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Chromatography Advisor # 4

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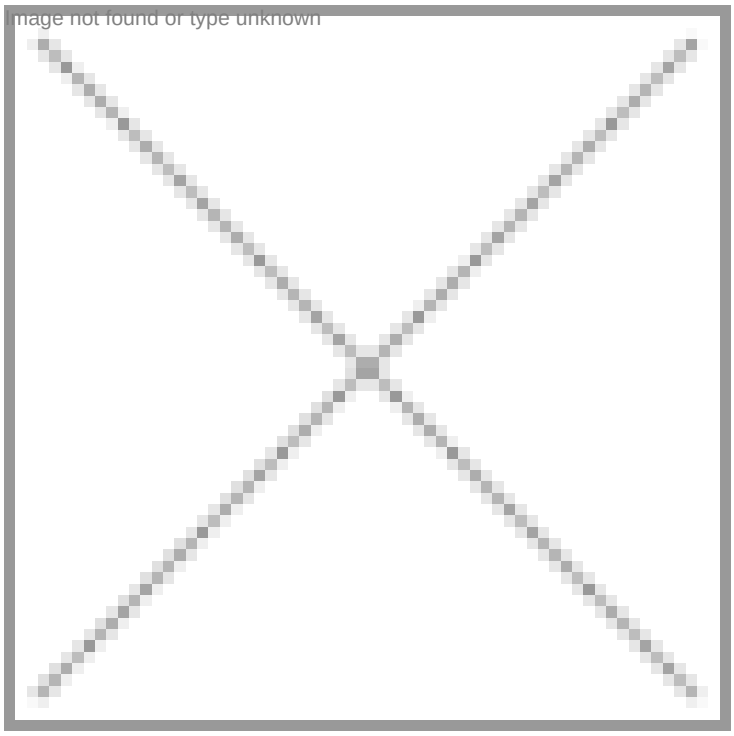

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Economic benefits of Protein A alternatives

Affinity-based chromatography using Protein A-type sorbents is the "industry standard" for purifying most antibody therapies today. There are several economic challenges with this approach; however Protein A is expensive, has low cleaning tolerances, and a limited lifetime compared to other sorbents. Additionally, elution steps often require highly acidic conditions that can result in substantial antibody aggregation. Several novel chromatography sorbents have proven to be efficient and more cost-effective alternatives to traditional Protein A, and they are giving drug manufacturers some new options to consider.

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

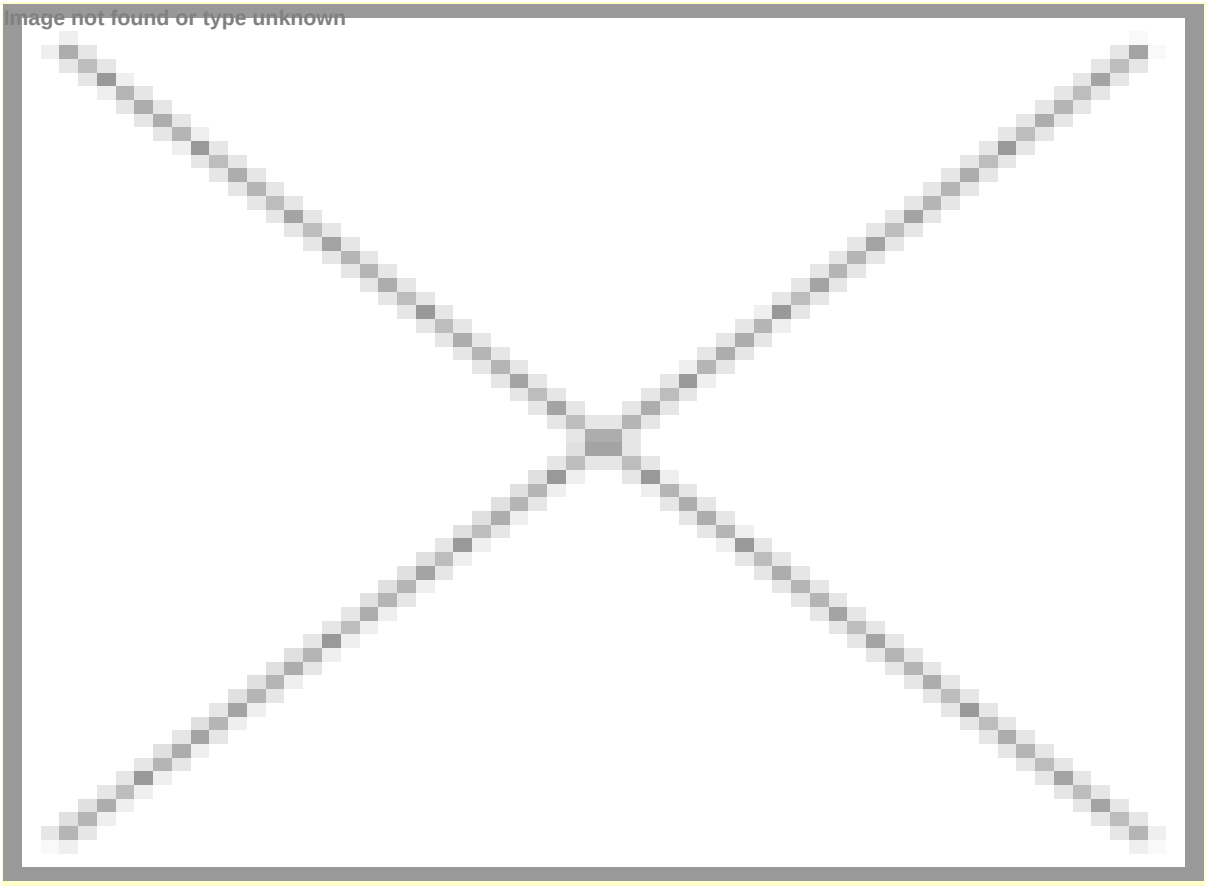
It's no secret, however, that Protein A-based sorbents are expensive, and this fact puts pressure on manufacturing to utilize these sorbents in the most efficient manner possible to keep overall production costs under control. The expense is compounded by other factors that limit the usefulness of Protein A-based sorbents. They have low cleaning tolerances compared to 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.

