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Top Tips to Optimize Downstream Processes

From resins to buffers to single-use technologies – there are many opportunities to improve downstream processes

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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

	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 1 (left): Process parameters used for simulation
Table 2 (above): Process output based on resin capacity

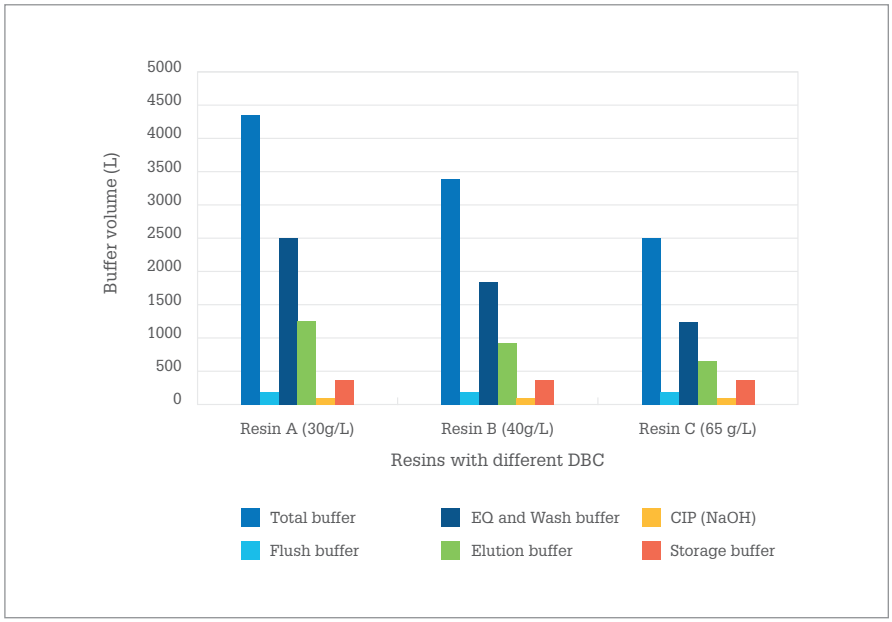

Figure 1: Buffer consumption of three protein A resins with different dynamic binding capacity (DBC) for processing of one 200L bioreactor batch

Emerging treatments, including cell and gene therapies, are exciting and are certainly starting to expand pipelines. However, traditional biologics (monoclonal antibodies) still dominate the world of biopharma. Research has shown that the clinical pipeline of antibody therapeutics grew by 30 percent over the past year (1) – excluding COVID-19 antibody therapies – highlighting the importance of these treatments and the need for their efficient production.

Given that 60–80 percent of mAb production costs can be attributed to downstream processing (2), removing downstream bottlenecks and improving yields will continue to be an important priority for mAbs manufacturers – especially amidst rising demand. Below, we offer a few suggestions.

Considering resins and buffers

In the capture step, protein A is the most widely used resin. Protein A is simple to implement as a standard purification process and holds a strong regulatory track record (3); however, the costs of the resin are substantial, making it important to optimize the process to maximize cost and efficiency. A key consideration in process optimization is understanding the role dynamic binding capacities (DBC) plays in overall protein A performance. Use of a resin with higher DBC can improve capture step productivity while maintaining column sizes and minimizing facility modification – especially when it comes to high titer cell culture processes.

To prove the point, we performed a simulation using BioSolve software,

"A lower volume of buffer consumption not only reduces raw material cost, but also buffer preparation time, buffer tank size and method of preparation."

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 and prevent damage to single-use buffer tanks — 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 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: Workflow improvements for each of the three options

calculating the number of bind/elute cycles, process time, and volumes of buffer required for a 2000 L bioreactor batch. We looked at three model resins with DBCs ranging from 30 g/L–65 g/L. Assumptions made for the calculations are summarized in Table 1. We maintained column size at 68.6 L for 2000 L cell culture reactor with 5 g/L titer value. We evaluated process productivity based on the number of cycles required per batch as well as process time.

What did we find? Higher DBC resins significantly reduce the number of cycles and total downstream processing time (see Table 2 and Figure 1). Notably, by reducing the number of cycles, one can also reduce operational risks and per-cycle costs for labor and consumables.

Similarly, a lower volume of buffer consumption not only reduces raw material cost, but also buffer preparation time, buffer tank size, and method of preparation. In our model, Resin C reduced total buffer consumption by approximately 40 and 30 percent when compared to Resin A and B, respectively.

