Astrea Bioseparations

Technical Note

HCPure™ host cell protein clearance resin: Mixed mode chromatography as a means of process intensification

Introduction

Since their introduction in the 1980s, many biotherapeutics have gone on to become blockbuster drugs. And now, thanks to advancements in biotechnology, new diseases that have previously been untreatable are being treated through a range of organism-derived recombinant therapeutics.

While the biotherapeutic industry began by using *E. coli* cells to express therapeutic proteins, there is an increased need to develop new ways to deliver therapeutics and identify the organisms that can express them. This broadening of potential therapeutic materials has led to many new modalities including bi-specifics and antibody drug conjugates. Development of these new modalities has highlighted the need to improve processes to respond to increasing demand, complexity, and competition.

To help meet industry demands, many companies are looking towards intensification methods to improve their existing processes. Bioprocess intensification looks at all aspects of the manufacturing process, and aims to find ways to increase productivity, decrease timelines, and reduce the cost of goods. Specific areas that intensification looks to improve include increasing production through changes in technology, or reducing energy consumption, OPEX, or CAPEX. Bioprocess intensification steps are typically significant changes to either the equipment or process, and in this paper, we will discuss how moving to single step mixed mode chromatography from a traditional two-step polish can reduce both OPEX and CAPEX by reducing material and labor costs while processing samples faster

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Review of current processes

The most common method of producing biotherapeutics is by expression in different cell systems. After cell harvest, downstream processes for therapeutic purification typically utilize an affinity chromatography step which involves a ligand with specific affinity to capture the target molecule. The eluate from the capture column is then further purified using polishing steps, often including multiple ion exchange (IEX) and hydrophobic interaction chromatography (HIC) steps. These different polishing steps are necessary to purify the target therapeutic from upstream impurities including host cell proteins, host cell DNA, ligand leachates, endotoxin, high molecular weight aggregates and fragments, among others.

Anion exchange chromatography is often used as the step following affinity chromatography to remove many impurities. While able to bind many different types of contaminants, ion exchange chromatography polishing is not always sufficient to remove all contaminants. In these cases, additional polishing steps, typically incorporating hydrophobic interaction chromatography (HIC) are required. However, HIC chromatography also offers limited selectivity due to the generalized nature of the binding interaction, which can contribute to poor overall yields.

Differences in binding interactions means that process conditions, such as salt content and pH, must be adjusted between steps. This can result in additional equipment, buffers, and labor hours to ensure that the product is ready for each stage. As an example, if we look at a typical Monoclonal antibody (mAb) workflow, Protein A is the capture step followed by IEX and HIC. However, because of the conditions required for IEX protein binding and the typically higher conductivity of Protein A eluate, the solution requires dilution before loading onto the IEX column. This poses a significant issue at manufacturing scales as the requirement to dilute Protein A eluates before IEX creates a labor bottle neck, significant buffer requirements and the need for large volume hold tanks. After IEX, the samples must then be concentrated to increase the salt content to utilize the hydrophobic regions of the HIC resin.

How can I intensify my downstream processes?

In a typical 3 step process there is ample opportunity to intensify the polishing steps. As we saw in the example workflow, after affinity chromatography, there are several dilution steps, chromatography steps and concentration steps. Each step in the process requires its own buffers, columns, resins, and time associated with packing and using the column.

There is a simple and easy way to reduce the time, costs, and equipment needed to polish a biotherapeutic, and that is to replace the two-step polishing train with a single mixed mode chromatography step. Whereas other forms of chromatography use only one interaction type to bind molecules, such as affinity, ion exchange or HIC, mixed mode chromatography utilizes multiple interactions. The most widely used is a combination of ion exchange and hydrophobic interactions. Unlike the previous workflow, where there were discrete steps for each purification type, using a mixed mode adsorbent allows for increased separation power by utilizing both interactions simultaneously without having to undergo the different manipulations in between each step.

How can Astrea Bioseparations help intensify downstream processes?

Astrea Bioseparations has designed and developed a resin specifically to remove host cell proteins from a range of starting feedstocks, the new HCPure™ host cell protein clearance resin. Utilizing mixed mode purification techniques, Astrea Bioseparations delivers a solution that offers several advantages over traditional workflows.

By using a mixed mode approach to purifying the primary capture eluate, multiple sample conditions and process parameters can be considered. HCPure's™ mixed mode binding interactions eliminate the need to dilute the high conductivity Protein A eluate, saving time and costs. Additionally, mixed mode demonstrates a higher specificity than HIC polishing, allowing for the removal of high molecular weight aggregates without decreasing yield.

