box on the next page). Full-service capabilities enable an OEM to decrease its number of suppliers, helping to improve the overall quality of finished products by reducing the number of systems and processes used and by enabling application of a single quality management system for a complete product assembly. Full service can help an OEM to lower its costs by reducing needs for audits, purchase orders, and shipping and receiving logistics. Vertical integration of services also enables a supplier to understand and rectify all design and manufacturing issues before its products go to market.

When OEMs spend less time and money managing such concerns, they can focus on research and development of materials for manufacture of novel drugs while remaining agile for the future. For instance, they can rely on Trelleborg's BioPharmaPro family of innovative products, materials, and services for single-use fluid-path equipment. The portfolio includes solutions from individual single-use components to assemblies and fully integrated systems for biopharmaceutical manufacturing.

A Weighty Situation

Traditionally, chromatography-column "shells" have been composed of unreinforced polypropylene (PP), stainless steel, or acrylic. However, such materials are heavy, creating mobility issues. Achieving and maintaining tight tolerances in associated components and seals for fluid distribution can involve significant expenses. Unreinforced PP, stainless steel, and acrylic also are difficult to manufacture at scales needed for commercial downstream processes. Thus, OEMs are seeking alternative column materials that can maintain pressure ratings at high scales without creating concerns for validation processes.

Trelleborg provides significantly improved hardware-material options for chromatography columns with its BioPharmaPro portfolio of products and services. The company's material experts use PP reinforced with continuous fiberglass, which creates a composite material that is lighter and thinner than traditional options (see photo above). The composite can be formed into large-diameter columns that maintain required pressures during chromatography processes. Because BioPharmaPro column shells are composed of the same product-contact material as that used in many columns for research and development and clinical trials (PP), users now can leverage a complete portfolio of PP-based columns for small-scale applications through to commercial production. Such fiber-reinforced composite columns also have a small fraction of the weight and wall thickness of traditional materials.

Trelleborg experts leverage a reinforced polypropylene (PP) composite material to make thin, light chromatography columns, using significantly less material than is needed for traditional options.

Now that single-use systems have become the standard in biopharmaceutical production, suppliers need to offer **CUTTING-EDGE** solutions and **VALUE-ADDING** services with an emphasis on sustainability.

Because Trelleborg uses an additive manufacturing process to produce the columns, they can be made to a breadth of sizes and can include machined features needed for assembly into a functional product. The inner surface finish provides strong sealing, and inner-diameter dimensional tolerances are designed to enable efficient manufacturing. With Trelleborg's cleanroom assembly space and manufacturing capabilities in extrusion, molding, and machining, each chromatography column and its associated components can be made and assembled under one quality and supply-chain system.

The Quest for Sustainability

Although they seem antithetical to a world trying to move away from disposable components and products, SUS promote sustainability in biopharmaceutical manufacturing by minimizing chemicals and resources (e.g., water and energy) needed to sterilize reusable systems (**2**). Single-use technologies also keep costs and preparation times low. Most important is that SUS nearly eliminate risks of cross-contamination because the product flow path is discarded and replaced after each batch.

Although SUS are a much more environmentally sustainable option than their stainless-steel precursors, biopharmaceutical OEMs are exploring more ways to increase their circularity or ease their end-of-life impacts. As the industry at large continues to grapple with sustainability, suppliers such as Trelleborg remain committed to finding sustainable solutions for single-use materials — e.g., by providing customers with recommendations on how to dispose of or repurpose their used components.

A Commitment to Driving Supply-Chain Efficiencies

Until recently, any components supplier that could provide single-use technologies to a biopharmaceutical OEM was a valuable partner. Now that SUS have become the standard in biopharmaceutical production, suppliers need to offer cutting-edge solutions and value-added services with an emphasis on sustainability to help OEMs compete in an increasingly demanding market. Proposing innovations in material formulations for existing systems — e.g., chromatography columns — is one example of how suppliers can demonstrate their expertise and value to OEMs. With the BioPharmaPro portfolio of products and services, Trelleborg is committed to being not only a provider of single-use fluid-path solutions, but also an innovator that helps drive supply-chain efficiencies so that patients can receive life-saving therapies more economically and sustainably than ever before.

