Research Article

Pharmaceutical BIOPROCESSING

Development of an acidic/neutral antibody flow-through polishing step using salt-tolerant anion exchange chromatography

For 'acidic' (pI < 7.0) or 'neutral' antibodies (pI: 7.0–8.0), it is challenging to operate traditional anion exchange chromatography in a product flow-through mode to achieve adequate clearance of HCP, DNA, leached ProA, HMW and viruses while maintaining high process yield. In this study, the authors developed a scalable mAb polishing step using a new salt tolerant chromatographic resin. Utilizing a combination of high-throughput condition screening in 96-well plates and optimization in small-scale column models, a polishing step was developed that demonstrated high process yield and efficient clearance of impurities for multiple acidic or neutral antibodies. Pilot scale production demonstrated scalability of the step. This polishing step can be easily integrated into most current Protein A/AEX two-column antibody purification platforms.

Keywords: acidic antibody • anion exchange chromatography • antibody purification • canine antibody • flow-through mode • neutral antibody • POROS XQ • salt-tolerant chromatography • two-column process

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Background

Monoclonal antibody (mAb) purification processes exist in different well-established platforms with extensive process performance histories for production of commercial mAbs [1–11]. In most two-column downstream processing platforms, the first column employed is Protein A, which binds the target mAb directly from the harvested cell culture fluid [3–7,11]. A low pH buffer is routinely used to elute the mAb product, which is followed by a viral inactivation step. Anion exchange chromatography (AEX), such as Q Sepharose Fast Flow (QFF) column chromatography [3–5,12–14] or Q membrane adsorber [15–18], typically serves as a second chromatographic step in flow-through mode operated at pH ≥ 7.0, binding trace impurities such as host cell proteins (HCP), DNA, leached Protein A (leached ProA), endotoxins, viruses, and in some cases, high molecular weight (HMW) species while the mAb product passes through. Traditional AEX chromatography is limited by the requirement for low loading buffer conductivity, often necessitating buffer exchange through tangential flow filtration (TFF) or dilution of the neutralized Protein A eluate for efficient impurity clearance. However, acidic and neutral antibodies (isoelectric point [pI]) ≤ 8.0), may have solubility issues at low ionic strength conditions. For these antibodies, it is often challenging to achieve adequate clearance for HCP and viruses when buffer conductivity is increased to favor antibody solubility (internal unpublished data). These challenges may be addressed by a salt tolerant interaction membrane adsorber or resin with a polyallylamine ligand [7,19–23]. Although the separation mechanism of salt-tolerant chromatography is not fully understood, we postulate that the salt tolerance property is due to selection of a proprietary surface chemistry along with optimization of the base bead structure and ligand density. Particularly, the salt tolerance properties based on a primary amine ligand offer the potential to capture proteins at higher conductivity than conven-

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tional chromatography through electrostatic and possibly hydrogen bond interactions as well as more binding sites available [19,22]. However, it has been observed that the primary amine ligand is not compatible with multivalent buffers such as phosphate and citrate, which limits wide application of primary amine-based salt-tolerant chromatography in mAb manufacturing [20].

A new chromatography resin, POROS XQ, with proprietary quaternary amines, is salt tolerant and compatible with multivalent buffers which would provide a practical alternative to traditional AEX resins. Efficient viral clearance has been demonstrated using two model viruses, MMV and XMuLV [24]. In this study, the XQ resin was evaluated as a polishing step in purification of acidic or neutral antibodies that previously proved challenging to purify using conventional AEX. Using a combination of high-throughput process development (HTPD) in a 96-well batch binding format and small-scale column optimization experiments in flow-through mode, we have developed an mAb polishing step successfully implemented at the pilot-scale, which demonstrates high step recovery and efficient clearance of impurities (HCP, host DNA, leached ProA, and HMW) for antibodies with relatively low pI values (≤ 8.0). Employing this new chromatography resin eliminates the need for a buffer exchange step such as tangential flow filtration or in-line dilution. This polishing step, which can be easily integrated into current mAb purification platforms, offers a viable alternative to traditional AEX in instances where acidic or neutral antibodies exhibit poor purification process performance. Finally, the methods described here for developing salt tolerant XQ operating conditions can be applied to purification process development of other chromatography resins.

Materials & methods

Cell culture

The mAbs used in this study were fully human IgG1, IgG4 or canine antibody produced in Chinese hamster ovary (CHO) cells grown in fed-batch mode using a serum-free medium in an overhead stirred bioreactor vessel (Bellco Biotechnology, NJ, USA). The cultures were harvested on day 14–15, with a typical mAb titer of 1.0–5.0 g/l.

