Overcoming Purification Challenges In Antibody Therapeutics Manufacturing

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Abstract

Complex alternative formats derived from conventional antibody structures, including Fab fragments, Fc-fusion proteins, and bispecific formats, are introducing new challenges into the antibody therapeutics manufacturing workflow. To overcome specific purification challenges associated with these molecules, antibody-subdomain-specific affinity chromatography resins have been developed for a wide range of these novel antibody formats. Complementing affinity capture resins with efficient polishing resins results in highly productive process-scale purification of biologic drugs at increased yields, allowing reduced time to market and a decrease in the overall cost of goods.

The landscape is changing for antibody-derived therapeutics

In the 35 years since the first monoclonal, antibody engineering has evolved dramatically and novel formats have been developed. Among the antibody therapies approved, there are now 100 FDA-approved monoclonal antibodies, a class that accounts for nearly one-fifth of all drugs approved each year (Mullard 2021).

These monoclonal antibodies, which are expressed in living cells, need to be purified to isolate the target from impurities, including host cell proteins (HCP), residual DNA, and product-related impurities co-expressed with the product or formed throughout the manufacturing process. Traditional non-affinity, multi-step purification processes have limitations for monoclonal antibody development; they can be lengthy, expensive, and result in low yields. Affinity chromatography is a successful technique for purifying biomolecules, solving the yield loss and purity challenges seen with non-affinity purification processes.

Affinity chromatography resins selectively capture the molecule of interest. The streamlined workflow, when combined with extremely effective polish steps, has numerous advantages, including:

- Fewer purification steps
- Greater product yield
- Shorter bioprocess development time
- Lower overall cost of goods
- Increased speed to market

Protein A affinity resins are the most commonly used resins in monoclonal antibody manufacturing. However, since protein A binds at the CH2-CH3 interface of the Fc region of IgG, these resins don't work for all antibody formats, including the more complex alternative formats such as Fab fragments, Fc-fusion proteins, and bispecifics, currently in either preclinical or clinical trials (Spiess *et al.* 2015). Often these biomolecules have an

altered or altogether missing Fc region, rendering the use of protein A purification inefficient or obsolete. As well, the acidic elution used during protein A chromatography can lead to aggregation of bispecific antibodies and Fc-fusion proteins and make the removal of product-related impurities more difficult.

Recent developments in affinity capture resins and polish solutions that remove impurities allow commercial production of novel antibody therapeutics that are proving valuable for the treatment of cancer and other conditions.

CaptureSelect affinity resin technology

This platform is derived from the unique heavy chain-only antibodies—also known as single domain antibodies or V_HH—found in the Camelidae family (Figure 1). The specificity and affinity of V_HHs can be tuned during the affinity ligand development process and these ligands are inherently stable, making them suitable for use in chromatography. Production in yeast makes the final chromatography resin animal-origin free (AOF) allowing use of the resins in clinical and commercial manufacturing.

The CaptureSelect™ technology is used to develop purification resins covering a wide range of therapeutic areas such as gene therapy, vaccines, and antibody therapeutics.

A wide array of affinity resins to capture many antibody formats

Thermo Fisher Scientific has a complete range of affinity resins that bind to different regions of an antibody thereby offering an effective affinity capture step for a wide range of antibody formats. These affinity resins are specific to human antibodies and do not cross react with bovine antibodies.

CaptureSelect CH1-XL

The CH1-XL affinity resin targets the CH1 domain (Figure 2, top right). It provides a high-purity one-step purification of correctly assembled kappa and lambda Fab fragments because there is no binding to free light chains, which can be over-expressed during production of Fab fragments. The resin has a high dynamic binding capacity and efficient elution at mild pH.

CaptureSelect FcXP

Unlike protein A, which binds to the interface of the CH2-CH3 domains, FcXP binds to the CH3 domain of the Fc region of IgG, thereby making this resin suitable for antibodies with an altered or absent Fc region (Figure 2, bottom right). Elution at mild pH 4.0–4.5 makes the resin very suitable for pH sensitive molecules such as Fc-fusion proteins.

CaptureSelect KappaXP and CaptureSelect LambdaXP resins

KappaXP and LambdaXP were developed for purification of all Fab fragments and bispecifics containing either a kappa or a lambda light chain (Figure 2, middle right). They have high dynamic binding capacity (Figure 3) and a large elution operating space that allows mild purification and elution for the target molecule. When light chains and light chain dimers are a challenge that cannot be solved in upstream development, CH1-XL is a better choice than Kappa or LambdaXP.

