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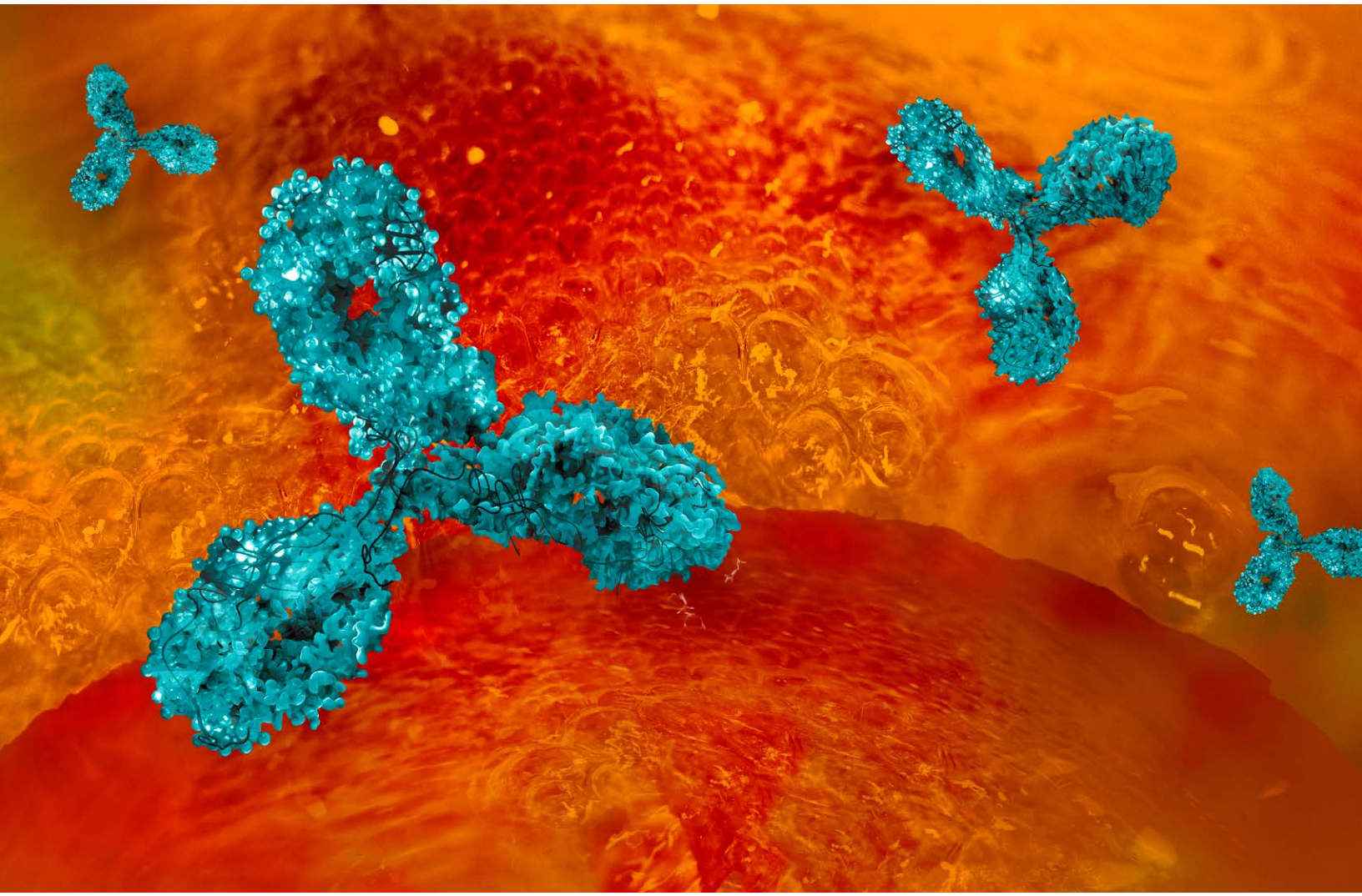
Downstream process intensification with novel chromatography resins for enhanced mAb purification



The evolution of the monoclonal antibody (mAb) market, since approval of the first mAb more than 30 years ago, has dramatically changed patient care. Advances in technology as well as knowledge about antibody engineering have brought forth a wide range of targeted therapies to address previously unmet medical needs. While early production processes rendered only low titers and poor purification yields, there has been substantial progress over the last several years to increase upstream process titer. Unfortunately, this has also led to a strain on existing downstream purification technologies. Although implementation of continuous unit operations has provided some relief, downstream processing (DSP) still presents a major bottleneck in the production of therapeutic antibodies.

