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White paper

# Flow-through Chromatography and Adsorptive Depth Filtration for Continuous Bioprocessing Applications

Current technologies for downstream processing such as clarification and chromatography can be adapted to make them more suitable for continuous bioprocessing applications. This white paper describes the use of these technologies to remove impurities in a manner compatible with a flow-through, continuous process. The utilization of these flow-through technologies can result in shorter lead times, reduced plant footprint, increased flexibility and a reduction in cost of goods (COGs).

## Adsorptive Depth Filtration

Depth filtration technologies are typically utilized during primary or secondary bioreactor harvest operations to remove particles from the feed stream based on size exclusion principles. Many types of depth filter media also have adsorptive behavior by design, which can be used during harvest to clear a host cell protein (HCP) and nucleic acid contaminants. This adsorptive behavior can also be specific for certain process-related contaminants such as aggregated product, and can be highly effective at their removal in downstream processes.

Depth filtration has a number of attributes which makes it appropriate for use in continuous and flow-through bioprocesses:

- Depth filter media is designed for particulate capture and as such the porosity is high and the pressure drop is low
- The media typically provides both adsorptive and size exclusion behavior, allowing removal of a range of contaminants
- The simple flow design of depth filters – an inlet, outlet and a vent – allows them to be easily inserted into the process stream
- At smaller scales the filters are quick to swap out without interfering with the flow within the bioprocess
- Depth filters are sized by constant flow, rather than constant pressure, making this technology easily compatible with continuous processes which are optimized by flow rate

Sieving is the mechanism by which a normal flow or tangential flow membrane filtration operates (**Figure 1**). Material remains on the top, barely entering the pore structure. While this gives a very good filtrate quality, it has very poor capacity; as the material sits on top, it will cake and create a significant resistance to flow.

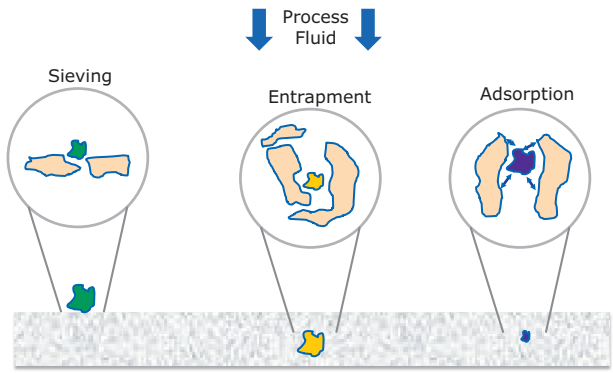



**Figure 1.** Different retention mechanisms on depth filters.

In contrast, depth filters are capable of trapping a substantial number of suitably sized particles within the media itself, rather than forming a cake on the surface. This mechanism is known as entrapment and allows depth filters to have a large dirt-holding capacity when it comes to heavy particulate feeds. The downside of the open depth filter media in this regard is that filtration is nominal, and if high pressure is applied for a long period breakthrough of particles will be observed.

The interplay of the two mechanisms, sieving and entrapment, allows a suitable pore size to be selected that provides an appropriate compromise between capacity and filtrate quality.

With adsorptive mechanisms, the materials inside the depth filter are not neutral. These materials, either by design or inadvertently, capture both small and large particles and molecules passing through the filter via hydrophobic, electrostatic and/or van der Waals interactions. Many modern depth filter media components such as diatomaceous earth (DE) and activated carbon are deliberately adsorptive, and can act like flow-through chromatography columns as well as having size exclusion properties.

## Depth filter media components

Activated carbon is carbon that has been activated with steam or chemical modification to create a highly porous structure with a surface area of more than 1,000 $m^2/g$. It has a wide range of particle geometry and a tortuous flow path. The pores are small enough to separate down to the molecular level, making it effective at removing small molecules such as protein fragments and phenolic or aromatic compounds, often found in mammalian cell culture feeds or any additives that are strongly colored. While small molecules are effectively removed during the wash steps of bind/elute chromatography operations, bind/elute is typically avoided when designing fully continuous or flow-through bioprocess; activated carbon can perform this function effectively and cheaply.