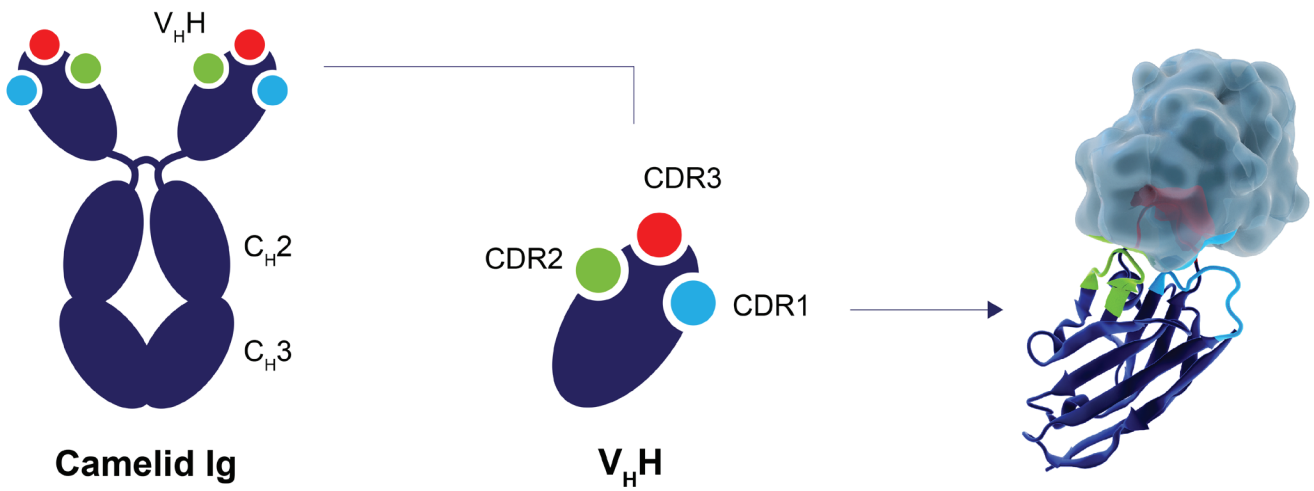

Figure 1. CaptureSelect ligands are derived from heavy chain antibodies. Left: A Camelidae heavy chain antibody. Middle: The V_HH portion, recombinantly expressed in yeast, is used in the CaptureSelect platform and is ideal due to its small size and robustness. Right: The CaptureSelect affinity ligand binds its target protein with great affinity and selectivity.

1. CaptureSelect FcXL was the precursor of CaptureSelect FcXP.

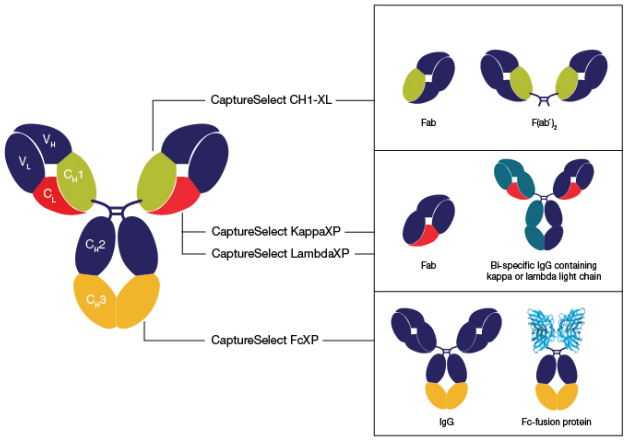

Figure 2. Representation of the different antibody binding sites of all four CaptureSelect resins. Each resin binds its own unique region on the antibody molecule.

CaptureSelect LambdaXP allows complete coverage of lambda sub-types and shows no cross reaction to kappa light chains. With this latest addition to the CaptureSelect antibody purification portfolio, manufacturers now have full coverage for all domains on the antibody. As with all these affinity resins, it is scalable and is AOF.

Case Study

Case study 1: Fab fragment purification using CaptureSelect CH1-XL

CH1-XL was used as an affinity product to purify ranibizumab (a Fab fragment) from HEK293 cells (Detmers *et al.* 2019). Despite the dirty feedstock load, flowthrough showed one elution band corresponding to an 86% yield of 98% pure Fab in a single step process (Figure 4). The lab chip analysis demonstrated all light chains and light chain dimers were removed from the feed material during the wash.

