An expanded toolbox for various MAb purification challenges

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An expanded toolbox for various MAb purification challenges

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Abstract

Monoclonal antibodies (MAbs) are commonly purified using platform approaches including two or three chromatography steps. Protein A affinity chromatography for direct capture of the MAb from clarified feed is typically followed by one or two polishing steps using ion exchange, multimodal, or hydrophobic interaction chromatography (Fig 1).

Even if MAbs are sharing many properties, all antibodies will not behave exactly the same. Some MAbs will be more challenging to purify, for example, due to a complex impurity profile or aggregate formation at certain conditions. In such cases, an expanded MAb purification toolbox with different options in primarily the polishing steps is beneficial.

In the work presented here, capture using high-capacity protein A medium (resin) was followed by polishing using a novel cation exchange chromatography medium. Advantages of working with a multimodal anion exchanger versus a traditional anion exchange chromatography medium for a "tricky" MAb prone to aggregation at pH values above 6, is presented.

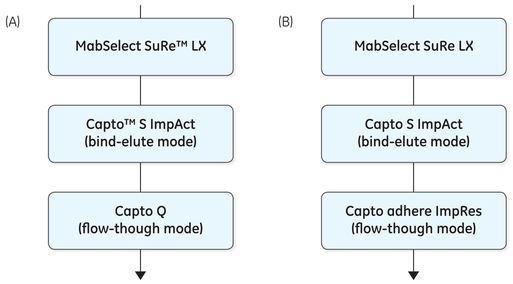

Fig 1. (A) Standard three-step MAb purification process. (B) Alternative tree-step MAb purification process.

Capture using MabSelect SuRe LX

Direct MAb capture from cell culture supernatant on MabSelect SuRe LX was performed using standard conditions. elution with 20 mM sodium acetate, pH 3.5 resulted in a narrow elution peak with a pool volume of 1.1 column volumes (CV) at a MAb concentration of approx. 40 g/L.

Variable loading concept for increased productivity

By applying the concept of variable loading, the productivity of a chromatography step can be improved (Fig 2). In a three-step loading procedure for MabSelect SuRe LX, for example, a productivity gain of close to 40% can be achieved, with maintained capacity and purification performance (1).

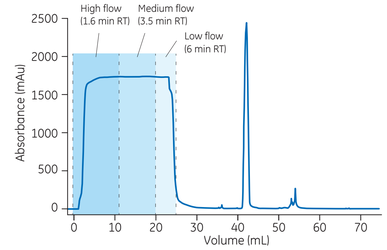

Fig 2. Variable loading on MabSelect SuRe LX medium. In a three-step loading procedure, sample was loaded at stepwise decreasing flow rate. RT = retention time.

Optimization of first polishing step using Capto S ImpAct

Dynamic binding capacity (DBC)

PreDictor™ RoboColumn™ Capto S ImpAct, 600 µL units were used for fast determination of DBC using a design of experiments (DoE) approach (2). As factors, pH and NaCl concentration were selected. DBC increased with increasing pH and NaCl concentration in the explored range (Fig 3).

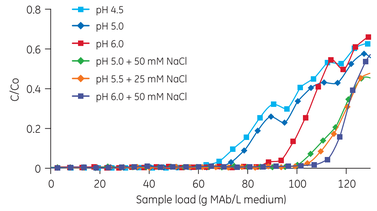

Fig 3. Overlay of breakthrough curves for the different binding conditions.

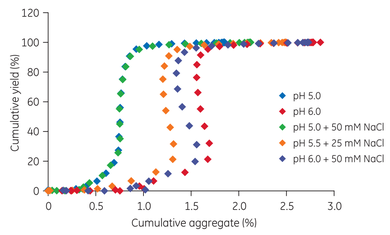

Fig 4. Cumulated monomer yield vs cumulated aggregate concentration for the different binding and elution conditions. Under the conditions tested, the most efficient aggregate removal was obtained at loading pH 5.0, irrespective of salt concentration.

