Tentacle cation exchangers for optimized mAb purification: Understanding the relationship between tentacle chemistries and resin performance

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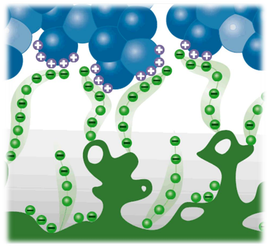
Indroduction

In the present work, protein binding characteristics as well as selectivities of different Fractogel® EMD tentacle cation exchange (CEX) resins have been evaluated in several mAb purification studies, focusing on mAb aggregate removal and HCP clearance. The goal was to obtain a comprehensive understanding of the relationship between the type of CEX tentacle chemistry and resin performance. This should facilitate optimization of the CEX separation step through the selection of the appropriate tentacle resin with respect to process-specific mAb purification objectives and in conjunction with appropriate experimental conditions obtained from screening experiments.

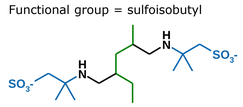
Working principle of tentacle resins



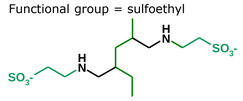
Fast mass transport properties:

Strong Cation Exchange

Fractogel® EMD SO_3^- (M) resin
Functional group = sulfoisobutyl



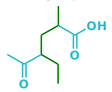
Fractogel® EMD SE Hicap (M) resin
Functional group = sulfoethyl



pK_A < 1

Weak Cation Exchange

Fractogel® EMD COO^- (M) resin
Functional group = carboxy



pK_A ~ 4.7

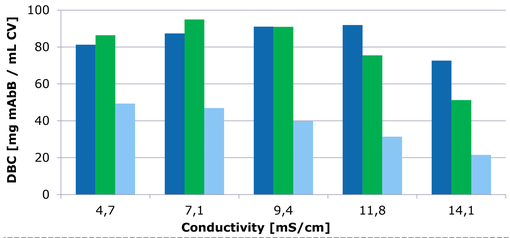
Resin	Typical DBC (mAb)	Operating Flow Rate*
Fractogel® EMD SO_3^- (M)	80-100 mg/mL packed resin	up to 200 cm/h at 1.5 bar
Fractogel® EMD SE Hicap (M)	80-100 mg/mL packed resin	up to 220 cm/h at 1.0 bar
Fractogel® EMD COO^- (M)	40-60 mg/mL packed resin	up to 300 cm/h at 1.8 bar

*Bed height: 20 cm; mobile phase: 150 mM NaCl
Compression factor: 1.33

Dynamic Binding Capacity

Dynamic monoclonal antibody binding capacities:

A) as function of conductivity

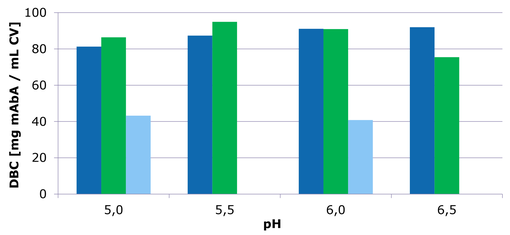


Residence time = 2 min
Column size = 40 mm x 10 mm i.d. (CV = 3.14 mL)
Sample: mAbB post-protein A pool, 5 mg/mL in 40 mM Na acetate + NaCl

Fractogel® EMD SO_3^- (M) resin
Fractogel® EMD SE Hicap (M) resin
Fractogel® EMD COO^- (M) resin

With the mAb investigated here, Fractogel® EMD SO_3^- (M) resin showed slightly higher and Fractogel® EMD SE Hicap (M) showed slightly lower binding capacities at elevated conductivities, Fractogel® EMD COO^- (M) resin showed lower binding capacities compared to strong CEX resins.

B) as function of pH



Column size = 20 mm x 8 mm i.d. (CV = 1 mL)
Sample: 5 mg/mL purified mAbA in 25 mM Na acetate + 25 mM Na_2HPO_4/NaH_2PO_4, conductivity 4 mS/cm

Fractogel® EMD SO_3^- (M) resin
Fractogel® EMD SE Hicap (M) resin
Fractogel® EMD COO^- (M) resin

With this particular mAb, Fractogel® EMD SO_3^- (M) and Fractogel® EMD SE Hicap (M) resins showed an optimum binding capacity at slightly elevated pH, whereas for Fractogel® EMD COO^- (M) resin, the capacity remained almost unchanged with pH variation.