Chroma — what?

Chromatography columns are systems that use a resin or other such medium to attract or repel molecules. Depending on chromatography-system design and medium chemistry, target proteins can be attracted to the resin while impurities are repelled and eluted, or vice versa. The formats described below represent three broad classes of chromatography.

Ion-exchange (IEX) chromatography exploits the reversible exchange of ions between a solid stationary phase and a liquid mobile phase. IEX techniques are particularly useful for separating and purifying charged compounds, such as ions and polar molecules, from complex mixtures.

Hydrophobic-interaction chromatography (HIC) involves separation based on target molecules' hydrophobicity. The approach often is used for separating proteins and other large biomolecules based on differences in their tendencies to repel or avoid water.

In **affinity chromatography**, target proteins are attracted to ligands immobilized onto a resin while impurities are repelled.

Affinity chromatography remains the most common approach to purification of protein-based biologics. By having target proteins bind with ligands immobilized on resin beads, impurities can be flushed out. Then, proteins are released from the resin using a buffer that disrupts the molecular interaction.

Chromatography-column hardware plays a key role in providing efficient purification. Flow distributors enable process fluids to disperse evenly around a column's entire circumference, and highly engineered sealing solutions protect valuable resins and fluids from escaping the system. Light-weight materials of construction allow for column prepacking and easy storage before use.

Over the years, significant developments in chromatography resins have created faster, more efficient purification processes. Such efforts will continue for years to come, and chromatography will continue to be a critical part of biologic manufacturing.

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Re-Imagining Chromatography

Navigating the Path to Downstream Intensification

Katy McLaughlin, Piergiuseppe Nestola, and Fabien Rousset

Global access to medication is a crucial driver in the pharmaceutical industry (**1**). Thus, drug manufacturers are encouraged to lower their production costs while increasing productivity to bring affordable drugs to market quickly.

Process intensification is a natural solution for improving facility output. So far, upstream processes have been the main focus of intensification efforts. Combined with high-performing cell lines, those strategies have created higher titers. However, manufacturers now face bottlenecks in their downstream processes, which must evolve to handle the improvements.

Downstream process intensification is an ideal solution for solving such issues. Process intensification can increase yield, decrease process timelines, reduce cost of goods (CoG), reduce footprint, and increase flexibility without making significant changes to process parameters. The relative importance of these drivers will inform the selection of an intensification approach. As the workhorse of downstream bioprocesses, chromatography is the focus of most intensification strategies.

Strategic Considerations for Process Intensification

For established modalities, such as monoclonal antibodies (MAbs), drug developers must try to supply a global demand while keeping up with competition from biosimilars. Therefore, being fast and responsive is critical. Downstream intensification strategies should prioritize flexibility and accelerate time to clinic.

For newer modalities, drug developers need to produce enough active pharmaceutical ingredient (API) when purity and yield are still challenged by relatively novel purification processes and an increasing demand for higher quality by regulatory authorities. But new modalities and platforms can create fresh opportunities for improving innovation and efficiency. Without access to existing platform processes, manufacturers of newer modalities might be more amenable to using innovative technologies to address their needs.

Finally, the increasing spotlight on sustainability in the biopharmaceutical industry (**2**) is particularly applicable to chromatography, the most water-intensive operation in an entire bioprocess (**3**). Process intensification strategies can address the environmental impact of a process with the goal of producing more product with the same or smaller facility footprint and less capital equipment. A smaller facility offers significant sustainability benefits due to reduced energy consumption (e.g., heating, ventilation, and air conditioning, HVAC). For all the above scenarios, a re-imagination of current chromatography approaches is essential.