Selectivity for MAb monomer and aggregates

The selectivity of Capto S ImpAct between MAb monomer and aggregates was determined using a DoE approach. As factors, loading pH and NaCl concentration were selected. Elution was performed with a gradient slope of + 350 mM NaCl for 20 CV. MAb aggregate concentration in the elution pool at 90% monomer yield was monitored as a response (Fig 4). The good selectivity of Capto S ImpAct between MAb monomer and aggregates can be seen from the chromatogram in Figure 5.

Sample: MAb in 50 mM sodium acetate, 50 mM NaCl, pH 5.0
Column: HiScreen™
Load: 76 mg MAb/mL medium (70% of Q_{B10})
Binding buffer: 50 mM sodium acetate, 50 mM NaCl, pH 5.0
Wash: 5 CV of binding buffer
Elution buffer: 50 mM sodium acetate, 50 to 400 mM NaCl in 20 CV
System: ÄKTA™ system

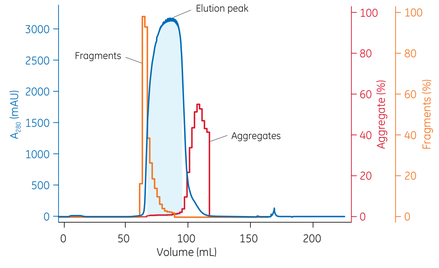

Fig 5. Initial polishing step. The fragments (orange histogram) elutes at the front of the elution peak (blue UV trace), whereas the aggregates (red histogram) elutes in the tail of the elution peak. The light blue area under the curve corresponds to pooled product fractions. Q_{B10} = DBC at 10% breakthrough.

Final polishing using the expanded MAb toolbox

As Capto adhere ImpRes can be operated at lower pH, this medium was evaluated as an alternative to Capto Q for the final polishing step (Fig 6). The overall results from the described three-step MAb purification process, shown in Table 1, are comparable with typical results from a traditional three-step process (3).

Sample: MAb in 25 mM sodium phosphate, 150 mM NaCl, pH 6.3
Column: Tricorn 5/50
Equilibration buffer: 25 mM phosphate, 150 mM NaCl, pH 6.3
Load: 150 mg MAb/mL medium at a flow rate of 0.5 mL/min
System: ÄKTA system

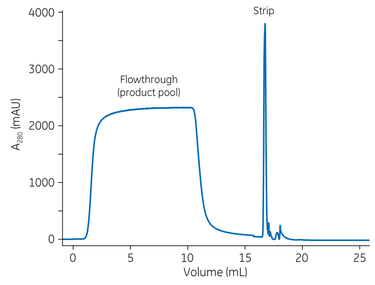

Fig 6. Final polishing step using Capto adhere ImpRes. The second peak contains mainly impurities that are stripped of the column with 100 mM acetic acid.

Table 1. Results from the three-step MAb purification process

Process step	MAb yield (%)	MAb concentration (mg/mL)	Aggregates (%)	HCP (ppm)	Leached ligand (ppm)
MabSelect Sure LX	99	37	2.9	298	3.6
Capto S ImpAct + buffer change	91	8.3	1.4	154	< 1
Capto adhere ImpRes	94	5.7	0.9	11	< 1
Total process yield	85				

Conclusions

A toolbox comprising modern chromatography media is useful in the development of effective MAb purification platforms. Here, we describe a three-step purification process for a challenging MAb prone to aggregation at pH values above 6.

- Despite the high binding capacity of MabSelect SuRe LX medium, the volume of the elution pool was not larger compared with the elution volume for a protein A medium with lower binding capacity.
- In the initial polishing step, Capto S ImpAct enabled separation of aggregates from the monomer fractions with good selectivity and high resolution.
- With Capto adhere ImpRes, aggregates, HCP, and leached protein A were efficiently separated from the target MAb, resulting in a MAb recovery of 94% and an aggregate content below 1%.

The total MAb recovery for the described three-step process was 85%.

References

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2. Application note: Optimization of dynamic binding capacity and aggregate clearance in a monoclonal antibody polishing step. GE Healthcare, 29145068, Edition AA (2015).
3. Application note: Three-step monoclonal antibody purification processes using modern chromatography media. GE Healthcare, 29132569, Edition AA (2015).

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