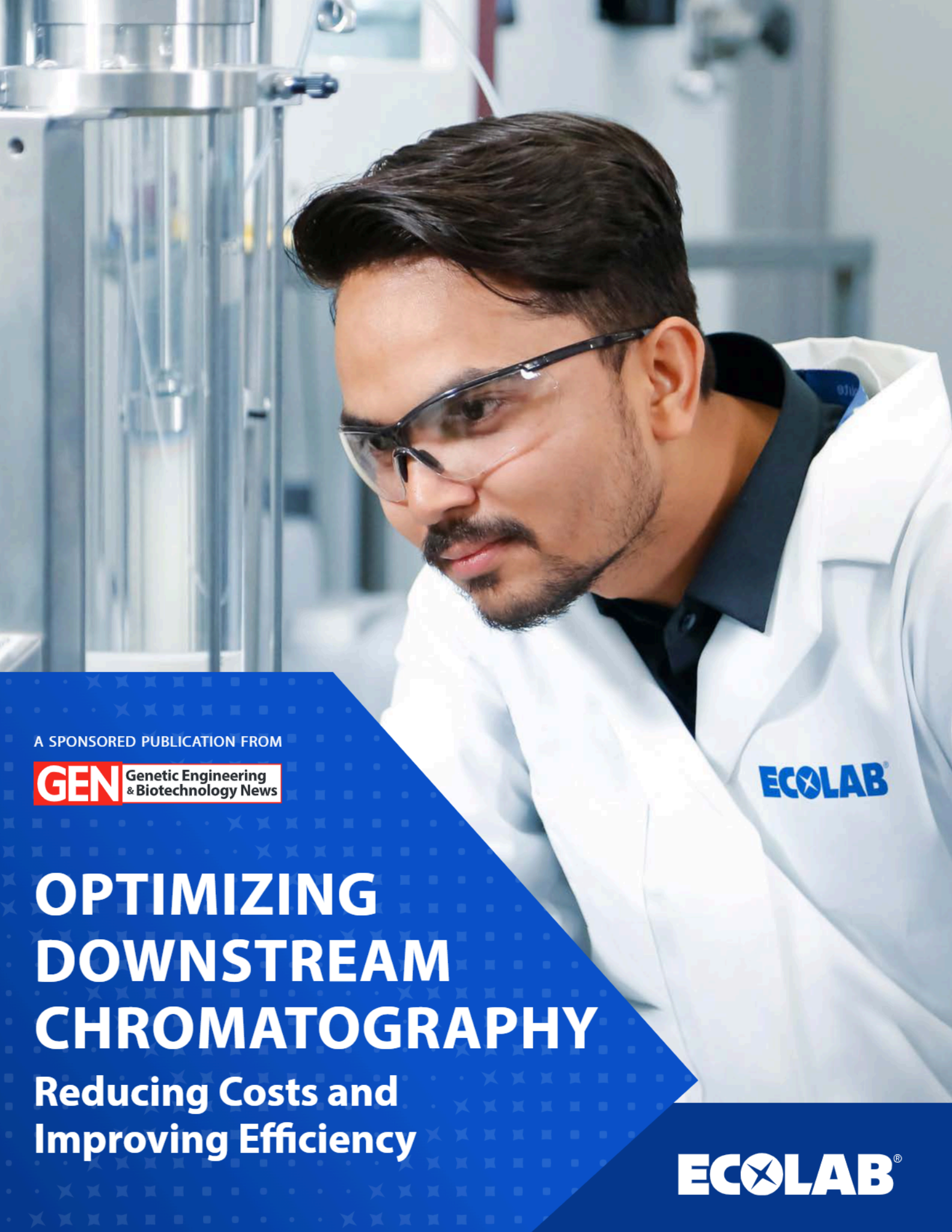




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OPTIMIZING DOWNSTREAM CHROMATOGRAPHY

Reducing Costs and
Improving Efficiency

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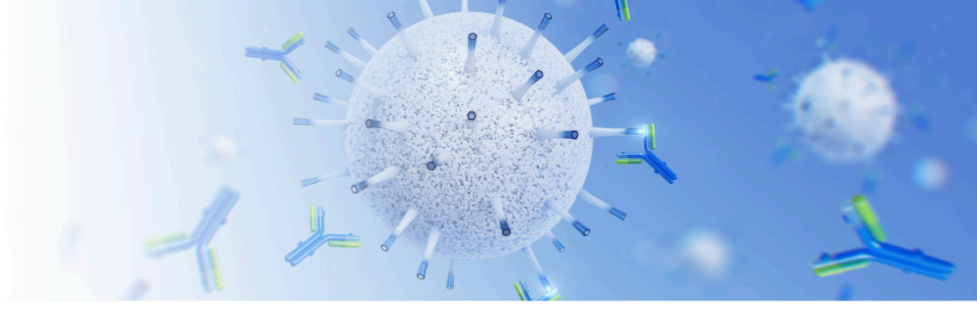


OPTIMIZING DOWNSTREAM CHROMATOGRAPHY

Reducing Costs and Improving Efficiency

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INTRODUCTION

The downstream chromatography industry is at the forefront of bioprocessing innovation, driving advancements in the purification and production of biopharmaceuticals. This ebook delves into the latest strategies and technologies revolutionizing downstream chromatography, offering insights into process intensification, optimizing chromatographic performance, and addressing host cell protein (HCP) challenges in monoclonal antibody (mAb) production.

Process intensification is a key focus area, aiming to enhance the cost-effectiveness and efficiency of protein A resins in bioprocessing. Traditional downstream chromatography represents a significant portion of manufacturing costs, with protein A steps contributing heavily to this expense. By implementing process intensification strategies, the industry can achieve substantial gains in productivity, reduce waste, and minimize facility space and energy consumption.