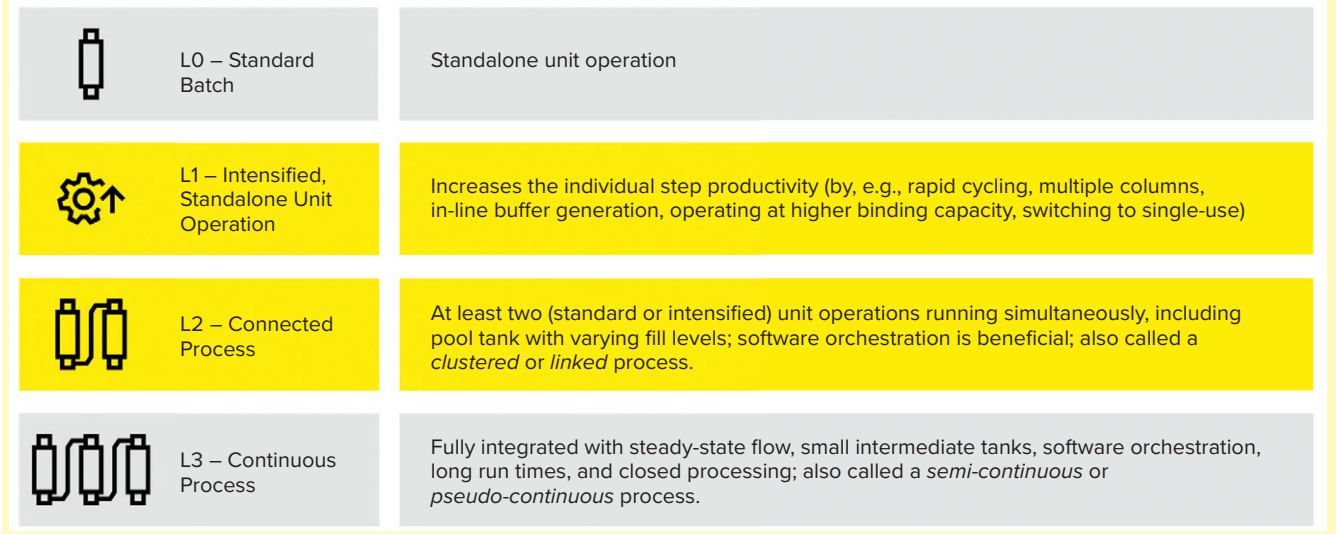
Re-Imagining Chromatography Eases Facility Bottlenecks

Increased Productivity with Minimal Process Changes: Process intensification often does not require significant changes to process parameters, workflow, or facility operations; a process can be largely unchanged but have increased productivity. Accumulated process understanding still is valid, and limited optimizations are required. For example, switching from multiuse systems to single-use consumables can increase reproducibility, accelerate timelines, and improve flexibility.

Relieve Supply-Chain Tensions: Improved efficiency offered by process intensification can help limit required consumables, creating a lean process with reduced operational expenditure and limited CoG. One example is to switch from resin to membrane chromatography in rapid cycling mode. Chromatography membranes such as the Sartobind Rapid A membrane improve consumable use, eliminating the task of ordering and storing significant volumes of expensive resins. This is extremely important for a contract development and manufacturing organization (CDMO) that stores different types of resins and columns for multiple customers.

Process Intensification Approaches Are Flexible: It is important to remember that there is no single route to process intensification. The pathway chosen depends on a facility's constraints, pipeline, strategies, and company goals. More and more options are available to couple upstream intensification with downstream intensification, helping manufacturers re-imagine their chromatography workflow. For example, switching from

Figure 1: Sartorius defines levels of process intensification from Level 0 to Level 3 (**4**).



multiuse batch to single-use multicolumn chromatography with the Resolute BioSMB system binds more product with more efficient resin use, shortening process times and reducing costs. Alternatively, if multiuse technologies are desired, the Resolute BioSC platform enables the performance of four steps on a single system, supporting a productive, continuous process.

Automation: Implementation of process intensification goes hand in hand with automation. A facility with intensified operations can operate with fewer personnel. Integrating process analytical technologies (PATs) can unlock novel, at-line data, contribute to more robust processes, and remediate traditional QC bottlenecks between unit operations (**5**).

Overcoming the Fear of the Unknown: Making changes to an already satisfactory process or adopting new technologies might be interpreted as inviting unnecessary risk. Often, capital expenditure in a manufacturing suite is already done (chromatography systems have been set up and large columns purchased), and intensification might require additional equipment and training. However, increased competition between technology suppliers has generated diverse solutions to solve downstream process intensification challenges, so it is possible to find a strategy to maximize productivity even with significant facility constraints.

