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Evaluation of novel CEX resin for continous processing of MAb purification

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Evaluation of novel CEX resin for continuous processing of Mab purification

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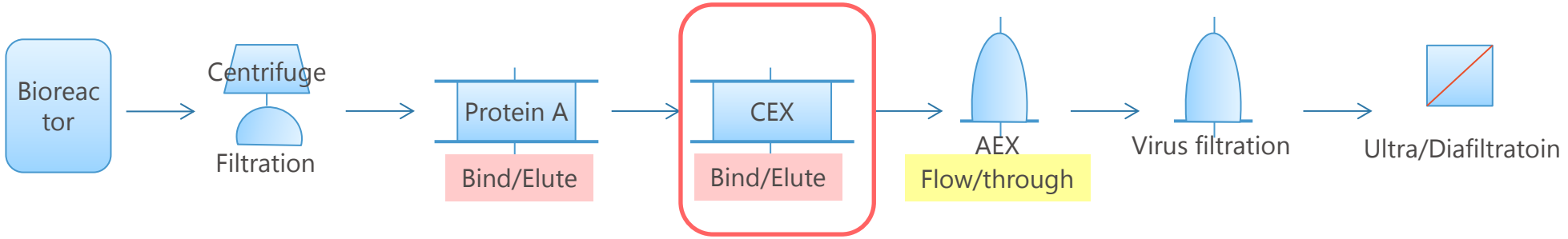
Abstract

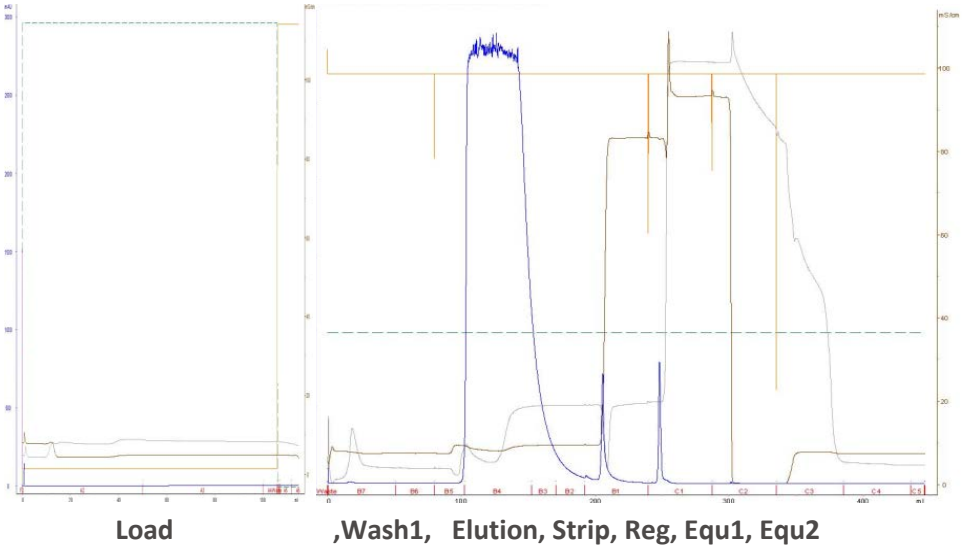
- We have evaluated novel cation exchange resin and membrane which is potentially suited for continuous process in biologics manufacturing.
- Typically cation exchange chromatography (CEX) is used in a bind/elute (B/E) mode for the MAb process since MAb act as cation at the buffer condition used for CEX due to the pI from neutral to weak basic. Generally, purpose of CEX chromatography is to remove aggregates and other impurities like HCP and DNA. Especially, aggregate removal is of interest to the industries. A continuous process in MAb downstream process can solve several bottlenecks in typical batch process. However its capability cannot be fully utilized if some step of chromatography is used in a B/E mode since it will take a longer process time due to the posing of product stream for binding and washing and it will take a more cost due to the requirement of large amount of resins as we use at the batch process. In such a case, chromatography in flowthrough mode has a potential to overcome those issues for both cost and time and enables us to develop more streamlined continuous process.
- We will present the result of our study to evaluate novel CEX resin developed by EMD Millipore (The Life Science division of Merck KGaA, Darmstadt, Germany) by comparing with the existing process of Mab A and we obtained a conclusion that this resin is fitted to the continuous processing very much. The novel resin showed a better impurity clearance than our existing process. For example, over 65% removal of aggregate by the novel resin was obtained in contrast to no removal by the existing process. A 20-fold better clearance for DNA was confirmed for the novel resin than the existing process. This indicates an additional polishing step can be omitted and this new chromatography can be a strong option if we need to reduce such impurities further. Also the resin cost is expected to be reduced down to 1/10 in maximum since those impurity clearance results were obtained about 10-times larger load than the typical B/E mode operation of CEX. Considering those aspects, we conclude that this resin showed a better fit for a continuous process. We will also discuss an expected effect of the novel CEX resin on cost and process time savings by a continuous process.

