Application Note

The use of Millistak+® Activated Carbon (AC) for downstream purification of monoclonal antibodies

Introduction

Following cell culture harvest clarification, the downstream purification of monoclonal antibodies involves a number of purification steps designed to remove host cell and product related impurities. The first of these steps is typically Protein A chromatography, which captures the product while providing very good overall impurity reduction. The product is subsequently eluted from Protein A using low pH and the resulting elution pool contains process and product related impurities which must be removed. Scientists rely on a toolbox of complementary purification technologies, the exact nature and order of which depends specifically on the product and impurities present in a given application. Most often combinations of ion exchangers (IEX) are employed to remove the remaining impurities. Cation exchange (CEX) may be used in flow-through or bind/elute mode, to remove host cell impurities, charge variants, and product aggregates. Anion exchange (AEX) is commonly employed in flow-through mode for removal of host cell impurities including HCP, DNA, and virus.

In order to use IEX chromatography effectively, one must ascertain suitable operating conditions (pH and conductivity) to achieve the desired separation of product and impurities. Since IEX steps rely on ionic interactions for adsorption, prospective impurities must carry the opposite charge to the adsorbent they are flowing through. This means solutions in the acidic pH range are required for CEX while solutions in the basic range are desired for AEX. In addition, IEX resins perform best under low salt conditions where solution conductivity is below 5 mS/cm, as salt ions interfere with charge interactions. Thus, a sequence of IEX steps (CEX followed by AEX) require solution adjustments be made before and/or after each step.

Activated carbon (AC) is compatible with all common solution conditions found throughout the downstream process. It is capable of operating in acidic and basic pH ranges as well as high and low solution conductivities. Since AC can be operated in flow-through mode under typical process pH and conductivities, the possibility to connect it with adjacent IEX chromatography steps becomes attractive.

AC's size selective properties allow it to remove impurities smaller than monoclonal antibodies making it most useful in cases where low molecular weight species are of concern or make up the bulk of the impurities.

Millistak+® CR40 media is formulated with AC retained in a rigid structure by a cellulose matrix. These materials, combined with a state-of-the-art manufacturing process, create a tortuous flow path that insures maximum impurity contact with the surface and pores of the AC, for optimum impurity adsorption. It is available in the Pod filter format offering flexibility because of its unique modular and disposable design, accommodating applications from lab to pilot to process scale.

The examples shown here demonstrate that AC has the ability to reduce HCP present in Protein A elution pools under various solution conditions. Along with state-of-the-art IEX chromatography resins, AC shows itself to be a worthy downstream purification tool capable of removing HCP and other impurities, adding to robust overall performance and productivity of downstream processes.

Materials and Methods

Cell Culture Production and Clarification

Experiments utilized Chinese Hamster Ovary (CHO) cell culture producing monoclonal antibody (MAb05). Cells derived from an expressing CHO-S cell line were grown in bioreactors to a density of 8.6 x 10^6 cells/mL and harvested at 86% viability. Cell culture suspensions were collected 12 – 15 days post-inoculation. The harvested cell culture was subsequently clarified using Millistak+® D0HC and X0HC depth filters. The clarified culture was then filtered using 0.22µm Millipore Express® SHC filters to lower bioburden.

Protein A Purification Process

The sterile 0.22µm filtered feed was purified using ProSep®-vA High Capacity Protein A resin. The product was loaded to a capacity of 28 mg/mL and the resin was washed with pH 7 25mM TRIS + 0.5M NaCl buffer to remove non-specifically bound species. The product was eluted from the resin using pH 2.5 25mM Acetic Acid + 25mM Glycine-HCl. The pH of the elution pool was subsequently adjusted to the desired pH using 2M TRIS Base and the conductivity was adjusted to the desired conductivity using a 5M NaCl solution. The resulting solution was filtered using 0.22µm Millipore Express® SHC filter. The elution pool antibody concentration was 22 mg/mL and the concentration was HCP of 5907 ng/mL or 269 PPM (ng/mg).

