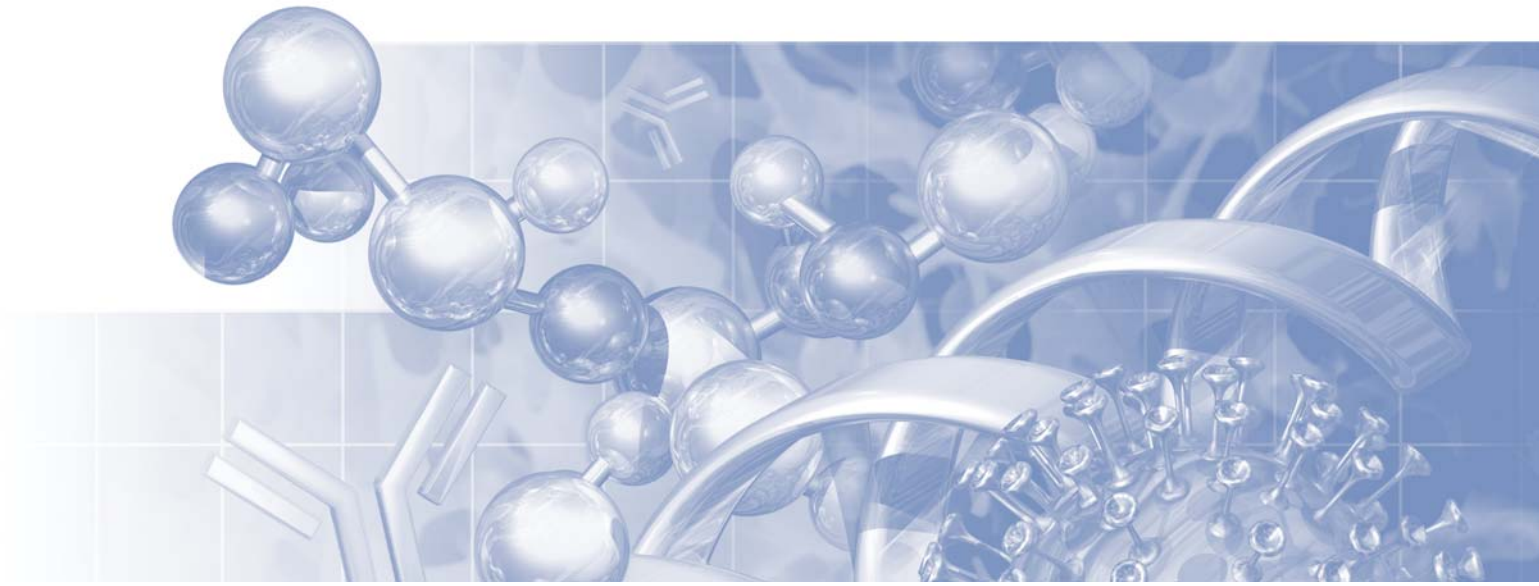
PALL Biotech

Application Note

USD 3317

A Universal Streamlined Three-Step Chromatography Platform for Monoclonal Antibody Purification

Flexible Use of CMM HyperCel™ Cation Exchange Mixed-Mode Sorbent for Host Cell Protein and Monoclonal Antibodies Aggregate Removal



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Summary

This application note describes a flexible and scalable chromatography platform suitable for the purification of monoclonal antibodies of different isoelectric points (pI) and physical properties.

The platform consists of three efficiently integrated purification steps:

1. Protein A capture (with KANEKA KanCapA♦ chromatography sorbent),
2. Anion exchange (AEX) membrane chromatography on Mustang® Q membrane (in flow through mode),
3. A final polishing/aggregate removal step on the mixed-mode CMM HyperCel cation exchange sorbent.

The properties of this unique mixed-mode sorbent allows load of the feedstream directly from the upstream anion exchanger, without any pH or conductivity adjustment, while keeping satisfactory levels of dynamic binding capacity (DBC). The platform is scalable and has been successfully tested on three different monoclonal antibodies (mAbs). The synergistic combination of the membrane AEX step and the mixed-mode cation exchange results in excellent impurity removal. Typically, the process results in ≥ 95% yield for all three steps within high mAb final purity (≤ 0.5% aggregate protein and ≤ 14 ppm of host cell protein [HCP]).