One area of focus is the challenges related to protein A affinity chromatography. While it has become the go-to capture step for the purification of mAbs, protein A resin is also one of the biggest costs during mAb manufacturing. Limitations related to process economy as well as efficiency call for innovative solutions that can help address these critical issues. Using its J.T.Baker® BAKERBOND® PROchievA™ protein A affinity resin with select additives, Avantor has designed an integrated mAbs downstream purification process to enable significant throughput improvement while removing critical impurities. When used in conjunction, these technologies represent a highly efficient process to obtain monoclonal antibodies for use as therapeutics.

THE ADVANTAGES OF PROCHIEVA

Improving upstream process titers during biopharmaceutical development offers many advantages, such as reduced manufacturing costs and better product quality; however, it simultaneously increases the burden on downstream operations that were historically designed to process lower amounts of antibody. More chromatography cycles must then be run to properly purify the product of interest. This can create challenges in the capture step where protein A chromatography is traditionally used due to its specificity. protein A is not only expensive but frequent use can lead to fouling due to any impurities in the column that are not properly cleaned after each use.

To resolve this issue, manufacturers use a high concentration of sodium hydroxide to clean their columns. Harsh solutions such as these can hydrolyze the resin, which reduces its lifetime by weakening the protein's alkaline stability. However, insufficient removal of impurities could carry over to the next phase of purification. There is also a risk of protein A ligands leaching out from the resin, increasing the burden of purification in subsequent processing steps.

One approach to overcoming this challenge is to increase the binding capacity of protein A resins, which allows more protein to be run per cycle, thereby reducing processing time and costs. Higher capacity resins can also significantly reduce the volume of buffer needed for that step as well as the costs associated with buffers, such as the labor and capacity required for preparation and storage. While this buffer volume change may not seem noteworthy in smaller volume phases, such as process development, the impact becomes greater as processes are scaled up for clinical manufacturing. Avantor considered these challenges and others when designing its J.T.Baker® BAKERBOND® PROchievA™ protein A derived affinity chromatography resin.

BETTER THROUGHPUT USING HIGH DYNAMIC BINDING CAPACITY

Dynamic binding capacity (DBC) is one of the most significant parameters affecting the throughput of a purification process. A high DBC enables more protein to be loaded into the column without the need to run more cycles, leading to improved throughput and time savings. Other resin manufacturers have improved DBC by reducing the particle size; however, this can create challenges when packing the column due to an increase in pressure. Figure 1 below shows BAKERBOND® PROchievA™ high DBC data at different residence times, which is based on an average particle size of 85 microns. When measured at 10% of the breakthrough point, the DBC is reached 65 mg/ml at eight minutes of residence time. This is a best-in-class performance for all protein A resins currently available in the market.

Figure 2 shows calculations using the theoretical number of cycles per batch based on different DBCs. When using a traditional resin with 35 mg/ml of DBC, more than six cycles per batch would be needed. Yet, when using BAKERBOND® PROchievA™ with the same conditions and column volume, only slightly more than three cycles per batch are needed to complete the process. Overall, compared to a traditional resin, BAKERBOND® PROchievA™ can reduce approximately 45% of the number of cycles needed per batch and buffer consumption by almost 40%.

ALKALINE STABILITY PROVIDES EXTENDED RESIN LIFE

Another critical parameter attribute of BAKERBOND® PROchievA™ resin is its alkaline stability. Figure 3 shows the result of cleaning the BAKERBOND® PROchievA™ column with 0.1 normal and 0.5 normal sodium hydroxide at 4°C, which is that more than 90% of initial capacity was retained after 100 cycles of cleaning in both conditions. This high alkaline stability provides extended resin lifetime over repeated cleaning of the column

