



Key Challenges and Potential Solutions for Optimising Downstream Bioprocessing Production

The term “optimise” is often applied to complex manufacturing, automation and business processes and implies that the most efficient function of all the elements of a process – technologies, sequences and procedures – has not been achieved. While no method can be perfect, every process can benefit from a careful consideration where new thinking, new techniques and new technology can yield significant improvements.

The dramatic growth in the use of biologics across multiple therapeutic applications and categories will only continue to increase. As the demand for these drugs accelerates, there are growing concerns about their cost and availability. Biologics manufacturers are investigating ways to address these concerns – and downstream production in bioprocessing operations is one such area.

Key Challenges in Downstream Production

Downstream production currently encompasses about 60 per cent of the total cost of producing a biologic drug. Finding ways to remove bottlenecks and improve yields in downstream could lead to more cost-effective production results, but there are several challenges associated with this goal:

Increased upstream yields: Significant investments have been made in the technologies and processes used in upstream processes with the goal of improving yields. Efforts to improve raw material characterisation, add single-use systems, perfusion systems and more precisely controlled bioreactors are leading to measurable increases in upstream yields. However, improvements in downstream throughput have not kept a similar pace to those for upstream, leading to potential bottlenecks in the end-to-end process.

A significant capital investment could be made to create larger chromatography systems to handle the increased production, but it would do little to accomplish the goal of cost-effectively aligning the productivity of downstream production with upstream yields. Loss from upstream to downstream: One of the fundamental structural challenges in biologic production is the approximately 30 per cent yields loss as harvest material goes through downstream purification. Any percentage of improvement in downstream recovery can contribute to improving the ultimate process yield for drug product of the target biologic.

Complexity of downstream production: Upstream productivity may be improving because it involves a more straightforward process. Once the target molecule and raw materials are loaded into the bioreactor, the process runs to completion with the appropriate testing and quality control.

Downstream production, on the other hand, involves multiple steps where the biological material is moved from harvest, centrifugation and polishing to multiple chromatography steps before reaching final fill and finish. Each step requires a unique

set of resins and buffers among other materials; storage and production systems at multiple steps; and analytical and quality control sampling activities in parallel.

Finding efficiencies and economies of scale across downstream processing steps involves more complex analysis and optimisation. Improvements may be reached after investigating key aspects of current purification steps and technologies, including:

- Expanding the use of mixed-mode and multimode chromatography resins, including resins with targeted ligands with increased selectivity to more efficiently process targeted molecules
- Exploring ways to make chromatographic buffers more effective by using new kinds of additives, as well as utilising prepackaged single-use buffer materials to streamline buffer exchange steps
- Making wider use of data analysis tools to develop deeper insight into complex material interactions in downstream process steps, particularly as they relate to raw material characterisation

Improving Process Chromatography Technology

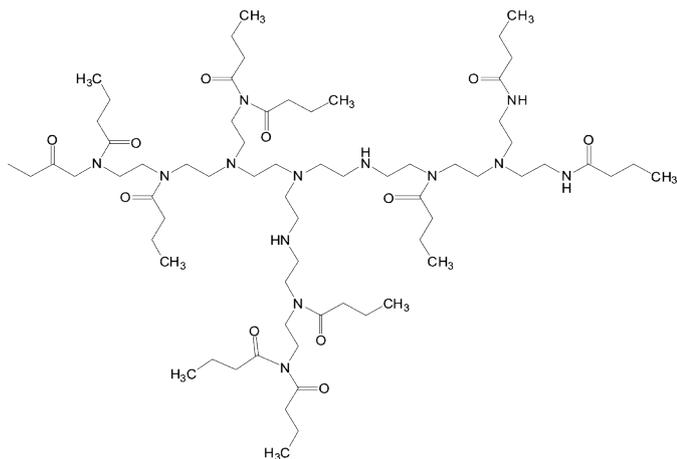
The ultimate goal of downstream optimisation is to improve recovery and therefore normalise, and potentially reduce, the cost per gram of protein produced. That means producing more drug product, using less time, with the same amount of resin and buffer material. One of the most effective ways to do this is by making better use of the newest generation of mixed-mode and multimode resins.