Optimizing Chromatographic Performance is another critical aspect covered in this ebook. The

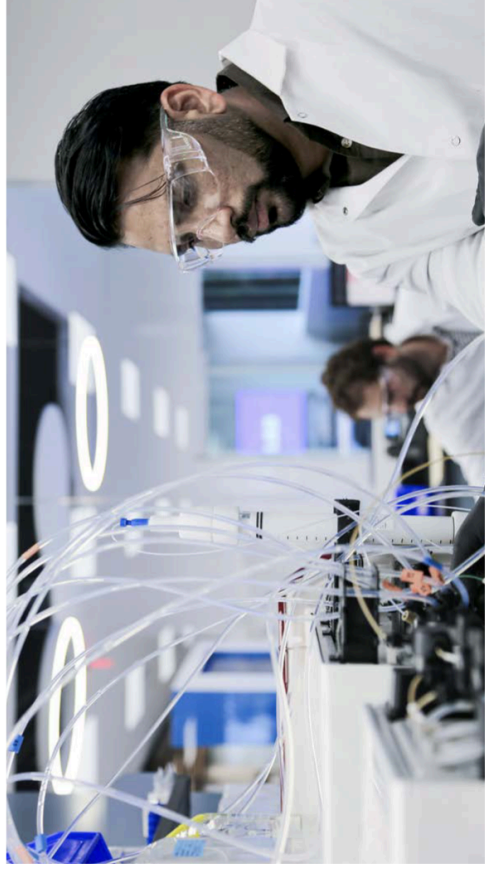
impact of packed bed height on efficiency and quality is explored, highlighting how modern chromatography resins with faster mass transfer can achieve higher dynamic binding capacities at shorter residence times. This optimization allows more cycles to be run per hour, improving productivity and reducing costs.

Addressing Host Cell Protein (HCP) Challenges in mAb production is essential for ensuring product quality and safety. Host cell proteins, expressed during the production of biotherapeutics, present significant obstacles in antibody purification. This paper discusses various approaches to mitigate HCP challenges, including the use of novel protein A-based agarose resins that enable purification under mild conditions, reducing aggregation and enhancing product purity.

In addition to these technical insights, the ebook also examines broader industry trends and challenges. Contract Development and Manufacturing Organizations (CDMOs) play a crucial role in adopting innovative bioprocessing technologies, but they must convince customers of the benefits of these new systems. Factors influencing HCP levels and the development of bioprocesses for cancer-fighting biosimilars are also discussed, providing a comprehensive overview of the current landscape in downstream chromatography.

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PROCESS INTENSIFICATION



Introduction

Downstream chromatography traditionally represents up to 80% of the costs involved in manufacturing a biological product, with protein A steps contributing to 70%. This financial strain is particularly impactful during pre-commercial work in Phase I and II trials, where significant amounts of resin capacity go unused. The rise of biosimilars and growing competition from alternative therapeutic modalities have reinforced the need to reduce the cost of goods, especially in clinical and commercial manufacturing processes. Process intensification aims to tackle this challenge by offering technical solutions to cost-related problems.

Process Intensification

Process intensification refers to strategies and technologies designed to improve operational efficiency and reduce costs in manufacturing processes. Beyond bioprocessing, it has been successfully applied in industries such as chemicals, petrochemicals, and food production, where it has led to significant gains in efficiency and sustainability. In downstream bioprocessing, strategies range from high-capacity pre-packed columns to single-use

consumables and advanced techniques such as continuous chromatography and Process Analytical Technology (PAT). These methods typically have a smaller footprint and use fewer materials to achieve equivalent purification outcomes, while enhancing efficiency and overcoming historic issues related to oversized chromatography columns. Given the significant cost burdens, the industry is focused on boosting cost-effectiveness by increasing productivity, reducing waste, minimizing facility space, and

lowering energy consumption. Process intensification methods can be implemented in several ways within an existing downstream process. Examples include multi-column, simulated moving bed (SMB) continuous chromatography, single-use systems such as pre-packed columns, and bed height adjustments. Several of these strategies are achievable when using chromatography resins with novel bed designs.

Process Intensification Strategies

- **Standard Column:** Industry standard, 20 cm bed height. Columns are often oversized, and lifecycle resin is underutilized, particularly in pre-commercial applications.

- **Reduced Bed Height:** Reduced bed heights have been adopted in industrial applications via the rapid cycling method. Multicolumn chromatography setups are exploring sub-10 cm bed heights. Reduced bed heights can increase productivity and reduce cost and facility footprint, particularly for pre-commercial applications with limited utility.

- **Reduced Column Diameter:** A narrower column (e.g., 60 cm instead of 80 cm) to reduce resin volume—often used in conjunction with a reduced bed height. Reduced column diameters can reduce cost, buffer consumption, and facility footprint.

- **Reduced Column Volumes:** Standard column setups with larger emulsified beads can use large amounts of buffer. Smaller, jetted beads may promote more

efficient buffer usage for non-loading steps via a reduction in voids and superior column packing. Lower non-loading column volumes translate to less buffer and therefore less water, additives, and waste, which can substantially improve sustainability metrics, particularly at scale or across multiple batches.

- **Variable Flow:** Splitting the loading phase into sections based on flow rate can reduce the average residence time. Here, loading is performed at a shorter residence time until the percent of dynamic binding capacity (DBC) limit is reached, after which the residence time is lengthened to achieve a higher DBC. With the residence time lengthened (and flow rate slowed), the available binding capacity is increased. Variable flow can increase column productivity versus single flow strategies.

