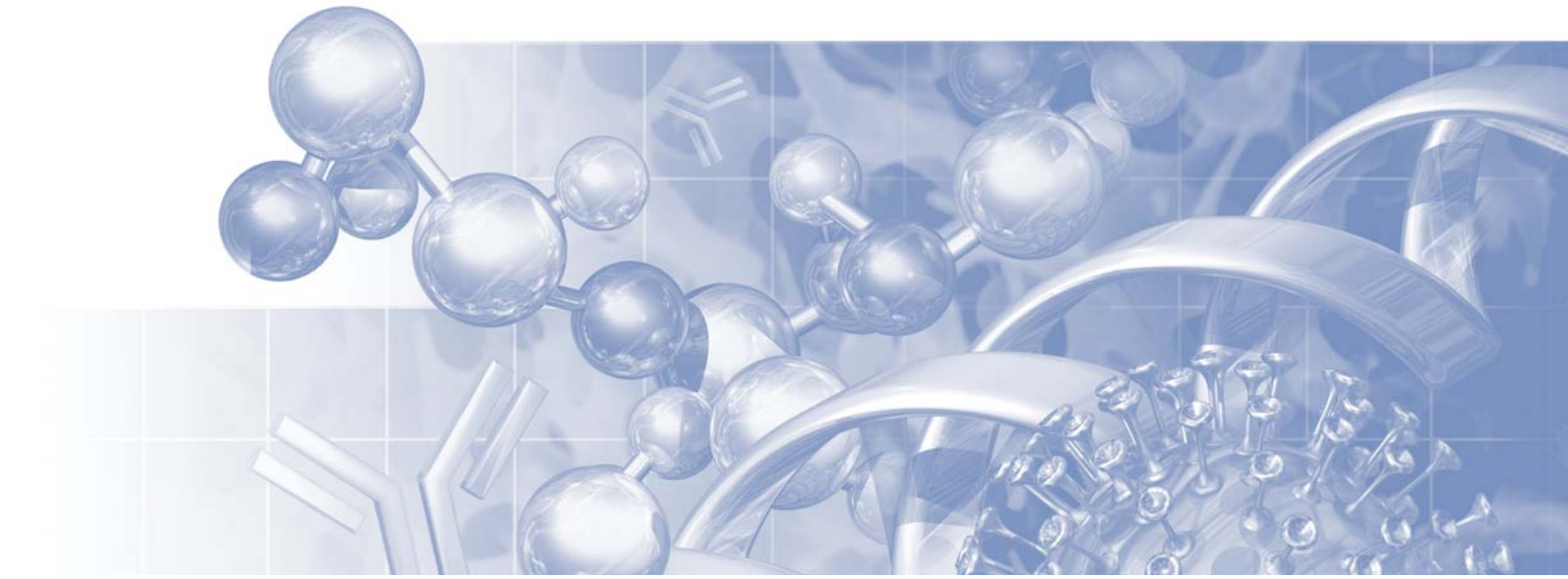
PALL Biotech

Application Note

USD 3319

Benefits of Mixed-Mode Cation Exchange Chromatography: CMM HyperCel™ Sorbent Used for Monoclonal Antibody and Other Recombinant Protein Purification



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Summary

This note addresses the performance of Pall's mixed-mode cation exchange (CEX) CMM HyperCel sorbent, which can be applied to both monoclonal antibody (mAb) polishing and recombinant protein purification. The study highlights the behavior of the CMM HyperCel sorbent versus conventional cation exchange (sulfopropyl groups) and a weak cation exchange multi-modal sorbent (cross-linked agarose) in terms of dynamic binding capacity (DBC) and selectivity (aggregate removal). Results demonstrate that compared to conventional cation exchangers, CMM HyperCel sorbent has a higher DBC (>60 mg/mL at pH 5.0, 25 mS/cm) over a broader range of pH (5.0 to 8.0) and conductivity (5 to 45 mS/cm). This capability provides maximal flexibility during process development, allowing streamlined operation with direct load of samples from previous process steps without feed adjustment. When used in a polishing sequence, the selectivity of CMM HyperCel sorbent allows good resolution of monomeric mAb from aggregates and removal of host cell proteins.

These unique properties can be of benefit for improved purification of engineered antibodies, antibody fragments, or various recombinant proteins with very close isoelectric points (pI) and/or degrees of hydrophobicity.