Diatomaceous earth is a powder held together in a rigid structure with cellulose matrices and a cationic resin binder. It is often referred to as “filter aid” because historically it was loaded on top of a membrane filter to act as a prefilter and increase capacity. DE has excellent aggregate removal capability as it is selective for very large molecules and particles, likely due to cumulative non-specific van der Waals forces. The resin binders are deliberately charged and cationic in nature, which means that it will also bind strongly to negatively charged contaminants such as nucleic acids. This can be a constraint with very large products with a net negative charge, such as lentiviruses, that typically bind to DE-based media. Cellulose only or polypropylene-based depth filters are an acceptable alternative and are readily available.

**Figure 2** shows an aggregate removal operation using a Millistak+® X0HC depth filter with the tightest pore size of diatomaceous earth. The starting high molecular weight percentage (HMW%), or amount of aggregates, was 9.3%.

A capacity of 60L/$m^2$ provides a filtration with a HMW% of only 3%, usually well within typical therapeutic molecule specifications. Aggregate removal steps are usually positioned downstream, such as after viral inactivation or before viral clearance filtration, so the process volumes are low enough that the capacity seen here is not a constraint.

**Figure 3** shows the removal of host cell proteins using the same approach. The starting value of 7,500 ng/mg is typical for the upstream operations of a bioprocess. The majority of host cell proteins were removed in the first tens of liters per $m^2$ coming out, increasing to 200 ng/mg by 60L/$m^2$.

The cationic resin binder inside the diatomaceous earth will bind to anything that is negatively charged and since a significant proportion of these host cell proteins could be negatively charged, they were successfully removed. DE also binds some of the larger components that can be expected in the starting material.

The resin binder is primarily present to add structural strength, allowing powder and fibers to be formed into a sheet that can withstand pressure; the electrostatic nature can however be leveraged very successfully for contaminant removal. A high ionic strength will greatly reduce the potential of this binding which is advantageous if the product has a small negative charge; by increasing the salt concentration, the product will cease to bind. If the product is strongly negatively charged, it will bind and will be unrecoverable. Negatively charged host cell proteins and DNA can, however, be effectively removed by this positively charged resin binder.

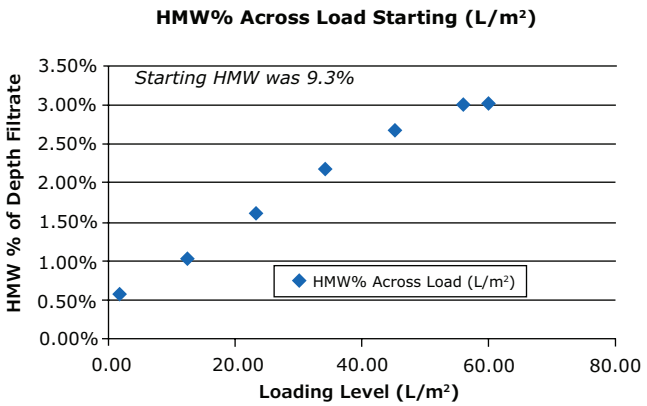



**Figure 2.** Reference: Schreffler et al, 2015; Characterization of postcapture impurity removal across an adsorptive depth filter; Bioprocess International, 13(3) p36

