Flow-Through Removal of mAb Aggregates with Eshmuno® CP-FT Resin

CEX Flow-Through vs. Bind/Elute Cation Exchange Chromatography

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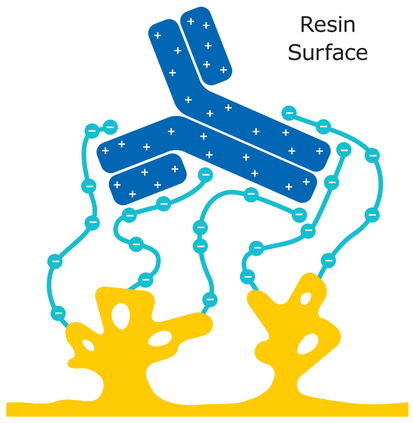
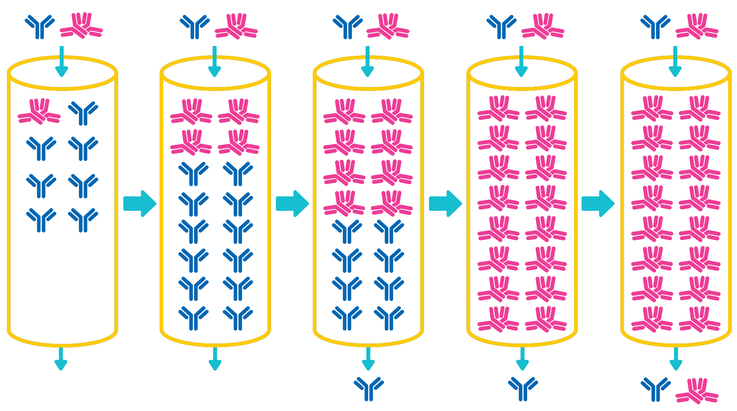
Introduction

Removing aggregates during the downstream purification of monoclonal antibody (mAb) therapeutics is imperative since these impurities increase the risk of an immunogenic response and can reduce efficacy. Aggregates are the most challenging impurity to remove in the downstream purification of mAbs as they are not removed by protein A chromatography and they have very similar isoelectric points and hydrophobicities to the monomeric protein. Eshmuno® CP-FT resin is the first CEX resin designed to efficiently remove aggregates using flow-through frontal chromatography.



Eshmuno® CP-FT resin has a novel tentacle ligand technology composed of both negatively charged ligands and neutral spacers that was optimized for the efficient removal of mAb aggregates. The unique tentacle structure facilitates displacement of the bound monomer by aggregates, which is the key process in a flow-through frontal chromatography mechanism.

During column loading both the monomer and aggregates will bind to the resin until the column is completely occupied. The monomer will break through the column first as it is displaced off the column by aggregates. When the column is entirely occupied by aggregates, they will also break through.



Frontal Chromatography Mechanism

Objective

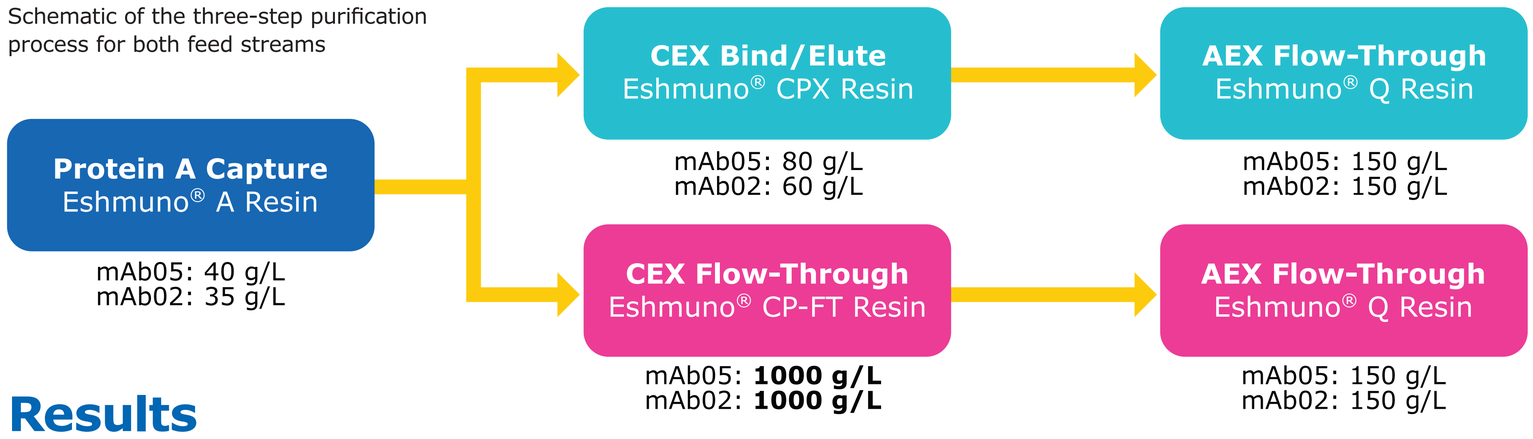
To demonstrate the advantage of using Eshmuno® CP-FT resin for the 3-step downstream purification of two mAbs when the CEX bind/elute chromatography step has been replaced with a CEX flow-through frontal chromatography step.

Experimental Plan

1. Two mAb feeds mAb05 (pI = 8.06), mAb02 (pI = 8.24) were enriched with aggregates using a high pH hold process (see Aggregate Enrichment Process below).
2. The feeds were then spiked into respective expressing clarified cell culture and then captured with affinity chromatography using Eshmuno® A resin.
3. Then the feeds were subjected to CEX bind/elute chromatography with Eshmuno® CPX resin or flow-through frontal chromatography with Eshmuno® CP-FT resin.
4. Finally the feeds were subjected to flow-through AEX chromatography with Eshmuno® Q resin.