Goals of Downstream Process Intensification

When evaluating process intensification strategies for downstream processes, manufacturers look for minimal disruption, low complexity, and a high return on investment. Most manufacturing facilities are set up to support single-column packed bed chromatography. Shifting towards a more novel setup, requiring a redesign or hardware refit to adopt continuous chromatography, could place a substantial cost burden on the manufacturer—not only in installation and hardware costs but also in downtime, commissioning, and validation. However, single-use materials provide a range of process intensification benefits, including reduced processing times, less capital investment in hardware, and fewer cleaning



and sanitization steps. Single-use materials and pre-packed columns have also enabled a significant reduction in cleaning and sanitization.

Cost of Goods (COGs) Modelling

Cost of Goods (COGs) modelling is a detailed method that analyses the performance and economics of a process or process step. It is particularly useful for protein A chromatography, which accounts for a large percentage of bioprocess costs. Elements of this can include resin, labor and materials costs, whilst accounting for DBC, residence times, life cycles, and campaign requirements. A useful way to express this in the context of protein A chromatography is via a purification cost, a cost-effectiveness metric. This allows various scenarios to be screened when evaluating different resins and column setups.

Dynamic Binding Capacity

Protein A capture steps typically utilize 20+ cm bed heights. This can reduce process efficiency and increase cost, particularly in pre-commercial development where the resin is under-utilized. This represents an opportunity to reduce resin volumes and to improve cost-effectiveness. A lower bed height is a simple way to reduce resin volume and increase utility. Until recently, operating with lower bed heights has not proven cost-effective or productive due to lower dynamic binding capacities achieved at shorter residence times. However, concerns about packing lower bed heights in wider columns (both standard and prepacked) have been largely overcome due to the improved flow distribution and packing consistency offered by more advanced axial compressive columns.

For protein A chromatography, cost of goods can be expressed as the cost to purify a unit mass of product, e.g., \$/g.

With conventional protein A resins, a lower bed height would require longer residence times to avoid increased cycle numbers as a result of the lower DBC. When accounting for buffer and labor costs, this process change would prove less efficient. However, the introduction of protein A resins with excellent mass transfer characteristics and high capacities at shorter residence times can overcome these limitations, allowing for the use of lower bed heights with a minor impact (or even improvement) on process time and productivity. Traditionally, the industry has targeted dynamic binding capacities up to 60 g/L at six-minute residence times. However, as demonstrated in Figure 1, Purolite™ Resins offer notably higher

dynamic binding capacities at two- and four-minute residence times due to their mass transfer characteristics.

Jetted Manufacturing for Enhanced Mass Transfer

The manufacturing method employed for resin production will impact a resin's properties and performance. Ecolab employs the patented method of Jetted manufacturing to produce PuroLite™ Resin base beads.

Unlike traditional batch emulsification methods, Jetted manufacturing produces beads that are more uniform in size, centering on a single mean particle size. This uniformity improves mass transfer and aids in target binding.

Demonstrating a narrower particle size distribution and uniformity coefficient, Jetted resin beads offer the potential for increased packing efficiency via a reduced void volume. This results in higher mass transfer rates, which improve binding capacities, buffer efficiency, impurity clearance, and resolution. For example, compared to larger beads of 85 microns or more, a 50-micron Jetted bead may achieve an approximate reduction in column volume of 25% during the elution step, improving facility fit. Furthermore, equilibration steps may require a lower salt concentration for column equilibration.

Cleaning Strategies

The use of sodium hydroxide (NaOH) has become the industry standard for cleaning in place (CIP) procedures to control microbial organisms during downstream purification. Due to these rigorous CIP procedures, it is

increasingly important for capture resins to be alkaline stable. Sodium hydroxide exhibits high efficiency in removing bound proteins, nucleic acids, and lipids from bioprocessing resins, reducing the risk of fouling on heavily burdened protein A columns. A high percentage of late-phase and approved molecules use the industry standard for CIP of 0.1M NaOH for a 15- to 30-minute contact time.

The following studies demonstrate the impact of varying contact times and NaOH concentrations on DBC and antibody yield using customer clarified cell culture fluid (CCCF). The exposure of Praesto™ Jetted A50 to different concentrations of 0.1M NaOH over an extended period highlights the stability of the ligand and of PuroLite Resins broadly to caustic conditions.

Process Cycling Lifetime for Praesto™ Jetted A50 (IgG1 mAb)

- Loading: 65.0 mg/mL (91% DBC) 10% at a 12-minute residence time). CIP: 0.3 M NaOH—15-minute contact time.
- Loading: 50.6 mg/mL (80% DBC) 10% at a 3-minute residence time). CIP: 0.1 M NaOH—60-minute contact time.
- Loading: 65.0 mg/mL (91% DBC) 10% at a 12-minute residence time). CIP: 0.1 M NaOH—75-minute contact time.

Process Intensification Example Scenarios

This section presents various process intensification strategies to drive productivity and cost-efficiency for protein A chromatography.

Bed Height and Column Internal Diameter Reduction

In this scenario, bed height was reduced in tandem with a reduction in the internal diameter (ID) of the column. Reductions in column ID can further drive cost savings but should be carefully balanced with time and cycle constraints, which may increase per batch. The bed height may need to be adjusted to balance these constraints when aiming to reduce a specific metric—in this case, cost-effectiveness.

Column Volume Reduction

Enhancing the efficiency of washing steps in downstream purification presents a promising approach to reducing water consumption and wastewater costs.

While this method has not been fully examined, growing attention to process efficiency has reignited interest in it. The introduction of Jetted beads, which provide better mass transfer, higher resolution, and lower void volume, could further improve residual clearance and buffer usage. Savings could be realized via a reduction in elution volume for instance, which would shorten the loading step for a successive chromatography step or reduce the amount of time/buffer spent during a ultrafiltration and diafiltration (UDFD) step. This reduction in eluate would also reduce the volume of hazardous waste generated—something many antibody drug conjugate (ADC) companies aim to do. A reduction in salt concentration—notably for the equilibration step, could also be achieved. Taken together, these savings, whilst limited at pre-clinical stages, could translate to larger savings at commercial scale, saving cost and improving sustainability metrics.