Benefits of Pall's mAb purification platform:

- Scalable and robust: adaptable to a variety of mAbs due to CMM HyperCel sorbent which allows to operate in a large design space.
- Avoids time and buffer consuming unit operations, e.g., dilution or diafiltration. The three chromatography steps are streamlined.

1. Introduction

Conventional industry-designed mAb chromatography purification platforms typically include a maximum of three steps, usually starting with a Protein A affinity capture. Important parameters for process economics include dynamic binding capacity, buffer consumption, overall process yield, and final purity achieved (HCP, DNA, virus and mAb aggregate removal). However, due to the variety of mAbs (as well as the development of mAb fragments and engineered antibodies) and chromatography sorbents characteristics, conventional purification processes (i.e., using cation exchangers) often require different pH and feed conductivity adjustments between chromatography steps to achieve sufficient levels of DBC and selectivity.

We describe hereunder a flexible platform, streamlined without buffer adjustments, that can meet the objectives of effective purification of mAbs with different isoelectric points and properties. The final step using a unique mixed-mode cation exchange sorbent (CMM HyperCel sorbent) is a key factor in the performance of the process.

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 the conductivity was adjusted with the addition of 5 M NaCl (as needed).

- **Chromatography Sorbents and Membranes**

Refer to Table 1. All runs were conducted using an ÄKTA♦ avant 25 system (GE Healthcare).

Table 1
Pall Sorbents and membranes used for small scale and scale-up studies

Sorbents / Membranes	Part Numbers (P/N)
KANEKA KanCapA columns, 1 mL, 0.5 x 5 cm	PRC05X050KANCAPA
CMM HyperCel columns, 1 mL, 0.5 x 5 cm	PRCCMMHCEL1 ML
KANEKA KanCapA columns, 40 mL, 20 cm bed height (BH), 1.6 cm internal diameter (ID)	26080-026
CMM HyperCel columns, 40 mL, 20 cm BH, 1.6 cm ID	20270-031
Mustang Q XT Acrodisc® units, 0.86 mL	MSTGXT25Q16
AcroPrep™ Advance 96-well filter plate with Mustang Q membrane	8071

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% or 10% breakthrough (BT).

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

Where: V5% = Volume at 5% BT (mL)
CL = mAb concentration in load (mg/mL)
DV = Dead volume (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. Optimization of Chromatographic Parameters on CMM HyperCel Sorbent

The mixed-mode CMM HyperCel cation exchange (CEX) sorbent described as a salt-tolerant cation exchanger is critical for the removal of aggregates in the final step of the process. CMM HyperCel sorbent is composed of a rigid cellulose matrix that has flow properties compatible with the needs of manufacturing scale protein production. The proprietary ligand, containing both a primary amine and a carboxyl group, confers cation exchange and hydrophobicity properties to the sorbent. At working pH (4 to 9), the amine group is never charged (pKa < 4). The carboxyl group is weakly charged at adsorption pH (4 to 6) to allow protein adsorption based on hydrophobicity. At elution pH (7 to 9), the carboxyl group is fully deprotonated and the elution will be based on negative charge repulsion. The flexibility of the ligand enables the separation of proteins with a large variety of isoelectric points and hydrophobicity levels (mAbs, antibody fragments and recombinant proteins). Fine tuning pH and conductivity conditions can be used to separate targeted molecules from closely related contaminants.

CMM HyperCel sorbent maintains a high DBC over a broad range of pH and conductivity conditions, circumventing the need to buffer exchange or dilute product before loading.

The first step in preliminary studies was to define DBC over a broad range of loading buffer conditions for CMM HyperCel sorbent with the specific mAb feedstocks used.