1. Introduction

Conventional industry-designed mAb chromatography purification platforms typically include three-steps, for example:
1. Protein A affinity capture
2. An intermediate polishing step using cation-exchange
3. And a final anion exchange (AEX) step in flow through (FT) mode

Mixed-mode or multi-modal chromatography offers alternatives to conventional methods such as ion exchange and has been increasingly used to purify proteins with closely related isoelectric points or hydrophobicities (engineered antibodies, fragments, charged variants or various recombinant proteins).

In Pall's mAb purification platform (refer to Pall application note reference USD3317), a unique mixed-mode cation exchanger, CMM HyperCel sorbent, was used as the third chromatography step, following mAb capture using Protein A (KANEKA KanCapA♦ sorbent), and anion exchange on a membrane chromatography adsorber (Mustang® Q membrane) operated in FT mode step. In this three-step platform, CMM HyperCel sorbent and Mustang Q membrane play a complementary role in the removal of DNA, host cell proteins (HCP) and aggregates. Note that a viral inactivation step at acidic pH (3.6) is also included prior to the intermediate step of the platform (see Figure 4).

Whatever the order of operation used, both DBC and selectivity (in this case aggregate removal) are key parameters to achieve drug product critical quality attributes, and should therefore be carefully evaluated during process development.

2. Materials and Methods

2.1. Materials

- **Monoclonal Antibody Feedstocks**

Adalimumab, Anti-Her2 (humanized IgG_1 monoclonal antibody that binds Her2), and Rituximab. mAb proteins were diluted to 2 g/L with the appropriate equilibration buffer. The pH was adjusted with 1 M acetic acid or 0.5 M Tris-Base, and conductivity was adjusted with the addition of NaCl.

- **Chromatography Sorbents**

Sorbents for comparative studies are listed in Table 1. mAbs were loaded on 1 mL prepacked columns and all experiments were performed using an ÄKTA♦ avant 25 system (GE Healthcare).

Table 1
Sorbents used for comparative studies

Sorbent	Base Matrix	Ligand	Particle Size (µm)
CMM HyperCel sorbent (Pall)	Highly cross-linked cellulose	Aminobenzoic acid	50-80
Multi-mode weak cation exchanger (MM-Agarose)		Multimodal weak cation exchange	75
Sulfopropyl cation exchange (S-CEX) resin	Cross-linked polystyrenedivinylbenzene	Sulfopropyl (— CH_2 CH_2 CH_2 SO_3 —)	50

2.2. Determination of Dynamic Binding Capacity

DBC was based on the volume (mL) of load material at a specific mAb concentration required to achieve 5% breakthrough (BT). The equation below was used to calculate the DBC at 5% BT:

$$\text{DBC at 5\% BT} = \frac{(V5\% - DV) \times CL}{CV}$$

Where: V5% = Volume at 5% BT (mL)
DV = Dead volume (mL)
CL = mAb concentration in load (mg/mL)
CV = Volume of the column (mL)

2.3. Quantification of HCP and Aggregate Analysis

- HCP quantification was conducted using CHO HCP ELISA kits, 3G (F550, Cygnus technologies).
- High performance liquid chromatography – size exclusion chromatography (HPLC–SEC) was chosen for soluble aggregate analysis (TSKgel♦ SuperSW3000 4.6 mm x 30 cm SEC column, Tosoh, P/N 18675) using a mobile phase of 20 mM sodium phosphate, pH 6.7, 0.5 M sodium perchlorate.
- Dip and Read♦ Protein A (ProA) biosensors (Fortebio, P/N 18-5010) and the ForteBio Octet♦ Red 96 biolayer interferometry (BLI) system were used to determine the mAb concentration of harvest cell culture fluid (HCCF) samples.
- A NanoDrop♦ 8000 spectrophotometer (ThermoFisher Scientific) was used to determine the mAb concentration of purified mAb samples by UVA_{280}.

3. Experiments and Results

3.1. Comparison of Dynamic Binding Capacities

A broad range of loading conditions were explored, from pH 5.0 to 8.0 and conductivity of 5 to 45 mS/cm. Chromatography steps and buffers are shown in Table 2, and DBC histograms in Figure 1.