Reducing the number of column volumes from five to three for two washing steps can impact

In each of the scenarios provided, Praesto™ Jetted A50 was screened in comparison to another agarose-based protein A resin at a standard 20 cm bed height. Each scenario aimed to optimize conditions for cost-effectiveness, although it is worth noting that performance may also be optimized for productivity. Data in the scenarios was normalized relative to the standard 20 cm benchmark. An improvement is represented by a value outside of this line. A decrease in performance is represented by values inside of this line.

Bed Height Reduction

In this section, when bed height is reduced from a 20 cm standard for a column with an internal diameter (ID) of 60 cm, the following benefits were observed:

- Productivity increases by 2.5x (10 cm) and by 1.5x (15 cm)
- Cost-effectiveness increases by 1.3x (15 cm) and by 1.5x (10 cm)
- Time savings are noted for both bed heights between 1.2–1.4x

It is important to note that these adjustments do come at the expense of more cycles per batch. However, depending on the resin, this can be offset by increased binding capacity. Balancing performance with operating constraints, optimal bed heights typically range between 10 cm to 20 cm.

Each strategy presented can be adopted individually or in tandem to deliver greater benefits. The aim of instituting process intensification strategies should be to balance optimal performance with feasibility.

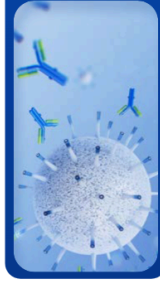


William Bowen, PhD is a field applications specialist for bioprocess chromatography resins at Purolite, an Ecolab Company.

buffer consumption. Improvements in cost-effectiveness will generate greater improvement across a higher number of batches per campaign, particularly at scale. The total cost savings will accumulate with the number of batches per campaign and the number of campaigns using this strategy. This will also translate to less water and less buffer additives, thereby benefiting sustainability metrics.

About the Author

Dr. William Bowen is a field applications specialist for bioprocess chromatography resins at Ecolab. Covering western Europe, he is responsible for providing technical support to customers in the use of Purolite resins at process development through to commercial manufacture. William completed his Master's and PhD in Chemical Engineering at Loughborough University. ■



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OPTIMIZING CHROMATOGRAPHIC PERFORMANCE

The Impact of Packed Bed Height on Efficiency and Product Quality

Introduction

When performing liquid chromatography, numerous process parameters impact chromatographic performance, process efficiency, and final product quality. While many of these parameters cannot be easily or immediately changed as they are related to specific hardware choices, several can be optimized for improved performance. This paper focuses on one such parameter—packed chromatography column bed height. In many instances bed height is selected on the basis of historical choices, but making small changes to this parameter can greatly improve process efficiency, especially when employing modern chromatographic resins.

Bed Height

Bed height refers to the physical height of the packed bed in the column—this is typically referenced in conjunction with the diameter of the packed bed to give the dimensions of the column. Typically, the diameter of the packed bed is set by the choice of hardware that is employed for the chromatographic column, whereas the bed height is a variable that may be altered as desired. Unsurprisingly, bed height is critical as a parameter that can strongly influence performance but is often ignored by chromatographers for the sake of convenience. Historically, a 20 cm packed bed height has been recommended for bind and elute liquid chromatography, based on the performance of early chromatographic resins and the complexity and interconnection of the parameters influencing column performance.

Mass Transfer

Legacy chromatography resins are known for their slow mass transfer characteristics. Mass transfer is the process by which substances move from one

area to another, typically from regions of higher concentration to those of lower concentration. In protein liquid chromatography, this involves proteins migrating from the surrounding process stream into the resin beads. The efficiency of mass transfer is primarily influenced by several factors, including the physical structure of the resin beads, the size of the protein molecules, as well as the temperature and viscosity of the liquid.

Due to the size of biomolecules, it takes time for a chromatography resin bead to become saturated with target molecules. If the resin is exposed to the process stream for too short a time, the resin will only be partially saturated and those binding sites deeper within the resin will not be accessed as illustrated in figure 1.

Residence Time

Due to the slow mass transfer of large proteins into resin beads, the residence time becomes a critical parameter. Residence time refers to the time in which the packed bed is exposed to the

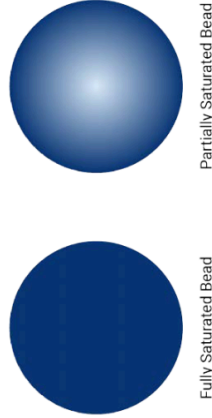


Figure 1. Fully and partially saturated beads.

sample. Long residence times give ample time for the protein feed solution to enter the beads in the packed bed and access the maximum number of binding sites, but of course long residence times result in longer chromatographic processes. Getting the residence time optimized can therefore have a large impact on the efficiency (in terms of rams of purified protein per minute) of the chromatographic process. During process development, residence times should be explored to understand the total capacity available at various residence times. A chart can then be plotted of dynamic binding capacity (DBC) as a function of residence time, as shown in figure 2.

As can be seen in the plot, capacity increases with residence time but starts to level off around four to five minutes, indicating that after this time the feed has fully penetrated the packed bed and the binding sites are fully occupied. Therefore, there is little benefit in using longer residence times beyond this point as no increase in capacity will be observed.

