Polishing of monoclonal antibodies using Capto adhere ImpRes in bind and elute mode

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Polishing of monoclonal antibodies using Capto™ adhere ImpRes in bind and elute mode

Capto adhere ImpRes is a strong ion exchanger with multimodal functionality designed for polishing of monoclonal antibodies (MAbs). In this study, the binding capacity for MAbs and the efficiency in the clearance of impurities using Capto adhere ImpRes in bind/elute (B/E) mode was evaluated. The study presents results from optimization of the loading conditions using the Design of Experiments (DoE) approach. The effects of buffer, pH, conductivity, and sample load were investigated. Two different MAbs were studied. The results showed high yields of monomeric MAb, as well as good clearance of aggregates, host cell proteins (HCP), and leached protein A.

Introduction

MAbs and MAb conjugates are today in great demand for use as biopharamaceuticals. As a result, more cost-effective, efficient, and flexible process purification schemes are one of the highest priorities for MAb manufacturers.

The relative homogeneity of MAbs makes them well-suited for platform processes, which are sets of unit operations, conditions, and methods applied to molecules of a given class. A platform approach is desirable as it saves both time and money in process development. GE Healthcare Life Sciences' MAb production toolbox employs protein A chromatography media such as MabSelect SuRe™ or MabSelect SuRe LX for capture of the target. After the initial protein A capture step, there is a wide range of options for intermediate and polishing purification steps. One of these options, Capto adhere ImpRes, is a cost-effective and flexible chromatography medium (resin) designed for high-resolution polishing of MAbs.

Capto adhere ImpRes is a multimodal anion exchange medium with a ligand (Fig 1) that displays high selectivity compared with traditional ion exchange polishing media. The medium enables operation in either B/E or nonbinding (flowthrough, FT) modes and results in either two- or three-step purification schemes. The small bead size of Capto adhere ImpRes enables high-resolution purification of target protein. The high resolution possible with Capto adhere ImpRes enables reduced buffer consumption and improved product yield compared with Capto adhere, a related product with the same ligand but with a larger bead size. Contaminants such as DNA, HCP, leached protein A, aggregates, and viruses are efficiently separated from monomeric MAbs[1] in B/E or FT modes.

This application note describes development of polishing steps for two different MAbs in B/E mode using Capto adhere ImpRes. The studies include measurement of static- and dynamic binding capacities at various binding conditions, as well as screening and optimization of gradient- and step-elution conditions.

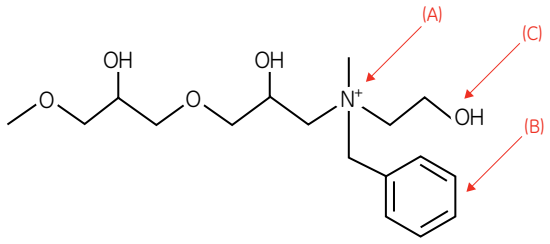

Fig 1. The Capto adhere ImpRes ligand exhibits many functionalities for interaction with a target molecule. The most pronounced are ionic interactions (A), hydrophobic interactions (B), and hydrogen bonding (C).

[1] Capto adhere ImpRes is also be used for purification of recombinant proteins and other biomolecules.

Materials and methods

Start material

The two MAbs used in this study were initially purified from CHO cell supernatant by protein A affinity chromatography. Some characteristics of the MAbs are shown in Table 1.

Table 1. Characteristics of the two antibodies used in the study

			DBC 10% (mg/mL)*	
Antibody	pI	Aggregate content (%)	Capto adhere ImpRes	Capto adhere
MAb A	7.3	2.5	71	56
MAb B	> 7.0	2.4	44	36

* Dynamic binding capacity (DBC) at 10% breakthrough (DBC 10%) for various antibodies measured at 4 min residence time.

Determination of static binding capacity

Static binding capacity (SBC) was determined in 6 µL PreDictor™ 96-well filter plates. Equilibration of wells in the filter plates was performed by addition of 200 µL of loading buffer per well followed by agitation at 1100 rpm for 1 min, after which the buffer was removed by vacuum extraction. The equilibration step was performed three times. MAb solution (200 µL volume, 4 mg/mL sample load, corresponding to 133 mg MAb/mL chromatography medium) was added to each well followed by agitation for 90 min. Unbound material (FT fraction) was removed by centrifugation for 3 min, and MAb concentration was determined by measurement of absorbance at 280 nm.

