Maximising mAbs Purification Efficiency: Focus Areas for Reducing Bottlenecks in Downstream Processing

Finding ways to remove bottlenecks and improve yields in downstream processing for monoclonal antibodies (mAbs) continues to be a key focus area for biopharma manufacturers. In downstream processing, the goal is to improve recovery and reduce the cost per gram of protein produced. Today, over 60% of the cost to produce a new mAb relates back to downstream steps.[1] Any percentage of improvement in downstream recovery can contribute to improving the ultimate process yield for drug product of the target biologic.

When compared to upstream processing, finding efficiencies and economies of scale in downstream steps involves more complex analysis and optimisation. Significant investments have already been made in the technologies and processes used in upstream processes. Improvements to raw material characterisation and the addition of single-use systems, perfusion systems and more precisely controlled bioreactors in upstream processing steps are all 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.

Expanding the use of mixed-mode and multimode chromatography resins – using resins to target ligands for increased selectivity can help to process targeted molecules more efficiently – and exploring ways to make chromatography buffers more effective – using new kinds of additives and prepackaged single-use buffer materials to streamline buffer preparation steps – are two potential areas for optimisation that could lead to significant downstream improvement.

How Resin Choice Impacts Overall Operations

Downstream processing generally takes place over a period of a few weeks. Multiple chromatographic steps, filtration steps, buffers and cleaning solutions are used as part of the process. A capture step is the first purification step where protein A has become the most widely used resin due to its highly specific nature, ease of use as a standard purification process and proven regulatory record.[2] The protein A step is one area where process efficiencies and cost savings may be gained by selecting a high-performance protein A resin and optimising buffer preparation.

When choosing a protein A resin, the resin dynamic binding capacity (DBC) is one factor that can impact overall process productivity. A resin with a higher DBC can improve the productivity of the capture step while keeping the column sizes the same. This in turn can minimise the need to modify facilities, specifically for high-titre cell culture processes.[3]

A simulation was performed with BioSolve software using three model resins having binding capacities ranging from 30g/L to 65g/L to calculate the number of bind/elute cycles, process time and amount of buffers required for a 2000L bioreactor batch. Assumptions made for the calculations are summarised in Table 1, where the column size was kept consistent as 68.6L for a 2000L cell culture reactor with a 5g/L titre value. The process's productivity was evaluated in terms of number of cycles required per batch and process time.

Cell culture volume	2000L
Titer	5g/L
Protein A column bed height	20cm
Protein A column volume	68.6L
Step yield	90%
Flow rate	150cm/hr
PROTEIN A PROCESS PHASE	**DURATION (COLUMN VOLUME)**
Flush (WFI)	3CV
Equilibrium	5CV
Load	N/A
Wash	5CV
Elution	5CV
CIP (0.5M NaOH)	2CV
Storage	5CV

Table 1. Process parameters used for simulation

Resins having higher DBC significantly reduce the number of cycles and total downstream processing time, as illustrated in Table 2. In addition to increasing productivity, reducing the number of cycles can also reduce operational risk and lead to lower costs for labour and consumables for each cycle.

	RESIN A	RESIN B	RESIN C**
DBC	30g/L	40g/L	65g/L
# of Protein A cycle/batch	4	3	2
Protein A column size	68.6L	68.6L	68.6L
Process time	18.8 hours	15.8 hours	12.8 hours
Total buffer consumption per batch	4,365L	3,429L	2,496L

* 2000L Bioreactor providing 5g/L titer
** DBC value of Resin C was taken from experimental value [3]

*Table 2. Process output based on resin capacity**

Reducing the amount of buffer consumed does more than impact raw material cost; it can contribute to verifiable savings in buffer preparation time, buffer tank size and method of preparation. In this model, total buffer consumption was reduced by approximately 30% with the use of resin with high DBC (Resin C) when compared to Resin B, and reduced by approximately 40% when compared to Resin A.