Background

Monoclonal antibody purification platform



- Typically in mAb purification process, CEX chromatography is operated in bind/elute mode due to the isoelectric point of mAb (pI 7-9).
- CEX removes aggregates, fragments, and HCP efficiently.
- Recently, CEX resin for flow/through mode has been developed in some companies.



Chromatogram of Current CEX step

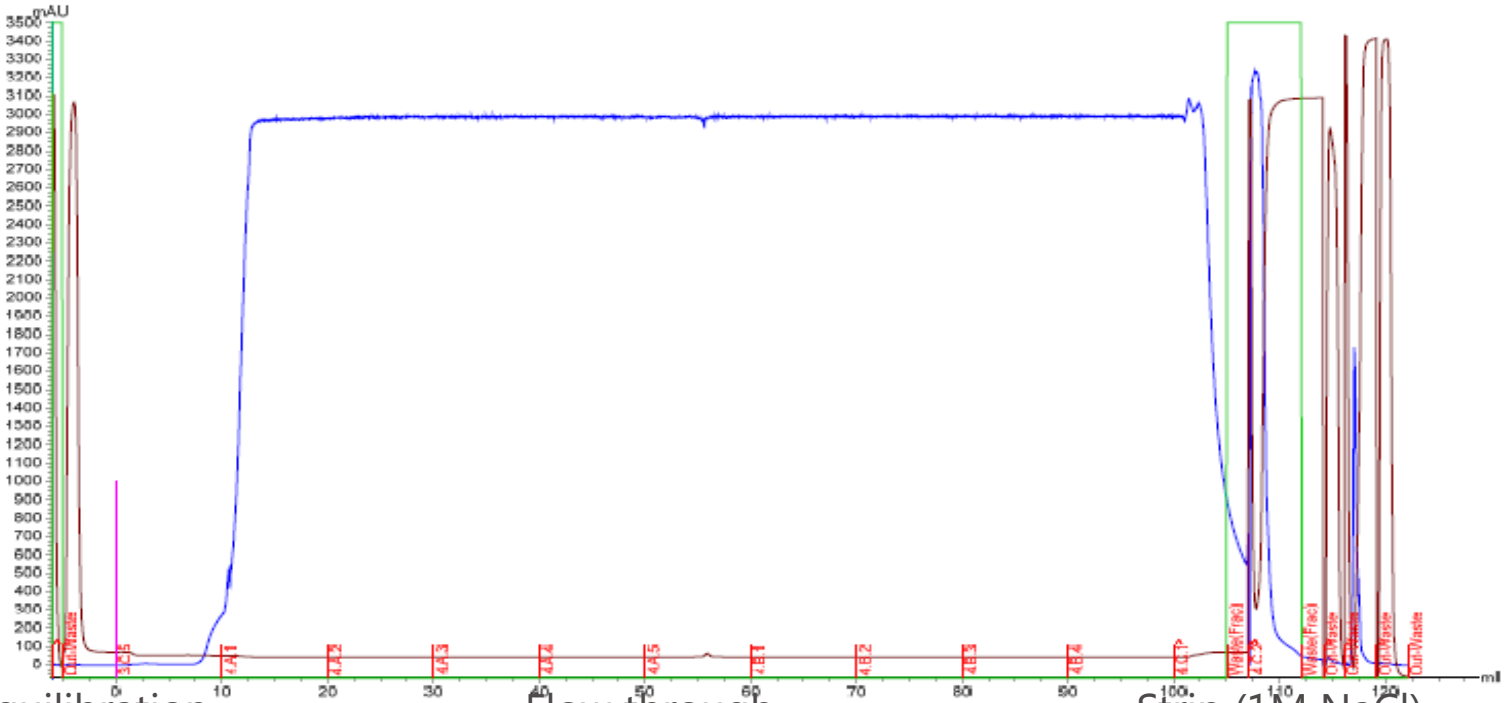
CEX Flow/through technology

- Bind impurities (HCP, DNA, Aggregate) → Smaller volume of resin or membrane
  Single-use compliant
- Less steps (no wash, elution step) → "Green" process
  Less process development needed
  Less buffer number (fewer steps)
  Reduced buffer consumption
- Easily applicable to continuous processing
  CEX → AEX → Virus filtration (tank less, hold less)
  Processing time and in-process holding tanks can be reduced
  Smaller column can be used