SBC was calculated according to:

$$MAb_{bound} = 0.2 \times (C_{in} - C_{out})\ [\text{mL} \times \text{mg/mL} = \text{mg}]$$

$$SBC = MAb_{bound}/V_{medium} = MAb_{bound}/0.006\ [\text{mg/mL}]$$

where C_{int} = MAb concentration in sample, C_{out} = MAb concentration in FT fraction, and V_{medium} = medium volume in each well (i.e., 6 µL).

Determination of dynamic binding capacity

Dynamic binding capacity (DBC) was determined by frontal analysis using ÄKTAexplorer™ 10 chromatography system. The UV-absorbance at 280 nm was used for determination of breakthrough. Before frontal analysis, the MAb solution was injected by-passing the column to obtain a maximum absorbance value. DBC was then calculated according to:

$$DBC_{X\%} = (V_{X\%} - V_0) * C_0/V_c$$

were $V_{X\%}$ = load volume (mL) at *x*% breakthrough, V_0 = void volume (mL), C_0 = MAb concentration in the sample (mg/mL) and V_c = volumetric bed volume (mL).

Screening of elution conditions

Measurement of yield at different elution conditions was performed in PreDictor 96-well filter plates. Equilibration of wells in the filter plates was performed by addition of 200 µL of loading buffer per well followed by agitation at 1100 rpm for 1 min, after which the buffer was removed by centrifugation. The equilibration step was performed three times. MAb solution (200 µL, 2.8 mg/mL, corresponding to 93 mg MAb/mL medium) was added to each well followed by agitation for 60 min. Unbound material was removed by centrifugation. Elution of bound material was then performed by addition of 200 µL elution buffer/well; the elution step was performed three times. MAb concentration was determined by measurement of absorbance at 280 nm. Yield was calculated according to:

$$Yield\ (\%) = 100 \times 200 \times (C_{eluate\ 1} + C_{eluate\ 1} + C_{eluate\ 1})/(200 \times C_{in}) = 100 \times (C_{eluate\ 1} + C_{eluate\ 1} + C_{eluate\ 1})/C_{in}$$

where C_{in} = MAb concentration in MAb solution and $C_{eluate\ 1, 2, 3}$ = MAb concentration in eluate 1 to 3.

Optimization of step elution conditions

Conditions for step elution were investigated in a packed column using ÄKTA™ pure chromatography system, DoE, and scouting functionalities included in UNICORN™ 6.3.

Determination of aggregates and aggregate clearance

Fractions from the chromatographic runs were collected and analyzed by gel filtration (analytical size exclusion chromatography) on a Superdex™ 200 5/150 GL column. The peaks were integrated and the dimer/aggregate concentrations (in percent) were estimated. Cumulated yield of monomers was plotted against cumulated aggregates (Fig 2).

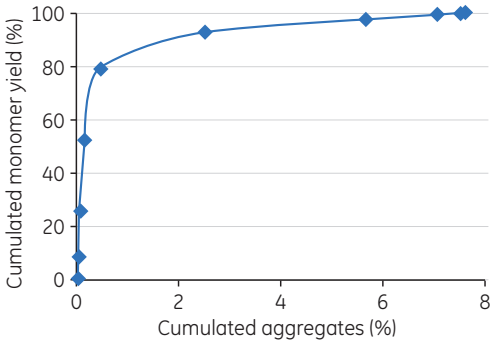

Fig 2. Evaluation of gradient elution was performed by gel filtration. The Figure shows an example of the resulting plot of cumulated yield of monomers vs cumulated aggregates derived from the gel filtration analysis.

Protein A and HCP ELISA

The protein A concentration in the start materials and flowthrough fractions was determined by Protein A ELISA kit (Repligen). Host cell protein concentration was determined by HCP ELISA (Cygnus Technologies).

Results and discussion

Case study, MAb A

The case study with MAb A shows a suggested workflow for method development including screening of conditions for SBC and DBC, screening of elution conditions, and optimization of conditions for step elution.