3.2. Dynamic Binding Capacity and Choice of Adapted Buffer Conditions

Table 2

Buffer conditions tested for CMM HyperCel sorbent DBC optimization

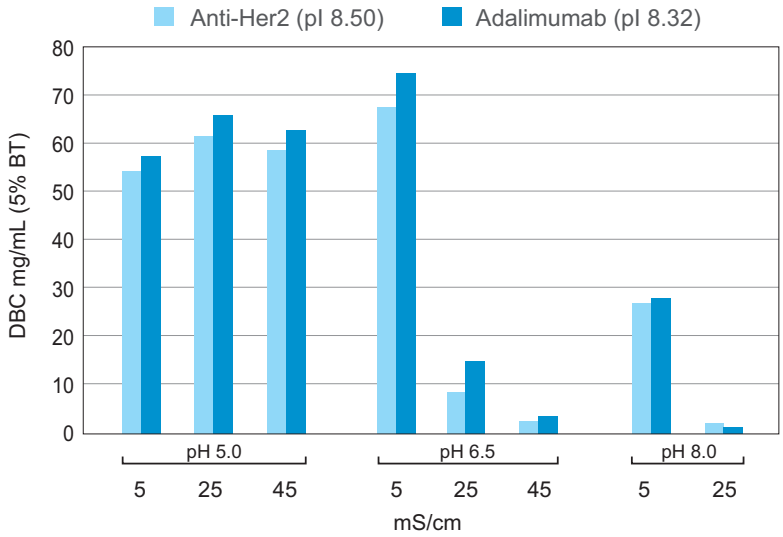
Step	Residence Time (min)	Duration	Buffer
Equilibration	1	5 or 10 column volumes (CV)	25 mM Na-acetate pH 5.0 at 5, 25, or 45 mS/cm 25 mM Na-phosphate pH 6.5 at 5, 25, or 45 mS/cm 25 mM Tris-HCl pH 8.0 at 5 or 25 mS/cm
Load	4	Variable	To 5% BT
Wash 1	1	2 or 5 CV	Equilibration buffer
Wash 2	1	10 CV	25 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
Equilibration	1	6 CV	Appropriate equilibration buffer

Studies confirm that CMM HyperCel sorbent can bind mAbs in a broad range of pH (5.0 to 8.0) and conductivity conditions (5 to 45 mS/cm), in contrast with conventional cation exchangers that typically operate in a pH range from 5.0 to 6.5.

As shown in Figure 1, a DBC >60 mg/mL can be observed for the two mAbs tested (62 and 66 mg/mL at pH 5.0, 25 mS/cm) which is a much higher conductivity than that currently used with conventional cation exchangers. This provides maximal flexibility for different process conditions. However, for the streamlined process described here, the eluate of the previous anion exchange membrane step must be directly loaded at pH 8.0 onto the CMM HyperCel column, which would not be possible with CEX. This buffer condition is also appropriate to maximize HCP removal by the previous membrane flow through step and the best compromise in terms of HCP removal and capacity on CMM HyperCel sorbent in this design space. Therefore, loading at pH 8.0 at a conductivity of 5 mS/cm was selected and still resulted in a good DBC level for different mAbs (>26 mg/mL).

Figure 1

DBC of CMM HyperCel sorbent for two mAbs, Anti-Her2 and Adalimumab, according to pH and conductivity



3.3. Selectivity (Aggregate Removal) Optimization

The CMM HyperCel sorbent unique mixed-mode mechanism allows discrimination of proteins with closely related isoelectric points and/or hydrophobicities. Optimization of pH and conductivity conditions is critical to obtain the best separation between monomeric mAb and aggregates. This optimization was performed by testing conductivity gradients over a fixed range of different pH values (constant pH of 6.5, 7.5 or 5.5). Three different mAbs, loaded at a residence time of 4 minutes were tested (Adalimumab, Rituximab, and Anti-Her2).

The results on the percent aggregate at 90% yield of monomer for each conductivity elution gradient for the three mAbs tested are shown in Table 3.

Table 3

Aggregate removal efficiency for three different mAbs. Summary of the percent aggregate mAb eluted proteins at 90% mAb recovery obtained from the conductivity gradient study

mAb	Aggregate in Load	Gradient Elution	Post CMM HyperCel Sorbent
Adalimumab	1.2%	pH 6.5, 5 mS/cm to 1 M NaCl	0.5%
Rituximab	2.0%		0.4%
Anti-Her2	1.0%		0.5%

