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Mixed-mode sorbents provide options for protein purification

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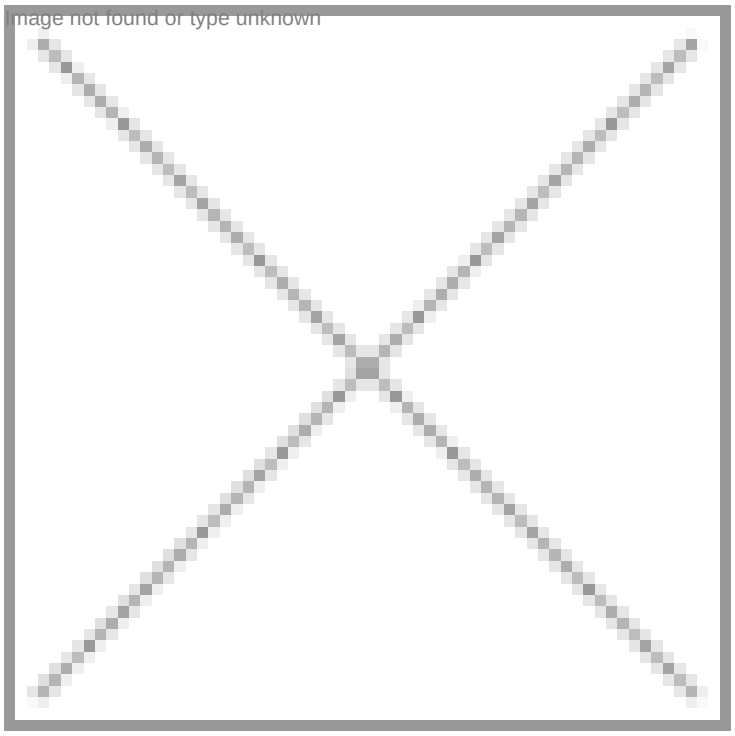
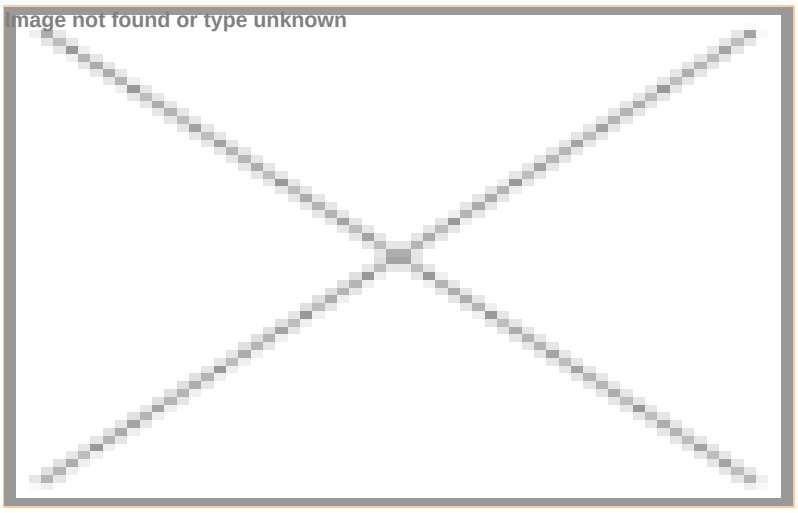


Chromatography Advisor # 6

Mixed-mode sorbents provide options for protein purification



Drug manufacturers have made improvements to upstream protein production, but the greater yields have created challenges for downstream purification processes. To avoid 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 range of approaches to protein purification. Unlike many sorbents used in hydrophobic interaction chromatography, they do not need to be used with significant quantities of salt additives-potentially increasing the efficiency of purification processes while reducing costs and the environmental impact of salt disposal.

To meet the growing demand for protein-based therapies, drug manufacturers have adopted technologies that have improved upstream biopharmaceutical production. But the resulting increase in yields has created challenges for downstream purification processes: more biomass means that there is 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. Recently some new tools have been added to help manufacturers select the optimum separation conditions to capture a target protein or resolve impurities. New, mixed-mode chromatography sorbents offer unique advantages to drug manufacturers by adding new chromatography selectivities that simplify the process of protein purification. Phenylpropylamino (PPA) and Hexylamino (HEA) HyperCel mixed-mode sorbents can be used in a range of approaches to purification since they combine hydrophobic and ionic components. Unlike conventional hydrophobic interaction chromatography (HIC), these sorbents do not require salt additives.

Based on a proven bead matrix currently used in production of clinical material for late-stage clinical trials, these new sorbents bring versatility to purification processes. Though they are new to the market, research shows that they have the potential to increase protein yield by preserving protein purity. And, because they eliminate the need to use salts, they may help to reduce costs and ameliorate environmental concerns related to recycling and disposal.

Versatility of mixed-mode sorbents

Proteins vary enormously in their properties, making it essential for drug makers to have a range of tools at their disposal to exploit these differences in separation and purification processes. PPA and HEA sorbents each combine the characteristics of HIC and some ion exchange features, providing a novel mechanism of mixed-mode separation that can work for a range of processing applications where conventional methods are
not effective.

HIC, commonly used for large-scale purification of biopharmaceutical products, is a technique for purifying and separating molecules based on differences in their surface hydrophobicity, or the physical property of molecules that are repelled by water. In an HIC process, the 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 2M. With such high concentrations of salt, the solubility of the proteins drops sharply, and the proteins precipitate out of the solvent.

