PEER REVIEWED

Improved Antibody Purification Process Through Use of Additives

Downstream biopharmaceutical processing utilises a series of interlocking and sequential operations, including filtration and process chromatography steps, that incrementally increases the purity of the target therapeutic molecule by exploiting the physical and chemical properties of that molecule.

It is critical for therapeutic monoclonal antibodies (mAbs) to have very high purity with low product-related and process-related impurities (host-cell proteins, DNA, leached protein A and aggregates) in order to be administered to patients safely.

In the production of mAbs, finding ways to improve yields and reduce downstream processing times, while sustaining low levels of impurities, remains a critical focus area for biopharma manufacturers. This need is becoming increasingly important, in part due to the significant improvements that have been made in upstream technologies and processes. Improvements in downstream throughput have not kept a similar pace with those made for upstream, leading to potential bottlenecks in the end-to-end process.

Downstream processing typically requires several weeks to complete prior to fill-and-finish steps. Not only does it involve multiple chromatographic and filtration steps, but depending on the specific process, more than a dozen buffers and cleaning solution steps may also be required. It is estimated that 60–80% of the total cost of producing a mAb can be attributed back to downstream processing.[1]

The article will outline a novel approach to improve impurity clearance efficiency of chromatographic steps by modulating the interaction of target protein and/or impurities with ligands on the resins. In this study, two chromatography resins were examined for the capture step of mAb purification: a Protein A resin with high dynamic binding capacity and a mixed-mode hydrophobic interaction chromatography (HIC) resin. Both were used for a capture step, and additives were screened to reduce the nonspecific binding of impurities. The goal of the study was to optimise chromatography steps and thus increase the capacity and removal of impurities such as residual HCPs, DNA, charge variants, aggregates and leached protein A.

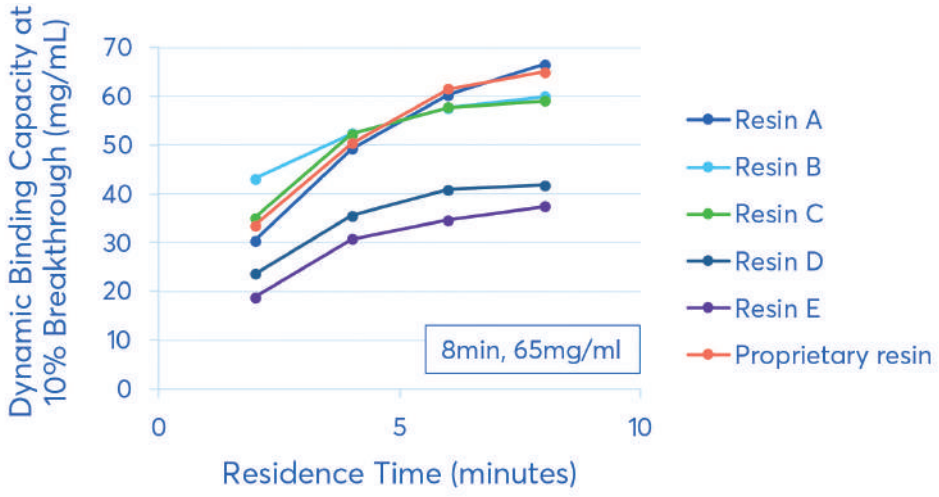
Assessing Two Different Capture Step Chromatography Techniques

To study the potential advantages of the use of additives to improve downstream capture step outcomes, two different chromatography techniques that can be used for capture step of mAb purification were examined.

The first is the protein A affinity chromatography step. In this study, a proprietary protein A resin was used as a model resin. The resin has a novel protein A ligand engineered for high specific affinity toward the Fc region of protein and features a standard-size agarose backbone for ease of use.

In this study, the importance of binding capacity and stability of the resin were analysed, as well as the use of additives to improve the protein A step; in addition, case studies of IgG purification using the selected proprietary protein A resin provided useful insights into optimising downstream capture steps.