Improving Buffer Preparation Workflows

Lower buffer solution requirements also provide flexibility to either make buffers in-house or utilise ready-to-use buffers. Buffers for the purification process can be prepared in multiple ways:

- Powder hydration in fixed stainless-steel tanks or single-use buffer prep reactors
- Multicomponent buffer concentrates with in-line dilution, or single component stocks with buffer stock blending

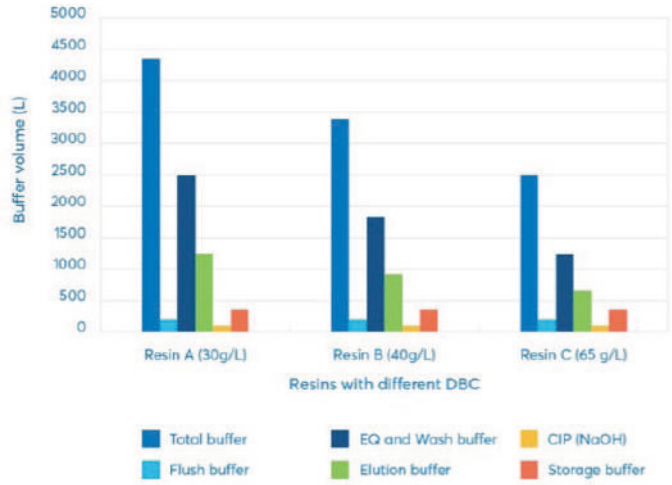

Figure 1. Buffer consumption of three protein A resins with different dynamic binding capacity (DBC) for processing of one 2000L bioreactor batch

Ready-to-use, cGMP 1x buffers

The most commonly used method for in-house buffer creation uses WFI (water for injection) grade water to hydrate powders in stainless-steel tanks. While this well-established method is ideal for large volumes, it requires significant and ongoing investment in infrastructure. For example, a biopharma manufacturer or its contract manufacturer (CMO) may need additional warehouse space for storing raw materials prior to their use, as well as a dedicated weighing and dispensing area – all of which need to be properly managed, kept clean and in accordance with cGMP practices. In addition, the footprint for the stainless-steel tanks within the facility must also be considered; in an existing facility, stainless-steel tanks take valuable space away from value-added operations and in a new facility, specifying additional square footage to a project could increase the size of the initial CAPEX request and construction time.

Additionally, new developments in single-use technology have added flexibility in buffer preparation methods, giving small- and medium-scale facilities the freedom to choose single-use tanks for buffer preparation. This can support faster changeovers and cleanouts in buffer preparation, saving both time and cost in manufacturing processes.[4]

Choosing a Hybrid Buffer Preparation Approach

Industry organisations, including BPOG, have offered insight into how buffer stock blending and in-line dilution enable overall improvements across unit operations.[4,5,6] The decision to select one option over the other (or a hybrid approach) will usually be dependent upon an economic analysis of items such as scale, batches of drug produced per year, raw materials used and other site attributes. Workflow improvements that can be implemented for each of the buffer prep options are listed in Table 3.

A hybrid approach using both in-house systems and outsourced buffers can streamline downstream purification unit operations significantly. As noted, the use of a protein A resin with a high DBC can reduce buffer usage to a more manageable level and the use of in-line dilution (ILD) systems will make the production of critical buffer components more efficient. Below are suggested buffer preparation methods for each buffer used in protein A step.

- *The cleaning buffer,* usually a fixed normality of NaOH, can be prepared in-house using concentrate or can be purchased as a 1X concentration due to the smaller volumes used to reduce safety concerns.

- *The storage buffer* (example: 20% ethanol) can also be managed in-house in the same way as described above due to low, consistent volumes that are typically required in the process, irrespective of the resin DBC.

- Volumes required of *equilibration buffers* and *wash buffers* (examples: 1X PBS or 50 mM Tris, pH 7) significantly decrease with an increase in resin DBC, as shown in Figure 1. Preparing these buffers using either in-house or single-use systems causes several operation challenges at lower DBC values due to high volume. For such buffers, the use of in-line dilution (ILD) systems using multicomponent concentrates (ex. 10X PBS) can provide operational advantages including facility footprint reduction, reduction in raw material management and availability of buffer on demand.

- *Elution buffers* (example: 0.1M acetate buffer, pH 3.4) usage can also be streamlined through the use of in-line dilution.