Conclusion

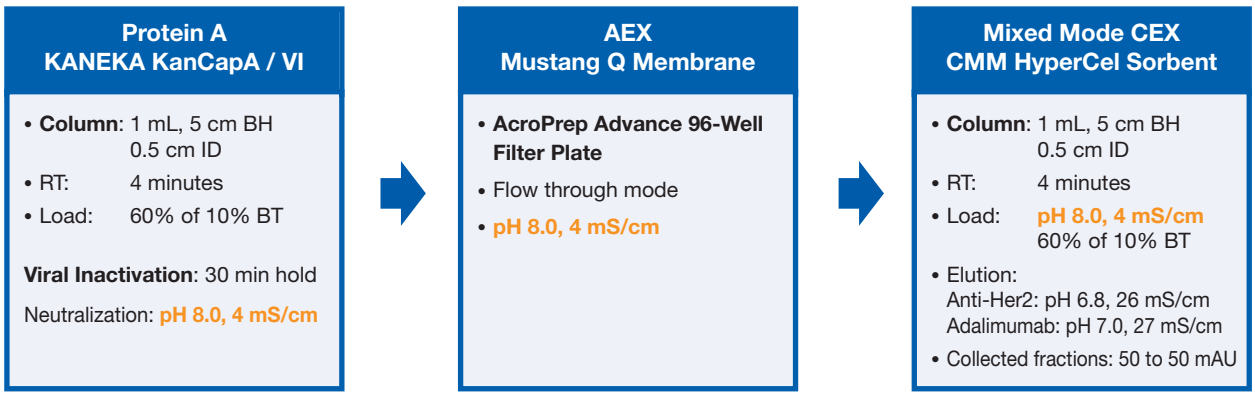
CMM HyperCel sorbent is effective for the removal of aggregates from a range of mAbs. By employing a simple conductivity gradient, up to 80% of aggregate can be removed without any further process optimization.

4. Purification of mAbs with the Streamlined Platform

To further refine the process, the gradient purification step was transitioned to a single step elution. This required a design-of-experiment (DoE)-based optimization and resulted in a unique elution buffer for the three mAbs. The optimized three-step purification platform was tested at laboratory scale (1 mL columns and 14 µL membrane per well in 96-well plates) and scaled-up to 40 mL columns and 0.86 mL Mustang XT Acrodisc devices. Parameters studied are HCP, percent aggregate protein, and percent yield. Process and parameters are shown in Figure 2 and Table 4.

4.1. Laboratory Scale Experiments

Figure 2
Streamlined process at laboratory scale



- **mAbs Capture**

Capture of mAbs was achieved using KANEKA KanCapA Protein A sorbent in 1 mL prepacked columns at the process development scale. This sorbent has a C-domain pentamer that exhibits high alkaline stability and negligible Fab binding. It achieves high DBC at 4 to 6 minutes residence time.

- **AEX**

AEX was conducted in flow through mode for impurity removal (as demonstrated with the decrease in HCP) on Mustang Q membranes in AcroPrep Advance 96-well filter plates of 14 µL.

- **Scale-up**

Scale-up was performed on Mustang Q XT Acrodisc devices of 0.86 mL.

- **Final Polishing**

Final polishing was achieved on CMM HyperCel as previously described.

Table 4
Process conditions

KANEKA KanCapA Sorbent	Buffers	Composition	CV
(Residence time = 4 min)	Equilibration	PBS	3
	Load	Adalimumab (ADA): Harvest (titer 0.7 g/L) Anti-Her2 (AH2): Harvest (titer 1.1 g/L) Rituximab (RIT): Harvest (titer 0.8 g/L)	To 60% of 10% BT
	Equilibration wash	PBS	3
	Intermediate wash	PBS pH 7.2, +0.5 M NaCl, 0.3 M arginine	4
	Pre-elute wash	ADA/AH2: 20 mM Na-phosphate pH 7.2, 4 mS/cm NaCl RIT: 20 mM Na-phosphate pH 5.8, 4 mS/cm NaCl	4
	Elution	50 mM acetic acid pH 3.0	4
	CIP	0.1 N NaOH	3

Virus Inactivation (VI)	Steps	Composition
	Low pH step	Addition of 1 M acetic acid to pH 3.6
	30 minutes hold step	N/A
	Neutralization step	Addition of 0.5 M Tris-base to final pH 8.4, 4 mS/cm

Mustang Q Membrane (Flow Through)		
	Membrane equilibration	20 mM Na-phosphate pH 8.0, 4 mS/cm