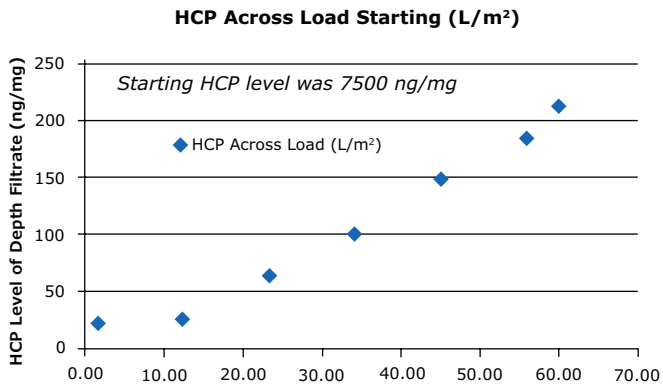



**Figure 3.** Host cell protein removal using Millistak+® X0HC depth filter with diatomaceous earth.

It should also be noted that depth filtration systems for primary clarification are always used in parallel flow mode, as the resistance to flow during plugging would impact the secondary filter unduly. If the media is used in an adsorptive application where particles are not present, it is possible to run the filters in series to increase residence time and improve contaminant binding capacity.

**Figure 4** shows the percentage of DNA removed by filtration.

DNA is negatively charged and binds effectively to the cationic resin binder. For the first hundred liters per $m^2$ there was nearly a complete removal of the nucleic material from the feed stream, at which point steadily rising breakthrough was observed.

**Figure 5** shows that the monoclonal antibody product is not adsorbing to the depth filtration media in substantial quantities.

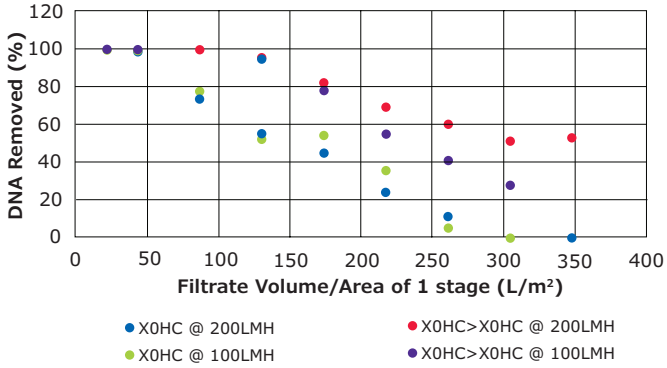



**Figure 4.** Percentage removal of DNA throughout filtration.

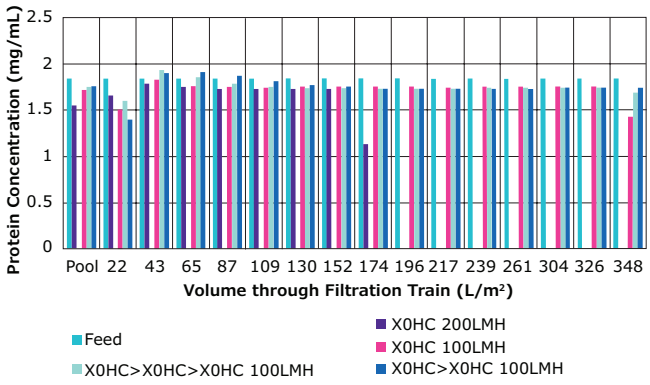



**Figure 5.** Adsorption of monoclonal antibody is minimal.

As diatomaceous earth and activated carbon are both natural products, there are a few caveats regarding their use in bioprocessing:

- They require substantial flushing to reduce extractables; newer synthetic equivalents of this depth filtration media need a much lower flush volume
- Change control is complex due to natural sources
- Binding is non-specific and as such a greatly oversized filter area can result in significant loss of yield

## Flow-Through Chromatography

For flow-through polishing steps, the focus is on impurity adsorption where the target molecule remains in the feed stream; bind and elute steps can be connected seamlessly, intensifying the process. There will be significantly less resin required to bind the impurities, higher binding capacities can be achieved and the size of the technologies can be reduced.

As shown in **Figure 6**, there is a range of orthogonal tools that can be used to purify antibodies in a flow-through mode including ion exchange (IEX), hydrophobic interaction (HIC) and size exclusion (SEC). Overall, the principle itself is based on combining technologies that work orthogonally to assure the platform robustness.