Protein A chromatography

MabSelect™ or MabSelect SuRe™ Protein A (GE Healthcare, NJ, USA) was used to purify antibodies present in the harvested cell culture fluid using an AKTA Explorer or Avant system under the control of UNICORN software (GE Healthcare), as described previously [7,25]. Following low pH treatment, the elution product pool was neutralized to the required pH with 1–2 M Tris base solution and clarified through a 0.22 µm filter (EMD Millipore, MA, USA), which served as the feed for AEX experiments. Different Protein A column wash strategies might be used to reduce the HCP burden to AEX polishing chromatography.

High-throughput AEX chromatography

Model mAbs were tested on POROS XQ (Thermo Fisher Scientific, MA, USA) in 96-well filter plates (Seahorse Biosciences, MA, USA) along with Q Sepharose Fast Flow (GE Healthcare) as a control, on a Tecan Freedom Evo200 system (Männedorf, Switzerland) at room temperature. The equilibration buffer conditions were evaluated with a full factorial design of experiment (DOE) at varying pH (6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0), and NaCl concentrations (0, 10, 20, 30, 40, 50, 75 and 100 mM). 20 mM MES was employed for equilibrations at pH 6.0, 6.5 and 7.0 while 20 mM Tris for pH 7.5, 8.0 and 8.5. An Eppendorf Motion 5070 liquid handler, equipped with an orbital shaker (Hauppauge, NY, USA), was used to transfer resin slurries into filter plates. Experiments were conducted at 270 µl working volume with 40 µl of resin per well. The antibody was loaded into each well at approximately 5 mg/ml-resin. Each experimental run consisted of three 10-min equilibration steps, one 60-min loading step and two 10-min strip steps. Incubations were performed at 1250 rpm on an orbital shaker. Liquid separations were conducted using vacuum or centrifugation. The flow-through and subsequent wash from each well was collected as the product pool.

Resin performance during HTPD has been traditionally evaluated by partition coefficient (Kp) value [26,27]. However, in order to better predict mAb flow-through performance in column runs, process yield was calculated in this study instead, using the following equation:

$$\text{Yield} = \frac{\text{mAb in flow-through (mg)}}{\text{Total mAb added to well (mg)}} \times 100\%$$

In addition, two other response parameters, HCP and HMW, were also assessed for each run.

POROS XQ column chromatography

A response surface study (central composite design, α = 1.414) for mAb-T on XQ was designed with three factors: load pH (6.5–7.5, axial points: 6.0 and 8.0), buffer conductivity (6.0–9.0 mS/cm, axial points: 4.5 and 10.5), and residence time (4–10 min, axial points: 1 and 13 min). Response parameters included flow-through process yield, purity (in terms of HMW for simplicity), HCP, DNA, leached ProA, and volume ratio of flow-through to feed. mAb-T was used as a model mAb here. All DOE runs were performed using Vantage L columns (5 ml bed volume with dimensions of 1.1 id × 5.3 cm, EMD Millipore). A confirmatory

Table 1. Model antibodies partially purified with MabSelect™ or MabSelect SuRe™ Protein A.

Antibody	Subtype	pI	HMW (%)	HCP (ng/mg)
mAb-A	Human IgG1	8.31	3.00–4.50	80
mAb-FL	Human IgG1	7.58	1.66	15
mAb-FY	Human IgG1	7.71	< 1.00	51
mAb-R	Human IgG1	7.64	3.90	21
mAb-T	Human IgG1	8.03	3.49–4.52	88–130
mAb-P	Human IgG4	6.89	2.02	31
mAb-C	Canine Igb	6.45	1.70–3.40	18

HCP: Host cell proteins; HMW: High molecular weight species.

run at the same scale was performed using optimal operating conditions while a pilot run was completed using 240-l harvested cell culture fluid. The BPG column (2.85 l with dimensions of 14 id × 18.5 cm, GE Healthcare) was utilized at pilot scale.

Antibody dynamic loading capacity (DLC) on XQ was determined at optimal buffer pH and conductivity conditions using Omnifit column (Diba Industries, CT, USA) at residence time of 4–8 min. Different flow-through fractions were collected and critical process impurity (HMW in most cases or HCP in some other cases) determined. The DLC value was mAb amount applied to the XQ column when HMW in the flow-through reached process criteria. Process and product-related impurities in XQ flow-through pool (purified product) were determined using different analytical techniques. The bound materials were eluted using 50 mM Tris, pH 7.5, 1.0 M NaCl and analyzed for the levels of impurities.

