Novel Technique for Quick and Easy Aggregate Removal of Monoclonal Antibodies Using Competitive Binding on CEX

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Background

Monoclonal antibodies (mAbs) are laboratory-produced molecules that can mimic the immune system's ability to fight off harmful pathogens such as viruses. They are widely used in the treatment of various diseases, including cancers, autoimmune disorders, and infectious diseases. However, one significant challenge in the development and use of monoclonal antibodies is the formation of aggregates.

Aggregates are clusters of monoclonal antibody molecules that have bound together. These can range from small oligomers to large insoluble particles. Aggregation can occur during various stages of mAb production, including expression, purification, storage, and even during administration. Aggregates are one of the Critical Quality Attributes (CQA) for monoclonal antibodies.

The aggregation of monoclonal antibodies is a critical issue that can impact the safety, efficacy, and stability of therapeutic mAbs. Understanding the factors that contribute to aggregation and implementing strategies to mitigate these effects are essential for the successful development and commercialization of monoclonal antibody therapies.

Causes of Aggregation

Physical Stress, Chemical Degradation, Formulation Components, Concentration

Consequences of Aggregation

Immunogenicity, Reduced Efficacy, Stability Issues

Strategies to Minimize Aggregation

Optimizing Formulation, Controlled Storage Conditions, Gentle Handling, Screening for Stability

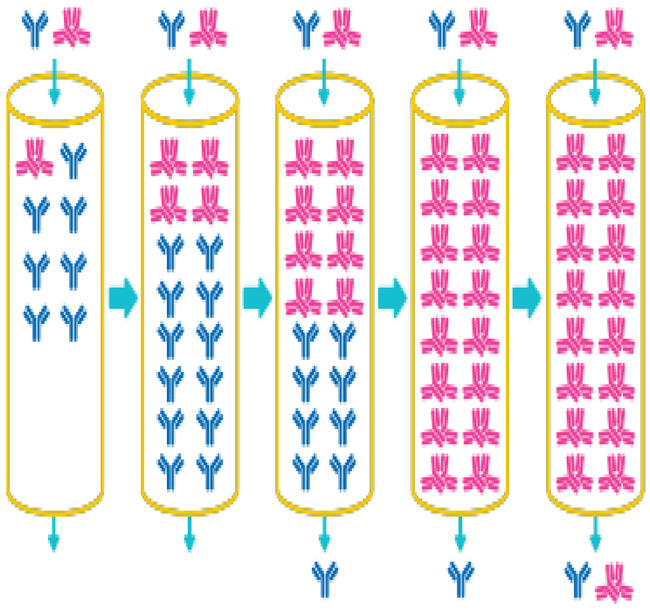
Methodology

In this study, we have demonstrated aggregate clearance using competitive binding techniques. Typically, cation exchange chromatography (CEX) is employed as a key step for aggregate removal in standard monoclonal antibody (mAb) purification processes. Our research focused on evaluating two distinct approaches: an extended CEX ligand resin and a CEX membrane.

In a typical bind elution mode CEX can capture up to 80mg/mL but in this case we have tested the resin and membrane which are negatively charged (CEX) in a negative mode purification to clear the aggregates. We have loaded up to 1000mg antibody per mL resin to see the clearance and recovery.

The protein was loaded on to the resin or membrane post viral inactivation step at pH 4.5, using low conductivity below 5mS/cm. Column was equilibrated using 50mM sodium acetate pH 4.5 buffer. These conditions allow the protein to capture on the resin, since the aggregates binds strongly it allows the monomer to come in flow through over a period of time due to displacement effect.

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 come in flowthrough. To get better recovery and maximum aggregate clearance we have generated breakthrough data for both resin and CEX membrane.