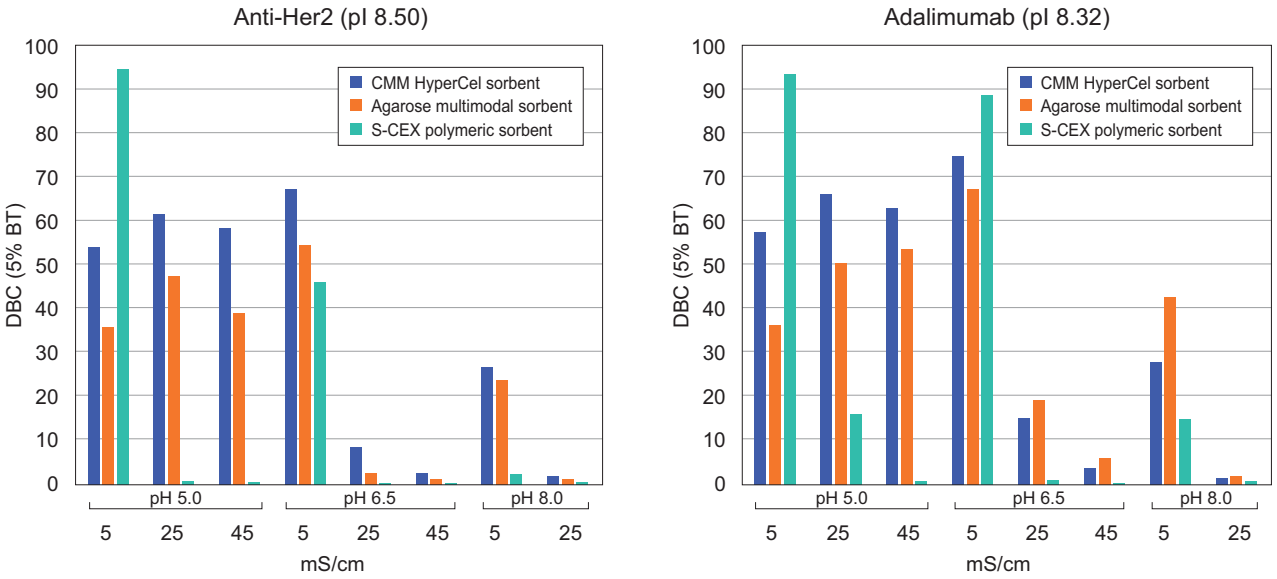
Table 2

Chromatography method used for DBC study

Step	Residence Time (min)	Duration	Buffer
Equilibration	1	5 or 10 column volumes (CV)	25 mM Na-acetate pH 5.0 at 5, 25, or 45 mS/cm 25 mM Na-phosphate pH 6.5 at 5, 25, or 45 mS/cm 25 mM Tris-HCl pH 8.0 at 5 or 25 mS/cm
Load	4	To 5% BT	
Wash 1	1	2 or 5 CV	Equilibration buffer
Wash 2	1	10 CV	25 mM Na-phosphate pH 6.5, 25 mS/cm NaCl
Strip	1	5 CV	25 mM Tris-HCl pH 8.0, 45 mS/cm NaCl
Cleaning-in-place (CIP)	1	5 CV	1 M NaOH

Figure 1

Comparative DBC study according to loading buffer pH and conductivity: DBC at 5% breakthrough (BT) for Anti-Her2 and Adalimumab using CMM HyperCel, agarose multimodal, and sulfopropyl polymeric cation exchange (S-CEX) sorbents



Results

As shown in Figure 1, the S-CEX sorbent exhibits higher DBC at low pH and conductivity (pH 5.0, 5 mS/cm). In contrast, CMM HyperCel sorbent – and to a lesser extent, the weak cation multi-mode agarose (MM-Agarose) sorbent – has a significantly higher DBC than S-CEX resin at higher conductivity (up to >60 mg/mL at pH 5.0, 45 mS/cm).

Therefore, CMM HyperCel sorbent allows operation over a broad range of pH (5.0 to 8.0) and conductivity (5 to 45 mS/cm), enabling process flexibility.

3.2. Comparison of Selectivities (Aggregate Removal Efficiency)

The selectivity performance of CMM HyperCel sorbent was compared to S-CEX and MM-Agarose sorbents. Selectivity is defined as aggregate removal with respect to yield. Conductivity elution gradient analysis was used to characterize the selectivity properties of the three sorbents. Elution gradients at different constant pH values (6.5 and 7.5) are shown in Figures 2 and 3. The elution peaks were fractionated and each sample subjected to mAb concentration analysis (by UV) and mAb aggregate analysis (by size exclusion chromatography). This enables the determination of accumulated recovery of mAb through the elution peak as well as the associated % aggregate. As aggregates typically elute later than monomer, this allows the identification of the % of product that can be recovered before a certain threshold level of aggregate is achieved. For example, in Figure 2C, if the target aggregate is below 0.5%, the yield from S-CEX sorbent will be <50%, from MM-Agarose <90% and from CMM HyperCel >90%.