AC Mechanism

AC is a complex, highly porous material occupying a wide range of particle geometry (Figure 1). Having available surface areas greater than 1000 m^2/g, AC has the highest volume of adsorbing porosity of any known material. Typical preparative chromatography beads have surface areas in the range of 30 – 50 m^2/g. Given the microporous structure of AC, small molecules are able to access more of the available surface area than large molecules. Adsorption is attributed to Van der Waals forces which are short range non-covalent interactions with the surface. Thus, the size of impurities to be adsorbed and residence time within the AC are important process considerations.[1]

Downstream Purification

Purification experiments were carried out using the powdered AC found in Millistak+® CR40 devices. The AC was equilibrated with the appropriate pH/conductivity buffer corresponding to the challenge conditions. Likewise, 1 mL columns of Eshmuno® Q (AEX) resin and a prototype CEX resin were prepared as controls and challenged in parallel with the AC for comparison.

Following equilibration, the adjusted Protein A elution solutions were flowed through the AC and IEX columns using a pump at 0.5 mL/min, corresponding to a residence time of 2 minutes. Fractions were collected at each column outlet over regular intervals and analyzed for HCP content. When the fraction concentrations are compared to the input feed concentration, a "breakthrough" percentage is revealed.

Figures 2-4 show the percent breakthrough of HCP, after flow through purification, as a function of MAb loading (mg MAb/m^2 AC surface area) or (mg MAb/mL resin) under four distinct solution conditions.

In addition to AC's ability to perform under all test conditions – its overall performance exceeds that of AEX and CEX in all experiments (except CEX at pH 5, 3.5 mS where performance is roughly equivalent) – in terms of HCP removal. This performance flexibility is a defining feature of AC and may be leveraged to add robustness to existing processes.

When fractions from each experiment are combined together to form a final process pool, total HCP removal is easy to compare.

Figure 1. (left)
Electron micrograph of AC.

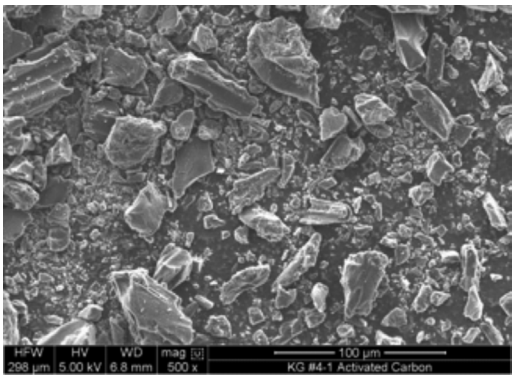

Figure 2. (right)
AC HCP Breakthrough. HCP breakthrough profile of AC. The data shows consistent purification in each experiment, tolerating the presence of salt and relatively insensitive to pH conditions.

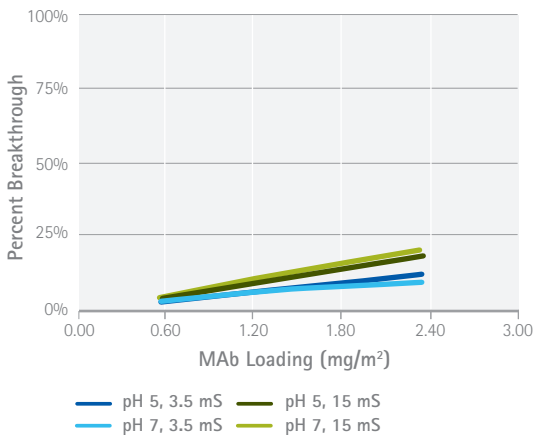

Figure 5 not only emphasizes AC's HCP removal advantage over IEX resins – but reveals how much AEX underperforms in comparison to AC and CEX – as the first purification step following Protein A. This type of data is useful in shaping downstream purification schemes, particularly in flow through mode. For example, one might select AC as the first process step, allowing it to remove the bulk of HCP, leaving CEX and AEX to do more specific tasks like aggregate and virus removal.