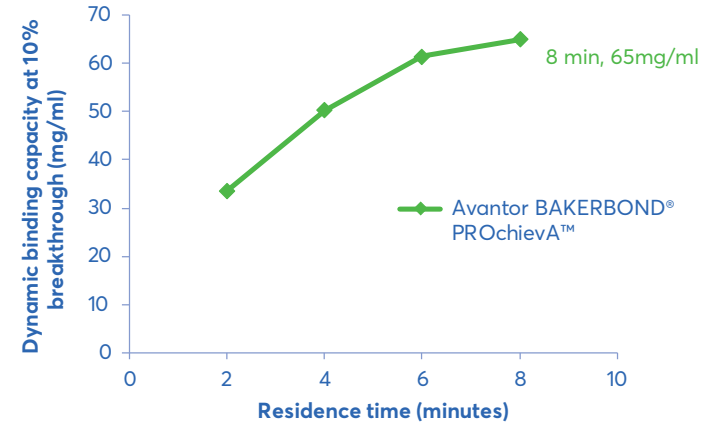


FIGURE 1: Dynamic binding capacity of BAKERBOND® PROchievA™

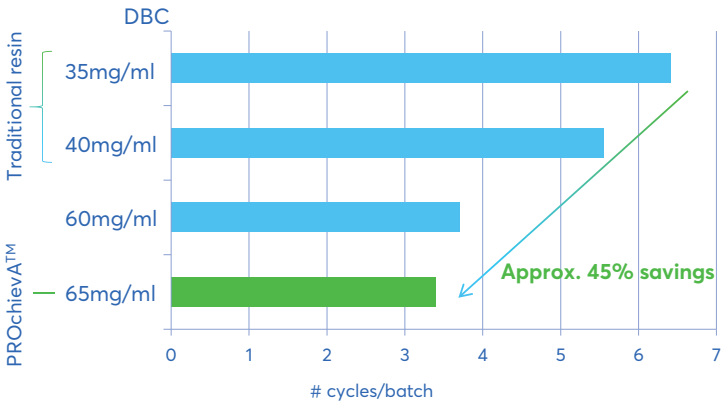


FIGURE 2: Effect of DBC on theoretical number of cycles per batch

with sodium hydroxide. It also reduces the process costs from replacing the resin, allows for better cleaning of the column, and reduces bioburden.

Based on the data in Figure 3, a resin with enhanced alkaline stability can give a lifetime of more than 100 cycles and, in the case of BAKERBOND® PROchievA™, can be used for more than 200 cycles with 25% of load safety factor.

In addition, since sodium hydroxide decreases the lifetime of a resin, Avantor screened different additives for cleaning, such as sucrose, ethylene glycol, and propylene glycol, to determine their impact on BAKERBOND® PROchievA™. The results indicated only a 10% loss in capacity compared to the 20% loss when using sodium hydroxide, even after 200 cycles.

EFFICIENT IMPURITY REMOVAL

The final parameter to consider for the protein A capture step is the efficiency of removing process related impurities, such as host cell protein (HCP), host cell DNA (HCDNA) and virus. The impurity removal test is done by purifying a model IgG1 produced from CHO cell and analyzing the impurity concentration of load sample and elution pool. Figure 4 shows the log reduction value of each impurity from a capture process using BAKERBOND® PROchievA™ resin.

The data with IgG1 shows high log reduction value of each impurity which reduces the impurity load on the subsequent polishing steps.

CAPTURE AND PURIFICATION OF FC FUSION PROTEINS

Fc fusion proteins are an emerging area of therapeutics where the Fc region of an antibody is fused to a protein of interest, creating a chimeric molecule. A frequent challenge in Fc fusion protein purification is the higher level of high and low molecular weight contaminants compared to traditional monoclonal antibody purification. Avantor assessed the performance of BAKERBOND® PROchievA™ with two unique Fc fusion proteins (Figure 5), where a 20% higher level of monomeric target protein purity based on SEC HPLC analysis was observed when compared to a currently available chromatography resin. A purity level of, for example, 90% compared to one with 70% will ultimately result in less stress on subsequent purification steps. Our team attributes this improvement to a specific affinity of our unique ligand to the Fc region.

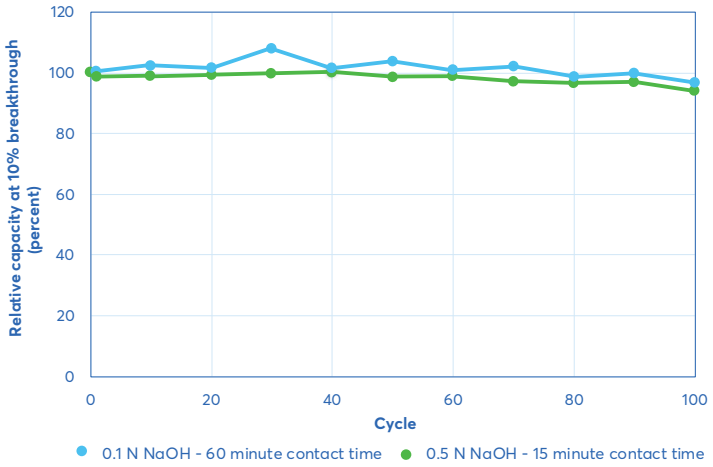
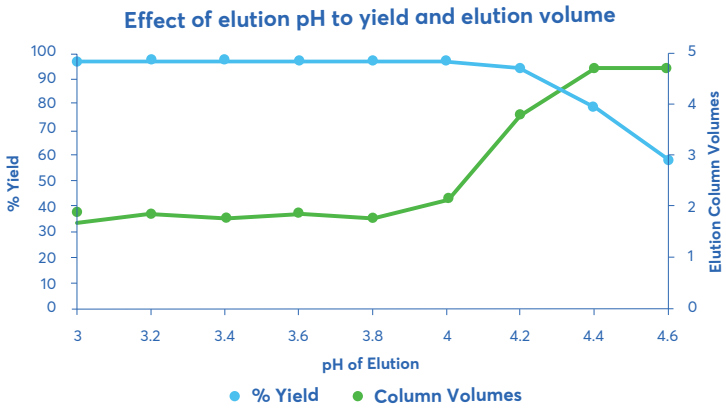


