Process-scale purification of monoclonal antibodies – polishing using Capto Q

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Process-scale purification of monoclonal antibodies – polishing using Capto™ Q

Summary

Anionic exchange media are an industry standard for large-scale polishing of monoclonal antibodies (MAbs). Polishing is typically the last step in the purification process after Protein A and cationic exchange chromatography. In this study, the reduction of contaminants (host cell proteins and Protein A) was evaluated using Capto Q in process purifications. The dynamic capacity of Capto Q for host cell proteins (HCP) and DNA was investigated and compared with other chromatography media and membranes. Capto Q showed significantly higher HCP capacity than did membranes. A cost analysis revealed that Capto Q is more economical than membranes when used in production and that the process economy of Capto Q increases with batch size and process frequency.

Antibody-based therapeutics are expected to continue to be a major source of new therapies for the next decade. MAbs are among the world's most expensive drugs and there is market pressure to decrease manufacturing costs considerably. The most significant improvement thus far has been increased expression levels. Higher titers and higher feed volumes have created a demand on enhanced capacity and speed in the downstream processes.

Introduction

Large-scale purification of MAbs usually consists of three chromatographic steps. The first step, Protein A affinity chromatography, generally delivers a product with a high purity, typically 99%, which can be further purified in a second step such as cationic exchange chromatography. Flowthrough anion exchange chromatography (AIEX) is often used as a final polishing step to remove contaminants (Fig 1). Around neutral pH and at low conductivity, most antibody species will not bind to the matrix but pass the column into the flowthrough fraction, while many viruses, DNA, and a large percentage of HCP are negatively charged and will bind to the matrix.

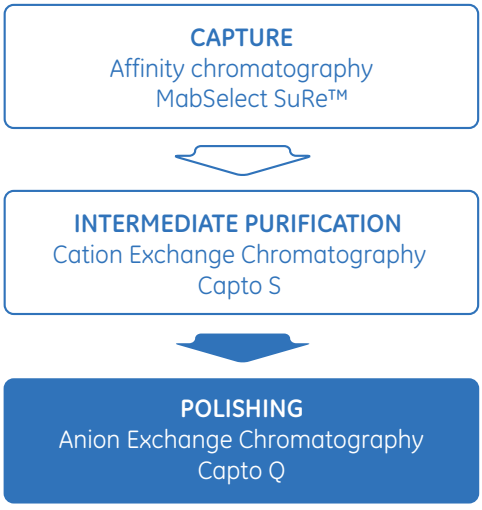

Fig 1. Procedure for large-scale purification of MAbs described in this application note.

Capto Q is a strong anionic exchange medium. Compared to other chromatographic media, Capto Q has high capacity in combination with high flow velocity and low backpressure, allowing reduced process cycle times and increased productivity. For a 10-cm bed, a linear flow rate of 700 cm/h gives a backpressure of less than 3 bar (Fig 2). Capto Q also has good cleaning-in-place stability and withstands all standard CIP procedures (e.g., 1 M sodium hydroxide, 2 M sodium chloride, or 70% ethanol; see reference 1).

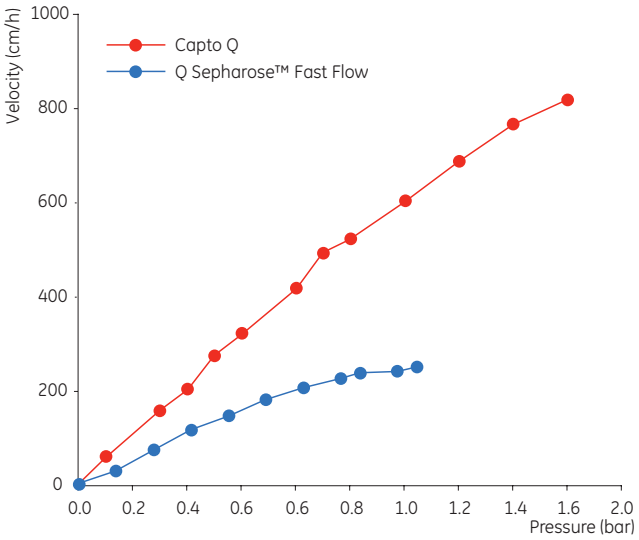

Fig 2. Comparison of backpressures at different velocities for Q Sepharose Fast Flow and Capto Q.