Aggregate Enrichment Process

Schematic of the three-step purification process for both feed streams



Results

mAb05 Process Evaluation

Chromatography Step	Resin	Loading (g/L)	Monomer Recovery	Dimer	Higher MW Aggregates	Total Aggregates	HCP (ppm)	mAb Concentration (mg/mL)
1. Capture	**Eshmuno® A**	40	88%	2.29%	0.77%	3.06%	47	15.1
2. CEX bind/elute	**Eshmuno® CPX**	80	87%	0.42%	0%	0.42%	3	15.8
3. AEX flow-through	**Eshmuno® Q**	150	>99%	0.43%	0%	0.43%	1	3.1
2. CEX flow-through	**Eshmuno® CP-FT**	1000	92%	0.55%	0%	0.55%	17	13.6
3. AEX flow-through	**Eshmuno® Q**	150	>99%	0.61%	0%	0.61%	3	8.7

mAb02 Process Evaluation

Chromatography Step	Resin	Loading (g/L)	Monomer Recovery	Dimer	Higher MW Aggregates	Total Aggregates	HCP (ppm)	mAb Concentration (mg/mL)
1. Capture (adjusted to pH 6.0)	**Eshmuno® A**	35	97%	2.34%	0.54%	2.88%	228	14.9
2. CEX bind/elute	**Eshmuno® CPX**	60	98%	1.84%	0%	1.84%	63	9.9
3. AEX flow-through	**Eshmuno® Q**	150	>99%	1.44%	0%	1.44%	4	3.1
1. Capture (adjusted to pH 4.0)	**Eshmuno® A**	35	97%	1.98%	0.45%	2.43%	302	15.4
2. CEX flow-through	**Eshmuno® CP-FT**	1000	91%	0.77%	0%	0.77%	181	13.7
3. AEX flow-through	**Eshmuno® Q**	150	>99%	0.98%	0%	0.98%	9	8.5

Discussion

Removal of Aggregates and HCP

Removal of aggregates from mAb05 feed: Similar amounts of aggregates were removed by flow-through chromatography with Eshmuno® CP-FT resin as was accomplished with bind/elute chromatography.

Removal of aggregates from mAb02 feed: The flow-through removal of aggregates with Eshmuno® CP-FT was superior to bind/elute chromatography.

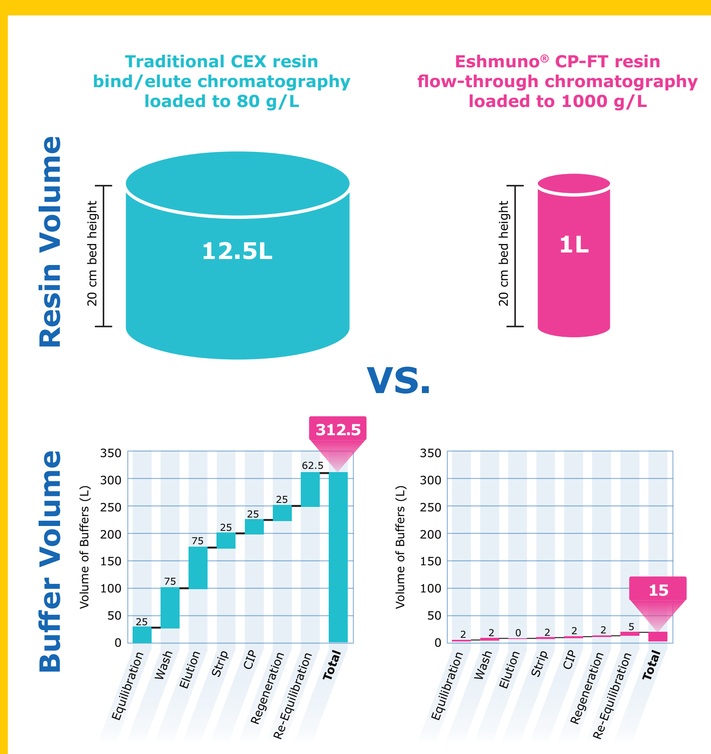
Removal of HCP from mAb05 and mAb02 feeds: CEX bind/elute chromatography was slightly more efficient at removing HCP, however the HCP levels for both feed streams were reduced below 10 ppm after the subsequent flow-through AEX step.

Feed Dilution Prior to AEX Flow-Through

mAb05 and mAb02 feeds: The feed from the CEX flow-through step had a much lower conductivity then the elution from the CEX bind/elute step. Therefore it did not need to be diluted prior to the AEX step.

Loading of CEX Columns

mAb05 and mAb02 feeds: Eshmuno® CP-FT resin was loaded to 1000 g/L in flow-through mode that is >12× than is possible in the bind/elute mode. The higher loading significantly reduces the volume of resin and buffers needed.



Summary

- Flow-through frontal chromatography using the Eshmuno® CP-FT resin removes similar amounts of mAb aggregates relative to traditional CEX bind/elute chromatography with significantly higher mass loading.
- After all three steps, similar amounts of HCP were removed for both the flow-through and bind/elute process streams.
- The lower conductivity feed from the CEX flow-through step did not require dilution prior to the AEX flow-through step.
- Significantly less resin and buffers are required for the flow-through CEX step relative to the bind/elute CEX step.