One of the most important specifications of protein A resin is dynamic binding capacity (DBC). A resin with higher DBC can improve the productivity of the capture step while keeping the column sizes the same and minimising the facility modification, specifically for high titer cell culture processes. Figure 1 shows a dynamic binding capacity comparison of different protein A resins that are available in the market. The binding capacity is measured at 10% breakthrough of purified IgG1 using 5 milliliter columns, where we use the loading



Column: Pre-Packed 5mL FPLC Column, **Sample: mAb** (2mg/ml) in 10 mM Na_2HPO_4, 2 mM NaH_2PO_4, 137 mM NaCl, pH 7.4, **EQ Buffer:** 10 mM Na_2HPO_4, 2 mM NaH_2PO_4, 137 mM NaCl, pH 7.4, **Elution Buffer:** 100 mM Sodium acetate, pH 3.4, **Cleaning Proprietary Resin:** 3CV of 0.5M NaOH, **Temperature:** 4°C

Figure 1: Comparison of dynamic binding capacity of multiple protein A resins.

condition of using 1X phosphate buffered saline (PBS).

As can be seen, the selected proprietary protein A resin yielded the best DBC performance, especially at eight minutes of residence time, with a DBC at 65 mg per mil. To demonstrate the effect of DBC on the process throughput, a theoretical number of cycles needed per batch depending on the DBC of that resin was calculated.

This comparison was based on assuming 60-liter protein A column volume with 2,000-liter bioreactor, with 5 gram per liter material titer. Using a traditional resin with 35 mg per mil of DBC, over six process cycles would be needed to purify this batch. Alternatively, use of a protein A resin with 65 mg per mil of DBC results in needing just over three cycles to purify the same amount of material, saving approximately 45% the number of cycles needed compared to traditional resin. (Figure 2) In addition to increasing productivity of the process, reduced number of cycles also allows for reduced operational risk and lower labour and consumables cost for each cycle.

Another important specification of protein A resin is alkaline stability. Protein A resins go through hydrolysis and lose capacity over time when it's cleaned with a sodium hydroxide solution. Thus, it is important to assess the alkaline stability of the resin at a defined cleaning condition. In this study, the proprietary protein A resin was tested using 1 mil prepacked column and cleaning the column with different concentrations of sodium hydroxide.

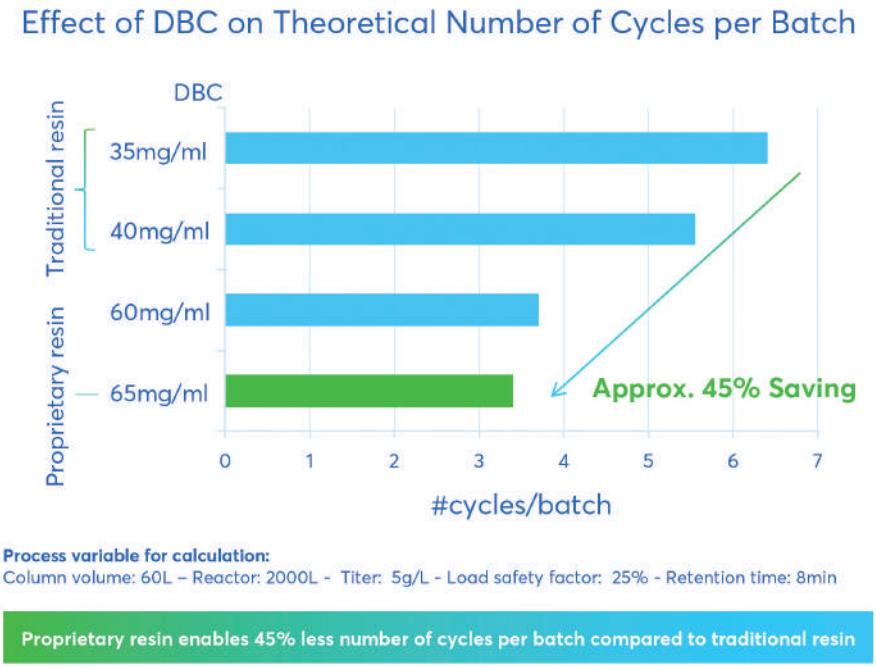


Figure 2: Importance of dynamic binding capacity on process throughput.