At constant pH, each process included a conductivity elution gradient (5 mS/cm to 1 M NaCl) spanning over 20 column volumes (CV). Runs were conducted with conductivity elution gradients at a constant pH 6.5 (S-CEX sorbent) and pH 7.5 (Anti-Her2, CMM HyperCel and MM-Agarose sorbents). These pH values were selected as more appropriate for each type of sorbent. To increase resolution within the elution gradients, the columns were underloaded with a total of 10 mg of protein on 1 mL columns with a residence time (RT) of 4 minutes.

Figure 2

Conductivity elution at constant pH 6.5, 5 mS/cm to 1 M NaCl: Accumulated percent aggregate versus percent yield across the conductivity elution gradients

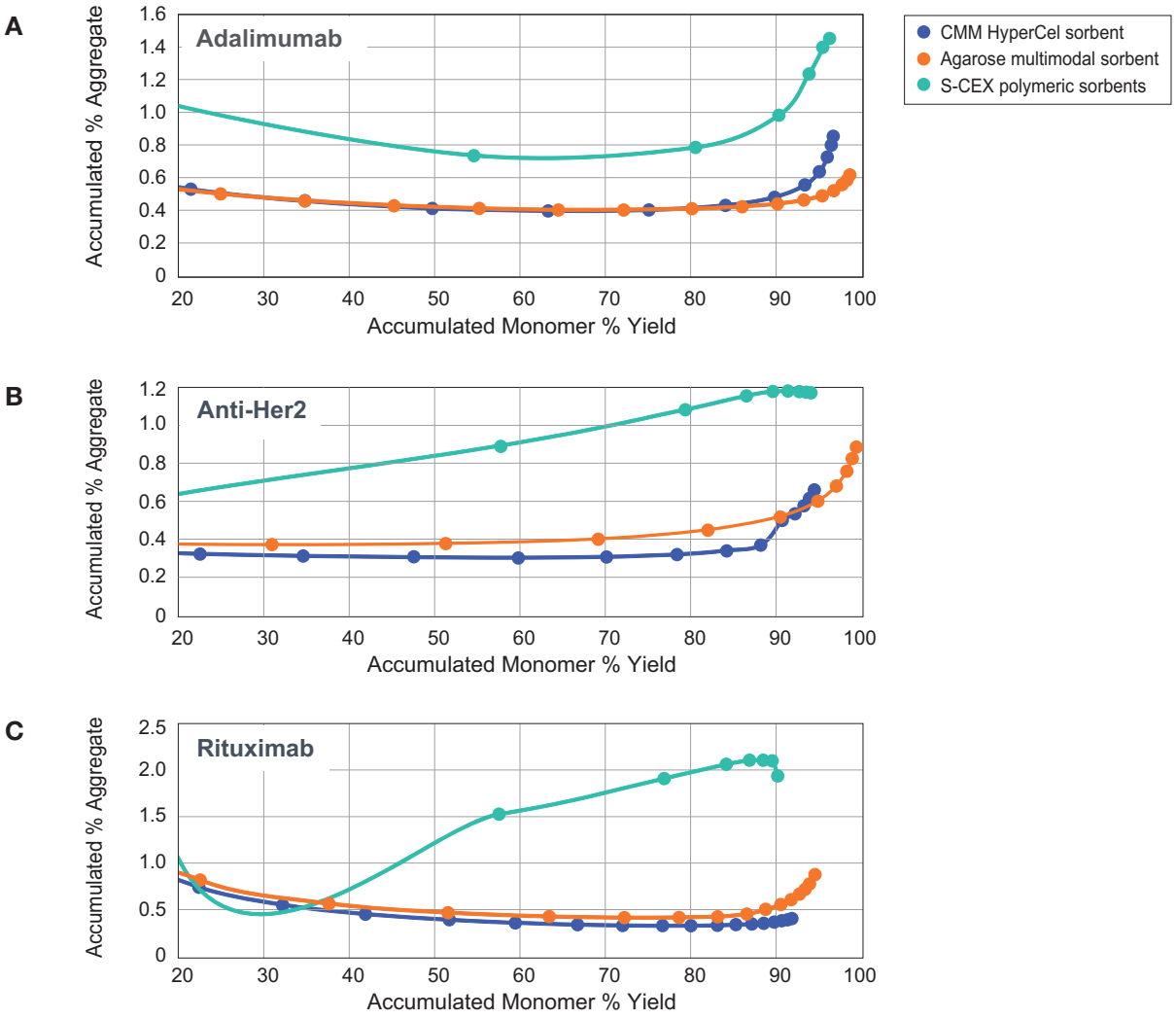
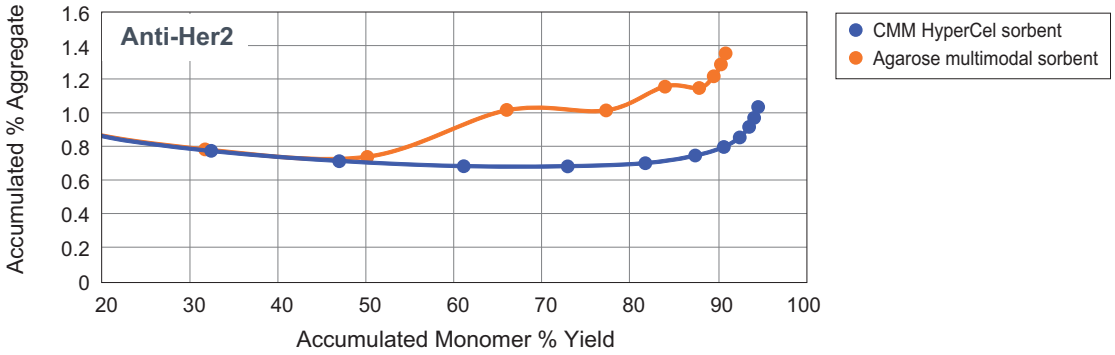