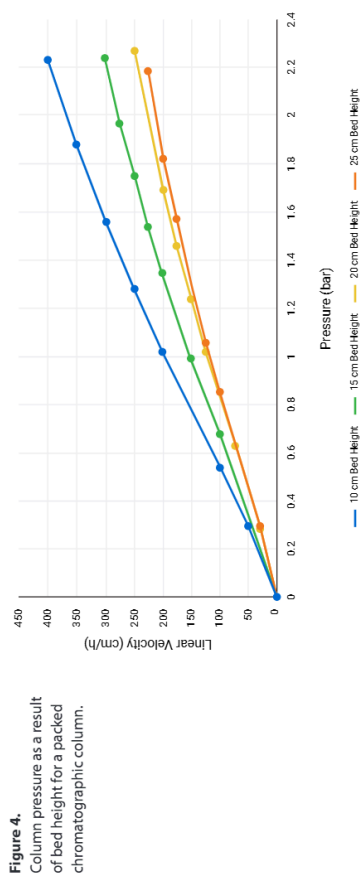
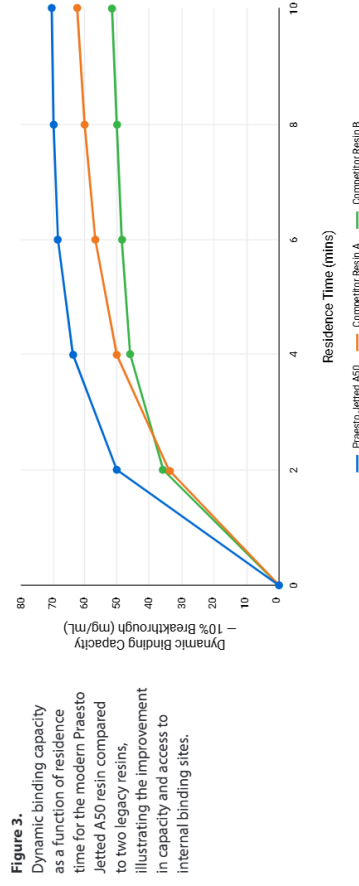
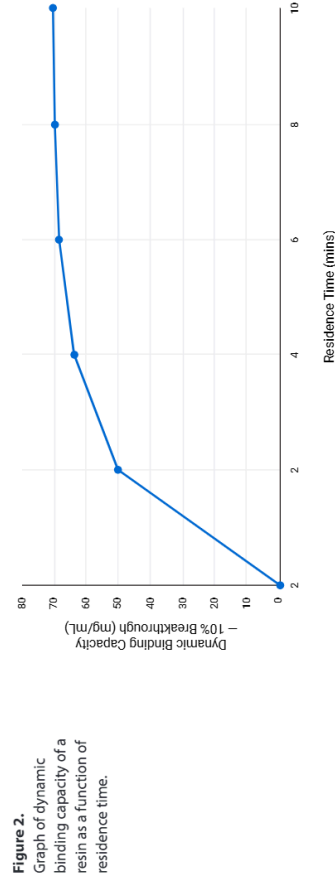
Modern chromatography resins tend to possess faster mass transfer characteristics. For example, Purolute™ Praesto™ Jetted A50 reaches high capacities at shorter residence times compared to alternative protein A resins as shown in figure 3.

Residence times can be shortened by processing at a higher flow rate, effectively pushing the protein solution through the column more quickly. However, high flow rates increase the pressure drop across the packed bed as more force is required to push the solvent through the column. Increased pressure can over-compress the packed bed and reduce the resolution of the column, giving poor chromatographic performance and in the worst case damaging the resin. In addition, the column hardware's pressure limit may be exceeded, resulting in the catastrophic failure of the column hardware.

The pressure in a packed bed is closely related to the height of the column bed. Lowering the bed height enables higher flow rates without surpassing the pressure limits of the packed bed as shown in figure 4.

Legacy Resins

All of these parameters may be altered to maximize performance, however ultimately it is the dynamic binding capacity characteristics of the resin itself that has the largest impact on the design of the chromatographic system. In liquid chromatography, the dynamic binding capacity offered by the early legacy protein A resins has traditionally required the use of large bed height of 20cm. Due to the comparatively lower DBC offered by legacy resins, large amounts of resins are required to capture the necessary amount of the target protein. As a result, with limited bench or floor space available in many facilities, increasing column bed height is commonly seen as the best way to maximize the total capacity of a column and therefore the separation efficiency in terms of grams purified in a given time frame, meaning that high



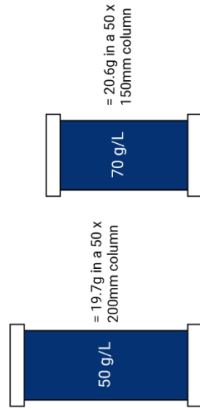


Figure 5. Illustrative example of the comparable total capacity of a column of reduced bed height but employing a resin with higher DBC, compared to a traditional 20cm bed height column packed with legacy resin of lower DBC.

pressure resistant resin and hardware becomes a necessity.

However, with modern affinity resins the situation has changed. Swapping a legacy resin for a material with higher DBC allows for the same amount of target protein to be purified with less resin and a reduced bed height. This is the Ecolab approach to bioprocess chromatography—leveraging the high binding capacity of modern resins allows an optimization of the chromatography for greater process efficiency.

The Ecolab Approach

Modern resins such as Praesto Jetted A50 have significantly higher dynamic binding capacities and shorter residence times than older legacy resins. As such, the bed height of a column can be reduced without losing overall capacity as illustrated in Figure 5.

The benefit of reducing the bed height of the column using a high capacity resin is that the flow rate can then be increased without experiencing pressure issues, especially if the resin reaches maximum DBC at short residence times. This can lead to impressive improvements in overall process

efficiency by significantly increasing the output over time. In effect the ability to perform protein purification at high speed—while maintaining DBC—allows for more cycles to be run per hour, and lowering bed height helps to facilitate this process. Table 1 shows the effect of these changes. In this example, high-speed chromatography was performed using a shorter, 10 cm bed height. In turn, this allowed for twice the number of cycles to be run with a smaller volume of resin. The study resulted in twice the productivity when compared to a 20 cm bed height.