Displacement of monomers by aggregates

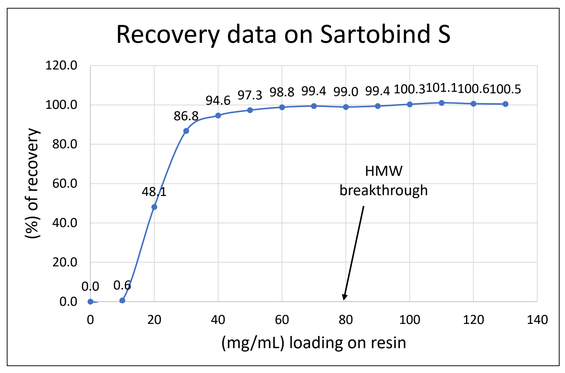
Results

Sartobind S (3mL) Recovery and Breakthrough table

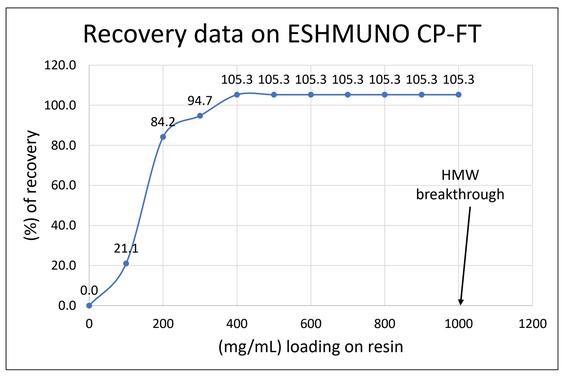
Sample Details	Conc. In g/L	Loading on membrane mg/mL	Residence time (min)	Total protein in FT	% Of break through	Total loading
Sartobind S load Load	1.0	0	0.2	0.0	0.0	0
Sartobind S FT Fraction-2	0.0	10	0.2	0.2	0.6	30
Sartobind S FT Fraction-3	0.5	20	0.2	13.7	48.1	60
Sartobind S FT Fraction-4	0.8	30	0.2	24.7	86.8	90
Sartobind S FT Fraction-5	0.9	40	0.2	27.0	94.6	120
Sartobind S FT Fraction-6	0.9	50	0.2	27.7	97.3	150
Sartobind S FT Fraction-7	0.9	60	0.2	28.2	98.8	180
Sartobind S FT Fraction-8	0.9	70	0.2	28.3	99.4	210
Sartobind S FT Fraction-9	0.9	80	0.2	28.2	99.0	240
Sartobind S FT Fraction-10	0.9	90	0.2	28.3	99.4	270
Sartobind S FT Fraction-11	1.0	100	0.2	28.6	100.3	300
Sartobind S FT Fraction-12	1.0	110	0.2	28.8	101.1	330
Sartobind S FT Fraction-13	1.0	120	0.2	28.7	100.6	360
Sartobind S FT Fraction-14	1.0	130	0.2	28.6	100.5	390
Sartobind S FT Fraction-15	0.6	140	0.2	16.9	59.3	420

ESHMUNO CPFT (0.3mL) Recovery and Breakthrough table

Sample Details	Conc. In g/L	Loading on ESHMUNO mg/mL	Residence time (min)	Total protein in FT	% Of break through	Total loading
CEX Load	0.67	0	0.2	0.0	0.0	0
ESHMUNO CPFT Fraction 1A1	0.2	100	0.2	6.0	21.1	30
ESHMUNO CPFT Fraction 1A2	0.8	200	0.2	24.0	84.2	60
ESHMUNO CPFT Fraction 1A3	0.9	300	0.2	27.0	94.7	90
ESHMUNO CPFT Fraction 1B1	1.0	400	0.2	30.0	105.3	120
ESHMUNO CPFT Fraction 1B2	1.0	500	0.2	30.0	105.3	150
ESHMUNO CPFT Fraction 1B3	1.0	600	0.2	30.0	105.3	180
ESHMUNO CPFT Fraction 2A1	1.0	700	0.2	30.0	105.3	210
ESHMUNO CPFT Fraction 2A2	1.0	800	0.2	30.0	105.3	240
ESHMUNO CPFT Fraction 2A3	1.0	900	0.2	30.0	105.3	270
ESHMUNO CPFT Fraction 2B1	1.0	1000	0.2	30.0	105.3	300

