Chromatography Advisor #6

Mixed-Mode Sorbents

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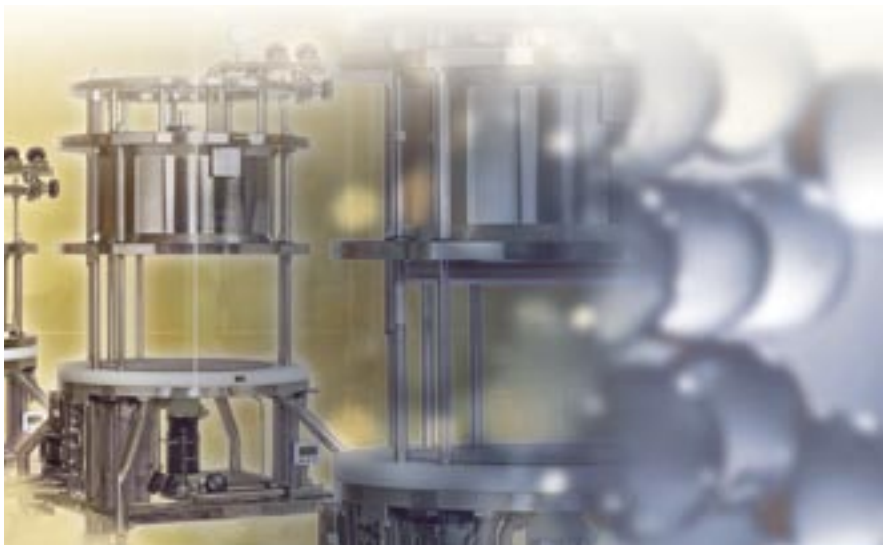
Biotech drug manufacturers have made many improvements to upstream protein production, such as cell-line engineering and adaptation to serum-free media, but the resulting greater yields have created challenges for downstream purification processes. To prevent process bottlenecks, the choice of chromatography method is critical. This advisor takes a look at new "mixed-mode" chromatography sorbents that combine hydrophobic and ionic components, making them useful for a number of approaches to protein purification. Unlike many sorbents used in hydrophobic-interaction chromatography, they need not be used with significant quantities of salt additives. That potentially increases the efficiency of purification processes while reducing costs and the environmental impact of salt disposal.



New environmentally friendly mixed-mode chromatography sorbents can improve efficiency and economics of drug manufacturing. (WWW.PALL.COM)

Meeting the Challenge

To meet a growing demand for protein-based therapies, biotech manufacturers have adopted new technologies that improve upstream biopharmaceutical production. But those improvements create challenges for downstream purification processes: more biomass leads to more product, often at higher concentrations, that needs to be purified.

Chromatography is one of the biopharmaceutical industry's most efficient and versatile methods for production-scale separation and purification. Recent chromatographic tools have been developed to help manufacturers select optimum separation conditions to capture a target protein or resolve impurities. Mixed-mode sorbents offer unique advantages to drug manufacturers by adding new selectivities that simplify protein purification. Phenylpropylamino (PPA) and hexylamino (HEA) HyperCel mixed-mode sorbents from Pall can be used in a range of approaches to purification because they combine hydrophobic and ionic components. Unlike conventional hydrophobic-interaction chromatography (HIC) media, these sorbents do not require salt additives.

Based on a proven bead matrix currently used in production of material for late-stage clinical trials, the new sorbents bring versatility to purification processes. Although they are new to the market, early research suggests that they could increase protein yield by preserving protein purity (see "For Further Reading"). Elimination of salts from purification processes may help to reduce costs and ameliorate environmental concerns related to recycling and disposal. The savings will be significantly magnified as process are scaled up.

Product Focus: All biologicals

Process Focus: Downstream processing

Who Should Read: Process development and manufacturing

Keywords: Chromatography, hydrophobic interaction, mixed mode, process optimization

Level: Basic

Versatility

Protein properties vary enormously, making it essential for makers of these complex active ingredients to have a range of tools at their disposal for exploiting the differences in separation and purification processes. PPA and HEA sorbents each combine characteristics of HIC with some ion-exchange features, providing a novel mechanism of mixed-mode separation that can work for a range of processing applications in which conventional methods are ineffective.

Commonly used for large-scale purification of biopharmaceutical products, HIC is a technique for purifying and separating molecules based on differences in their surface hydrophobicity (the physical property of molecules that are repelled by water). In an HIC process, removal of water molecules during binding and elution is effected by adding lyotropic salts such as ammonium sulphate or sodium sulphate, often in concentrations up to 2 M. At such high concentrations of salt, protein solubility drops sharply, and the molecules precipitate out of the solvent.

Using that much salt introduces challenges. In addition to the purchase of significant quantities of reagent salts, processors must follow environmental regulations for their disposal after the adsorption and wash stages of each chromatography process. In large-scale production, that can add significant cost and even have a negative environmental impact. High concentrations of salt also create issues related to columns and chromatography equipment such as hardward damage due to corrosion.

In an IEX process, proteins are isolated based on their charge at a certain pH. This is a useful technique for the final stages of a purification process — sometimes referred to as the "polishing" of the product — as well as in the early capture and intermediate stages.