Biologic drugs are becoming more diverse, with more complex molecular structures. However, producing these precisely targeted drugs can also yield by-products that are very closely related chemically or biologically to the target molecule, with no therapeutic value.

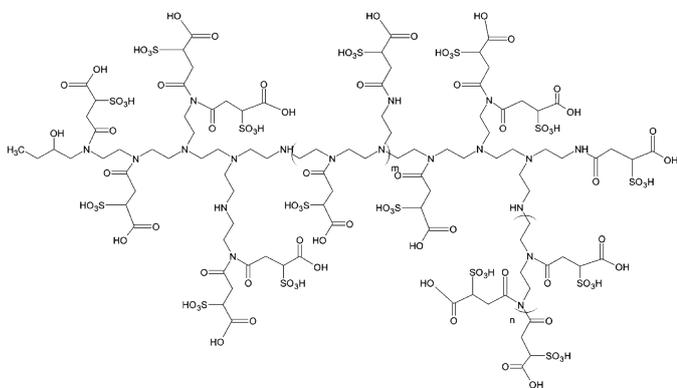
Separating undesired glycosylated molecules and aggregates presents major challenges, as they are likely to have limited differential binding to traditional ion exchangers and can coelute. Thus, the combination of increased upstream yields and more complex molecules calls for new approaches to chromatography resins. In response, chemistry suppliers have been focusing their efforts on process chromatography selectivity and efficiency.

The traditional solution to this type of challenge is to utilise multiple downstream ion exchanges. While it yields the targeted drug, the cost per gram of this approach can become prohibitive. More advanced methods of achieving effective selectivity are based on new ligand chemistries engineered to achieve very precise, selective interaction with the targeted protein. This approach increases selectivity while reducing the required number of steps, helping control process complexity and costs.

Targeted affinity chromatographic media are based on ligands tailored to interact with specific proteins, offering high selectivity for a target drug molecule. This can be a time-consuming approach if implemented for every new molecule; instead, mixed-mode and multimode approaches offer advantages to be considered.



Mixed-mode ligand structure: Mixed-mode media offer more interaction possibilities with the targeted drug molecule.



Multimode ligand structure: Multimode resins have the capacity to simultaneously interact with different sites or regions of the protein molecule.

Mixed-mode chromatography media are based on ligands that offer two or more interaction possibilities with the targeted drug molecule. The mixed-mode approach has proven to be effective and more productive in applications, such as intermediate and polishing steps, for purifying proteins based on differential salt-induced hydrophobicity. The advantage with the mixed-mode approach is that the same media can be used for different purification steps, modulated by solution conditions, such as using multiple buffers or multiple elution steps. However, newer mixed-mode resins have ligand chemistry that enable use of multiple, sequential interactions during the normal chromatographic process.

Multimode resins offer greater potential for efficiencies and improved yields. Rather than requiring multiple chromatographic purification steps, the simultaneous interaction makes it possible to separate very closely related proteins in a single step. This means it is possible to have the primary, secondary and tertiary interactions all happening at the same time. This simultaneous purification can occur without requiring additional intermediate steps, such as buffer exchange, buffer titration or dilution.

There have been studies showing that using a multimode or mixed-mode ligand with multiple interactions has the potential to boost chromatography yields while merging two process steps – and the ancillary time and costs associated with each step – ultimately impacting cost per gram.

For example, typical chromatography processes may first use a separate cation exchange step, then an ion exchange step. The yield is about 80 per cent pure after the first step, reaching upwards of 95 per cent after the second step.

With a multimode resin, it is possible to reach the 95 per cent purity in one step. Even with using a multimode resin in the column, it is more efficient to process 70 grams in one batch versus running 100 grams through separate cation and ion exchange steps. This is due to the overall higher throughput and the lower cost of materials, since this approach reduces the buffer consumption, types of filtration systems used, and ancillary costs associated with each chromatographic step. And since each step typically takes up to two hours, costly production time and labour can be cut in half.

Another method for optimising process chromatography is using the continuous chromatography method. In continuous multicolumn chromatography, the large column is effectively split into a number of much smaller columns that operate in series over a larger number of cycles. While product is loaded onto some columns, other columns in the set are going through the wash, elution and regeneration phases. Combined with resin optimisation and merging two chromatography steps into one, there is the potential for a three-fold improvement over traditional processes.