CMM HyperCel Sorbent	Buffers	Composition	CV
(Residence time = 4 min)	Equilibration	25 mM Tris-HCl pH 8.0, 4 mS/cm	10
	Load	Post Mustang Q	To 60% of 10% BT
	Wash	ADA/AH2: 25 mM Tris-HCl, pH 7.6, 4 mS/cm RIT: 25 mM Tris-HCl, pH 8.0, 4 mS/cm	7
	Elution	ADA: 50 mM Na-phosphate pH 7.0, 27 mS/cm AH2: 50 mM Na-phosphate pH 6.8, 26 mS/cm RIT: 50 mM Tris-HCl pH 7.0, 26 mS/cm	15 (50 to 50 mAU)
	CIP	1.0 N NaOH	3

Table 5

Small laboratory scale streamlined three-step purification: HCP and aggregate removal

	mAbs	Harvest	Post Protein A / VI	Post Mustang Q	Post CMM HyperCel
HCP (ppm)	Anti-Her2	160,000	240	39	8
	Rituximab	750,000	380	28	13
	Adalimumab	160,000	130	15	7
Aggregate (%)	Anti-Her2		1.1	1.3	1.0
	Rituximab		0.8	0.8	0.7
	Adalimumab		0.8	0.7	0.4
Step yield (%)	Anti-Her2		75	99	90
	Rituximab		91	96	94
	Adalimumab		87	94	88

4.2. Scale-Up to 40 mL Columns

Figure 4

Scale-up

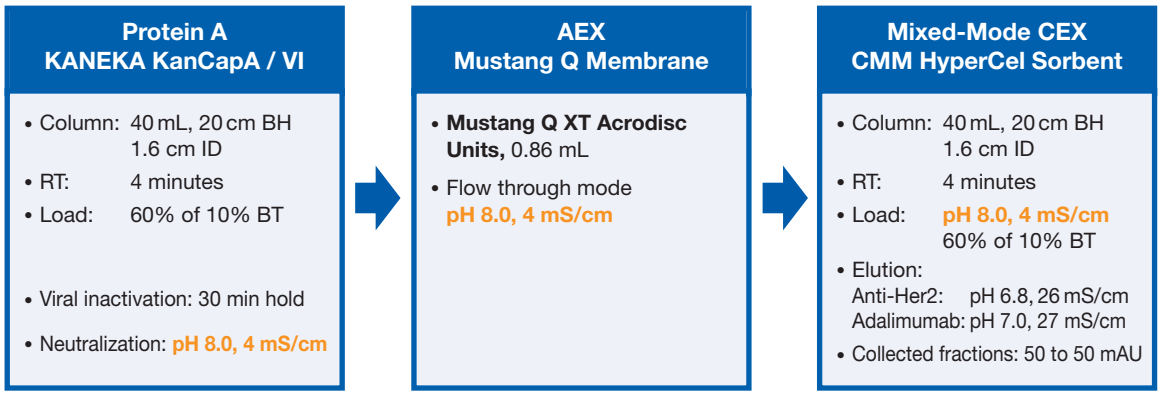


Table 6

Scale-up of streamlined three-step purification: HCP and aggregate removal

	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

Conclusions

The results of these studies in Table 5 (small scale) and Table 6 (scale-up) show a high yield (mAb recovery) and purity (HCP and aggregate protein removal).

In addition, the process performance was maintained and aggregate removal was even improved with the scale-up chromatography process: purification of Adalimumab and Anti-Her2 resulted in ≥ 95% yield for all three steps within the process and final products with high purity (≤ 0.5% aggregate protein and ≤14 ppm HCP).

General Conclusions

The three-step chromatography process described in this note provides a robust and flexible platform to purify mAbs that can be implemented in batch or continuous mode.

All sorbents and Mustang Q membrane are designed for industrial operations, are scalable, can be cleaned in place using standard NaOH treatment, and are supported by extensive Pall validation documentation.

- **Protein A capture on KANEKA KanCapA sorbent** provides high step purity and DBC
- **Mustang Q membrane chromatography** is processed on a plug-and-play single-use device, minimizing footprint and avoiding cumbersome packing/unpacking operations
- **CMM HyperCel mixed-mode cation exchange sorbent** used for final polishing results in efficient HCP and aggregate removal, and enables product capture over a very broad range of pH and conductivities while maintaining high DBC
- **The three chromatography steps are smoothly streamlined** (no buffer exchange or pH adjustments) avoiding time and buffer-consuming unit operations such as dilution or diafiltration
- **Other mAbs have been purified using this platform, confirming its scalability and robustness**. The platform can easily be adapted to a variety of mAbs that exhibit a broad range of isoelectric points and hydrophobicities

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