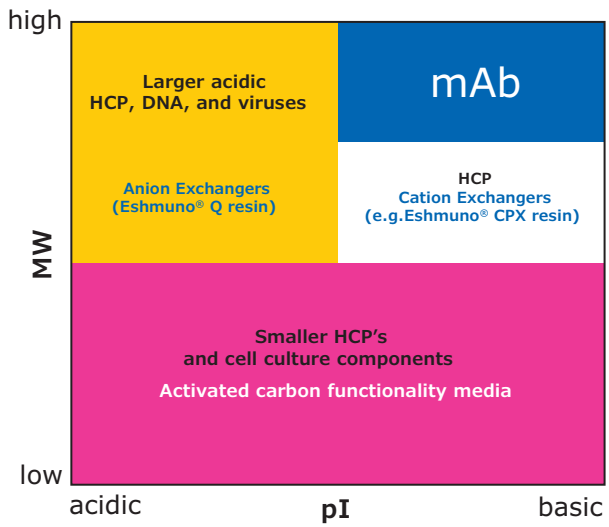



**Figure 6.** A range of orthogonal purification tools are available for antibody polishing.

In order to make the polishing as robust as possible, two main principles are considered: size and charge. The impurities typically found in cell culture feed streams are both low and high molecular weight; for the low molecular weight impurities, the technology of choice is a highly-selective adsorber with very high surface area such as activated carbon. In terms of charge, ion exchange used in the flow-through mode adsorbs acidic impurities including host cell protein, DNA and leached protein A. Cation exchangers can also be used to help and protect the other technologies.

**Figure 7** provides an example of this approach. The feed from a non-expressing CHO cell line was concentrated using a cation exchanger and applied to a 2D gel to map the host cell proteins. The x-axis is the separation according the charge, with acidic host cell proteins on the left and basic host cell proteins on the right.