Figure 3

Conductivity elution gradients at a constant pH of 7.5, 5 mS/cm to 1 M NaCl: Accumulated percent aggregate versus percent yield across the conductivity elution gradients



Selectivity Comparison Results

- With conductivity elution gradients at a constant pH of 6.5, an increased aggregate removal was achieved with CMM HyperCel and the MM-Agarose sorbents compared to the S-CEX resin, for three different mAbs (Figure 2).
- With conductivity elution gradients at a constant pH of 7.5, both CMM HyperCel and MM-Agarose sorbents showed superior aggregate removal compared to the S-CEX resin. However, CMM HyperCel sorbent outperformed the MM-Agarose sorbent (Figure 3).

Aggregate removal results are summarized in Table 3, showing the accumulated percent aggregate at 90% yield of mAb protein for each conductivity elution gradient.

Table 3

Selectivity: comparative aggregate removal performance

		Eluted: % Aggregate Protein vs. Monomer (at 90% mAb Recovery)			
mAb		Gradient Elution	S-CEX	CMM HyperCel	MM-Agarose
Adalimumab	(load: 1.2%)	pH 6.5, 5 mS/cm to 1 M NaCl	1.0	0.5	0.4
Rituximab	(load: 2.0%)		2.0	0.4	0.6
Anti-Her2	(load: 1.0%)		1.2	0.5	0.5
Anti-Her2	(load: 1.0%)	pH 7.5, 5 mS/cm to 1 M NaCl	–	0.8	1.2
		pH 5.5, 5 mS/cm to 1 M NaCl	1.1	–	–

Contaminant Removal Performance of CMM HyperCel Sorbent in a Three-Step Platform

Total process contaminants (HCP and aggregates) removal results for a three-step streamlined process (Figure 4) are shown in Table 4 for three different mAbs. Parameters included HCP, percent aggregate protein, and percent yield for the following samples: harvest, post Protein A / virus inactivation (VI), post Mustang Q membrane, and post CMM HyperCel sorbent.

Overall, this process resulted in a final product with <14 ppm HCP and <0.5% aggregate protein with >95% yield for all three steps, in line with regulatory expectations.