Creating buffers in-house is a well-established method suitable for manufacturing large volumes; however, preparation of buffers often involves utilities and resources, such as Water for Injection (WFI), which may be constrained due to demand in other systems such as clean-in-place or other process lines. Further, the sheer number and volume of buffer solutions required for the entire downstream purification process may cause scheduling issues for the buffer prep team trying to meet the demands of the production schedule. Reduced buffer solution requirements offer additional flexibility as these operations require significant infrastructure, including warehouse space for holding raw materials prior to their use, a weighing and dispensing area for raw materials, and space to store the prepared solutions which are often stored in corridors due to lack of space. In addition, the stainless-steel tanks themselves can require a considerable footprint in the facility and frequently

experience corrosion issues due to the caustic nature and high chloride content of commonly used buffers.

New developments in single-use technology have added flexibility in buffer preparation methods, allowing small- and medium-scale facilities to move to single-use tanks for buffer preparation. This has enabled faster changeovers in buffer preparation, saving both time and cost in manufacturing processes (4). Single-use fluid handling systems can help reduce bottlenecks, particularly in cell therapy manufacturing where downstream processing is often slowed by a lack of the suitable closed manufacturing systems. The closed, automated systems that are available are often unsuitable for large volumes of allogeneic cell therapies. If a biomanufacturer uses single-use equipment, a reputable supplier with a multiple-source supply chain is key to avoid disruptions.

A hybrid approach

Combining both in-house systems and outsourced buffers in a hybrid approach can help streamline downstream purification unit operations. Moreover, in-line dilution (ILD) systems can improve the efficiency of critical buffer component production.

- Clean-in-place solutions: Usually a fixed normality of NaOH, it can be prepared in-house using concentrate or purchased as a 1X concentration thanks to the smaller volumes needed, lowering safety concerns.
- Storage buffer: Due to low consistent volumes typically required (irrespective of resin DBC), storage buffers, such as 20 percent ethanol, can be managed in-house in the same way as the cleaning buffer.
- Equilibration and wash buffers: Volumes of these buffers (for example, 1X PBS or 50 mM Tris, pH 7) significantly decrease with an increase in resin DBC, as shown in Figure 1. Whether these buffers are prepared using in-house or single-use systems, high volumes can cause several operation challenges. When preparing these buffers, inline dilution (ILD) systems using multicomponent concentrates (for example, 10X PBS) can provide operational advantages. For example, ILD can help minimize facility footprint, reduce raw material management, and increase availability of buffer on demand.
- Elution buffers: Use of these buffers (for example, 0.1M acetate buffer, pH 3.4) can also be streamlined through the use of an in-line dilution system.

Workflow improvements in buffer preparation

Broadly, there are three options for buffer prep system/process in downstream purification:

1. Single-use buffer prep reactors or chemical hydration in fixed stainless-steel tanks
2. Multicomponent buffer concentrates with in-line dilution or single component stocks with buffer stock blending
3. Ready-to-use cGMP 1X buffers

BioPhorum Operations Group (BPOG) and other industry organizations have offered insight into how buffer stock blending and in-line dilution generate overall improvements across unit operations (4, 5, 6). Choosing the right option will usually depend on an economic analysis of several factors, including scale, batches of drug produced per year, raw materials required, and other site attributes.

The flexibility and productivity of the mAb capture process step can be improved by using high DBC resins along with optimal buffer management. High-capacity resin decreases process time by allowing fewer numbers of cycles required per batch – saving cost, mitigating risk, and reducing labor costs.

In addition, implementing a high DBC resin decreases the volume of process buffers significantly, which allows the flexibility to adopt different buffer preparation processes based on facility requirements. Each facility and downstream process has unique requirements and bottlenecks, so having flexible process optimization options is important.

As innovative biologic treatments continue to emerge, manufacturers will almost certainly face even more hurdles – but, in every situation, the development of highly efficient, high yield manufacturing processes will be a key factor for success.

References

1. *H Kaplon et al., MAbs 14 (2022). DOI: 10.1080/19420862.2021.2014296*
2. *BioProcess International, "Key challenges and potential solutions for optimizing downstream bioprocessing production," (2019). Available at https://bit.ly/3GlBxzi*
3. *T Pabst, J Thai, A Hunter, Journal of Chromatography A 1554, 45-60 (2018). DOI: 10.1016/j.chroma.2018.03.060*
4. *P Vengsarkar, N Deorkar, BioPharm International 33, 17-20 (2020).*
5. *BioPhorum, "An economic evaluation of buffer preparation philosophies for the biopharmaceutical industry" (2019). Available at https://bit.ly/3GDKOUN*
6. *BioPhorum, "Nimble-Biophorum buffer stock blending system: A more advanced concept for buffer manufacturing" (2019). Available at https://bit.ly/3GJpgX6*