Chromatography Advisor #3

Economic Benefits of Protein A Alternatives

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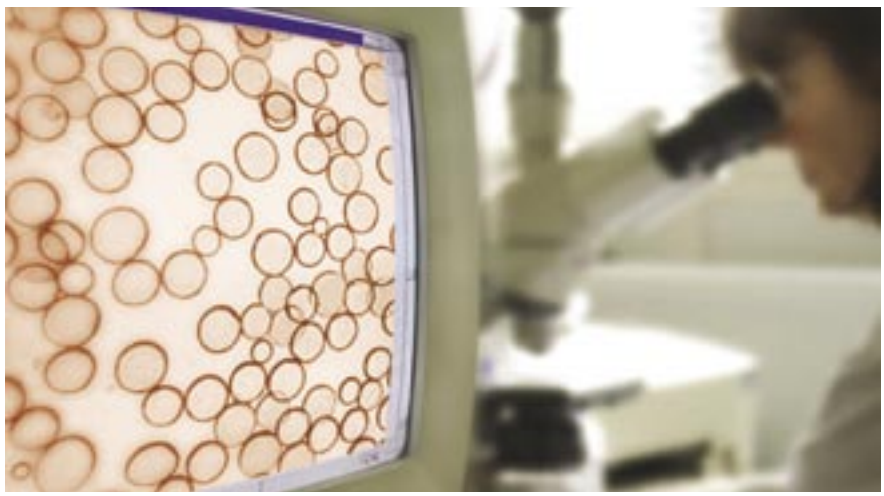
Affinity chromatography (AC) on protein A–based sorbents has been used for more than a generation by drug manufacturers to purify protein therapeutics. AC is based on the ability of a protein or other biopolymer to bind with a natural or synthetic ligand. When used in conjunction with other chromatography steps, it provides an effective way of isolating desired antibodies for downstream drug production.

It's no secret, however, that protein A–based sorbents are expensive, a fact that puts pressure on manufacturing to use such sorbents as efficiently as possible to keep overall production costs under control. The expense is compounded by other factors that limit the usefulness of protein A–based sorbents. They have low cleaning tolerances compared with chemical-ligand (e.g., ion-exchange) sorbents, and the leaching of protein A fragments during antibody purification remains a significant problem despite attempts to improve engineering.



Pall Corporation (www.pall.com)

Chemically Stable IgG-Selective Sorbents

A different purification approach using two novel sorbents is proving to be an effective alternative for some manufacturers. Both sorbents share structural and functional features, but they operate by different chromatographic modes. Carrying a mercaptoethyl-pyridine ligand, MEP HyperCel operates by hydrophobic charge induction chromatography (HCIC). It captures antibodies through a mild hydrophobic interaction and affinity interaction with the immunoglobulin-selective ligand. Carrying a mercaptobenzimidazole-sulfonic acid ligand, MBI HyperCel operates by a "mixed-mode" chromatography, capturing antibodies through ionic interaction and molecular recognition on the immunoglobulin-selective ligand. In some applications, the two sorbents can be used effectively in tandem. MEP sorbent was introduced to the market in 1999 and was issued a drug master file number in 2001. MBI sorbent was released commercially in 2004.

Antibody desorption results from a charge repulsion induced by reduction (for MEP ligands) or elevation (for MBI ligands) in the eluant buffer pH. For MEP sorbents, antibody binding is achieved with a neutral pH, and desorption is possible by decreasing the pH to a relatively mild 4.0–5.5, depending on the pI (isoelectric point) of the antibody. Because this pH is mild, it reduces aggregate formation and preserves antibody activity. MBI ligands have been engineered for higher pH values, which is useful for antibodies that are sensitive to acidic pH values below 5. The alternative ligand structure can also provide useful, alternative selectivity. Figure 1 illustrates the adsorption and desorption mechanisms.

In most applications, antibody binding with these ligands is independent of salt concentration, suggesting that typical feedstocks can be loaded onto MEP and MBI sorbents without extensive dilution or diafiltration. For nonantibody proteins, MEP HyperCel may serve as a useful alternative to traditional hydrophobic interaction chromatography (HIC). In such applications, binding on MEP HyperCel is typically achieved at significantly lower salt concentrations than those required with traditional HIC sorbents.

Product Focus: Proteins (antibodies)

Process Focus: Purification

Who Should Read: Production and development

Keywords: Chromatography, sorbents, ligands, process economics, cleaning agents

Level: Intermediate

Increased Service Lifetime

By some estimates, MEP and MBI ligands offer three to five times longer service life compared with protein A–based sorbents. The reason for that is the linkage chemistry of protein A. When exposed to protease-containing feedstocks and caustic cleaning agents, protein A sorbents lose their stability. Cleaning validation protocols therefore must be carefully adjusted to extend the service lifetimes of such resins, and analytical tests must be performed to monitor and quantify the levels of protein A fragments that are leached. Those fragments also must be effectively removed from product streams.

Similar to the ligands used for ion-exchange sorbents, MEP and MBI sorbents are chemical ligands that remain stable at pH values up to 14, and they can be cleaned regularly with sodium hydroxide solutions at 1M concentrations. They can withstand biological feedstocks and the NaOH solutions typically used for clean-in-place (CIP) procedures. They also eliminate any danger of protein A leakage. These factors add up to considerable cost-savings and ease-of-use for antibody production.

Broad Use

MEP-based sorbents have proven effective for purifying a wide range of biological streams. Although they capture IgG directly from mammalian cell culture supernatants at 95% purity, they are also effective for more challenging feedstocks that contain more impurity proteins, including albumin. Unlike protein A, they enable binding for IgG variants from different species. Human, rat, goat, sheep, mouse, and even murine IgG_1 antibodies can be effectively purified with MEP-based sorbents. Table 1 provides examples of purification rates. When isolating antibody from albumin-containing feedstocks, the IgG fraction isolated on MEP HyperCel can be freed of residual albumin impurity (the principal impurity in examples below) by anion-exchange chromatography on DEAE ceramic HyperD.

MBI-based sorbents also effectively bind human IgG without salt, enabling them to be used at physiological ionic strength to achieve optimal binding with only a minor adjustment of pH. Because binding is possible in an alkaline solution for desorption, acidic-sensitive IgGs can preserve their biological activity.

Viral clearance also has been studied with these sorbents, with results similar to protein A. In one example, MEP sorbent was used with minute virus of mice (MVM) in the presence of IgG. A viral clearance reduction of ~4 log was reported, equivalent to that of the viral clearance performance of protein A in the same test. When combined with other orthogonal steps (for example ion-exchange), these sorbents can remove virus and host-cell protein at levels similar to protein A, compatible with the requirements for therapeutic-grade IgG purity.

The introduction and use of MEP and MBI sorbents in the marketplace has clearly added new options for manufacturers seeking alternatives to protein A. These products add to a growing list of options and strategies aimed at helping to keep drug processing costs in check.

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Table 1: Purification examples for MEP-based sorbents

	Protein	IgG content	IgG initial purity	Final IgG purity	Recovered IgG	Overall IgG yield	Purification factor
Feedstock	Mg	Mg	%	%	Mg	%	
CCS with FBS	1740	30	1.7	69	23	76	40
Protein-free CCS	330	17	5	99	17	99	19
Bovine serum	440	121	28	75	105	87	3
Ascites fluid	55	9	16	83	7	79	5

Figure 1: Adsorption and desorption — a neutral or mildly acidic pH at ionic strength typical of cell culture supernatant is used to capture antibodies with MEP and MBI sorbents; desorption occurs by modulating the pH.