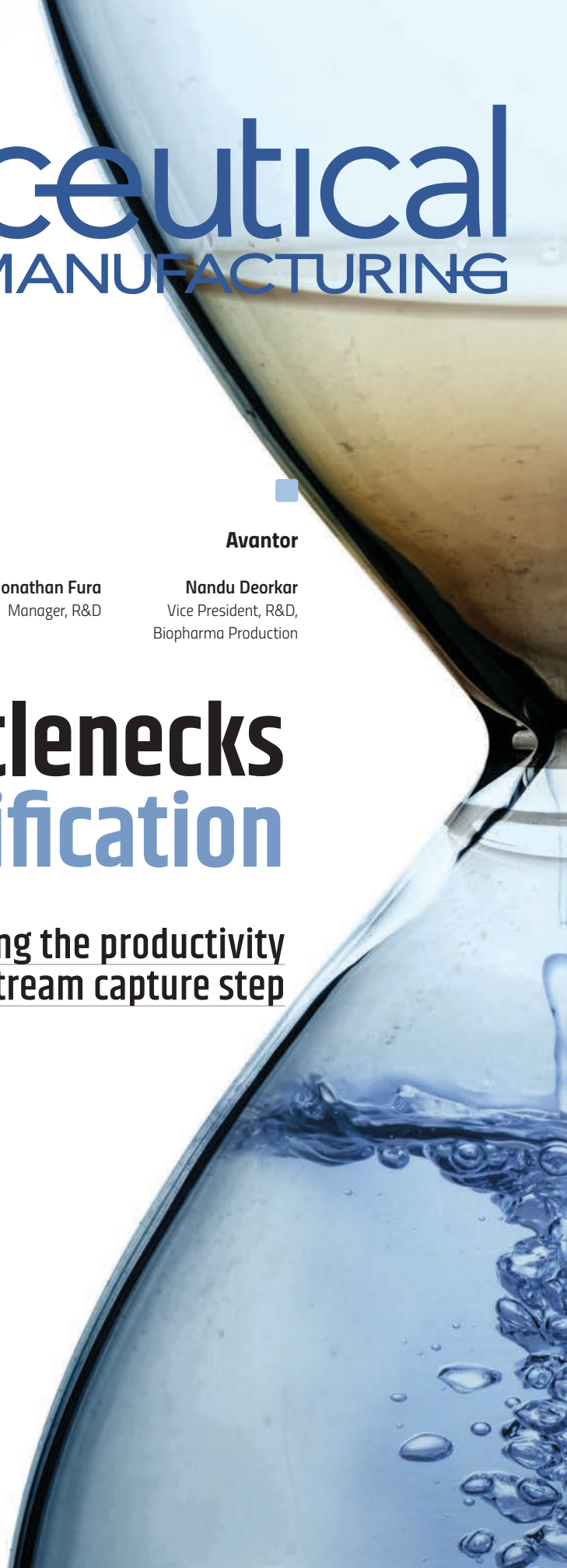
Pharmaceutical MANUFACTURING



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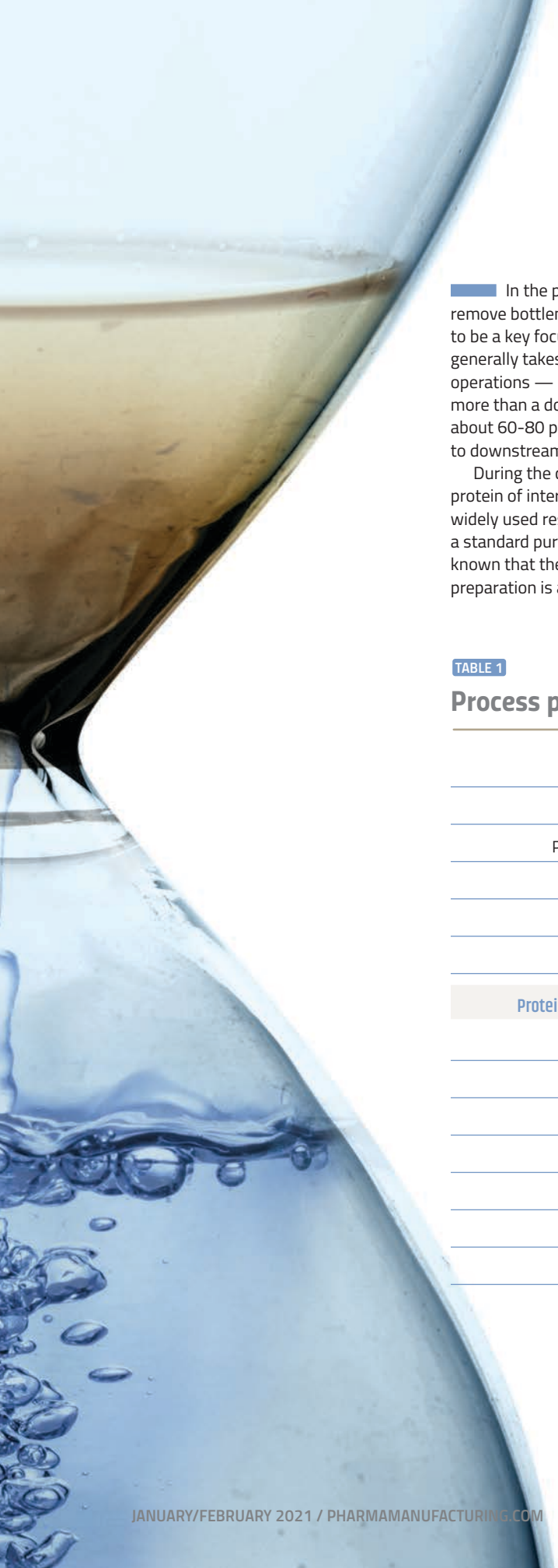
Removing bottlenecks from mAb purification

Strategies for increasing the productivity of the downstream capture step



In the production of monoclonal antibodies (mAbs), finding ways to remove bottlenecks and improve yields in downstream processing continues to be a key focus area for biopharma manufacturers. Downstream processing generally takes place over a period of a few weeks and involves many unit operations — including multiple chromatographic steps and filtration steps, and more than a dozen buffers and cleaning solutions as part of the process. In fact, about 60-80 percent of the total cost of producing a mAb can be attributed back to downstream processing.[1]

During the capture step, the goal is to isolate, concentrate and stabilize the protein of interest. During this purification step, protein A has become the most widely used resin due to its highly specific nature, ease of implementation as a standard purification process, and strong regulatory track record.[2] It is well known that the cost of the protein A capture step is substantial, and that buffer preparation is an area for improvement to help improve both cost and efficiency.

TABLE 1

Process parameters used for simulation

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

In this article, we will highlight strategies to improve the productivity of the capture step through the selection of a high-performance protein A resin and optimization of buffer preparation. Buffer preparation will be further discussed by comparing in-house buffer preparation and ready-to-use buffers, or buffer concentrates utilizing latest technologies and supply chain options.