FIGURE 1:
PPA and HEA HyperCel - Effect of temperature on protein binding

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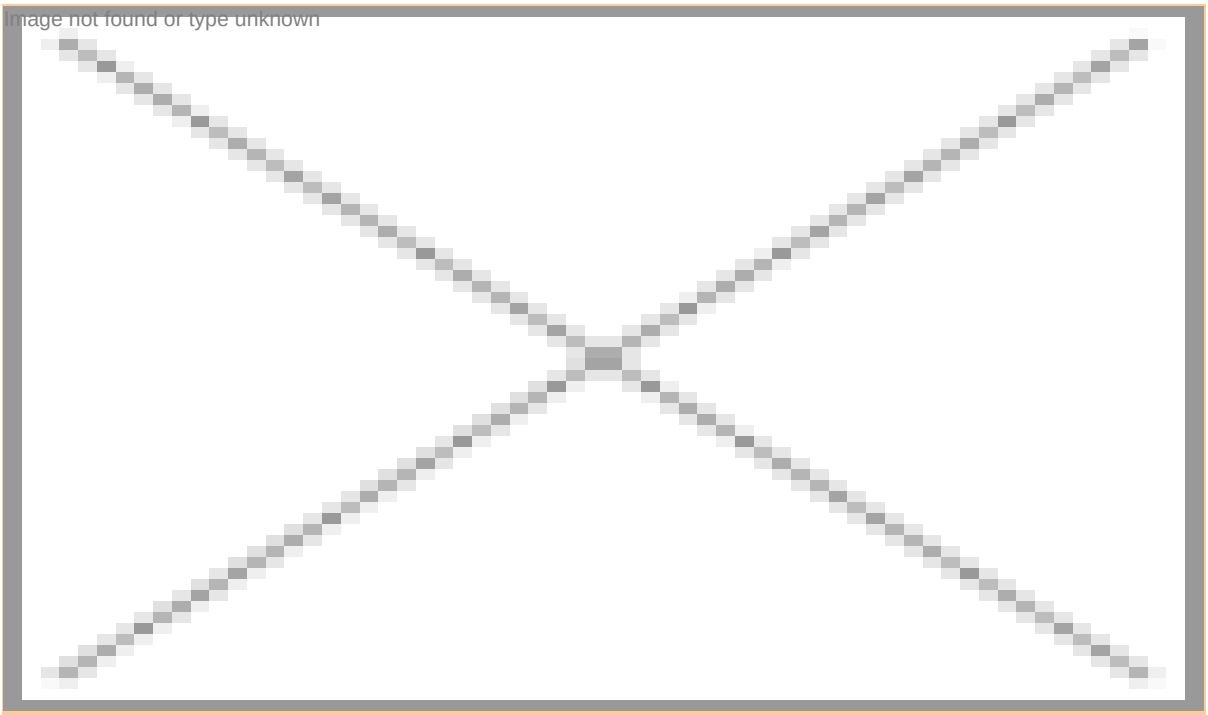

Protein binding capacity increases with increasing temperature. This indicates a hydrophobic interaction adsorptive process even in the absence of lyotropes.

But utilizing this much salt introduces challenges. In addition to purchasing significant quantities of reagent salts, processors are required to follow regulations for their disposal after the adsorption and wash stages. In large-scale production, this recycling can add significant costs and have a negative environmental impact. High concentrations of salt also create issues related to the chromatography equipment and columns.

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

Combining dominant "low-salt" hydrophobic binding mechansism with some ion exchange properties the new 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 dilute buffer, or buffer of moderate conductivity. This mechanism applies to a broad range of proteins, including monoclonal antibodies (MAbs), enzymes, vaccines, recombinants, and plasma fractions. Because the sorbents are based on a mechanically and chemically stable matrix, they can be easily packed and unpacked in columns and operated at flow rates up to 1,000 cm per hour. The sorbent can also withstand repeated harsh alkaline treatments (1 M NaOH during 200 cycles) for effective sanitization and cleaning in place.

Used in a resin screening process, PPA & HEA HyperCel sorbents offer an addition to the process chromatography purification toolbox: an alternative or orthogonal option to well-known 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. It compared the properties of these mixed-mode sorbents with those of anion exchange, HIC, and hydrophobic charge induction chromatography (HCIC) sorbents. Tests have demonstrated a distinct selectivity of the new sorbents compared with the other methods.

Data show that the new sorbents have superior binding capacity in physiological (0.14 M NaCl) buffers like PBS, compared with conventional HIC sorbents. For example, they demonstrated a binding capacity of about 50mg/ml for BSA, compared to less than 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 simply achieved in PBS

at pH 7.4, without addition of salt, and elution is obtained by pH decrease, in a gradient or a step-elution mode (i.e. pH 7.4 to pH 3.0).

The study found that the process binding capacity increased as temperature increased (Figure 1), indicating that a hydrophobic interaction adsorptive process was taking place, without the addition of lyotropic salts.

This research shows that, under certain conditions, these 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. They offer an additional process purification tool by bringing distinct selectivities to capture protein or resolve impurities. As an alternative approach to conventional HIC, this "no-salt/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.

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Note: This article has earlier appeared in Bioprocess International