Practical Considerations for Process Scaling

The use of shorter bed heights—in conjunction with a high DBC resin—significantly benefits productivity. Ideally, these benefits would be maintained when scaling production. Typically, when scaling, column bed height remains consistent while column diameter is increased. In addition, a constant linear flow rate is used at each scale. Maintaining bed height and linear flow rate when scaling keeps residence time—and capacity—constant.

However, practical issues, such as the limitation of available column sizes, must be considered when scaling.

While chromatography columns are available in a variety of diameters, size limitations may prevent exact scaling. Using a column that is not exact to scaling without making the proper alterations may result in some binding capacity remaining unused.

In addition, efficiency is critical when processing large volumes of sample. Utilizing all required

Resin	Residence Time (mins)	DBC (g/L)	Cycles	Column Dimension (ID x BH cm)	Buffer Volume (L)	Total Processing Time (h)	Total Resin Cost (\$)	Productivity (g/L/h)
Praesto Jetted A50	2	50.4 (40.3)	8	60 x 10	3845	7	\$465,004	51.3
Competitor A	4	45.9 (36.7)	4	60 x 20	3845	7	\$1,252,023	25.7
Competitor B	4	50.2 (40.2)	4	60 x 20	3845	7	\$1,252,023	25.7

Table 1. Improvements in productivity when employing a 10cm bed height and a high DBC resin compared to a 20cm bed height column with an older, legacy low DBC resin.

capacity is preferred to improve process efficiency—four cycles is more efficient than 4.5.

One strategy commonly utilized in membrane chromatography is to scale based on column volumes per hour (CV/h). This strategy allows the same residence time to be used when scaling, with CV/h remaining constant rather than centimeter per hour (cm/h).

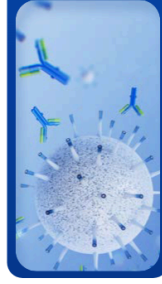
In addition, the aspect ratio (bed height : column diameter) can be easily adjusted—provided the flow rate is adjusted to maintain the same CV/h. The main obstacle when using this strategy is ensuring the operation is performed under the same pressure flow constraints for the packed bed.

Conclusion

In conclusion, optimizing chromatographic processes by adjusting parameters such as packed bed height can significantly enhance the productivity of bioprocessing. By changing the mindset that a fixed bed height is always required and leveraging the high performance characteristics

of modern chromatography resins with higher dynamic binding capacities, operators can reduce column bed height while maintaining effective purification performance. This shift allows for increased flow rates and shorter residence times, ultimately leading to more efficient processing and greater output over time.

Ecolab recommends this approach to bioprocess chromatography as a means to maximize efficiency in protein purification. By focusing on optimizing bed height and employing high-performance resins, bioprocessing facilities can achieve impressive improvements in productivity, enabling them to run more cycles per hour and minimize the amount of resin required. This strategy not only enhances the overall workflow but also contributes to cost-effectiveness and a more streamlined production process. Embracing these modern techniques will ensure that operators stay at the forefront of bioprocessing technology while delivering high-quality products in a more efficient manner. ■



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PUROLITE JETTED A50 HIPH

Addressing Host Cell Protein Challenges in Monoclonal Antibody Production

YAIR PERES, PHD, FIELD APPLICATION SCIENTIST AT ECOLAB



PuroLite Jetted A50 is engineered to purify monoclonal antibodies and associated protein constructs, supporting various FDA-approved antibody-based therapies.

Host cell proteins (HCP) have a serious impact on downstream processes in antibody manufacturing, and their removal presents a significant obstacle in antibody purification. To address this issue, Ecolab has developed PuroLite™ Praesto™ Jetted A50 HipH, designed to elute at higher pH, thereby reducing both aggregation and host cell protein levels as discussed in this paper.

Host Cell Proteins and Their Impact

Host cell proteins (HCP) introduce many challenges in monoclonal antibody downstream manufacturing. HCP are expressed during the production of biopharmaceuticals by the host organism, typically by Chinese Hamster Ovary (CHO) cells. This standard mammalian host cell produces over 6000 proteins, of which HCPs are directly secreted into the cell culture fluid or released from lysed host cells during culture and harvest.¹ The presence and levels of HCP depend on various factors including host cell type, clone type,

the product being expressed, and upstream conditions. HCP levels in CHO clarified cell culture fluids (CCCF) of 30 monoclonal antibodies range from 5 to 6 logs ppm, showcasing the complexity of HCP in the process feed stream and creating considerable challenges for downstream processes.²

HCP Characterization and Regulation

Various methods are employed to measure and characterize HCP, such as immunoassays (ELISA) and mass spectrometry. HCP levels are considered a critical quality attribute—while regulatory agencies do not specify allowed levels of HCP in the final drug product, conventional practice aims to limit the levels of HCP to a maximum of 100ppm due to potential adverse immune responses in patients. Proteolytic HCP may also impact product quality of the final drug by cleaving the product during storage, highlighting the importance of reducing HCP to the lowest reasonable level. Previous studies have found

that marketed monoclonal antibodies and derivatives exhibit an average HCP level of 20 ppm, indicating an ongoing effort to manage HCP levels.³

Approaches to Mitigate HCP Challenges

Industry and regulatory standards utilize prior knowledge and a Quality by Design (QbD) approach to ensure product quality and manage risks, as guided by the ICH Q6B guideline. Unit operations upstream and downstream are designed to decrease HCP presence or remove it from the feed stream.⁴ For instance, post-harvest clarification utilizing depth filters can effectively remove a subset of HCP through size exclusion or charge interaction.