Comparison to a Charged Depth Filter

Research has been conducted indicating that charged depth filters, such as the Millistak+® X0HC filters, are suitable replacements for expensive AEX resins in downstream purification.[2] We investigated the use of a Millistak+® CR40 µPod® filter in direct comparison with a Millistak+® X0HC µPod® filter to purify a post Protein A elution pool.

While Millistak+® X0HC does in fact offer HCP removal, its removal capacity did not compete with CR40 when loaded to typical processing ranges (presented here in L of fluid / m^2 of depth filter frontal area). In addition the Millistak+® CR40 µPod® filter shows similar characteristics as the AC data shown previously (Figure 2) – in both % HCP breakthrough and pH robustness (Figure 6).

Antibody Recovery and Fragments

Product recovery is an essential aspect of a successful downstream purification process. AC has the ability to remove impurities without having a negative impact on recovery. Collecting breakthrough fractions during these experiments allows one to analyze samples for antibody concentration (recovery) alongside impurities like antibody fragments. Analysis was done using size exclusion chromatography to differentiate the species of antibody found throughout the process. While AC does not typically remove aggregated forms of antibody (due to their size, data not shown), it is able to remove miscellaneous fragments of antibody.

Figure 7 shows how MAb05 can be purified using a Millistak+® CR40 µPod® filter. Notice the MAb reaches full breakthrough while HCP and fragments lag behind. AC's sieving property drives these phenomena and is expected as fragments are closer in size to MAb while HCPs tend to be smaller. Antibody fragments are not always present and are generally found at very low concentrations compared to the product. AC is able to provide fragment removal, which is an attractive feature because fragments are not easily removed through IEX due to their molecular similarity to intact MAb molecules.

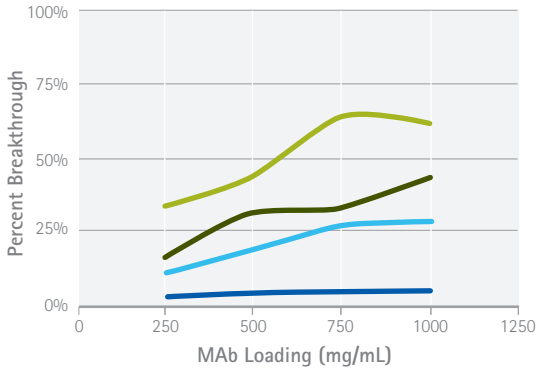

Figure 3.
CEX HCP Breakthrough. HCP breakthrough profile of CEX. The data indicate performance is best in low salt / low pH environments.

- pH 5, 3.5 mS
- pH 7, 3.5 mS
- pH 5, 15 mS
- pH 7, 15 mS

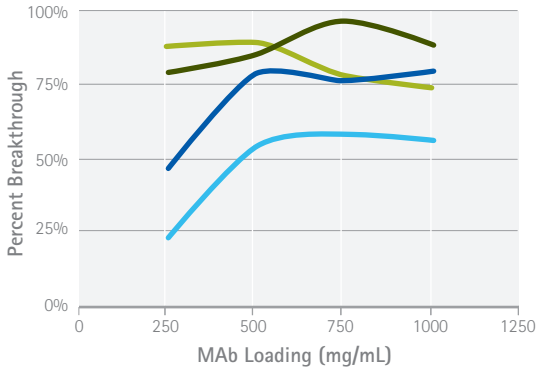

Figure 4.
AEX HCP Breakthrough. HCP breakthrough profile of AEX. The data indicates performance is best in low salt / high pH environments.