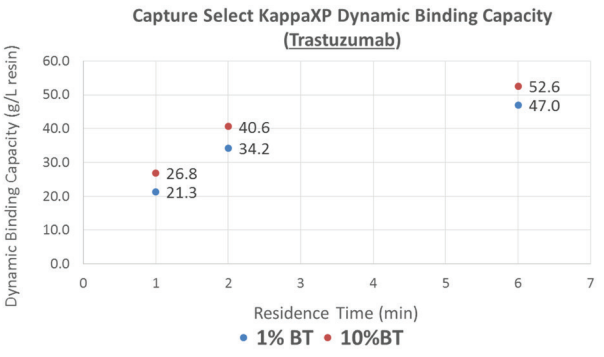

Figure 3. Dynamic binding capacity of KappaXP measured at 1% and 10% breakthrough using a recombinant humanized IgG1 monoclonal antibody (Trastuzumab) feed.

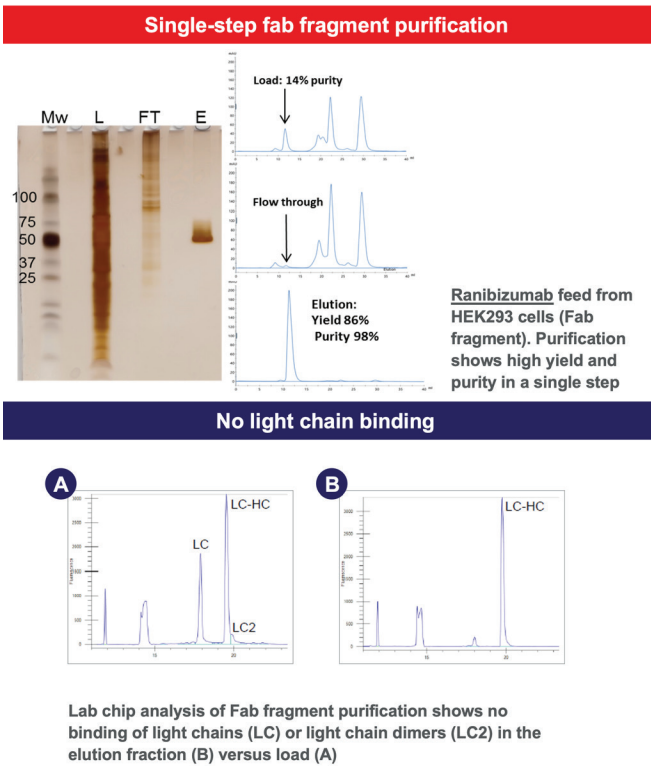

Figure 4. Purification of ranibizumab from HEK293 cells using CaptureSelect CH1-XL resin. Left: A single-step Fab fragment purification run showing the flow through fraction (FT) and the elution (E). Right: Lab chip analysis of Fab fragment purification with peaks corresponding to light chains (LC) and light chain dimers (LC2) in the elution fraction (B) versus load (A).

Case study 2: Rapid implementation of CaptureSelect FcXL resin for a novel antibody format

A large pharmaceutical customer was looking for an effective purification solution for a novel antibody format to replace its existing process (Detmers *et al.* 2019). The new molecule's Fc region had been altered, resulting in poor protein A binding and the company didn't have a suitable alternative affinity resin qualified as a GMP-compliant raw material. The customer was looking for a process to overcome these limitations, improving efficiency and yield while fitting into its commercial next-generation manufacturing facility.

The existing process used an affinity resin followed by three polish steps. The affinity resin had many disadvantages, including low binding capacity, low pH elution that affected product stability,

elevated fragment levels, and an environmentally unfriendly regeneration solution.

Screening for a more efficient capture resin

Three affinity resins, including CaptureSelect FcXL,[1] and one non-affinity resin were compared to the existing affinity resin under scaled-up conditions (Figure 5). CaptureSelect FcXL showed low levels of both high-molecular-weight (HMW) and low-molecular-weight (LMW) impurities compared to the other test resins and the existing process. It also exhibited a high load density and a comparable yield to the existing process. While the non-affinity resin had very high load density and good removal of HMW and LMW impurities, its host cell protein reduction and yield were too low to be an effective alternative to the existing process.

CaptureSelect FcXL was selected because it scored best on all of the necessary attributes.

Improved manufacturing process

	Existing Affinity	Affinity B	Affinity C	Non-Affinity	Capture Select FcXL
Load Density	med	low	med	very high	high
CHOP Reduction	high	high	very high	low	med
Yield %	high	high	low	low	high
Sum HMWs rel%	med	med	med	med	med
Main Peak rel%	med	med	med	high	high
Sum LMWs rel%	high	high	high	med	low

Figure 5. Comparison of purification criteria for various capture resins including client's existing affinity resin. CaptureSelect FcXL led to increased purity as shown by yield, Main Peak relative percentage and lower levels of low molecular weight (LMW) impurities compared to all other resins tested. CHOP is Chinese Hamster Ovary Protein.