Characteristic features of tentacle ion exchange media compared to conventional resins:

- enhanced surface area of the bead thus providing increased dynamic binding capacity (DBC)
- multi-point ligand-protein interactions delivering better selectivity
- improved resolution due to enhanced pore diffusion

Impurity Removal

HCP clearance

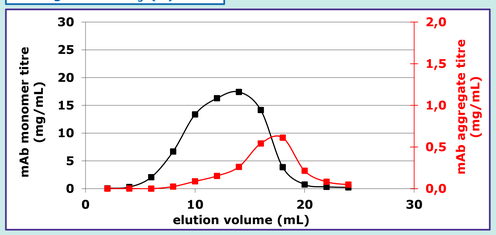
In order to better differentiate HCP clearance capabilities between the different strong CEX functional groups, the resins were challenged with cell culture supernatants (CCS) of various HCP starting levels:

Feed	Loading conditions	Resin	HCP in Load (ng/mg)	HCP in CEX eluate (ng/mg)	HCP removal factor
mAbA CCS	pH 5.0, 4 mS/cm flow rate: 150 cm/h protein load: 10 mg/mL CV	Fractogel® EMD SO_3^- Fractogel® EMD SE Hicap	26666 24974	515 428	52 58
	pH 6.0, 4 mS/cm flow rate: 150 cm/h protein load: 10 mg/mL CV	Fractogel® EMD SO_3^- Fractogel® EMD SE Hicap	21639 26850	199 189	109 142
	pH 6.0, 4 mS/cm flow rate: 300 cm/h protein load: 17 mg/mL CV	Fractogel® EMD SO_3^- Fractogel® EMD SE Hicap	155349	218 84	713 1849
mAbC CCS	pH 6.0, 4 mS/cm flow rate: 150 cm/h protein load: 10 mg/mL CV	Fractogel® EMD SO_3^-	8378	66	127
mAbD CCS	pH 6.0, 4 mS/cm flow rate: 150 cm/h protein load: 8 mg/mL CV	Fractogel® EMD SO_3^- Fractogel® EMD SE Hicap	97051	2169 1902	45 51

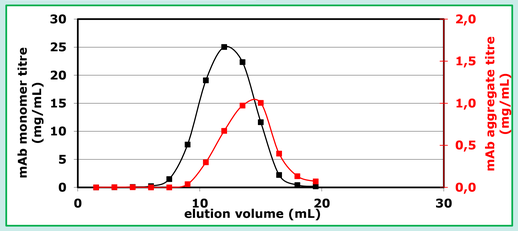
Aggregate Removal

Post Protein A capture pools of a mAb feed with 2.5% aggregate level were purified using three different tentacle CEX resins and linear salt gradient elution:

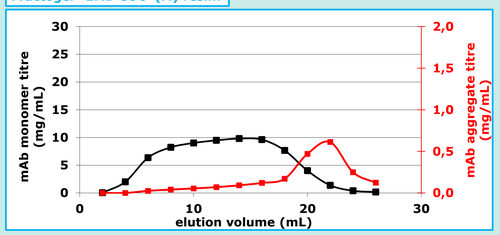
Fractogel® EMD SO_3^- (M) resin:



Fractogel® EMD SE Hicap (M) resin:



Fractogel® EMD COO^- (M) resin:



Summary of results:

Resin	mAb pool* yield (%)	mAb pool* volume (CV)	Load conductivity (mS/cm)	Conductivity at peak max. (mS/cm)
Fractogel® EMD SO_3^- (M)	89	3.3	6	19
Fractogel® EMD SE Hicap (M)	42	2.0		19
Fractogel® EMD COO^- (M)	94	4.9		16

*Pooling criteria: final mAb pool aggregate level ≤ 1.5%

Experimental Conditions:
- Feed: mAbE, post-protein A pool, 2.5% aggregates
- Column size: 200 mm L x 5 mm i.d. (CV = 3.9 mL)
- Operating buffer: 50 mM acetate + 24 mM NaCl, pH 5.0 (ca. 6 mS/cm)
- Linear gradient elution: 0 - 1 M NaCl in 40 CV
- Flow rate: 200 cm/h
- Load: 40 mg/mL CV

Summary
Among the tentacle cation exchangers tested, Fractogel® EMD SO_3^- (M) resin showed slightly higher mAb binding capacities at elevated conductivities compared to Fractogel® EMD SE Hicap (M) resin, indicating a higher degree of salt tolerance of sulfoisbutyl tentacles compared to sulfoethyl tentacle structures. Even though the binding capacity of the weak cation exchanger Fractogel® EMD COO^- (M) resin is generally lower compared to strong CEX tentacle resins, it provided the highest selectivity in the separation of mAb aggregates and monomers, especially when it comes to challenging cases of mAb aggregate separations.

When comparing strong cation exchangers, Fractogel® EMD SO_3^- (M) resin showed a significantly higher performance with respect to aggregate separation than Fractogel® EMD SE Hicap (M) resin, which can be attributed to the higher selectivity of the sulfoisobutyl ligand compared to the sulfoethyl ligand. Regarding HCP removal, however, Fractogel® EMD SE Hicap (M) resin holds great potential, as in individual cases the removal factor at the high end was more than double compared to Fractogel® EMD SO_3^- (M) resin.

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