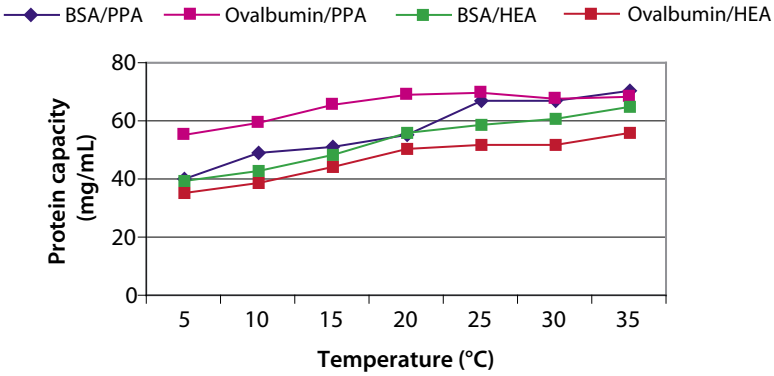
Combining a dominant "low-salt" hydrophobic binding mechanism with some ion-exchange properties, mixed-mode chromatography sorbents can be "fine-tuned" to capture proteins or discriminate between impurities based on differences in relative hydrophobicity and isoelectric points. The purified proteins can be recovered in either dilute buffer or buffer of moderate conductivity. This mechanism applies to a broad range of proteins: monoclonal antibodies (MAbs), enzymes, vaccines, other recombinant proteins, and plasma fractions. Because the sorbents are based on a mechanically and chemically stable HyperCel matrix, they are easily packed and unpacked in columns and can be operated at flow rates up to 1,000 cm/h. The sorbents can also withstand repeated harsh alkaline treatments (1 M NaOH during 200 cycles) for effective sanitization, cleaning in place, and reuse.

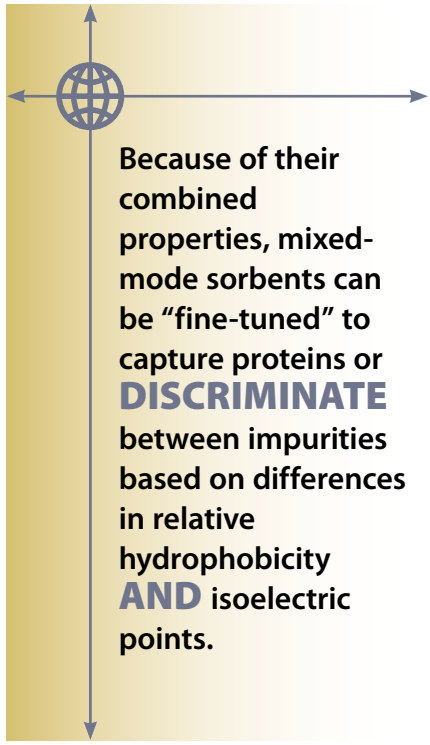
Used for resin screening, PPA and HEA HyperCel sorbents represent a useful addition to the process chromatography purification toolbox: an alternative or orthogonal option to individual separation methods such as ion exchange.

Effectiveness without Salt Additives

A recent study evaluated the binding and elution properties of PPA and HEA sorbents with a variety of proteins (1). It compared the properties of these mixed-mode sorbents with those of anion exchange, HIC, and hydrophobic charge-induction chromatography (HCIC) sorbents. Tests demonstrated a distinct selectivity of the new sorbents compared with the others.

Figure 1: Protein binding capacity increases with increasing temperature, which indicates a hydrophobic-interaction absorptive process even in the presence of lyotropes.





Data show that the new sorbents have superior binding capacity in physiological (0.14 M NaCl) buffers such as phosphate-buffered saline (PBS), compared with conventional HIC sorbents. For example, they demonstrated a binding capacity of about 50 mg/mL for BSA compared with <2 mg/mL for conventional HIC resins under similar low-salt conditions. The sorbents also proved capable of interacting with a range of proteins. Typically, protein binding is achieved using PBS at pH 7.4, without addition of salt, and elution is effected by pH decrease in a gradient or a step-elution mode (pH 7.4 to pH 3.0).

The study found that process binding capacity increased with temperature (Figure 1), indicating that a hydrophobic-interaction adsorptive process was taking place without addition of lyotropic salts. Protein binding capacity also increases with temperature, which similarly indicates a hydrophobic-interaction adsorptive process in the absence of lyotropes.

This research shows that, under certain conditions, the new sorbents can provide a greater yield and recovery of

protein products. Their effectiveness and versatility can help streamline the purification stages of drug processing, potentially reducing the costs of downstream production. So they represent an additional process purification tool that can bring distinct selectivities to capture protein or resolve impurities. As an alternative approach to conventional HIC, this "no-salt or low-salt" capture option minimizes the risk of protein aggregation and recovery loss, and it provides an environmentally friendly method that can contribute to the overall reduction of purification costs, with no waste recycling necessary.

References

1 Levison P, et al. Physiological Hydrophobic Interaction Chromatography: A New Technique for Process Applications. Recovery XII Congress, Litchfield, AZ, 2–6 April 2006; www.versamatrix.com/page/en/1142.aspx.

For Further Reading

1 Bengio S, et al. A New Mixed-Mode Platform for Large-Scale Protein Separation. bioLOGIC Europe Congress, Amsterdam, The Netherlands, 15–22 June 2006; www.terrapinn.com/2006/bio_CH.

2 Guerrier L, et al. A Dual-Mode Approach for the Selective Separation of Antibodies and Their Fragments. *J. Chromatography B,* 755, 2001: 37–46.

3 Weatherly GT, et al. Initial Purification of Recombinant Botulinum Neurotoxin Fragments for Pharmaceutical Production Using Hydrophobic Charge Induction Chromatography. *J. Chromatography A*, 952, 5 April 2002: 99–110.

4 Schwartz W, et al. Comparison of Hydrophobic Charge Induction Chromatography with Affinity Chromatography on Protein A for Harvest and Purification of Antibodies. *J. Chromatography A* 908, 26 January 2001: 251–263.

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