Static binding capacity

To find optimal binding capacity for MAb A, SBC was determined in 6 µL PreDictor 96-well filter plates. Binding pH was varied between pH 4.0 and 8.0[2,3] and the salt concentration from 0 to 500 mM NaCl. All samples and buffers were prepared automatically using a TECAN® robot. The results show that the highest SBC was obtained at high pH and low salt concentration (Fig 3, orange region). Based on these results, a narrower range of pH and NaCl concentration was used for further investigation of conditions for DBC.

[2] Binding buffers were citrate, pH 4; acetate pH 4.6 and 5.7; phosphate pH 5.7, 6.3, and 6.9; and Tris pH 7.4 and 8.0. The ionic strength from the buffer salts was kept constant at 40 mM.

[3] To avoid deamidation of the MAb, pH should normally be maintained below pH 8.0.

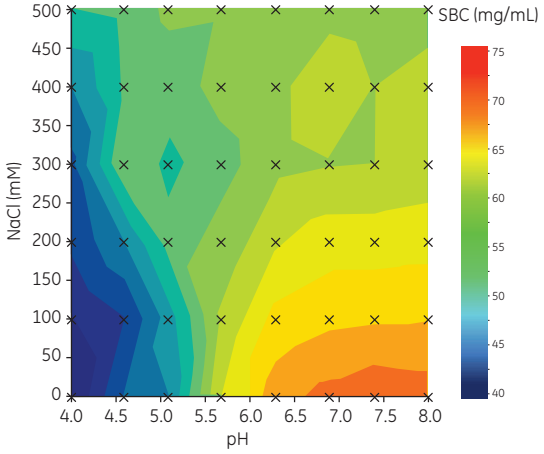

Fig 3. Contour map showing screening of SBC for Capto adhere ImpRes.

Dynamic binding capacity

The influence of pH and salt concentration on DBC was measured by DoE using Capto adhere ImpRes packed in a Tricorn™ 5/50 column. Based on the results for SBC, binding pH was varied between pH 6.0 and 7.8[4] and salt concentration from 0 to 200 mM NaCl. In addition, the residence time was varied from 2 to 8 min.

The results from the DoE are shown in Figure 4. Modeling of data was performed using MODDE™ v9.0 software, resulting in a good model fit and predictive power (data not shown). In accordance with the trend for SBC, an increase in pH and decrease in salt concentration resulted in higher DBC, while lower capacity was obtained at short residence time. Further experiments described below were performed using binding with 40 mM sodium phosphate, pH 7.8.

[4] Binding buffers: Sodium phosphate, 0 to 200 mM NaCl, pH 6 to 7.8. The ionic strength from the buffer salts was kept constant at 110 mM.

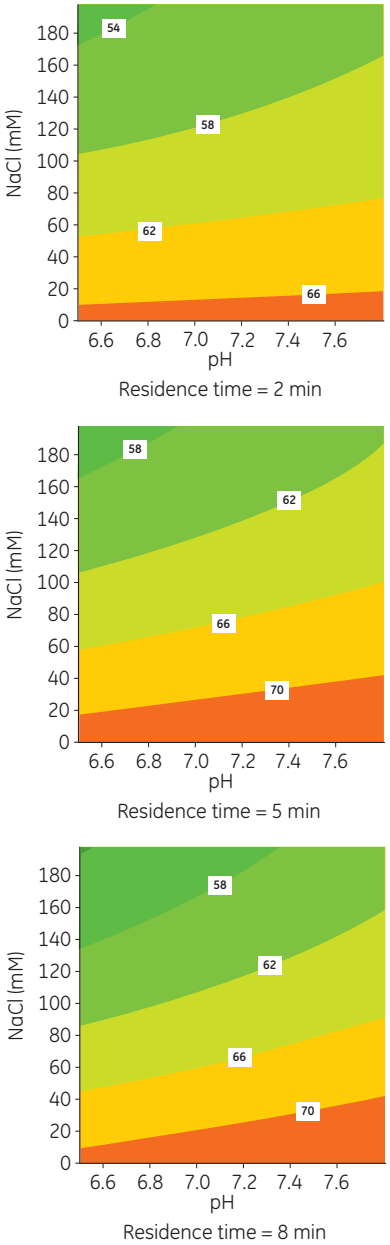

Fig 4. Contour maps for DBC at 10% breakthrough and different residence times on Capto adhere ImpRes.