Ultimately, making such changes is critical to remaining competitive and reducing costs, especially with the upcoming cost pressure on drug prices and increased competition from biosimilars. The implementation of downstream process intensification requires a culture change and willingness to re-imagine chromatography operations, whether that involves small steps and modifications to existing processes or building a new, intensified process.

Where Do I Start?

Normally, a stepwise approach is taken to minimize disruptions, digest these innovations, and build a technology experience.

Identify Your Main Bottlenecks: A good starting point is identifying the major constraints in the process and manufacturing suites. If supply chain tension, stock challenges, and high CoGs are major roadblocks, it is advisable to focus more on consumables and single-batch options. Alternatively, if reducing the time to clinic is a key driver, the main opportunities will be to reduce downstream process times, ideally by choosing connected or continuous options. If perfusion systems are in place to adapt to product demand during clinical phases, coupling upstream process steps to the first downstream process steps (clarification and capture) can reduce process timelines significantly.

There is no single route to process intensification. More and more OPTIONS are available to couple upstream intensification with downstream intensification.

Take It Step-By-Step: Fortunately, process intensification does not necessitate an "all or nothing" approach. Ideally, manufacturers should consider the short-, mid-, and long-term process improvements they want to make. Some equipment facilitates an incremental implementation of process intensification strategies. For instance, the Resolute BioSC system can be operated in batch or multicolumn mode and offers a large range of flow-path configurations to design a tailored multistep system.

An incremental approach allows you to build the necessary expertise and grow confidence within the operational and quality teams (by carrying out engineering runs, determining the scale-up strategy, performing process validation, and defining the batch). Starting by improving the batch sequence through working with new consumables like novel

Intensified Chromatography Advice Column

My product is early in its life cycle. Are there specific intensification strategies I should implement now? Ideally, scale-down models should be used as soon as possible in process development. Some intensification options are less dependent on the molecules you are developing; for example, continuous harvest, in-line dilution/conditioning, and sterile filtration. Other steps including chromatography capture and intermediate polishing are more challenging because titers, impurity levels, and biomolecular stability can significantly influence the suitability of an intensification strategy.

Production bottlenecks primarily come from the beginning of a downstream process as soon as we try to increase its scale and volume. Therefore, process development equipment and scaled-down consumables can be used to study the impact of intensification strategies on critical quality attributes (CQAs) at the capture step.

Exploring membrane-based chromatography in rapid cycling mode with the Sartobind Rapid A system could increase productivity by supporting higher flow rates and eliminating column-handling activities while simplifying future scale-up activities.

My facility has a limited footprint. What options do I have to intensify? In such circumstances, your goal is to produce the same or higher titer in less space. That means incorporating compact equipment, ready-to-use devices, and multistep systems where possible. The Resolute BioSMB and BioSC platforms are perfect examples of systems that can reduce downstream footprints. One system can manage several process steps, and working in a connected or continuous mode can avoid the need for many tanks in a manufacturing suite. Ready-to-use or plug-and-play devices are also powerful when a footprint is limited. For example, membrane chromatography or convective materials provide high productivity (in grams per liter per hour) and reduce the consumables footprint considerably.

When space is limited, a potential solution is transitioning to single-use technologies. Single-use equipment generally requires less operational space (because system cleaning is not required, minimizing floor space needed for extra buffer). However, some stainless-steel solutions – such as the Resolute BioSC platform – also can help reduce a footprint because multiple steps can be operated on a single platform. Alternatively, if a fully single-use solution is desired, the Resolute BioSMB system is a valuable option to reduce footprint and resin use.

chromatography membrane formats (such as Sartobind Rapid A in rapid cycling conditions to optimize the use of your consumables) will initiate your journey to the single-batch strategy from early phase trials to commercial production (Figure 1, Level 1).

Consider Single-Use: Process intensification and single-use systems often work harmoniously to create a next-generation facility. Switching from multiuse to single-use technologies might enhance flexibility and accelerate the setup of a new facility, which to some extent can be considered the first step toward process intensification.

Connecting the Parts

The next step is to consider parallel batch and connected process options. One example is to couple an upstream process with clarification, clarification with chromatography (capture), capture with virus-removal steps, or all chromatography steps with ultrafiltration/diafiltration steps (Figure 1, Level 2).