New Approaches to Buffers

Optimising the resin chemistry presents significant opportunities to improve downstream production. Improving the ways buffers are formulated and delivered to the end user can also positively impact productivity.

Traditionally, buffers have been very targeted: One type of buffer targets one type of pH in a column, then a different buffer is used to target a different pH. There is a move to more universal buffers that can be used in multiple process steps; while it's not possible to reuse the buffer, having to acquire, store and manage fewer buffers can help control costs.

There is also a greater focus on the use of additives to improve buffer performance. For example, in hydrophobic interaction chromatography (HIC), bioprocessors are working to fine-tune the selectivity of HIC functional groups. One method being explored is to use a select range of additives in the chromatographic media to improve the retention and selectivity of proteins as they move through the media, modulating their hydrophobic interaction and improving separation efficiency with decreased retention time, thereby improving throughput.



The advent of mixed-mode and multimode media and the increased selectivity they offer presents significant potential operational cost savings by eliminating intermediate purification steps that require additional time, materials, equipment and personnel.



Taking a more advanced approach to additives is one way buffers can be improved to optimise production. Another innovative advancement in buffer technology is the standardisation of buffer packages, pre-designed for specific applications and delivered ready-to-use in single-use packaging.

Leading buffer suppliers are now implementing “buffer-on-demand” programmes designed to eliminate buffer-related costs in terms of labour, time and capital expenses from downstream production. Buffers are supplied in single-use packages – either pre-weighed and ready to use in solution, or as concentrates that can then be diluted and used in the columns.

This can eliminate capital expenses associated with buffer preparation tanks and equipment as well as storage space. It can also eliminate multiple buffer preparation, testing and validation production steps, directly impacting labour costs, time and management and documentation activities.

Chemistry suppliers who are providing these buffers on demand can work with the manufacturer to recommend and formulate very specific buffer materials, with stringent and well-documented materials characterisation, so that the biologics manufacturer can be assured that the buffer’s performance in the chromatographic step is always on-target.

Enhancing Use of Data Analytics Tools

One area where the biopharmaceutical industry lags behind other manufacturers is the aggressive use of data and predictive analytics to mine for and uncover ways to improve productivity, process yield and costs. The industry is making significant investments to improve its use of data.

Currently, much of the focus is on using process data to optimise a process, then reliably repeat that batch by reaching the perfect balance of process parameters. These efforts are being conducted for both upstream and downstream production; however, much of the focus is on the process data itself, somewhat in isolation.

There is an opportunity to expand the application of data analytics beyond the process to more precisely and completely characterise the raw materials used in both upstream and downstream production, then integrate the data into overall optimisation efforts. In many cases, the biologics manufacturing sites are simply using standard certificate of analysis (CofA) data received with the delivery of chromatography resins, buffers and other production materials.

Raw material variability presents serious issues impacting downstream efficiency, resulting in long investigations and potential delays in making drugs available for patients. Leading chemistry suppliers are implementing more advanced characterisation efforts that provide manufacturers with further insights into the variability of materials within the integrated supply chain. These include supplying e-datasets, such as:

- CofA data for all raw material lots manufactured
- Manufacturing in-process data
- In-test actuals for conforming specs
- Stability testing interval data

This approach could enable a biologics manufacturer to more accurately assess and predict the process performance of any given raw material ahead of its use. This is a more holistic and data-driven way to assess the total impact of other downstream optimisation steps, such as adopting more targeted resins or implementing the use of novel additives in buffers.



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New Potential for Downstream Optimisation

The ultimate goal for downstream optimisation is clear: controlling or even reducing the cost per gram of value biologic drugs. Many of the more expensive elements of current downstream process steps, such as chromatography media, can be utilised more efficiently by investigating how newer, state-of-the-art materials offer ways to condense and streamline process steps.

Each downstream process has requirements unique to each target drug. Working with suppliers of downstream materials who have deep insights into how process chemistries and raw materials perform can be a highly productive way to advance downstream biologic optimisation.



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