Screening of elution conditions

Measurement of yield at different elution conditions was performed in 96-well filter plates as described in Materials and methods. Binding was performed in 40 mM sodium phosphate, pH 7.8. Elution pH was varied between 4.5 and 8.0 and salt concentration between 0 and 1 M NaCl. The result, Figure 5, shows that the highest yield was obtained at low pH and low salt concentration. Based on this result, further studies of elution conditions were performed by gradient elution in packed columns.

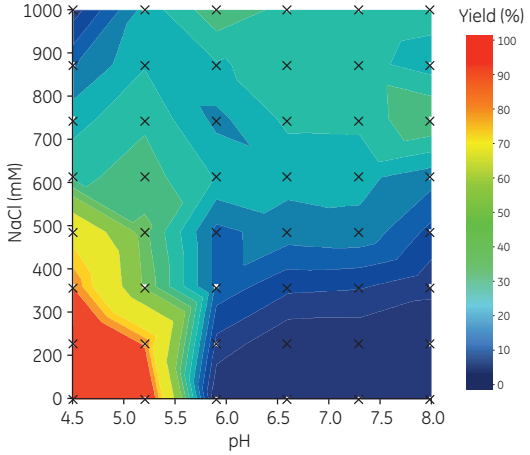

Fig 5. Screening of elution conditions in PreDictor plate. Yield obtained by varying elution pH between 4.5 and 8.0 and NaCl concentration between 0 and 1 M. Evaluation performed by Assist software for PreDictor plates.

Gradient elution

Gradient elution was performed from 40 mM sodium phosphate pH 7.8 to 20 mM sodium phosphate, 20 mM citrate, pH 4.0 with or without addition of 100 mM NaCl[5]. Chromatograms are shown in Figure 6. Fractions were collected and analyzed by gel filtration. Cumulated concentration of aggregates (%) vs cumulated yield of monomeric MAb (%) was calculated according to Materials and methods. The results showed that addition of 100 mM NaCl in the elution buffer resulted in slightly lower elution pH, lower aggregate content, and a broader elution peak than elution buffer without NaCl (Table 2).

[5] A mixed buffer with ionic strength that is too high might result in elution of MAb during the wash step or early in the gradient.

Table 2. Results from gradient elution on Capto adhere ImpRes using elution buffer with and without NaCl

NaCl (mM)	Elution pH (peak maximum)	Aggregate at 90% yield (%)	Elution volume (CV)
0	4.87	0.5	8.9
100	4.77	0.4	9.8

Column: Tricorn 5/50, column volume ~ 1 mL
Medium: Capto adhere ImpRes
Sample: MAb A, partially purified by protein A chromatography
Sample load: 43.4 mg MAb/mL chromatography medium
Start buffer: 40 mM sodium phosphate pH 7.8
Elution buffer: 20 mM sodium phosphate, 20 mM citrate, pH 4.0 (blue curve); 20 mM sodium phosphate, 20 mM citrate, 100 mM NaCl, pH 4.0 (green curve)
Gradient: 0% to 100% elution buffer in 20 CV
Residence time: 4 min
System: ÄKTAexplorer 10

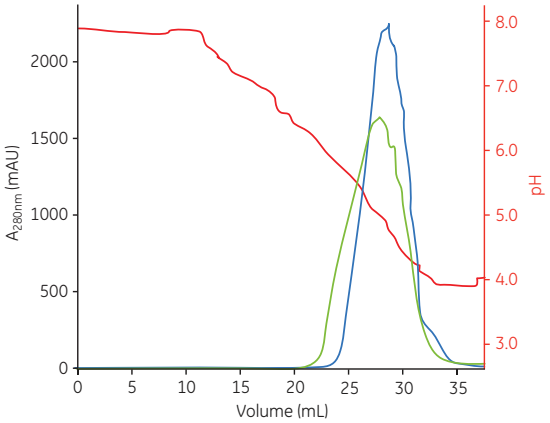

Fig 6. Gradient elution on Capto adhere ImpRes using elution buffer with NaCl (green curve) and without NaCl (blue curve) of MAb A, which was partially purified by protein A affinity chromatography.