After 100 cycles of cleaning, the resin retained more than 80% of its initial capacity, regardless of sodium hydroxide concentration. This stability is important because it will dictate how often the new resin needs to be purchased or repacked in a new column. The savings in resin cost for production is proportional to the lifetime of the resin; thus, it is possible to save half of the resin cost by selecting resin with two times longer life cycle in sodium hydroxide.

The use of different additives to increase protein A resin stability was then studied. The additives included ethylene glycol, propylene glycol and sucrose at 4°C and room temperature to 1 normal sodium hydroxide at 4°C cleaning study. (Figure 3)

The best stability was achieved when 1M sucrose was added to sodium hydroxide solution, resulting in more than 90% of initial capacity being retained after 200 cycles of cleaning. In addition, the study also assessed room temperature stability data. As Figure 3 shows, a similar study was performed at room temperature, where the column was stored in 1N sodium hydroxide and 1M sucrose mixture for 24 hours. After 24 hours of storage in room temperature, the resin retained more than 90% of the initial capacity.

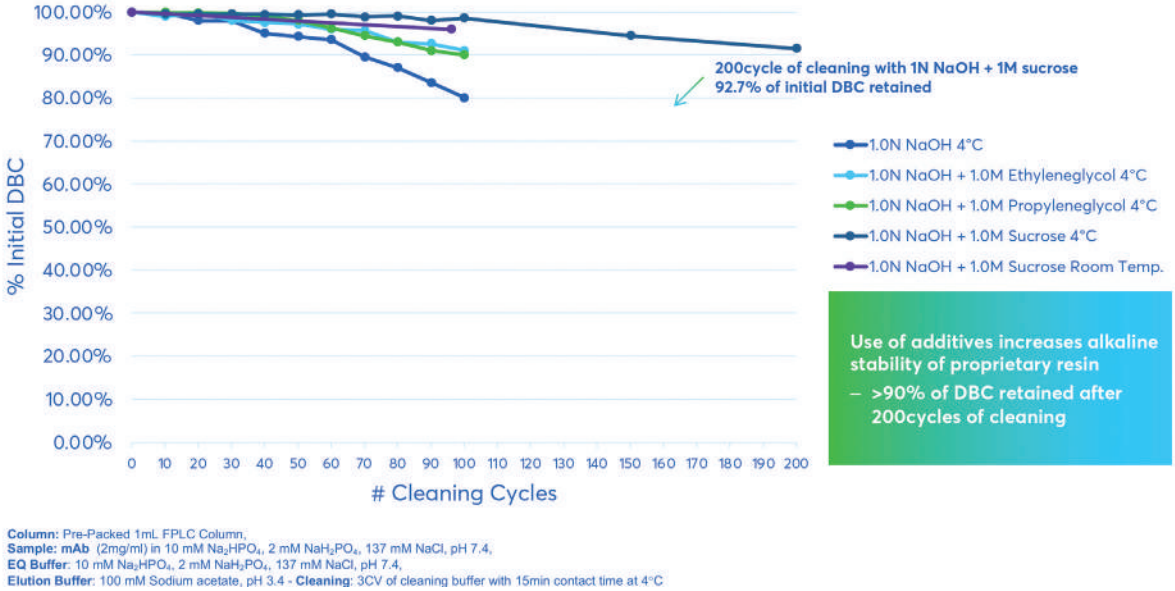
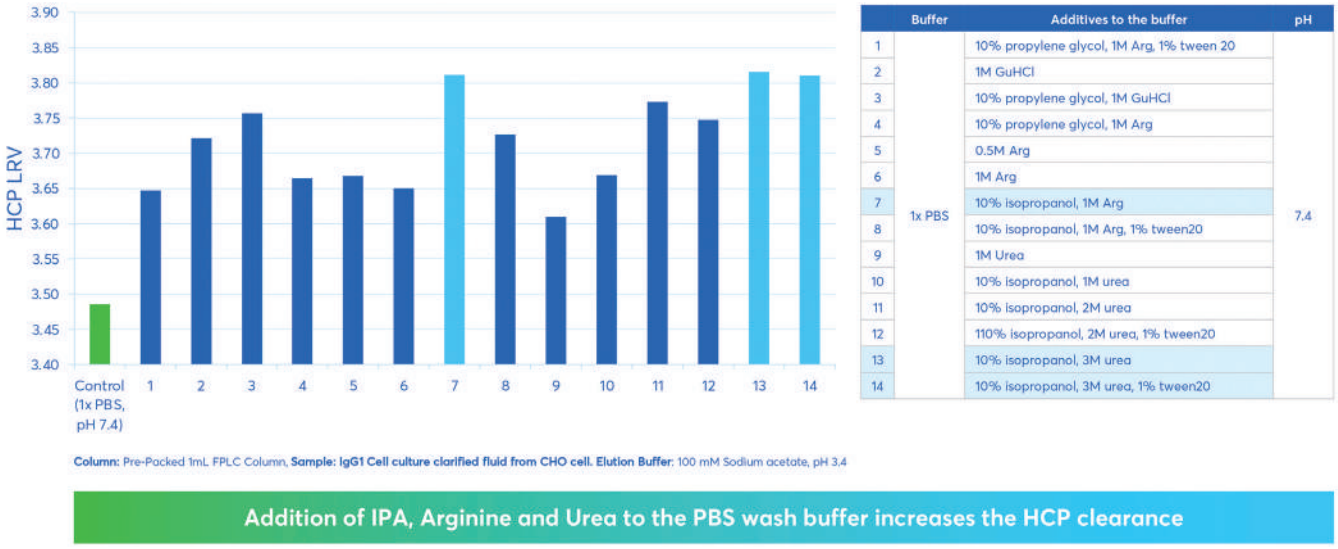