FIGURE 3: Resin life of PROchievA. All testing conducted at 2°C to 8°C.



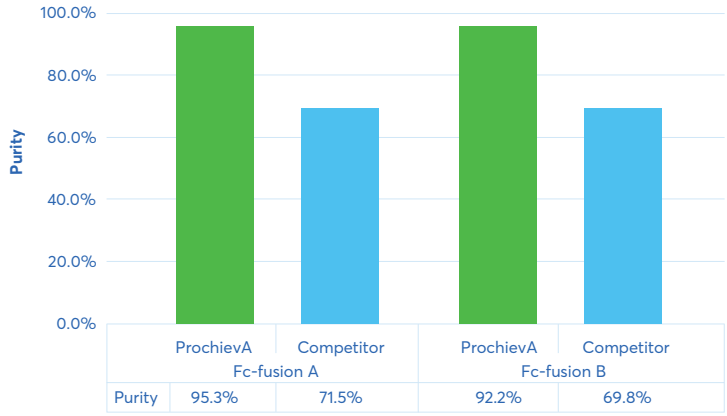


FIGURE 5: Fc-fusion protein purification using PROchievA and commercially available protein A resin.

A COMPLETE DOWNSTREAM SOLUTION PROVIDER

Avantor understands that improving efficiency in order to reduce costs and bring life-changing therapies to market faster requires continual advances in bioprocessing productivity. That is why, in addition to the benefits of using BAKERBOND® PROchievA™, Avantor experts can assist in optimizing customer specific downstream processes by screening resins, buffers, and additives to customize a solution most appropriate for each customer's product and its unique needs.

As a trusted global partner in the life sciences, advanced technology, and applied materials industry, Avantor enables breakthroughs in life-changing biologics. We possess an extensive portfolio of cGMP production chemicals, excipients, and chromatography solutions, single-use and fluid-handling solution, and cell culture media as well as services. Our goal is to help biopharmaceutical manufacturers streamline their processes, improve their yields, and ultimately reach the market faster with novel therapies that help make a better world.

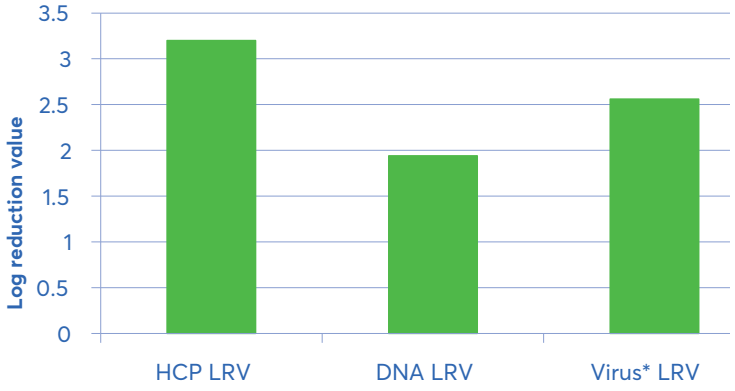


FIGURE 4: Impurity removal rate of PROchievA

ABOUT THE AUTHORS

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In her current role, Jungmin leads product and process development projects with multiple biopharmaceutical industry partners, including customised product development for cell and gene therapy customers. She holds a MS and Ph.D. in chemical engineering, specialising in the optimisation of a continuous chromatography system.

Jonathan Fura
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In his current role, Jonathan focuses on developing technologies that are utilised to advance the development and manufacturing of antibody-based biopharmaceuticals and diagnostics. Jonathan holds a Ph.D. in chemistry with a focus on developing immunomodulation therapeutic strategies.

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