Step elution

Based on results from screening in 96-well filter plates and gradient elution, conditions for step elution were further investigated in a packed column using DoE, varying sample load between ~ 50% and 70% of DBC (37.2 to 49.6 mg MAb/mL chromatography medium). Elution pH was varied between 3.5 and 4.5, and salt concentration between 0 and 100 mM NaCl. The responses from the design were yield, aggregate concentration, pool volume, HCP, and protein A concentration. The results from the design are shown in Table 3.

Modeling of the experimental data was performed with MODDE v9.0 software. Good models were obtained for all responses except for protein A[6]. The model showed that the only significant factor was elution pH. Thus, a higher elution pH resulted in lower yield, lower aggregate concentration, higher pool volume, and lower HCP concentration (Fig 7).

[6] As the values and the variation of protein A concentration in the elution pools were very low, no model could be obtained for this response.

Table 3. Results from DoE evaluation of step elution on Capto adhere ImpRes

pH	NaCl (mM)	Sample load (mg/mL)	Yield (%)	Aggregates (%)	Pool (CV)	HCP (ppm)	Protein A (ppm)
3.5	0	37.2	95.2	3.33	1.88	1233	1
4.5	0	37.2	83.6	0.61	5.24	319	Below LOQ†
3.5	100	37.2	92.9	4.11	1.90	973	2
4.5	100	37.2	85.4	0.74	5.59	405	Below LOQ
3.5	0	49.6	94.9	3.35	1.96	713	2
4.5	0	49.6	85.4	0.80	5.48	306	Below LOQ
3.5	100	49.6	93.4	4.01	2.50	1103	3
4.5	100	49.6	87.1	0.55	5.86	661	Below LOQ
4.0	50	43.4	93.6	2.26	3. 45	684	1
4.0	50	43.4	92.5	1.83	3.54	577	1
4.0	50	43.4	93.5	1.99	3.52	666	1
3.3	50	43.4	92.6	4.94	1.66	ND*	ND
4.7	50	43.4	83.4	0.29	6.89	ND	ND
4.0	50	43.4	92.6	2.11	3.53	ND	ND

* ND = Not determined.

† LOQ = Limit of quantitation.

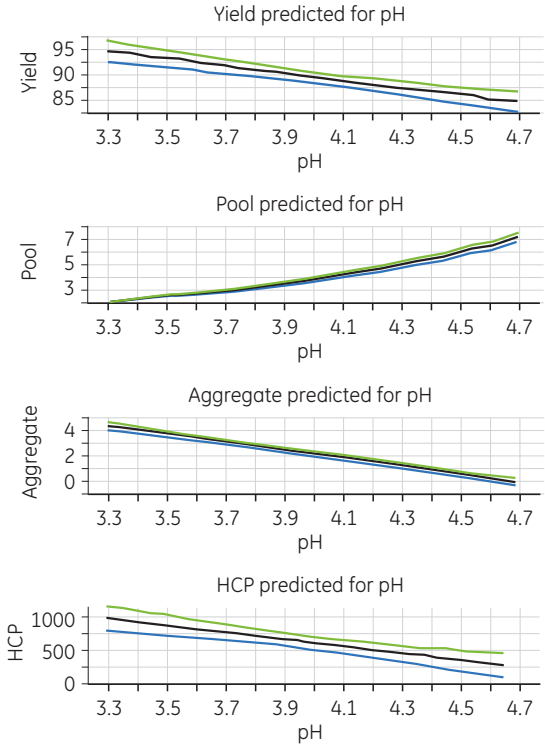

Fig 7. Response plots for yield, pool volume, aggregate-, and HCP concentrations.

Verification of the design

The model suggested an elution pH of 4.5 (0 M NaCl) and a sample load of 70% of DBC (≈ 50 mg/mL). Column verification of the method was performed in a Tricorn 5/50 column. The obtained result was in good agreement with the expected result for yield, pool volume, aggregate-, and HCP clearance (Table 4). The relatively high initial HCP level in the sample used accounts for the high HCP level after polishing. HCP levels could be further reduced, either by including a wash step before elution of the MAb or by addition of a third purification step.