The next step was to qualify the FcXL resin as a raw material to be used in the customer's cGMP manufacturing. The campaign results showed high consistency between development, pilot, and GMP scale-ups (Figure 6). The yield and HCPs in pool were consistent going from the pilot plant to the GMP runs. Introducing FcXL resin simplified the purification process and removed one polish step. FcXL had a higher binding capacity, resulting in a better facility fit, an improved impurity profile with virtually no LMW components, and better pool stability because the elution asset was milder (pH 4.2). The data demonstrated excellent scalability and an environmentally friendly regeneration solution.

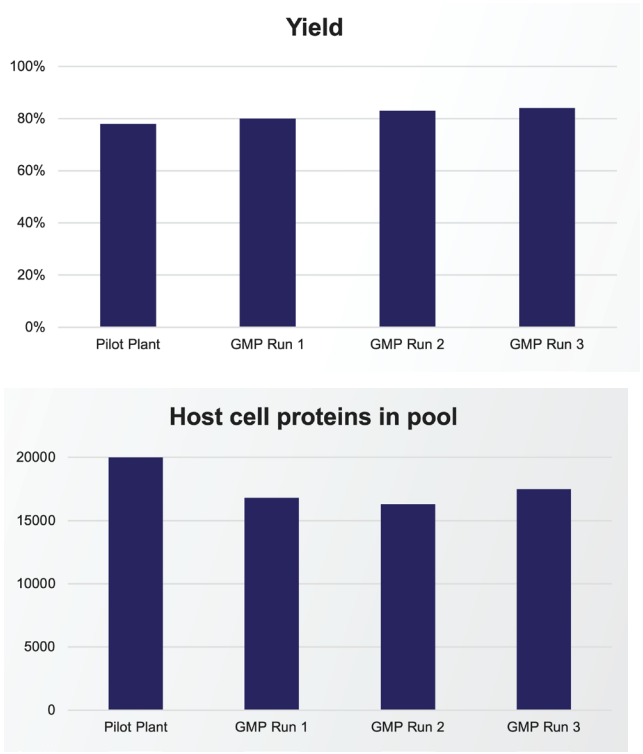

Figure 6. GMP manufacturing campaign results with FcXL resin.

Removing impurities with a range of polish resins

Process-related impurities, including HCPs, nucleic acids, aggregates, and other product-related impurities need to be removed to levels that ensure adherence to regulatory guidelines. Polish solutions to remove impurities during downstream purification come in a range of formats (Figure 7). These include anion and cation exchange resins, as well as a line of products that take advantage of hydrophobic interaction chromatography (HIC).

POROS™ resins exhibit high capacity and have excellent resolution properties independent of flow rate (Figure 8). The rigid bead structure results in linear and predictable performance at any scale and allows for stringent cleaning when needed. In addition, the beads have large throughpores leading to a reduced mass transfer resistance. The smaller size of the beads results in excellent resolution properties, making these resins extremely suitable for the separation of closely related product forms. POROS resins can be used at higher flow rates, thereby increasing process throughput and overall productivity.

Antibody-drug conjugate purification using POROS HIC resins in bind/elute mode

Antibody-drug conjugates (ADC) are another expanding class of antibody-derived therapeutics. As the drug-antibody ratio (DAR) of an ADC can alter efficacy (Hamblett et al. 2004) and pharmacokinetics (Sun et al. 2017), it is essential to purify the ADC with the ideal DAR.

POROS Resin	Type of AEX Resin	AEX Applications
D50	Weak	Bind/Elute: Protein, virus, plasmid DNA purification
PI50	Weak	
HQ50	Strong	Flow Through: Trace impurity removal by binding impurities (DNA, viruses, HCP, aggregates, endotoxin)
XQ	Strong	
POROS Resin	**Type of CEX Resin**	**CEX Applications**
HS 50	Strong	Bind/Elute: Polish of many biomolecules (mabs, vLP/viruses, fusion proteins, high pI rProteins
XS	Strong	Flow Through: Polish for Mabs by binding impurities under normal B/E conditions: Impurity removal (aggregates, HCP, DNA, viruses)
POROS Resin	**Level of hydrophobicity**	**HIC Applications**
Ethyl	Moderate	Bind/elute mode of moderately to considerably hydrophobic molecules
Benzyl	Medium	Bind/elute or flow-through mode depending on molecule
Benzyl Ultra	High	Flow-through mode in lower salt to bind impurities such as aggregates

Figure 7. Overview of all POROS Ion Exchange and HIC resins.