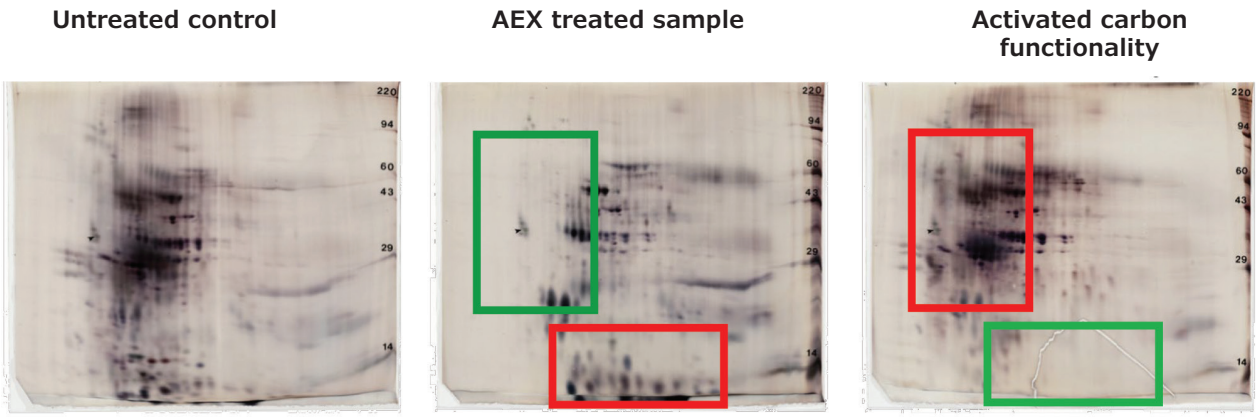


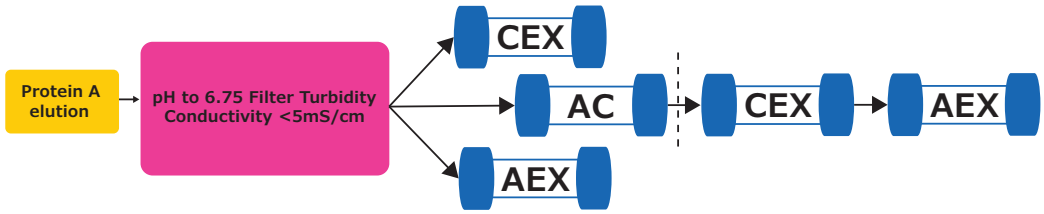

**Figure 7.** Example of orthogonal purification for antibody polishing.

The y-axis shows separation according to molecular weight. The same sample was then treated with anion exchange. As seen on the center image, the acidic proteins were effectively adsorbed; neutral small proteins were still present after the treatment as seen in the red rectangle. If the same test solution is treated with activated carbon, low molecular impurities were adsorbed. The larger acidic impurities do not penetrate into the pores on the natural activated carbon.

Low molecular weight compounds can get into the porous network of activated carbon and be adsorbed, mainly by the van der Waals interactions. In contrast, the product – usually a much larger molecule – is not able to enter the porous network but rather stays only mildly adsorbed to the surface or in the flow-through solution.

Activated carbon can also help in the removal of cell culture components. In perfusion-based processes many additives are used and some, such as antifoaming agents, can present difficulty for downstream processes. Activated carbon has a substantial capacity towards those low molecular weight substances and can ensure effective removal of abundant impurities.

**Figure 8** shows the comparison of individual technologies for single-step purification.




| | HCP level (ppm) after dynamic flow-through experiment with various materials | | | |
|---|---|---|---|---|
| Loading (mg/mL) | Activated carbon | Eshmuno® CPX resin | Eshmuno® Q resin | All combined in one |
| 350 | 55.2 | 72.1 | 11.2 | 2.0 |
| 500 | 75.4 | 63.3 | 12.5 | 2.9 |
| 650 | 89.3 | 73.3 | 10.4 | 3.9 |
| 750 | 123.5 | 78.3 | 12.9 | 4.6 |
| 900 | 200.3 | 89.7 | 10.3 | 7.8 |

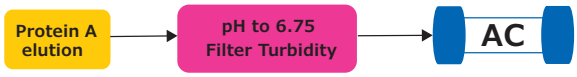
**Figure 8.** Single-step purification using individual technologies.

A Protein A elution pool was adjusted to pH 6.75 and applied to a cation exchanger, activated carbon and an ion exchanger. The results showed a reduction of host cell protein level with each individual technology; the most powerful technology in this experimental setup was the Eshmuno® Q resin. When these technologies were combined into a single train, a very low host cell protein level was achieved at very high loadings. 900 milligrams of antibody was applied for a one milliliter column which is approximately 15x more than one would typically use for the bind and elute applications.

The following four case studies demonstrate the utility of this orthogonal approach.

## Case Study #1: Reduce low molecular weight impurities with high yield

As shown in **Figure 9**, activated carbon was used. It works for a broader range of pH and conductivity and allows to overcome the lack of information on the target molecule. An efficient host cell protein removal was achieved, reducing levels from 1,000 ppm to 369 ppm. Protein A which had leached from the protein A column was also removed.




| | Feed | After AC FT |
|---|---|---|
| HCP (µg/mL) | 4,41 | 1,46 |
| PA (ng/mL) | 177,00 | 122,00 |
| mAb (g/L) | 4,28 | 3,95 |
| PPM HCP | 1030,37 | 369,62 |
| PPM PA | 41,36 | 30,89 |

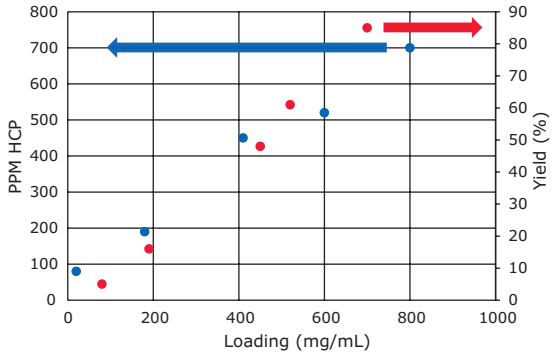
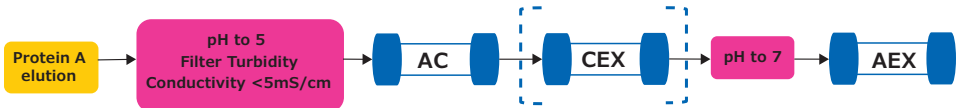