- Evaluation of novel CEX adsorber

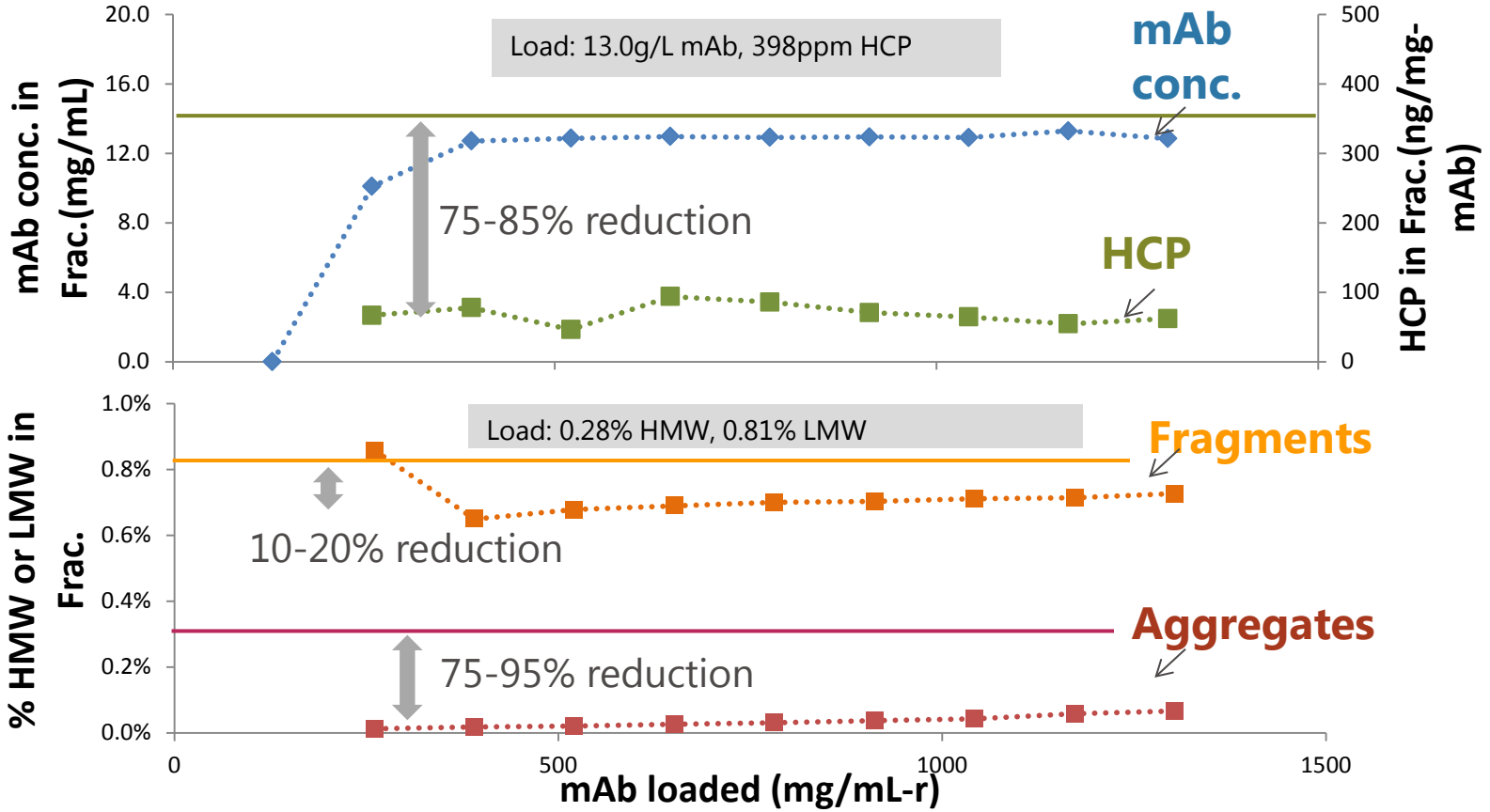
Chromatogram of novel CEX resin

Column volume: 1mL (0.8 cm ID x 2 cm H)
Load sample: Mab A CEX Load 25 mg/mL (2-fold dilution according to conditions vendor recommended, pH5, 3.2 mS/cm)
Load amount: **1300 mg/mL-resin**
Flow rate: 0.33 mL/min (Residence time: 3 min)



Equilibration Flow through Strip (1M NaCl)