As a member of the BioProcess™ media family, Capto Q meets the demands of industrial biotechnology with validated manufacturing methods, security of supply, and comprehensive regulatory support to assist process development, validation, and submission to regulatory authorities.

Because the purity after the Protein A step is so high, often above 99%, membrane adsorbers derivatized with Q groups have become an alternative to column chromatography in MAb purification processes.

In this study, the capacity of Capto Q for DNA and HCP was investigated and compared with other matrices and membranes. Reduction of contaminants using two different MAbs was also investigated. A cost analysis (based on the comparison in reference 3) demonstrated that column chromatography using Capto Q is a cost-effective alternative to membranes.

DNA and HCP capacity

Laboratory-scale studies were performed to evaluate the dynamic binding capacity for DNA and HCP. A comparison was carried out between Capto Q, Q Sepharose Fast Flow, and two membrane adsorbers, Mustang™ Q and Sartobind™ Q.

Sheared DNA (sonicated salmon sperm DNA, GE Healthcare code no. 27-4565-01) was used as sample. The DNA was dissolved in running buffer (100 mM sodium phosphate, pH 6.5) to a concentration of 0.1 mg/ml. Capto Q and Q Sepharose Fast Flow were packed to a bed height of approximately 10 cm. The membrane capsules were connected directly to the ÄKTAexplorer™ 100 system. The absorbance of the sample solution was determined in column bypass mode and sample was loaded onto the column until ≥ 30% of the initial absorbance was observed at the column outlet. The dynamic binding capacity at 10% breakthrough was then calculated (Q_{B10}) and the results are summarized in Table 1.

At a flow rate of 300 cm/h, Capto Q has a dynamic binding capacity of 1.47 mg/ml (10-cm bed height), and is superior to Q Sepharose Fast Flow, which has a much lower capacity (0.15 mg/ml) and cannot be used at flow rates higher than 300 cm/h. Capto Q, on the contrary, has a capacity of almost 1 mg/ml even at a flow velocity of 1200 cm/h (a flow recommended only for low bed heights).

Table 1. Dynamic binding capacity at 10% breakthrough (Q_{B10}) for DNA using different media and membranes

Medium	Flow velocity (cm/h)	Residence time (min)	Q_{B10} (mg/ml medium)
Q Sepharose Fast Flow	300	2.2	0.15
	600	N.A.	N.A.
Capto Q (10-cm bed height)	300	2.1	1.47
	600	1.0	1.54
	1200	0.5	0.96
Mustang Q	300	0.007	4.10
	600	0.004	4.07
Sartobind Q	300	0.006	0.86
	600	0.003	0.93

The capacity of the Mustang Q membrane at a flow rate of 300 cm/h was 4.1 mg/ml, which is 2.8 times higher than the capacity of Capto Q. Sartobind Q had only 60% of the capacity of Capto Q (0.86 mg/ml). At the higher flow rate (600 cm/h), the ratio between Capto Q, Mustang Q, and Sartobind Q was essentially the same.

A solution of CHO proteins obtained from CHO-cells not expressing antibody (Boehringer Ingelheim Pharma) was used to test the capacity for binding HCP. In this experiment, Q Sepharose Fast Flow was not evaluated. The capacity values were determined in a similar manner as described for DNA capacity, but calculated at the 30% breakthrough level. The results are summarized in Table 2.

Table 2. Dynamic binding capacity at 30% breakthrough for HCP using Capto Q and Q membranes

Column	Flow rate (cm/h)	Residence time (min)	Capacity (mg/ml medium)
Capto Q (2.5 cm bed height)	156	1	14.5
Mustang Q	6	0.28	6.8
Sartobind Q	6	0.36	12.3

The dynamic binding capacity for Capto Q was 14.5 mg/ml at a contact time of 1 min. Both membranes showed lower capacities than Capto Q (47% for Mustang Q and 85%, for Sartobind Q), but the contact times were shorter. Conversely, contact time does not significantly influence the capacity of the membranes as no mass transport occurs inside a membrane.

Contaminant reduction

There are no absolute guidelines given by the authorities about acceptable levels of contaminants in MAb formulations. Important classes of contaminants are DNA, Protein A, aggregates, and HCP. The current WHO standard (see reference 2) requires a residual DNA contamination of < 100 pg/dose and in industrial applications levels below 10 pg/dose are commonly achieved. For other contaminants, typical specification values are < 5 ppm for Protein A, < 50 ppm for HCP and < 1% of aggregates.