**Figure 9.** Removal of host cell protein in a single step. Antibody post Protein A feed (pH 5.0, conductivity 4.8 mS/cm, ~1000 ng/mL HCP amount) loaded on packed 1 mL Activated Carbon device @2 min RT after equilibration with 50 mM Acetate buffer pH 5.0, conductivity of 5 mS/cm for 20 column volumes

## Case Study #2: Purify post-Protein A pools to <100 HCP ppm

This study sought to purify post-protein A pools at the host cell level to below 100 ppm (**Figure 10**). To enhance the binding of the host cell protein, two different pH windows were chosen for the cation and the ion exchanger.




| | Capacity (g/L) $OD_{280}$ | Fraction | V (mL) | $OD_{280}$ | Yield (%) | LMW | HCP (ppm) |
|---|---|---|---|---|---|---|---|
| Activated Carbon pH5 | 1379 | Load | 190 | 9.36 | 115 | 1.8 | 2755.2 |
| | | FT | 235 | 8.74 | | 1.7 | 1202.7 |
| Eshmuno® Q resin pH7.0 | 1085.4 | Load | 270 | 5.18 | 100.6 | 2.3 | 82.85 |
| | | FT | 260 | 5.34 | | 2.1 | 24.81 |

**Figure 10**. Removal of HCP using full template. Antibody post Protein A feed (pH 5.0, conductivity 5.2 mS/cm, ~3000 ppm HCP amount) loaded on packed 1 mL Activated Carbon device @2 min RT after equilibration with 50 mM Acetate buffer pH 5.0, conductivity of 5 mS/cm for 20 column volumes. The flow-through collected and loaded on CEX 1 mL device @2 min RT. Flow-through collected and adjusted to pH 7.0, conductivity of 4 mS/cm and loaded on 1 mL Eshmuno® Q column @2 min RT after equilibration with 50 mM Phosphate buffer pH 7.0, conductivity of 4 mS/cm for 10 column volumes

Using a combination of activated carbon and cation exchanger, the study started at pH 5, followed with an ion exchanger and a pH increase to 7. The activated carbon reduced the host cell protein level from 2,700 to 1,200 ppm at loadings higher than 1 kilogram of antibody per liter of resin. The Eshmuno® Q resin completed removal of host cell protein from 82 to 24 ppm, four times below the requested certification at the window of 1 kilogram per liter allowed.

## Case Study #3: Standard purification versus the new template

The final case study compares batch processing against continuous flow-through polishing (**Figure 11**). For the batch processing, a bind and elute cation exchanger and anion exchanger were used to purify the antibody to 24 ppm. For the flow-through approach, activated carbon, Eshmuno® CPX and Eshmuno® Q resins were used. With the flow-through approach, the same recovery levels were obtained at the same purity levels but reducing the amount of resin needed by 10 – 15x.

- High binding capacity for HCP (>800 mg/mL loadings)
- Efficient removal of HCP (<30 PPM)
- Reduction in necessary resin quantities

| | Batch | Continuous |
|---|---|---|
| HCP | 24.7 PPM | 26.6 PPM |
| mAb conc. | 7.5 (g/L) | 5.98 (g/L) |
| Activated Carbon | - | 4.5 mL |
| Eshmuno® CPX resin | 75.5 mL | 4.5 mL |
| Eshmuno® Q resin | 37.5 mL | 4.5 mL |