How resin choice impacts overall operations

When choosing a protein A resin, the resin dynamic binding capacity (DBC) is a critical factor that impacts the productivity of the overall protein A process. A resin with higher DBC can improve the productivity of the capture step while keeping the column sizes the same and minimizing the facility modification, specifically for high titer cell culture processes.

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 2000L bioreactor batch. Assumptions made for the calculations are summarized in Table 1 where the column size was kept consistent as 68.6L for 2000L cell culture reactor with 5g/L titer value. Productivity of the process was evaluated in terms of number of cycles required per batch and process time.

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 of the process, reduced number of cycles allows for reduced operational risk and lower labor and consumables cost for each cycle.

A lower volume of buffer consumption not only impacts raw material cost, but buffer preparation time, buffer tank size and method of preparation. In this model, total buffer consumption was reduced by approximately 30 percent with the use of resin with high DBC, which was resin C when compared to resin B, and reduced by approximately 40 percent when compared to resin A. Lower buffer solution requirements also provide flexibility to either make buffers in-house or utilize ready to use buffers.

Buffer preparation options

Buffers for the protein A chromatography step can be supplied to a chromatography skid in multiple ways. An established method to generate buffers in-house is with the use of WFI (water for injection)-grade water to hydrate powders in stainless steel tanks. This approach is suitable when large amounts of buffer are needed.