Because therapeutic proteins are produced from a variety of starting feedstocks and conditions, HCPure™ host cell protein clearance resin has been specifically developed to be the foundation of a platform technology, achieving highly purified end products with minimal process adjustments. This eye towards flexibility and high purity allows the HCPure™ resin to produce a clean end-product from a range of starting feedstocks.

HCPure™ helps to intensify Protein A based workflows.

HCPure™ allows for increased selectivity by utilizing both hydrophobic and hydrogen bonding interactions simultaneously. Unlike many mixed mode resins available, the primary interaction of HCPure™ is through hydrophobic interaction, with hydrogen bonding being secondary. This puts HCPure™ at an advantage over other mixed mode resins and traditional ion exchangers because of its capability to retain functionality at relatively high conductivity load conditions compared to ion exchange polishing steps.

To demonstrate the ability of HCPure™ mixed mode chromatography resin to replace a traditional two step polishing train, CHO cells were used to express IgG before being purified using Protein A. The traditional two step polish discussed earlier was replaced by a single step using HCPure™ mixed mode chromatography adsorbent.

Neutralized protein A elutes containing CHO IgG samples were passed through HCPure™ across a range of pH and conductivities including the protein A elute with no adjustments (pH 6, 12 mS/cm). The host cell protein concentration and IgG recovery was determined. The results showed an average of 84% reduction in HCP as well as an average IgG recovery of 87%. The high purity and yields obtained across a wide range of pH's and conductivities, demonstrate HCPure™ mixed mode chromatography's ability to achieve the performance of a traditional three step process while being robust enough to tolerate a wide range of conditions. Furthermore, the ability to use the post protein A elute with no pH or conductivity adjustments offers time and cost saving benefits.

Conditions	IgG recovery (%)	HCP reduction (%)
pH 4, 8 mS/cm	84	87
pH 4, 18 ms/cm	85	90
pH 6.5, 13 ms/cm	86	86
pH 6.5, 13 ms/cm	85	86
pH 6.5, 13 ms/cm	86	84
pH 9, 8 ms/cm	89	74
pH 9, 18 ms/cm	92	84
pH 6, 12 ms/cm	89	78

Figure 1 - HCPure™ was able to achieve 74 - 90% reduction in HCP and 84 - 92% IgG recovery across a range of pH and conductivity conditions, demonstrating effectiveness as a single polishing step. The pH 6 12 mS/cm condition represents the neutralized protein A eluate with no further buffer adjustments, offering good HCP removal without a buffer exchange step requirement.

HCPure™ helps to intensify non-Protein A based workflows.

New biotherapeutics can be bi-specific antibodies, antibody drug conjugates or antibody fragments. These biomolecules do not have the Fc region that binds to Protein A, and as such require a different capture method; in many cases Protein L is chosen because it binds to the kappa light chains on the antibody.

Despite differences in binding sites, samples purified using Protein L typically use the same two step polishing train as Protein A eluates, providing an opportunity for process intensification. To demonstrate the non-Protein A workflow can also be improved by bioprocess intensification, *E. coli* cells were used to express the antibody fragment V_k before being purified using Protein L.

After elution from the Protein L column, the samples were split and then polished using either a single HCPure™ step or a two-step polish of Q PuraBead® HF followed by HCPure™. The two different polishing trains were then compared for host cell protein clearance, host cell DNA removal and V_k yield. Comparison of the two and three step polishes showed that both effectively cleared both HCP and HCDNA while maintaining high yields.

Fraction	% PPM Reduction	% HCP bound	% DNA bound	% Vk bound	% Vk yield
HCPure™ (2-step)	99	99	91	1.2	98.8
Q & HCPure™ (3-step)	99	99	92	0.8	99.2

Table 3- Comparison of 2 and 3 step processes using HCPure™ after Protein L

Conclusion

By utilizing the principles of bioprocess intensification, significant savings are achieved by combining the typical two-step polishing process into a single step using mixed mode chromatography. Converting to a single step creates significant material cost savings associated with purchasing and maintaining additional columns, resins, and buffers required for operation. Elimination of additional processing steps brings products through the manufacturing process faster and eliminates the labor costs associated with additional column packing and sample processing.

Astrea Bioseparations' HCPure™ host cell protein clearance resin is a mixed mode chromatography resin that can be incorporated into a wide range of workflows. By being flexible enough to be effective, independent of starting feedstock or capture method, HCPure™ can be the foundation of an intensified process that can cost effectively bring products to market faster.

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