Protein A Affinity Chromatography and HCP Removal

Protein A affinity chromatography, commonly used in monoclonal antibody manufacturing, offers selective capture of target molecules while allowing other impurities to flow through. Modified pH washes and additives have been utilized to increase HCP removal during the protein A step, but residual HCP quantities, including “difficult to remove” HCP, have been reported to persist, potentially due to non-specific binding to chromatography media or co-elution with antibodies.⁵

Furthermore, the acidic elution (pH 3.5-4) commonly employed to release antibodies from Protein A resins increases the propensity for protein aggregation, particularly in Bi-Specific Antibodies (BsAb), Fc-fusions, and other mAb derivatives.^{6,7} Aggregation has been identified as a key factor in the persistence of HCP, complicating efforts to clear aggregates with current methodologies.^{8,9}

Decreasing the levels of HCP, aggregates and other

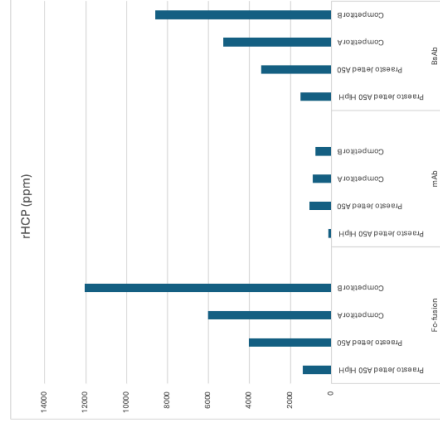


Figure 1. Residual host cell protein present after the capture of three types of monoclonal antibody using four different affinity resins—Praesto Jetted A50, Praesto Jetted A50 HipH, a traditional competitor protein A (competitor A) and a high-performance protein A (competitor B).

impurities in the protein A eluate pool alleviates the challenge in purification and enables a lower chromatography matrix (resin, membrane) volume for clearing HCP impurities in subsequent steps. In a conventional three-step chromatography, process aggregate removal is typically restricted to a single polishing step, thus reducing their level also enhances process robustness.^{9, 10}

Thus, mitigating protein aggregation during the protein A capture step can increase product yield, enhance product quality, and improve process economy.

Praesto Jetted A50 HipH for Enhanced HCP Removal

PuroLite's Praesto Jetted A50 HipH, a novel protein A-based agarose resin, addresses these challenges by enabling the purification of Fc-containing

molecules under mild conditions. The design of the Praesto Jetted A50 HipH protein A ligand introduces enhanced pH sensitivity to the ligand -antibody binding, allowing for the disruption of the protein A-Fc interaction and dissociation of the product from the resin at a higher pH compared to traditional protein A resins. Furthermore, the milder elution conditions preserve the integrity of acid-labile proteins, mitigate aggregation, and can leave tightly bound impurities adsorbed by the resin thereby contributing to an increase in product purity from both product-related and process-related impurities, including HCP.

Studies using Praesto A50 HipH resin as a capture step across molecular modalities measured by ELISA is shown in figure 1, demonstrating a 2.3 to 8.6 fold increase in HCP clearance compared to a traditional protein A resin (competitor A), and a high performance protein A resin (competitor B).

Conclusion

Host cell proteins are process-related impurities that pose challenges for producing monoclonal antibodies and derivatives. Removing these impurities is crucial for product quality, and introducing sufficient log reduction in HCP levels during a whole chromatographic workflow is paramount to developing a robust and efficient process.

Purilite's Praesto Jetted A50 HipH resin has been developed to allow elution of bound monoclonal antibodies at a higher pH than typically seen with Protein A affinity resins. This leads to a lower level of aggregation, particularly with complex antibody architectures like bispecific antibodies. Reduced aggregation effectively facilitates robust and superior clearance of HCP compared to other

commercial protein A resins, significantly enhancing product purity and quality. Combining Praesto Jetted A50 HipH with polishing resins that give good HCP clearance can lead to unprecedented levels of host cell protein removal. ■

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CDMOS NEED TO CONVINCE CUSTOMERS OF NEW TECHNOLOGY BENEFITS

BY GARETH JOHN MACDONALD



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CDMOs are willing to invest in innovative bioprocessing technologies, but the economics need to make sense. Platforms that can be used for multiple projects are worth the money, but bespoke, product-specific systems can be harder to justify.

In biopharma, willingness to adopt innovative manufacturing technologies varies depending on the type of company involved and the product being made. For larger companies with established products, the regulatory burden of switching out traditional stainless-steel systems for disposable

tech is often deemed too great. For smaller and mid-sized firms with newer products, using more modern systems is the more logical, more affordable option.

For contract manufacturers, deciding whether to invest—or not—in innovative technologies can be even more complicated according to Priya Baraniak, PhD, chief business officer at Miami, FL-based cell and gene therapy CDMO, OrganaBio.

"New technologies often require significant upfront

investments in equipment, infrastructure, and personnel training, not to mention costs associated with validations and documentation to ensure regulatory compliance. In addition, new technologies need to be seamlessly integrated with existing manufacturing processes and IT systems. This can be challenging, as it may require modifications to existing workflows and data management practices," she tells GEN.

The key factor determining many CDMOs' technology investment decisions is whether the contractor can convince the customer of the benefits of a particular system, Baraniak says.

"CDMOs rely on their clients to adopt new technologies and processes. Convincing clients to embrace new technologies and pay for the associated costs can be a major challenge. Thus, early technology adoption can be a major financial burden for CDMOs, especially smaller ones with limited resources."

She added: "This burden is even more pronounced for technologies that are not universal and are highly bespoke or specialized. Platform technologies where costs can be defrayed across multiple clients are more easily implemented by CDMOs."

Intelligent Choices

Innovative technologies that have the potential to save manufacturers time and money are likely to be an easy sell, says Sarah Alter, PhD, the CDMO's senior director of scientific affairs, citing artificial intelligence-based modeling as an example.