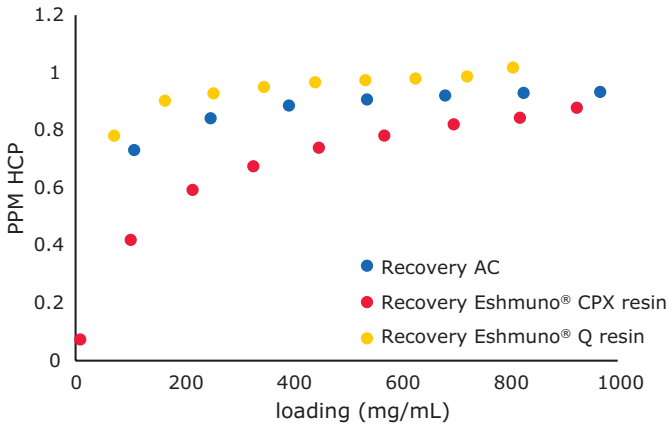



**Figure 11**. Comparison of batch versus continuous flow-through polishing

As shown in these case studies, impurity removal using a flow-through polishing template was highly successful:

- Activated carbon provides a robust, widely applicable orthogonal technology for antibody polishing efficiency adsorbing impurities
- Host cell protein specification in antibody feed streams can be reached using combinations of cation exchange, anion exchange and activated carbon functionality in a flow-through mode
- Target specification of <50 ppm is obtained with loadings up to 1.5 kg/L for the activated carbon, Eshmuno® CPX, Eshmuno® Q and Eshmuno® S resins sequential flow-through train assuring >90% antibody yield
- Significant resin reduction is enabled using flow-through purification mode

A flow-through aggregate removal approach based on the cation exchange principle which works optimally at pH 5 was also developed. This technology needs to be compatible with an activated carbon and Eshmuno® CP-FT resin, so it has to adsorb aggregates at high loadings.

Compared to other available technologies, Eshmuno® CP-FT resin is designed for aggregate removal. As shown in **Figure 12**, the experiment started with 8% aggregates and a specification of less than 1% aggregates at the loading of 878 milligrams per milliliter resin was achieved.

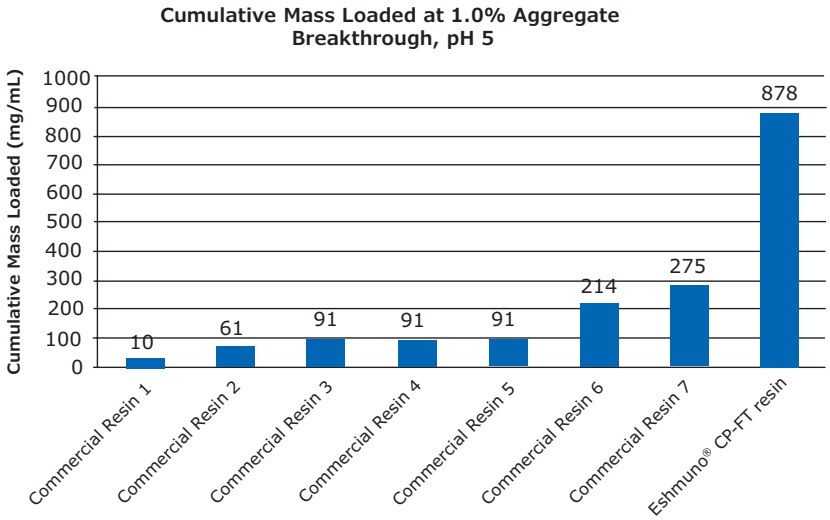



**Figure 12**. Eshmuno® CP-FT resin has the highest capacity for aggregate removal in a protein A pool compared to a panel of resins evaluated. Feed: Aggregates ~ 8% and host cell protein 1650 ppm.

## Conclusion

A central element in continuous processing is the implementation of flow-through approaches in which the target molecule remains in the feed. A number of technologies are available and can be combined orthogonally using different separation principles in order to effectively remove impurities. As described in this white paper, reduction in host cell protein, DNA and aggregates can be effectively achieved using technologies based on activated carbon, diatomaceous earth and standard ion and cation exchangers.

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