Figure 3: Use of additives increases alkaline stability with more than 90% of DBC retained after 200 cleaning cycles.



	Buffer	Additives to the buffer	pH
1	1x PBS	10% propylene glycol, 1M Arg, 1% tween 20	7.4
2		1M GuHCl	
3		10% propylene glycol, 1M GuHCl	
4		10% propylene glycol, 1M Arg	
5		0.5M Arg	
6		1M Arg	
7		10% isopropanol, 1M Arg	
8		10% isopropanol, 1M Arg, 1% tween20	
9		1M Urea	
10		10% isopropanol, 1M urea	
11		10% isopropanol, 2M urea	
12		110% isopropanol, 2M urea, 1% tween20	
13		10% isopropanol, 3M urea	
14		10% isopropanol, 3M urea, 1% tween20	

Figure 4: The addition of IPA, arginine and urea to the PBS wash buffer increases HCP clearance.

Together, these show the advantages of improving protein A resin alkaline stability through use of selective additives with sodium hydroxide cleaning solutions.

Another use of additives to improve protein A step efficiency is screening different additives for wash buffer to increase the host cell protein removal rate. In a study, fourteen additives were screened with different combinations and concentrations. Those additives are added to the control wash buffer, which was 1X PBS, and the experiment was performed by loading the IgG cell culture clarified fluid from the CHO cell system, with the column being washed with 1X PBS and the different additives mixture. (Figure 4)

Compared to a control without additives, all fourteen additives demonstrated beneficial impact on increasing the host cell protein removal, with the most significant improvements shown with the combination of isopropanol with arginine, isopropanol with urea, or isopropanol with urea and polysorbate.

Using Additives to Improve HIC Separation Performance

In addition to studying the impact of additives to the protein A step, the use of additives to increase the separation of the HIC process was also studied. In downstream processing, HIC resins have been used for both the capture step as well as the polishing step of mAb purification. HIC processes are useful to separate the aggregates, closely related proteins and process-related impurities based on the hydrophobic interaction that is induced by the high salt concentration.