Table 4. Verification of the suggested design

Result	Yield of monomer (%)	Pool volume (CV)	Aggregates (%)	HCP (ppm)
Expected result	86	5.6	0.7	400
Experimental result	85	5.5	0.8	400

Case study, MAb B

The related multimodal anion exchanger, Capto adhere, has been successful for MAb polishing in FT mode. However, Capto adhere has also found use in B/E mode, even though the particle size is not optimal. In a case study using MAb B, the performance of Capto adhere in B/E mode was compared to that of Capto adhere ImpRes, considering DBC at various residence times, and gradient and step-elution conditions.

Static and dynamic binding capacity

SBC and DBC for MAb B were determined using the same methodology as shown in the first case study. Highest SBC and DBC were obtained at high pH and low ionic strength (i.e., 20 mM sodium phosphate, pH 7.8[7]).

[7] To avoid deamidation of the MAb, pH should normally be maintained below pH 8.0.

Dynamic binding capacity vs residence time

DBC at 10% breakthrough for Capto adhere ImpRes and Capto adhere was measured at different residence times (linear flow rates) in the range of 1 to 10 min. As seen in Figure 8, DBC for Capto adhere ImpRes is higher and less sensitive to residence time than Capto adhere. Capto adhere ImpRes can therefore be operated at shorter residence times (i.e., higher flow rates) while maintaining process robustness with regard to capacity[8].

[8] Due to pressure-flow limitations, a maximum bed height of 10 cm is recommended at 2 min residence time.

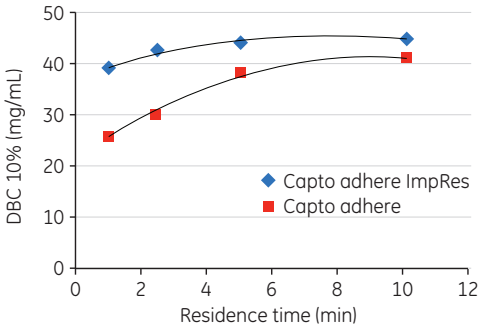

Fig 8. Dynamic binding capacity vs residence time. DBC at 10% breakthrough measured in 28 mM sodium phosphate, pH 7.75.

Gradient elution

Gradient elution by pH was performed on Capto adhere ImpRes. Unlike the example with MAb A, addition of NaCl to the elution buffer resulted in a narrower elution peak (Fig 9, green curve). Collected fractions were analyzed by gel filtration and cumulated yield of monomer was plotted against cumulated concentration of aggregates. The result shows good separation between monomer and aggregates, and that separation was improved on Capto adhere ImpRes compared with Capto adhere (Fig 10).

Column:	Tricorn 5/50, column volume ~ 1 mL
Medium:	Capto adhere ImpRes
Sample:	MAb B, partially purified by protein A affinity chromatography
Sample load:	30 mg/mL
Start buffer:	28 mM sodium phosphate, pH 7.75
Elution buffer:	30 mM sodium phosphate, 25 mM citrate, pH 4.1 (blue curve) 30 mM sodium phosphate, 25 mM citrate, pH 4.1 + 250 mM NaCl (green curve)
Gradient:	0% to 100% elution buffer in 20 column volumes (CV)
Residence time:	4 min
System:	ÄKTAexplorer 10

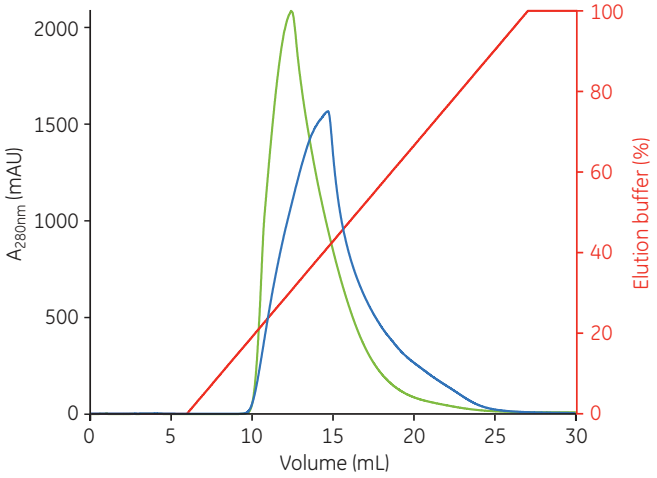

Fig 9. Gradient elution of MAb B from Capto adhere ImpRes with (green curve) and without (blue curve) NaCl in elution buffer.