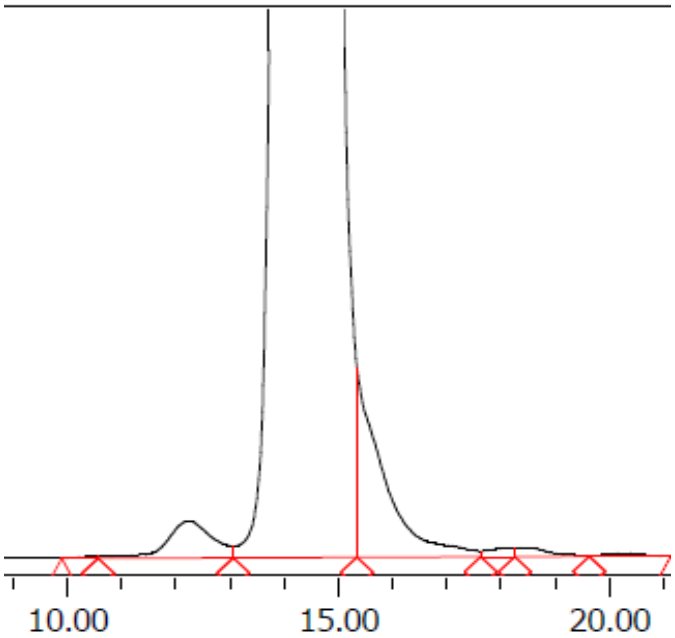
Impurity removal property (HCP, Aggregates, and Fragments)



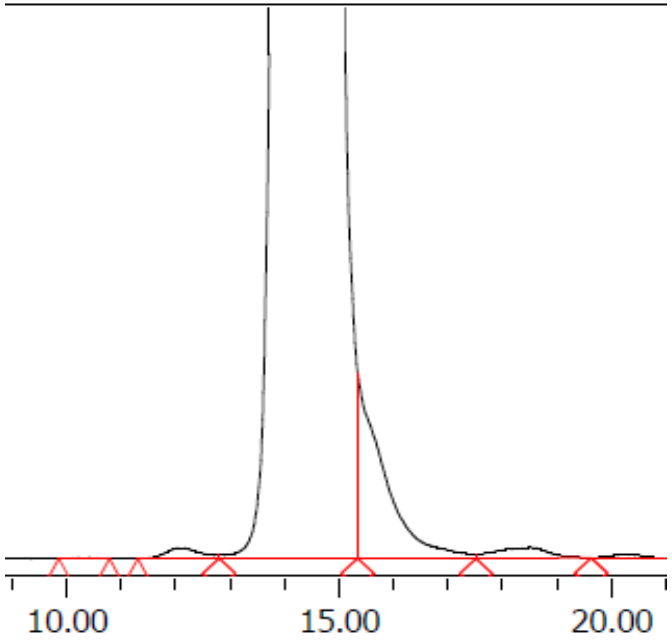
- Efficient reduction of impurities was observed as the manufacturer's information.
- Aggregates and Fragments gradually increased as amount of load. However, significant breakthrough of impurities was not observed under the tested condition.