BUFFER PREPARATION METHOD	POWDER HYDRATION IN STAINLESS-STEEL OR SINGLE-USE TANKS	MULTICOMPONENT BUFFER CONCENTRATES WITH IN-LINE DILUTION (OR SINGLE COMPONENT STOCKS WITH BUFFER STOCK BLENDING)	READY-TO-USE cGMP 1X BUFFERS
Workflow improvements	— Supply of pre-weighed cGMP powdered raw materials in pails and drums, or in single-use powder delivery systems, to eliminate solid subdivision steps and streamline pre-buffer prep operations — Delivery and use of free-flowing powdered raw materials to eliminate de-clumping steps and prevent damage to single-use buffer tanks — Supply of pre-weighed cGMP powdered raw materials in single-use powder delivery systems to enable faster charging into tanks and quicker turnaround time — Implementation of rapid ID systems in the warehouse to speed up incoming material release into production — Hot WFI usage in dissolution to speed up dissolution in single-use tanks with poor heat transfer rate (cooling or heating)	— Extractable & Leachable (E&L) data on single-use packaging which enables longer shelf life — Single-use in-line dilution systems to reduce cleaning validations and enable faster batch changeovers — Stability studies on buffers made using buffer concentrates to analyze shelf life — pH/conductivity sensitivity to temperature of buffers for in-line dilution system (for example, TRIS buffers are extremely sensitive to temperature) to reduce rejected buffers — Harmonized concentrates/stocks across unit operations to improve flexibility of concentrates — Robust supplier agreements and forecasting of demand to prevent supply chain issues — Standardized single-use connectors for process use to enable more flexibility across unit operations	— Stability studies available on buffers to analyze shelf life (for example, 1x buffers are typically susceptible to pH/conductivity changes over time, leading to shorter shelf life) — Robust supplier agreements and forecasting of demand to prevent supply chain issues — Implementation of rapid ID systems including refractive index and Fourier transform infrared (FTIR) testing for quick release of buffer solutions

Table 3. Suggested workflow improvements for various buffer preparation methods

Conclusion

There are a number of areas where streamlining downstream processing steps can help improve overall mAbs processing efficiencies and help downstream productivity match the improved efficiencies achieved in upstream processing.

Focusing on new approaches to the protein A step is one area where significant opportunity exists. The flexibility and productivity of the mAb capture process step can be improved by utilising high-capacity affinity resins, along with optimal buffer management. A high-capacity resin reduces the process time by allowing less numbers of cycles required per batch, resulting in reduced process and labour costs, as well as reduced risk. Moreover, the implementation of a high-DBC resin decreases the volume of process buffers significantly. This reduced buffer volume provides flexibility to adopt different buffer preparation processes based on the facility requirements. Since each mAb production process may have its own requirements and bottlenecks, it is important to have flexible process optimisation options so that unique solutions can be applied to various mAb products. However, by investigating and investing in these types of new technologies and new approaches, the ability to create and deliver these valuable, in-demand biologics more cost-effectively can help make sure that patients and communities worldwide benefit from these therapies.

REFERENCES

1. Deorkar, N., Berron, C. (2019). Key challenges and potential solutions for optimizing downstream bioprocessing production. International Biopharmaceutical Industry 2(2), 10-12.
2. Pabst, T., Thai, J. & Hunter, A. (2018). Evaluation of recent protein A stationary phase innovations for capture of biotherapeutics. Journal of Chromatography A 1554, 45-60.
3. J.T.Baker BAKERBOND PROchievATM protein A resin. vwr.com, accessed October 2020.
4. Vengsarkar, P., Deorkar, N. (2020). Improving mAb manufacturing productivity by optimizing buffer and media prep process flow. BioPharm International, July 30, 2020.
5. Gibson, K. et al (2019). An economic evaluation of buffer preparation philosophies for the biopharmaceutical industry. BioPhorum Operations Group, December 2019.
6. Schrott, C. et al (2019). Nimble-Biophorum buffer stock blending system: A more advanced concept for buffer manufacturing. BioPhorum Operations Group, December 2019.

Nandu Deorkar

Nandu Deorkar, PhD, MBA, is the Vice President of Research & Development for Avantor. His expertise in materials technology research & development includes chemical/polymer R&D, drug development, formulation, drug delivery technologies, process development and technology transfer. Dr. Deorkar earned his PhD in chemistry from the Indian Institute of Technology, Bombay, and his MBA from Fairleigh Dickinson University, New Jersey (USA).

Jungmin Oh

Dr. Oh leads product and process development projects at Avantor, where she is responsible for solving customer-centric problems with multiple biopharmaceutical industry partners. She holds M.S. and Ph.D. degrees in Chemical Engineering focusing on the optimization of a continuous chromatography system.

Pranav Vengsarkar

Dr. Vengsarkar is focused on product and process development for new cGMP products and excipients, and development and design of single-use raw material delivery systems at Avantor. He holds a Bachelor's in chemical engineering ICT Mumbai and a Ph.D. in Chemical Engineering from Auburn University.