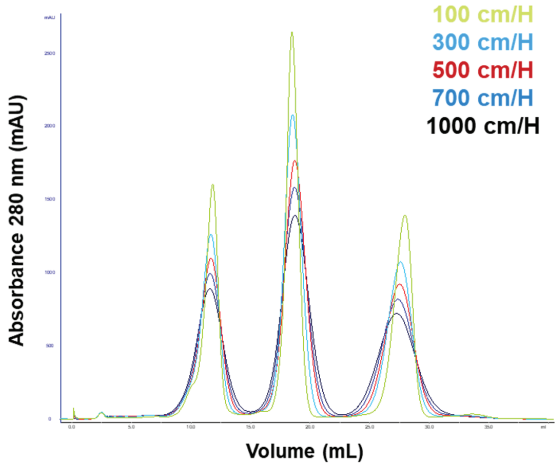

Figure 8. POROS resins show excellent resolution properties independent of flow rate.

POROS HIC resins were compared directly to alternative resins for their ability to resolve ADCs based on their DAR. Alternative resins showed little to no resolution between ADCs with DAR 0, 1, and 2 (Figure 9, top). The butyl-based resin showed poor resolution (top left) and the ethyl-based resin showed no resolution at all (top right).

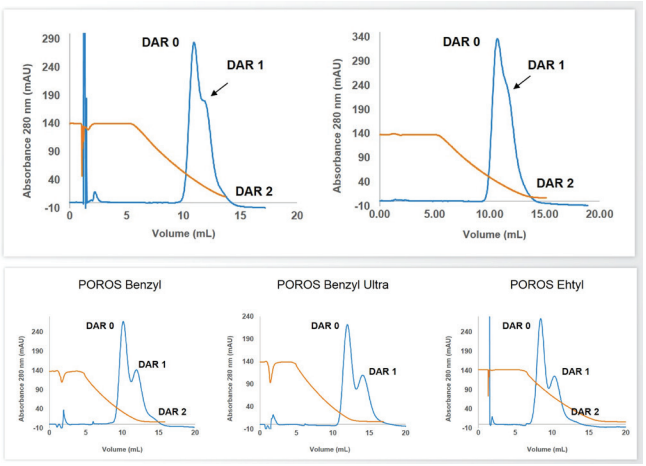

Figure 9. Performance of various HIC resins to selectively purify ADCs based on DAR. Comparison of alternative resins (top) with three POROS HIC resins (bottom). Top left: Butyl-based resin. Top right: Phenyl-based alternative resin. Bottom from left: POROS Benzyl, Benzyl Ultra, and Ethyl resins.

Alternatively, all three POROS HIC resins have excellent resolution properties, with POROS Ethyl being the best of the three (Figure 9, bottom).

This case study demonstrates the applicability of POROS HIC resins for the selective purification of ADCs containing the most effective DAR.

Host cell protein removal with affinity polish resins

An efficient means of removing impurities is essential and there are several difficult-to-remove HCPs from CHO-produced monoclonal and bispecific antibodies (Singh *et al.* 2019). These include subsets of product-related HCPs, expression-related HCPs, and HCPs that co-elute with the target molecule. There are a few HCPs that combine all these attributes and these are the ones that are notoriously difficult to remove, one of which is clusterin.

A CaptureSelect affinity ligand specific to clusterin was developed and coupled to the POROS base bead, creating a scavenging resin to remove this specific HCP from the final product. The resin is designed to be used in flowthrough mode, leaving the impurity in the column while the product flows through. Customer feedback demonstrated that the POROS CaptureSelect ClusterinClear affinity matrix removed more than 95% of the clusterin in a single pass, along with more than 65% of the other top HCPs.

Given the success of this resin, it can be used as an add-on to conventional polish steps.

Conclusion

For complex alternative antibody formats, including those that do not bind protein A, there are efficient affinity purification technologies

providing high purity and yield in a single capture step. Combining these with a POROS polish can result in a reduction of process steps or progress from a bind/elute to a flowthrough polish process. CaptureSelect resins are available as pre-packed process scale columns that can be used in cGMP processes. All of the products in this white paper are seamlessly scalable for manufacturing purposes.

These capture and polish affinity resins should help solve the challenges of purifying novel antibodies that are transforming the therapeutic landscape.

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