SEC analysis
Load, Flow through Pool, Strip sample

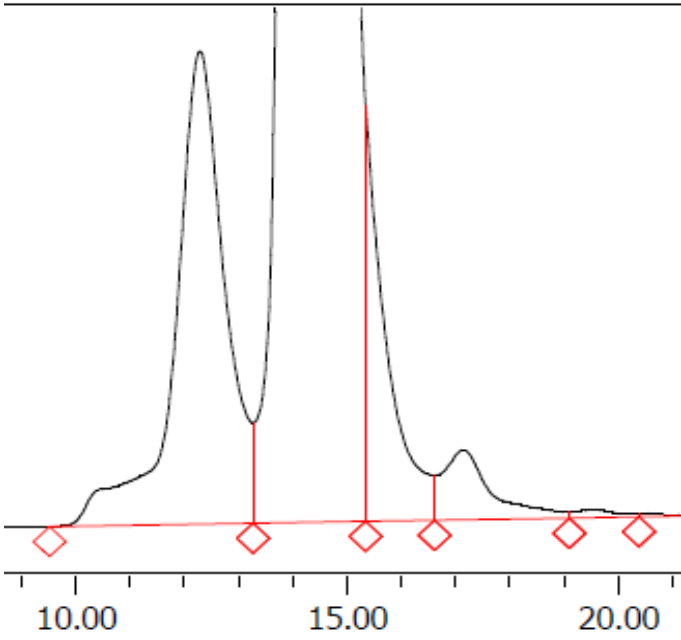
Load



Flow through Pool



Strip



Load:	98.9% monomer, 0.3% HMW, 0.8% LMW
Flow through:	99.1% monomer, 0.06% HMW, 0.9% LMW
Strip:	94.5% monomer, 4.0% HMW, 1.5% LMW

Comparison of CEX Bind/Elute mode and Flow/through mode

	Recovery (%)	SEC (%)			HCP (ppm)	DNA (ppb)
		HMW	Monomer	LMW		
Current process -Bind/Elute-						
Load	-	0.2	99.0	0.7	1200	66
Eluate	97	0.3 (not reduced)	99.0	0.6	230 ($\frac{1}{5}$)	0.3 ($\frac{1}{220}$)
Novel resin –Flowthrough-						
Load	-	0.3	98.8	0.9	400	160
FT	95	<0.1 ($\frac{1}{3}$)	99.1	0.8	70 ($\frac{1}{6}$)	0.03 ($\frac{1}{5000}$)

Summary

- Impurity removal capability by novel CEX F/T resin was comparable or better than by current CEX process with vendor-recommended conditions as the first evaluation.

- Resin volume can be reduced for about 1/10
  - Current CEX process is operated in Bind/Elute mode with Capacities < 100 g/L-resin. Merck CEX resin is operated in Flowthrough mode with Capacities > 1000 g/L, which allows for a smaller CEX column (up to 90% reduction)
    - For example, 60 cm ID x 20 cm → 20 cm ID x 20 cm
- Reduce buffer number and fluid volume depending on column size

Lower costs and save process time in CEX process can be achieved.

Future plan

- ✓ Optimize sample loading condition (pH, conductivity, flowrate, etc.)
- ✓ Continue to explore further candidates for CEX F/T mode
- ✓ Application to continuous processing
  - ➢ Coupling of CEX and AEX step
  - ➢ Buffer selection which is suitable for in-line conditioning (pH and conductivity)