DNA, HCP, and Protein A clearance

Contaminant removal on Capto Q was compared to Q Sepharose Fast Flow using a sample of Protein A-purified MAb (Table 3). The DNA clearance was found to be similar to that of Q Sepharose Fast Flow with respect to HCP, Protein A, aggregates, and DNA.

The column was loaded with approximately 65 mg of MAb/ml resin. The sample contained 0.01 ppm of DNA and the flow through fraction < 3×10^{-4} ppm DNA, corresponding to at least a 30-fold reduction. In the same experiment, reduction of Protein A was more than 15-fold, from 33 ppm to < 2 ppm. HCP decreased from 808 to 16 ppm, a 50-fold reduction. All contaminant levels were well below those required by either the WHO or the industry.

Interestingly, the reduction of all contaminants was essentially the same at all flow rates tested: 150, 300 and 700 cm/h. Such high flow rates are ideally suited to deal with the large volumes present in today's Mab processes, where fermentation volumes of 10 000 L are now common. At the same column size, using Capto Q instead of Q Sepharose Fast Flow would allow for a 3 to 4 times higher flow rate, while achieving the same contaminant clearance. As shown in the capacity section the Q_{B10} value for Capto Q is much higher than that of Q Sepharose Fast Flow. This implies that the column sizes could be smaller as well, saving money in buffer consumption, resin consumption and hardware investments. All of these facts indicate that a more economic process is possible by using Capto Q instead of Q Sepharose Fast Flow.

The aggregate level of 1% was unchanged after passing the column. This is not unexpected as the classical way of removing aggregates is based on HIC (Hydrophobic Interaction Chromatography).

Table 3. Contaminant reduction comparison at different flow rates

Sample	HCP (ppm)	% Aggregate	DNA (ppm)	Prot. A (ppm)
Load	808	1	1×10^{-2}	33
Q Sepharose FF 150 cm/h	16	1	$< 3 \times 10^{-4}$	< 2
Capto Q 150 cm/h	13	1	$< 3 \times 10^{-4}$	<2
Capto Q 300 cm/h	16	1	$< 3 \times 10^{-4}$	<2
Capto Q 700 cm/h	17	1	$< 3 \times 10^{-4}$	<2

Column: 1.6 × 9 cm, 18 ml
Mode: Flowthrough
Sample: Protein A pool adjusted to pH 8.0, approx. 65 mg/ml medium

Column: Tricorn™ 5/100 packed with 2.06 ml Capto Q; bed height 10.5 cm
Sample: BioInvent MAb (BioInvent International), eluate from Capto S in 25 mM sodium phosphate, pH 7, 7.5 or 8
Sample load: 1 mg IgG/ml medium
Starting buffer: 25 mM sodium phosphate, pH 7, 7.5, or 8
Elution buffer: 200 mM sodium phosphate, pH 8
Flow velocity: 500 cm/h
System: ÄKTAexplorer 100