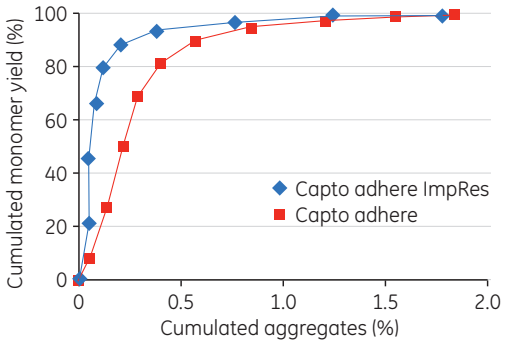

Fig 10. Cumulated aggregates vs cumulated MAb monomer yield after gradient elution using Capto adhere ImpRes and Capto adhere.

Step elution

From the gradient elution results above, step elution from Capto adhere ImpRes and Capto adhere was performed at pH 6.5 and 62.5 mM NaCl (i.e., 25% of elution buffer, Fig 11). The sample load was 70% of DBC 10%. Fractions were pooled and analyzed for yield, aggregate-, and HCP concentration. Despite 20% higher load, step elution from Capto adhere ImpRes resulted in higher yield and improved aggregate clearance compared to Capto adhere (Table 5). HCP levels were below the detection limit for ELISA.

Column:	Tricorn 5/50, column volume ~ 1 mL
Sample:	MAb B, partially purified by protein A affinity chromatography
Sample load:	30 mg/mL
Start buffer:	28 mM sodium phosphate, pH 7.75
Elution buffer:	30 mM sodium phosphate, 25 mM citrate, 250 mM NaCl, pH 4.1
Step elution:	25% elution buffer (pH 6.5, 11.3 mS/cm)
Residence time:	4 min
System:	ÄKTA pure

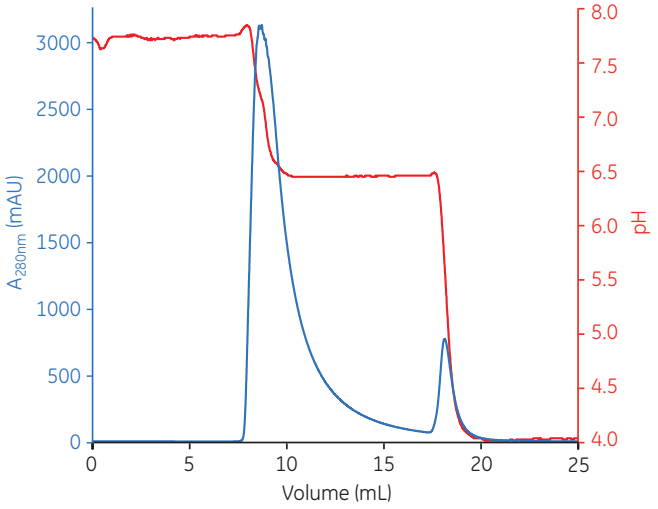

Fig 11. Step elution of MAb B using Capto adhere ImpRes.

Table 5. Results from step elution

Medium (mg/mL)	Sample load (mg/mL)	Yield (%)	Pool volume (CV)	Aggre-gates (%)	HCP* (ng/mL)
Capto adhere ImpRes	30	91	4.4	0.5	Below detection limit (< 20 ng/mL)
Capto adhere	25	79	6.1	0.8	Below detection limit (< 20 ng/mL)

* Measured with general ELISA from Cygnus Technologies.

Conclusions

In this work, we present results from two case studies using Capto adhere ImpRes, a multimodal anion exchanger designed for polishing. Two different MAbs were purified in B/E mode. The results show high yields of MAb monomers, good clearance of aggregates, HCP, and leached protein A.

Ordering information

Product	Code number
Capto adhere ImpRes, 1 L*	17-3715-03
Capto adhere, 1 L*	17-5444-03
Superdex 200 5/50 GL, 1 × 3 mL	28-9065-61
Tricorn 5/50 column	28-4064-09

* Several pack sizes available. Visit www.gelifesciences.com for more information.

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GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

imagination at work

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare Europe, GmbH
Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Japan Corporation
Sanken Bldg., 3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

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