Modular equipment can ease the transition toward faster and more continuous production. The ultimate goal is to have a continuous process from upstream to fill–finish in which biomolecules are not handled during a process, improving safety and quality (Figure 1, Level 3). However, a fully continuous process is not a suitable objective for every product; the end goal is driven primarily by the features of a biomolecule and commercial demands for it.

Embracing New Technologies

There is no universal solution for intensifying downstream bioprocesses. However, many technologies and implementation strategies are adaptable to essentially all process and business needs. The decision about which to use typically is driven by the type of molecules manufactured, their foreseen commercial scale and market demands, and the time a drug is envisioned to reach the market and its potential growth.

Manufacturers should embrace new technologies and innovations in chromatography and engage the help of a trusted partner to overcome fears of change and mitigate risks.

Are you ready to re-imagine your chromatography process? Learn more at https://www.sartorius.com/en/products/process-chromatography.

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Efficient Capture of a Low-Titer Fusion Molecule Using a Novel Protein A Membrane

with Florian Knoll

The importance of monoclonal antibodies (MAbs) as therapeutics is growing constantly. Protein A affinity chromatography usually is performed to purify monoclonal antibodies (MAbs) and other molecules with Fc moieties. Often, such processes leverage gel- or bead-based resins with immobilized ligands. But such materials work slowly, creating processing bottlenecks. In a recent BPI Ask the Expert webinar, Bibitec scientist Florian Knoll presented results from his work with membrane chromatography at the FH Bielefeld University of Applied Sciences.

Knoll's Presentation

A membrane's specialized structure and wide pores allow for convecdiff flow, which handles higher volumes and titers than gel- and bead-based chromatography media can. Membranes can achieve a dynamic binding capacity of ~40 g/L at residence times measured in seconds rather than minutes. Thus, Knoll and his university colleagues experimented with rapid cycling of a membrane-chromatography process to purify a low-titer Fc-fusion protein from Chinese hamster ovary (CHO) cell-culture supernatant.

First, the team performed characterization studies at small scales, optimizing parameters such as membrane volume and flow rates. Runs were performed on an ÄKTA avant chromatography system using a Sartobind Rapid A Nano 1-mL membrane. The team was able to use most of the values recommended by the device's supplier, Sartorius, excepting application speed. Sartorius specified that MAb purification could result in yields of >90% at an application speed of 5 MV/min. However, because the given Fc-fusion protein was about half the size of a full MAb, purification could be performed at twice the speed. After optimization of parameters, Knoll's team achieved yields of 90% at an application speed of 2.5 MV/min. Compared with a resin-based process, the optimized membrane process generated comparable yields but in 15× less application time.

After initial characterization, Knoll's team sought to scale up the process. To obtain similar results to those from a resin-based process, the team needed to purify 76 g of fusion protein over two days. The primary questions, then, were how large of a membrane to use and over how many cycles. Based on values from the characterization runs, Knoll's team implemented a 10-mL Sartobind Rapid A mini device on an ÄKTA pilot 600 benchtop chromatography system over 16 cycles.

Chromatograms from the resin- and membrane-based processes matched perfectly, indicating that the processes provided comparable recoveries. Results showed deviations of <7% in key parameters. Yield and purity were consistently >90% and comparable to results from both the university's resin-based process and Sartorius's recommended membrane-based process.

Knoll's team scaled up by a factor of 10, applying 15 L of supernatant containing about 4 g of product for 16 cycles. The resulting chromatograms were not as consistent as in previous runs. Nevertheless, the elution peaks deviated by <10%. Thus, Knoll's team successfully scaled-up the membrane-based process and achieved robust performance, even before adjusting feed parameters.

A smaller-scale process gave similar results with some fluctuations over 16 cycles. Pressure increased after cycle 7, when Knoll applied the second half of the supernatant. He hypothesized that the 0.2 M NaOh wash was unable to remove remaining impurities, but that consistency could be achieved with optimization of the wash step.

Knoll's team evaluated whether linearity could be retained during scale-up. In three out of four cases, the process gave an expected scaling factor of about 10, but it was twice that in one case. Data showed that the difference is attributable to a larger tailing on one elution peak during scale-up. Knoll set a cutoff at 10 mAU and said that the scaling factor would drop with a setting of 100 mAU.