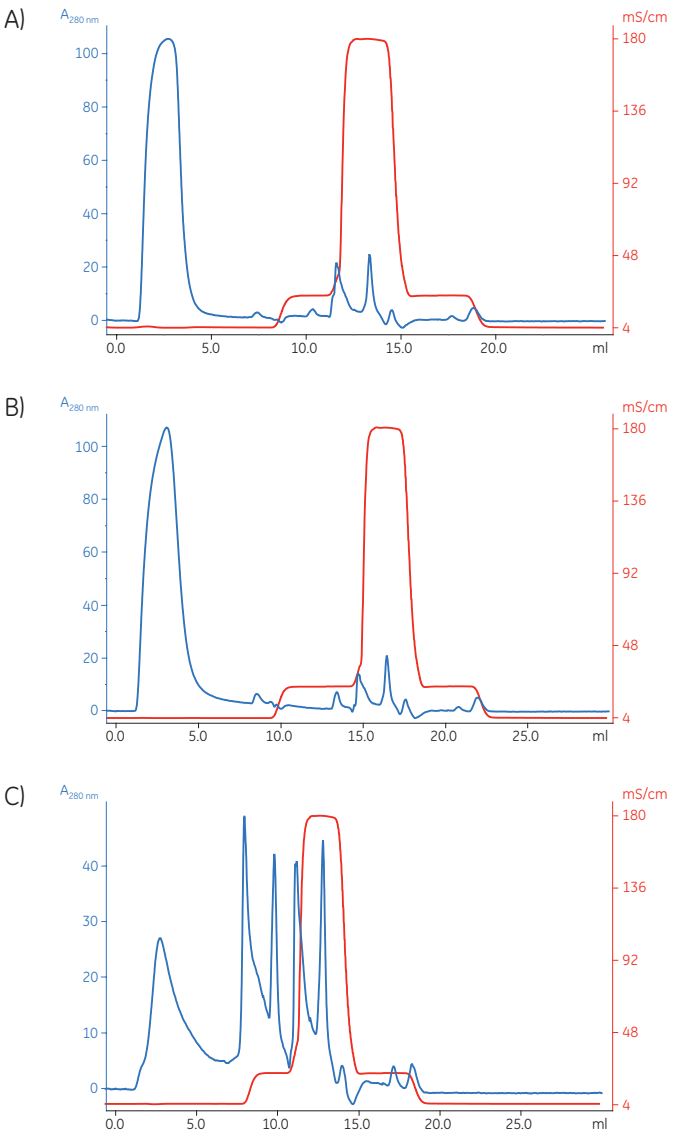

Fig 3. Optimal pH for purification of BioInvent MAb, chromatograms at **A)** pH 7.0, **B)** pH 7.5, and **C)** 8.0.

Purification of BioInvent MAb

A monoclonal antibody from BioInvent that had been purified from culture supernatant with MabSelect SuRe (Protein A ligand) followed by Capto S (cationic IEX) was used as sample to demonstrate the use of Capto Q in the polishing step of a MAb purification.

Because of the relatively low pI of the BioInvent MAb, an optimum pH for separation on Capto Q needed to be determined. Purifications were performed at three different pH values (7.0, 7.5 and 8.0) and the runs were compared in order to find the highest pH where the antibody yield was acceptable (Fig 3). At pH 7.5, the yield was high (96%, Table 4), and contamination minimized.

Table 4. BioInvent MAb yields in the flowthrough fraction at different pH

pH	7.0	7.5	8.0
Yield (%)	99	96	66

The sample solution had a concentration of 10.5 mg/ml after Capto S and buffer change; 23.8 ml of this solution was applied to the Capto Q column, corresponding to a load of 121 mg/ml medium. The flow rate was 500 cm/h, giving a residence time of 75 s. The chromatogram is shown in Figure 4.

The yield in the collected flowthrough fraction was 94%. HCP levels were determined by ELISA using a standard commercial kit (Cygnus Technologies). The HCP content was decreased from 35 to 5.4 ng/mg IgG, which is more than a six-fold reduction and is well below typical reduction specifications. After the Capto S step, the Protein A concentration was below the lower detection limit, and was still undetectable after the Capto Q column. Gel filtration analysis (Fig 5) revealed a total of 0.7% of aggregates after the Capto Q step, which is under the normal security limit of 1%

Cost analysis

A cost analysis comparing Q Sepharose Fast Flow with Sartobind Q in a large-scale MAb process has been published (3). An economic evaluation of Capto Q as an alternative for the polishing step was performed using the same process and cost data as in the reference above. Mustang Q was not evaluated in the published study and was therefore excluded from further analysis. The comparison was made under the following conditions:

1. Calculation was based on a 10-yr operation with 40 batches/yr
2. For Capto Q and Sartobind Q, two different batch sizes were evaluated; 13.5 and 50 kg
3. Binding capacities were assumed to be 140 g/l for Capto Q, 3000 g/m^2 for Sartobind Q, and 70 g/l for Q Sepharose Fast Flow

The comparison of Capto Q, Q Sepharose Fast Flow, and Sartobind Q is shown in Table 5. The membrane cost is considerably higher than the cost for the chromatography medium. However, the column approach requires more buffers and has higher labor costs. Moreover, the column approach has initial costs for development (column lifetime studies, column packing studies, etc.), as well as validation of column cleaning and lifetime.