While these methods are established and ideal for large volumes, these operations need significant infrastructure, including warehouse space for holding raw materials prior to their use and a weighing and

EXHIBIT 1

Buffer consumption of protein A step

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

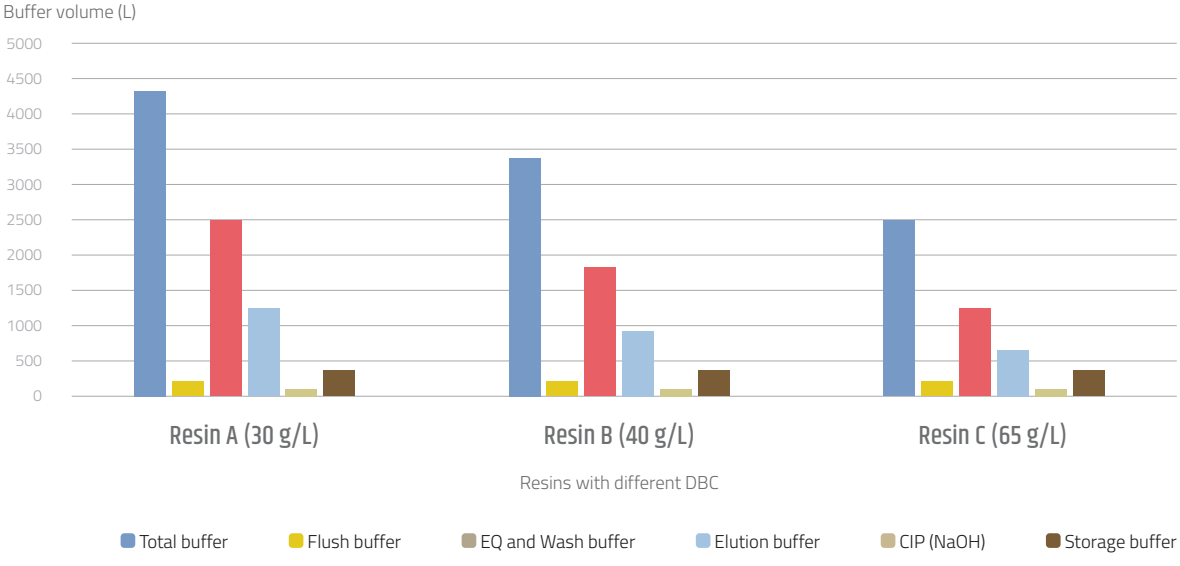


TABLE 2

Process output based on resin capacity*

	Resin A	Resin B	Resin C **
DBC	30 g/L	40 g/L	65 g/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 5 G/L TITER
** DBC VALUE OF RESIN C WAS TAKEN FROM EXPERIMENTAL VALUE

A hybrid approach using both in-house systems and outsourced buffers can streamline downstream purification.

dispensing area for raw materials. The footprint for the stainless steel tanks themselves within the facility must also be considered.

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 and cleanouts in buffer preparation, saving both time and cost in manufacturing processes.[3]

Choosing a hybrid buffer preparation approach

A hybrid approach using both in-house systems and outsourced buffers can streamline downstream purification unit operations significantly. Additionally, 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 productions of critical buffer components more efficient. Here are some of the keys to this hybrid approach:

- 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 (such as 20 percent 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 increase in resin DBC as shown in Exhibit 1. Preparing these buffers using either in-house or single-use systems cause several operation challenges at lower DBC values due to high volume. For such buffers, the use of 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 (ex. 0.1M acetate buffer, pH 3.4) usage can also be streamlined through the use of in-line dilution.

Workflow improvements for buffer preparation

Different options of a buffer prep system/process for downstream purification can be broadly split into three groups:

- 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)
- Ready to use cGMP 1X buffers

Industry organizations, including the BioPhorum Operations Group (BPOG) have offered insight into how buffer stock blending and in-line dilution enable overall improvements across unit operations.[3-5] 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.

TABLE 3

Suggested workflow improvements for various buffer preparation methods

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 apowder 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 spreed 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 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 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, or Fourier transform infrared (FTIR) testing for quick release of buffer solutions

Workflow improvements which can be implemented for each of the options are listed in Table 3.

Conclusion

We have shown how the flexibility and productivity of the mAb capture process step can be improved by utilizing high-capacity affinity resin along with optimal buffer management. High-capacity resin reduces the process time by allowing less numbers of cycles required per batch, which results in process cost savings, reduced risk and labor costs. Moreover, implementation of high DBC resin decreases the volume of process buffer significantly. This reduced buffer volume provides flexibility to adopt different buffer preparation processes based on the facility requirements. Based on the volume of each buffer, combination of 1) in-house hydration, 2) in-line dilution of buffer concentrate or 3) ready-to-use buffer can be applied to the process. Each facility and downstream process has unique requirements and bottlenecks. It is important to have a flexible process optimization options so that unique solution can be applied to various mAb products.

References

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