- pH 5, 3.5 mS
- pH 7, 3.5 mS
- pH 5, 15 mS
- pH 7, 15 mS

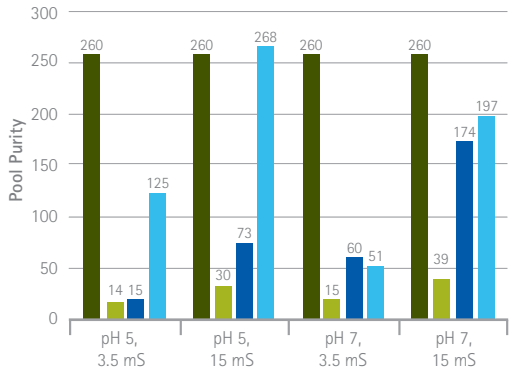

Figure 5.
Purification Pool Purity (HCP PPM). Fraction pools from Figures 2 - 4.

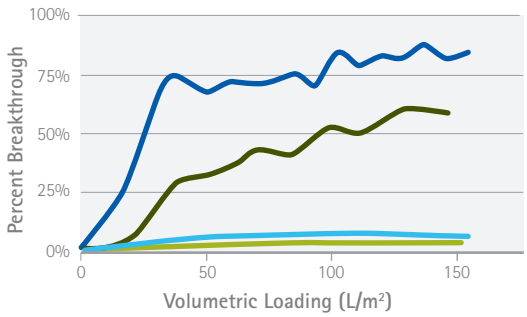

Figure 6.
Millistak+® µPod® Filter HCP Breakthrough. HCP Breakthrough on Millistak+® X0HC vs. CR40 filters.

- X0HC pH 5
- CR40 pH 7.5
- X0HC pH 7.5
- CR40 pH 5

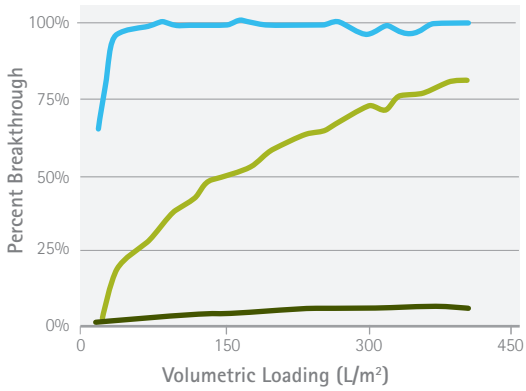

Figure 7.
Millistak+® CR40 µPod® Filter Performance. Complete breakthrough profiles of MAb, HCP, and antibody fragments.

- MAb
- Fragments
- HCP

Conclusions

The data presented in this application note provide guidance on the properties, usage, and potential performance of AC in Millistak+® CR40 filters. Using AC downstream of Protein A may alleviate some of the demands on subsequent IEX steps by reducing process impurities (particularly HCP), potentially reducing the size and cost of IEX purification required to reach purity targets and increasing the robustness of entire processes. This added layer of purification may be used to reshape the entire downstream process by reducing pH changes, hold tanks, dilution requirements and other process disruptions while increasing connectivity possibilities between steps. In comparison with typical IEX resins, AC shows a unique ability to perform under a variety of solution conditions without sacrificing impurity removal. This flexibility allows AC to be used virtually anywhere during downstream processing without step specific solution adjustments normally required for IEX — making it an ideal candidate for use in connected flow through applications. The Millistak+® CR40 filter can be used directly on Protein A elution pools as the first step in downstream purification — out performing the Millistak+® X0HC filter in a side by side comparison. AC's size selective properties allow it to remove impurities smaller than monoclonal antibodies, even antibody fragments, effectively without suffering significant product losses. Because the Millistak+® CR40 filter is available in the Pod filter format, it is scalable, disposable, and does not require packing validation like chromatography resins. Since the Millistak+® CR40 filter exists in a depth filter type format, it may offer potential turbidity and particle removal utility as well as the impurity removal demonstrated here. Overall, AC is an easy to use, inexpensive plug-and-play tool that can be exploited in a variety of downstream applications without complex process development.

References

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2. Schreffler J, Bailley M, Klimek T, et al. 2014.
   Characterization of Post-Capture Impurity Removal across an Adsorptive Depth Filter.
   Bioprocess International

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