Column: Tricorn 5/200 packed with 4 ml Capto S to a bed height of 20 cm
Sample: MabSelect SuRe eluate after virus inactivation and filtration
Sample load: 100 mg MAb/ml medium
Starting buffer: 0.02 M sodium citrate, 0.012 M sodium chloride, pH 5.3
Intermediate wash: 6 CV of 40 mM sodium phosphate, pH 6.5
Elution buffer: 0.1 M sodium phosphate, pH 7.0
Flow rate: 500 cm/h (residence time of 2.4 min)
System: ÄKTAexplorer 100

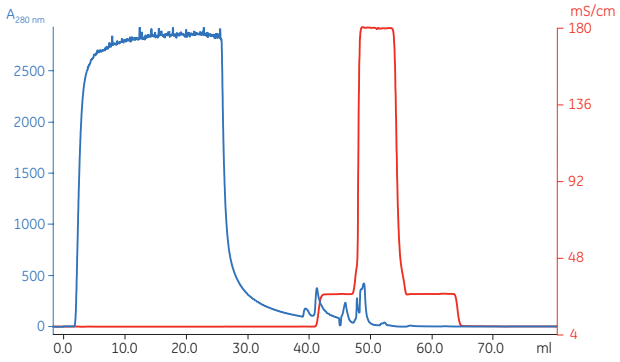

Fig. 4. Purification of the BioInvent MAb, Capto Q step.

Column: Superdex™ 200 10/300 GL
Sample: Flowthrough fraction from Capto Q
Sample load: 50 µl (0.41 mg)
Elution buffer: 10 mM phosphate buffered saline, pH 7.4
Flow rate: 0.5 ml/min
System: ÄKTAexplorer 100

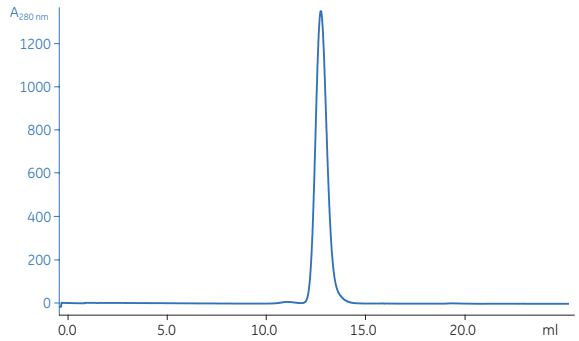

Fig 5. BioInvent antibody purification. Gel filtration analysis of the flowthrough after the Capto Q step.

For processes that will be run frequently, such costs are averaged over more runs. The Q membranes are ready to use, and as the membranes are disposable, no validation of cleaning and lifetime is needed.

At a batch size of 13.5 kg, the total cost for Capto Q is 8% lower than Sartobind Q. If the batch size is increased to 50 kg/run, the difference is more pronounced: the cost when using Capto Q is only 65% of the cost for Sartobind Q.

Compared with Q Sepharose Fast Flow, the higher binding capacities of Capto Q together with the higher operating flow rates result in significantly higher productivity for Capto Q (up to six-fold higher).

Conclusions

This study assessed the performance of Capto Q in flowthrough mode during MAb purification process. The yield obtained in process runs is high, typically more than 95%. Compared to Q Sepharose Fast Flow, Capto Q allows a 3 to 4 times higher flow rate while achieving at least the same contaminant reduction. This suggests that Capto Q is a more economic alternative to Q Sepharose Fast Flow for process purification of MAbs.

A cost analysis demonstrated that Capto Q is more economical than Q membranes, and that cost saving from using Capto Q increases with batch size and process frequency.

References

1. Data File: Capto Q, Capto S and Capto DEAE. GE Healthcare, 11-0025-76, Edition AG (2012).
2. WHO *Technical Reports* **878**, Annex 1 (1998).
3. Zhou, J.X. and Tressel, T. Basic concepts in membrane chromatography for large-scale antibody production. *Biotechnol. Prog.* **22**, 341-349 (2006).

Table 5. Cost comparison among Sartobind Q, Q Sepharose Fast Flow, and Capto Q

	13.5 kg batch			50 kg batch	
	Sartobind Q	Q Sepharose Fast Flow[1]	Capto Q	Sartobind Q	Capto Q
Load	3000 g/m^2	70 g/l	140 g/l	3000 g/m^2	140 g/l
Development costs kUSD[2]	0	700	700	0	700
Manufacturing costs kUSD					
Hardware	0	400	300	0	300
Medium/filter	3600	440	650	13320	2405
Labor	280	800	630	280	630
Buffer	840	3500	1750	3108	6475
Validation costs kUSD	0	310	310	0	310
10-yr operation cost comparison kUSD	4720	6150	4340	16708	10820

[1] Data from Zhou and Tressel (3)

[2] US dollars (× 1000). Development costs (chromatography media) could be divided into column lifetime (200 kUSD), assay development (200 kUSD) and column packing studies (300 kUSD).

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