He concluded that the Sartorius chromatography membrane provides 14× higher productivity than protein A affinity resins with comparable binding capacity. Flow rates can be higher with membranes because of their pore structure. Although a membrane-based process requires 3× more buffer than would a resin-based process, Knoll still recommended using membranes. "They are easy to handle with no need for cleaning, validation, or column packing."

Question and Answer

Is membrane use more sustainable than resin use? It depends on how the membrane is used and how your process or molecule performs with the membrane. The membrane is faster, with less material use, and it can be less expensive. That said, membranes require 3× more buffer than resins. But because resins and associated materials require regeneration, using the membrane may be more sustainable.

Find the full webinar online at www.bioprocessintl.com/category/webinars.

Professional Attire Guidelines for Bioprocessing

Steven Cumper

The global biotechnology industry has undergone a significant period of growth over the past three to four years. The COVID-19 pandemic accelerated the size and importance of an already growing sector, compounding the responsibility that falls on developers and manufacturers to deliver products that are uncontaminated and safe. Ensuring compliance with industry regulations is essential to safety, and professional attire for laboratories and cleanrooms is an integral part of adhering to standards.

Biomanufacturing businesses often handle living cells, cell components, live viruses, and other potentially sensitive or harmful substances. Such work involves adhering to strict safety and cleanliness standards. Because of the potential hazards of bioprocessing, the industry is heavily regulated. Depending on the nature of their work, companies may be subject to rules imposed by the US Department of Agriculture (USDA), Environmental Protection Agency (EPA), Occupational Safety and Health Administration (OSHA), and Food and Drug Administration (FDA). Agency guidelines must comply with the US Coordinated Framework for the Regulation of Biotechnology.

Guidelines for Appropriate Professional Attire

Bioprocessing industry guidelines are far-reaching and include specifications for professional attire. Appropriate clothing differs depending on the work of the person in question. For example, a senior executive might wear a business suit, whereas employees doing hands-on laboratory work will have to meet different expectations. In most laboratories, hard-wearing scrubs are the best choice of garment. Medical scrubs have roots in the early 20th century when medical professionals learned more about and emphasized the importance of hygiene.

However, in some bioprocessing environments, scrubs offer inadequate protection. *Cleanrooms* require an even higher level of control to preserve the integrity of processes and products. Such environments are common in the bioprocessing and pharmaceutical industries. Cleanroom attire may resemble a hazmat suit, including a full-body gown, shoe covers, a face mask, and gloves. Such attire enables wearers to remove their suits without compromising their own clothing or skin.

Cleanrooms are regulated by the International Organization for Standardization (ISO). ISO 14664 defines 10 classes of cleanrooms, each category specifying a type of protective clothing (**1**). Class 1 refers to the strictest of cleanrooms, with professional attire that resembles the hazmat suits mentioned above. The remaining nine levels are somewhat less stringent in their requirements.

The Benefits of Following Guidelines

Professional attire guidelines benefit employers and employees alike. Employees benefit from increased workplace safety because the guidelines are designed to keep workers in laboratories and similar locations safe. That protection also extends to their families once employees leave their places of work. And just as professional attire keeps workers safe, it also protects manufactured products.

Industry leaders have recognized that employee health and well-being are closely tied to company growth and profitability. Providing a safe workplace is an integral part of corporate social responsibility. Complying with legal requirements helps companies mitigate risk and limits liability in cases of unexpected incidents and accidents.

By following regulations imposed by organizations such as OSHA and ISO, employers can mitigate employee exposure to risks arising from the nature of their business. Professional attire in bioprocessing keeps employees and their families as safe as possible by preventing cross-contamination of cleanroom products with external substances. The same is true for potentially harmful substances that might be removed accidentally from laboratories and contaminate home environments.

Maintaining regulatory compliance in bioprocessing attire starts by understanding current guidelines for specific environments and ensuring that a company's procedures and protocols are up to date. Businesses need to understand that different areas of each company will be subject to different regulations. Although financial concerns are understandable, leadership teams must recognize the importance of workplace safety in bioprocessing. Initial cost, disposability, and sterilization requirements are all important considerations, but none is more critical than employee safety.

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