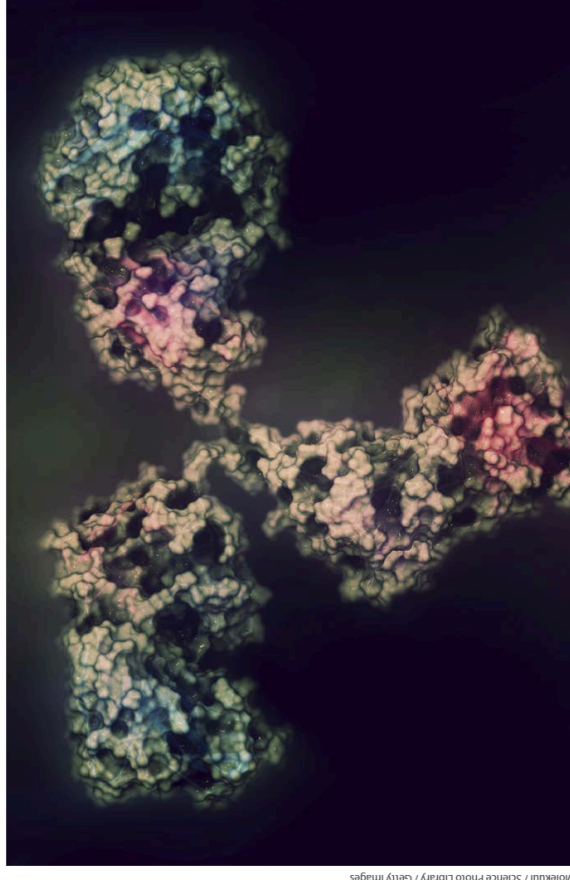
"Biologic and protein therapeutics discovery and optimal formulation development are time- and resource-intensive. AI and ML are emerging as predictive and generative tools in protein engineering and can also be employed to model multiple biophysical and chemical degradation properties."

CDMOs are also more likely to be able to convince a customer to invest in technology that solves specific problems, Alter says.

"The innovation in protein technology has brought forth a host of challenges related to the purification of these therapeutic proteins. Some manufacturing aspects that need improvements include controlling protein reduction, protein capture, ensuring stability, eliminating aggregates, removing host cell proteins, and optimizing protein recovery." ■

MULTIPLE FACTORS INFLUENCE HOST CELL PROTEIN LEVELS

BY MIKE MAY, PHD



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The production of many therapies based on monoclonal antibodies relies on Chinese Hamster Ovary (CHO) cells. Unfortunately, these cells also make host cell proteins (HCPs), which can make a drug less effective and even set off immune responses in patients. So, manufacturers try to minimize HCPs in therapies developed with CHO, but it's a difficult task. Nonetheless, Sri Madabhushi, PhD, a principal scientist and director of biologics process R&D at Merck & Co. in Rahway, NJ, and her colleagues recently proposed a novel approach to the HCP battle.

Today, manufacturers often use cell viability as an indicator of lower levels of HCPs. The idea here is that lower viability goes together with cells releasing more HCPs, but that's not what all studies find. As Madabhushi's Merck team put it, "viability alone does not reliably predict HCP levels."

Instead, these scientists used transcriptomics to analyze the HCPs, especially the problematic ones, in CHO cultures, and looked for an upstream solution. They found that the level of HCPs in a CHO

depends on many factors. For example, different media used in culturing can change the kind of HCPs produced. The concentration and composition of HCPs in a culture also varied with the temperature at specific stages of the bioprocessing, the percentage of dissolved oxygen, and so on. By controlling these factors, the Merck scientists reduced the HCPs generated in a CHO-derived therapy.

As often happens in biology, one measurement is not enough to tell a story. Plus, as scientists

delve deeper into a question, unexpected results can emerge. As Madabhushi and her colleagues concluded: "Surprisingly, our findings debunked the notion of viability as a primary indicator for controlling HCP levels, highlighting instead the significant influence of factors such as clone selection, host cell line, media, and process parameters."

In short, a more accurate story in biology is often a more complicated one. That's the case for controlling HCPs in CHO-developed therapeutics. ■

BUILDING A BIOPROCESS FOR A CANCER-FIGHTING BIOSIMILAR

BY MIKE MAY, PHD



Marcin Klaczynski / Getty Images

In the next few years, atezolizumab will go off patent in the United States and Europe. This monoclonal antibody is a checkpoint inhibitor that allows a person's immune system to kill cancer cells. To pave a pathway to producing a biosimilar version of atezolizumab, Mehmet Inan, PhD, director of the Izmir Biomedicine and Genome Center in Turkey, and his colleagues developed a bioprocess for producing this drug in recombinant cells created from the Chinese hamster ovary (CHO) DG44 cell line, which is often used by the pharmaceutical industry.

According to Inan's team: "There is no documented production process of atezolizumab biosimilar in the literature except one in the plant platform." So, this team of scientists developed a method for manufacturing a biosimilar of atezolizumab in mammalian cells.

First, Inan and his colleagues used different methods of transfecting CHO DG44 cells with viral genes that would produce atezolizumab. By screening the resulting cell lines, the scientists selected one that produced the most atezolizumab.

Using a batch-fed bioreactor and testing eight chemically defined media that are commercially available, plus supplements, Inan's team selected the most productive conditions for culturing the cells. From this, the process produced yields of the antibody as high as 800 mg/L.

After a purification process composed of three forms of chromatography—affinity, cation exchange, and ion-exchange—Inan and his colleagues compared the performance of the

biosimilar to the commercial form of atezolizumab. The results showed that both forms of atezolizumab produced similar binding of the intended target, which was the programmed death-ligand 1 (PD-L1).

To move this biosimilar into clinical trials, though, industrial scientists would need more thorough testing, including toxicology studies. As this example shows, a long path lies between any approved biological drug and creating a biosimilar that can be used in patients. ■



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