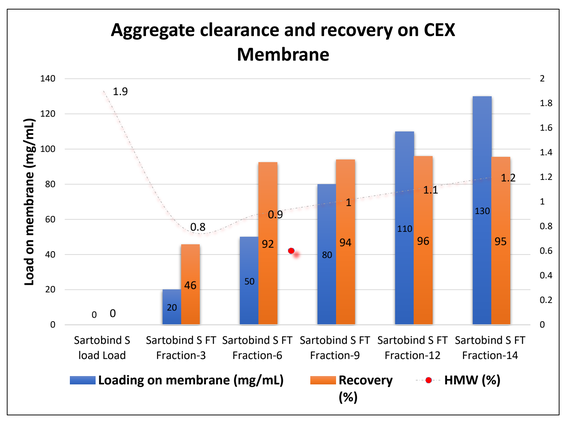


% of recovery on Sartobind S

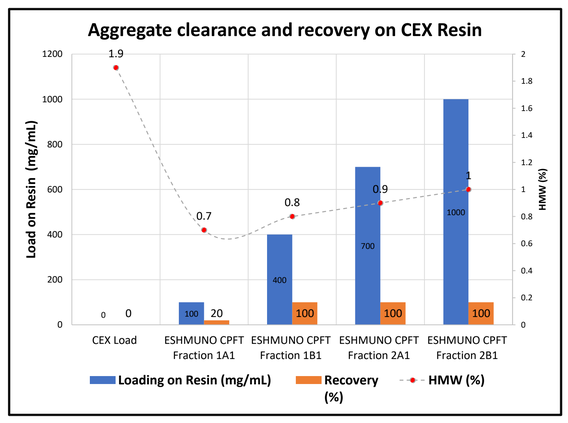


% of recovery on Eshmuno CP-FT

- Breakthrough data was generated on both resin and membrane.
- Eshmuno CP-FT resin was showing better recover compared with membrane.
- Aggregate removal is very effective on CEX resin compared with membrane.
- Total loading capacity is 10x higher on the resin compared to membrane. .



Aggregate Clearance Vs Recovery And Loading On Membrane (mg/mL)



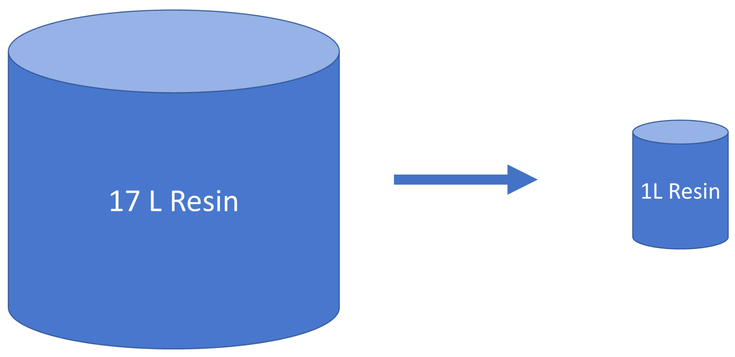
Aggregate Clearance Vs Recovery And Loading On Resin (mg/mL)

- On the CEX membrane, aggregate breakthrough occurred at a loading of 80 mg per mL membrane, while on the CEX resin, breakthrough was observed at 1000 mg antibody per mL resin.

Conclusion

The Eshmuno CP-FT exhibited superior performance in aggregate removal compared to Sartobind S. While both initially showed lower recovery, overall recovery significantly improved in flow-through mode using displacement methodology. Eshmuno CP-FT successfully cleared 1000 mg protein per mL resin, maintaining HMW below 1%, whereas Sartobind S experienced HMW breakthrough at 80 mg protein per mL. This displacement methodology can be applied to other CEX-based resins or membranes to reduce aggregate percentages in negative mode chromatography, offering the biopharma industry a valuable opportunity to evaluate and optimize CEX chromatography for novel molecules or existing processes, ultimately minimizing cost, footprint, and facility occupancy time.

Comparison Between Conventional Bind And Elute CEX Vs Negative Mode Aggregate Removal



Bind and Elute (80mg/mL) **FT Mode (1000mg/mL)**