Figure 4

Three-step streamlined process

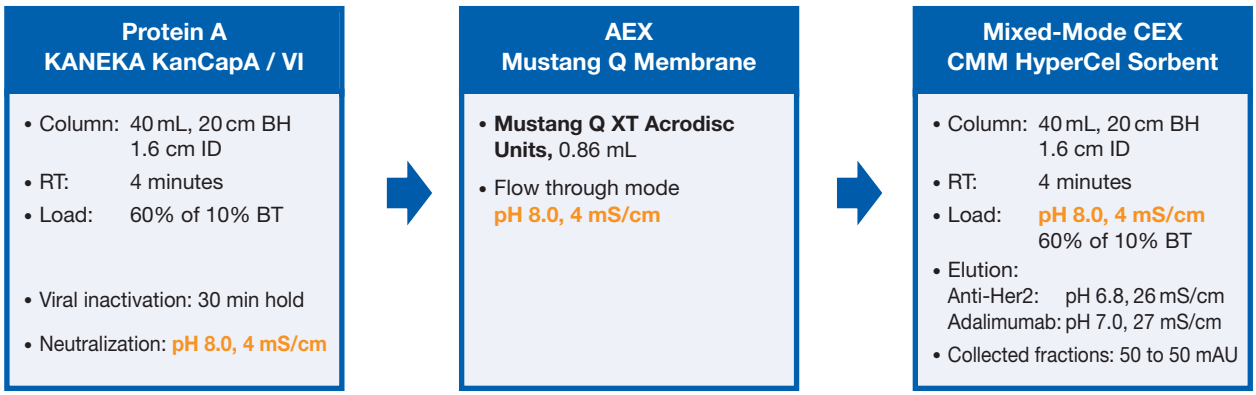


Table 4

Contaminant removal and yield for a three-step streamlined process

	Mabs	Harvest	Post Protein A/VI	Post Mustang Q	Post CMM HyperCel
HCP (ppm)	Anti-Her2	140,000	290	85	14
	Adalimumab	250,000	840	38	9
Aggregate (%)	Anti-Her2		0.8	0.8	0.5
	Adalimumab		1.1	0.7	0.3
Step Yield (%)	Anti-Her2		96	97	99
	Adalimumab		>100	95	98

4. Discussion: More Flexibility and More Process Options

As shown in this study, the CMM HyperCel mixed-mode cation exchanger maintains a high DBC over a broad range of pH and conductivity. This feature allows for more flexible process development, enabling different options according to previous steps in the process:

Option 1: High pH Load, Streamlined (Three Step Smoothly Integrated Process Without Buffer or pH Adjustments)

Protein A capture, (KANEKA KanCapA sorbent), virus inactivation, intermediate step on Mustang Q AEX membrane adsorber in FT mode, and CMM HyperCel sorbent for final polishing. In this case, one condition of pH 8.0, 5 mS/cm could be used to both maximize HCP removal by the AEX step, and still achieves acceptable DBC during the subsequent CMM HyperCel sorbent step. Such an integration cannot be performed with a conventional S-CEX resin (i.e., with two mAbs at pH 8.0, 5 mS/cm, a DBC >26 mg/mL obtained with CMM HyperCel sorbent versus 2 or 15 mg/mL with S-CEX sorbent according to the mAb).

Option 2: High Conductivity Load

This option can be used for other proteins than mAbs, for example to process an eluted product from a preceding step within a multi-step purification. As an example, recombinant proteins, Fab fragments, engineered antibodies purification processes may include multiple bind and elute steps (no initial Protein A capture). Such chromatography steps may require high conductivity elution, or to keep high conductivity to preserve protein stability. These feedstocks with high conductivity may then be directly loaded onto CMM HyperCel columns without buffer exchange or pH adjustments, which would not be possible with conventional sorbents. At the same time, this high conductivity load would not significantly jeopardize DBC, leading to improved process economics and throughput.

General Conclusions

- CMM HyperCel sorbent has a high DBC (>60 mg/mL at pH 5.0, 25 mS/cm) over a broad range of pH (5.0 to 8.0) and conductivity (5 to 45 mS/cm).
- These unique features provide maximal flexibility during process development, allowing streamlined operation with direct load of samples from previous process steps without feed adjustment.
- This contributes to enhanced process economics, saving unit operation costs such as ultrafiltration/diafiltration (UF/DF) and buffers.
- CMM HyperCel mixed-mode sorbent exhibits better selectivity compared to the S-CEX and multi-mode sorbents, leading to improved mAb aggregate removal, which is in line with regulatory expectations for mAb product.
- These properties can be exploited to purify other « non-mAb » proteins or engineered antibodies and fragments.
- The three-step streamlined platform uses scalable sorbents that can be cleaned by usual NaOH CIP treatment and disposable membrane devices which eliminate column packing hurdles and associated costs.

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