In this additives study, a mixed-mode HIC media was used. It is a primary hydrophobic interactive resin that also has weak anion exchange sites of secondary and tertiary amine groups. This mixed-mode design offers improved selectivity and efficiency compared to other HIC resins.

The first additive, labeled AD1, was tested at different concentrations, and the separation and retention of four proteins – cytochrome c, myoglobin, lysozyme and alpha-amylase – was analysed. For the study, a column with 10-centimeter bed height was used, and an ammonium sulfate buffer was used for binding. For the elution step, a linear gradient of decreasing ammonium sulfate concentration was used, with AD1 being added to the elution buffer, and the total elution time was 40 minutes.

The study that achieved the best separation with the highest efficiency was when 0.85% of additive was used in the elution buffer compared to no additive being used and the retention time of strongly retained protein was decreased by adding the additive. However, it's important to note that, while the retention time has been decreased, the separation of four proteins is still very distinctive.

To further clarify the impact of the use of the AD1 additive in the HIC process, separation factor, resolution and retention factor of four proteins at two conditions, with and without the AD1 additive, were studied. (Figure 5)

For amylase, the retention factor is lower when the additive is used in the elution buffer. While the process efficiency is increased by reducing the retention time, the resolution and separation factors were not affected. Thus, when the separation efficiency is calculated based on the retention factor, the amylase efficiency is improved significantly through the use of

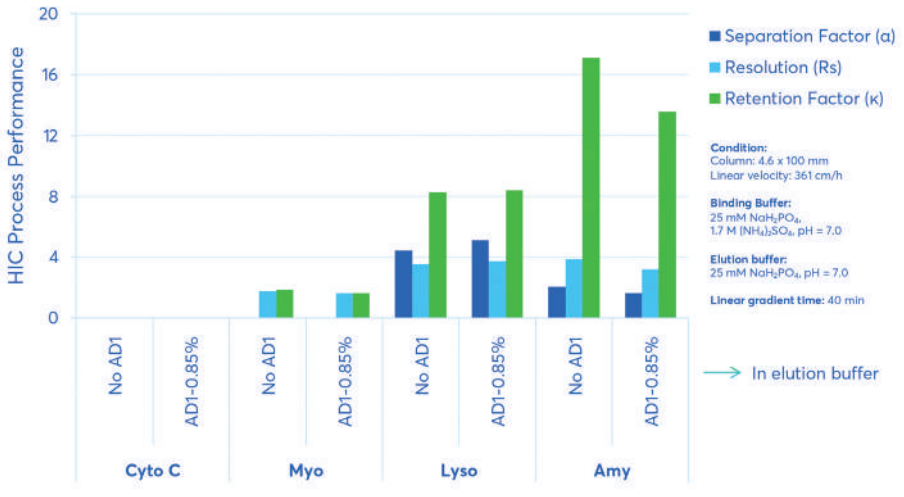
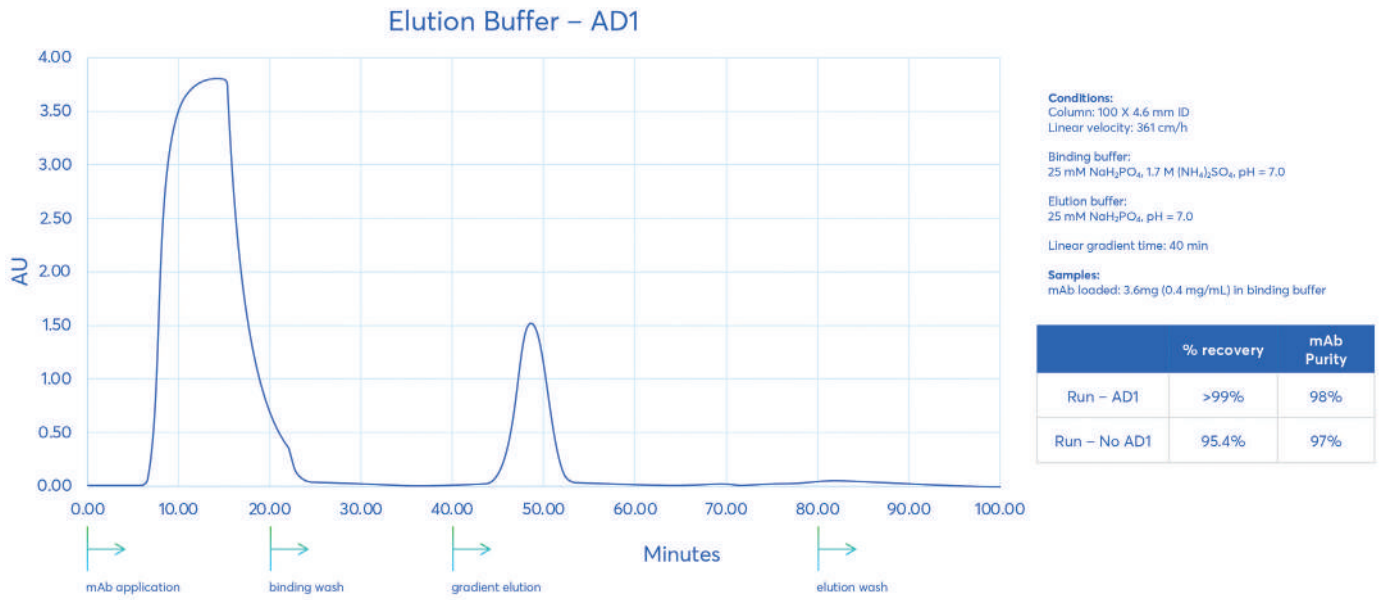