Chemically stable IgG-selective sorbents

A different purification approach using two novel sorbents is proving to be an effective alternative for some manufacturers. Both of these sorbents share structural and operational features, however they operate by different chromatographic modes. Carrying a mercaptoethyl-pyridine ligand, MEP HyperCel operates by hydrophobic charge induction chromatography (HCIC), and captures antibodies via 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 and captures antibodies through ionic interaction and molecular recognition on the immunoglobulin-selective ligand. In some applications, the two sorbents may be used effectively in tandem. MEP sorbent was introduced to the market in 1999 and 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 to 5.5, depending on the pI 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. In Figure One, the adsorption and desorption mechanisms are illustrated.

Figure One



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.

In most applications, antibody binding with these ligands is independent of salt concentration, which suggests that typical feedstocks can be loaded onto MEP and MBI sorbents without extensive dilution or diafiltration. For non-antibody 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.

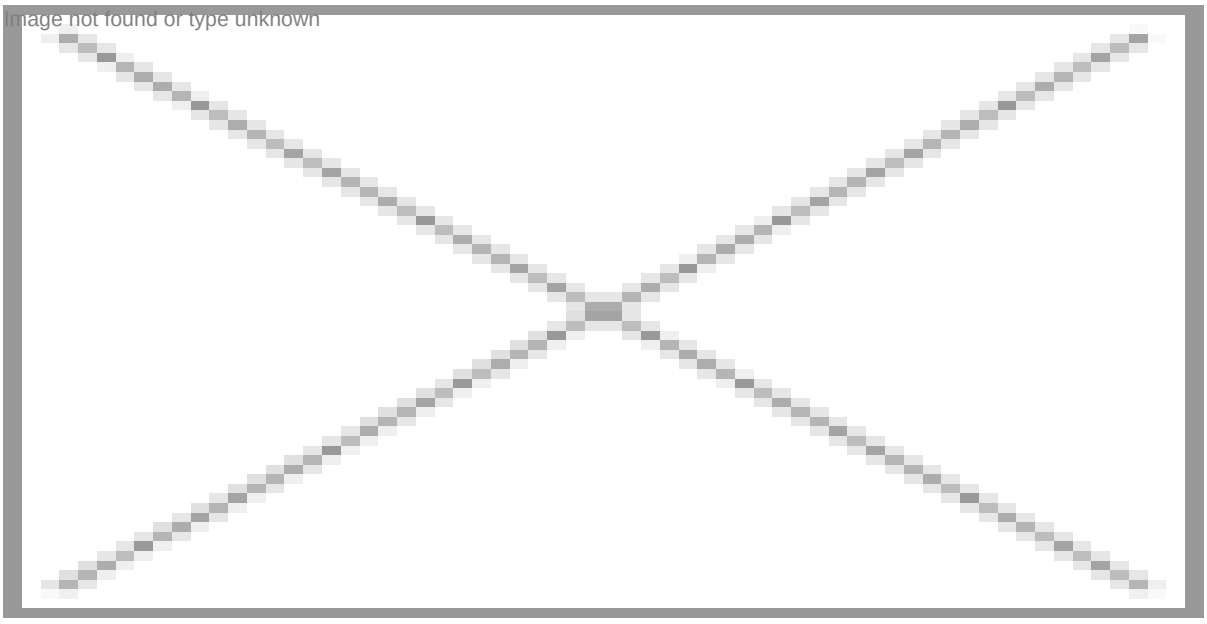
Increased service lifetime up to 500 percent

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

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 an be cleaned regularly with sodium hydroxide solutions at 1M concentrations. They are able to 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 for a wide variety of biological sources

MEP-based sorbents have also proven effective for purifying a wide range of biological streams. While they capture IgG directly from mammalian cell culture supernatants at 95 percent purity, they are also effective for more challenging feedstocks that contain higher levels of impurity proteins, including albumin. Unlike Protein A, they enable binding for IgG variants from different species. Human, rat, goat, sheep, and mouse and even murine IgG1 antibodies can be effectively purified with MEP-based sorbents.
Figure Two 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 are also effective in binding human IgG without salt, enabling them to be used at physiological iconic 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 are able to preserve their biological activity.

Viral clearance has also 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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Note: This article has earlier appeared
in Bioprocess International.