Figure 5: AD1 reduces amylase retention without affecting protein separation factor and retention.



	% recovery	mAb Purity
Run – AD1	>99%	98%
Run – No AD1	95.4%	97%

Figure 6: Use of additive with elution buffer improves both recovery and mAb purity.

additives in the elution buffer. This impact of efficiency improvement will magnify when the additive is used in a higher-scale process where the process time becomes more critical and more expensive as the scale increases.

Further investigations on other additives used with the HIC resin were also conducted. Four different additives with different molecular weights were used. While each additive required different concentrations for optimal result, all the additives showed increases in resolution and reductions in the retention factor, which will increase the efficiency of the process while not sacrificing the separation of proteins.

A final study using the HIC resin with additives was conducted, in this instance performing actual purification of mAb as a capture step of the process. (Figure 6) Clarified cell culture feedstock was loaded onto a 10-centimeter bed height column, and it was eluted with and without additives in the elution buffer. Both the recovery and mAb purity were very good for both conditions, but both showed some improvement when the additive was used. Recovery was higher than 99%, and the final mAb purity was 98%.

Additives Offer Opportunities to Help Optimise Downstream Process Steps

The global demand for biologic-based therapeutics based on mAbs is expected to continually grow in the coming years. This places greater urgency on the need to find ways to reduce costs and processing times so that valuable, life-changing treatments can be supplied more readily and cost-effectively to communities across the globe.

Innovative efforts, like the use of additives to enhance capture steps, such as protein A and HIC as described above, can provide one more tool to achieve these goals. To improve the capture step of mAb purification, it is critical to select a resin with high binding capacity to increase the process throughput. Alkaline stability of protein A resin needs to be considered to reduce the process cost.

Different additives have shown to be effective at increasing the alkaline stability of the proprietary protein A resin that was studied and optimising the host cell protein removal during the wash step. For hydrophobic interaction resins, mixed-mode HIC media process efficiency can be improved using different additives in the elution step.

REFERENCES

1. Deorkar, N., and Berrón, C. (2019). "Key challenges and potential solutions for optimizing downstream bioprocessing production," International Biopharmaceutical Industry 2(2) 10-12.

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