Analytical techniques

The in-process samples and purified mAbs were analyzed for product concentration, purity and residual impurities as described previously [7,25]. Briefly, antibody concentration in cell cultures, was determined by Octet Protein A titer assay (Pall Life Sciences, NY, USA). Protein A or AEX-purified antibodies were quantified through the absorbance at 280 nm, using a Nanodrop system (Thermo Scientific, DE, USA). Size exclusion high performance liquid chromatography (SE-HPLC) was used to monitor the size heterogeneity of mAbs under native conditions on an Agilent HPLC system using Chemstation as the controlling software (CA, USA). A TSK-Gel G3000SW$_{XL}$ column (Tosoh Bioscience, PA, USA) was utilized to separate HMW impurities, monomers and fragments. The HCP level was measured using the ELISA developed at Lilly with a quantification limit of 6.25 ng/ml. The samples from HTPD were analyzed using a Gyrolab™ workstation model xP on a Gyrolab Bioaffy™ 1000 CD (Gyros U.S., Inc., NJ, USA) with in-house reagents developed for CHO cell line.

The leached MabSelect™ or MabSelect SuRe™ Protein A ligand in antibodies was determined using Repligen's ProA ELISA kit (MA, USA) with a quantification limit of 0.1 ng/ml according to the manufacturer's protocol. Residual CHO DNA in antibodies was measured by quantitative PCR developed at Lilly using in-house DNA standards. The quantification limit of the assay was 0.1 pg/ml.

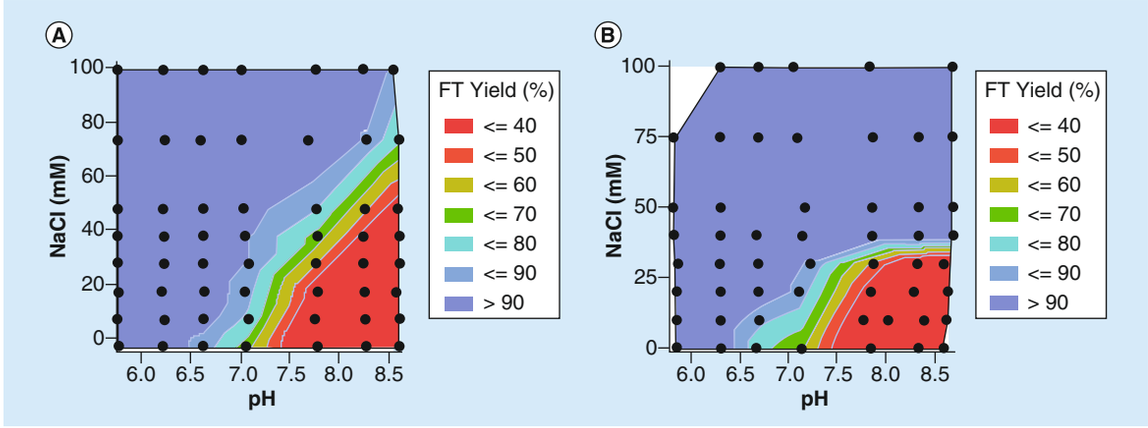

Figure 1. Contour profiles of the flow-through process yield generated from the high-throughput screening of mAb-A using QFF (A) and XQ (B).

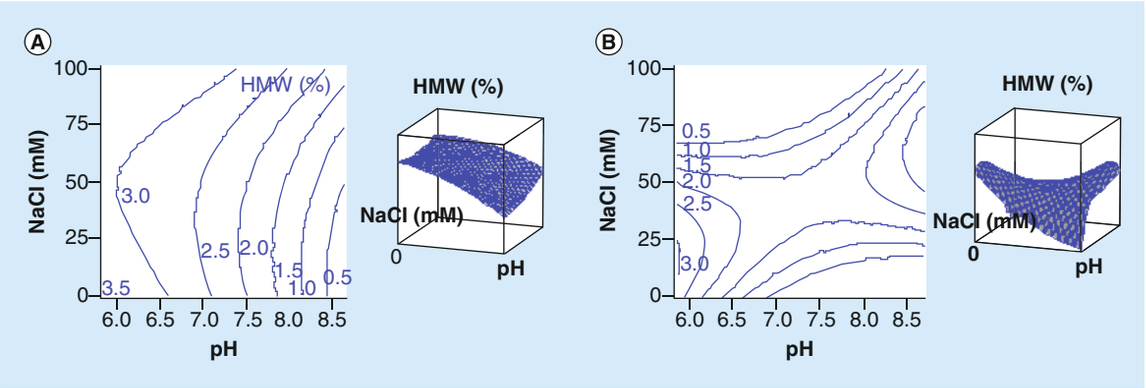

Figure 2. Plots of residual high molecular weight species generated from the high-throughput screening of mAb-A using QFF (A) and XQ (B). Load: 5 mg-mAb/ml-resin with an mAb concentration of 1.0 mg/ml.

Data analysis

All experimental design and data processing were performed using JMP version 9.0.3 software (SAS Institute, NC, USA), unless stated otherwise.

Results

Seven unique mAbs with pI ranging from 6.4 to 8.3 were selected for this study (Table 1). All seven antibodies exhibited significant downstream processing issues specifically related to the performance of the polishing QFF step run under standard platform conditions. Less than 80% of antibody product was recovered in the flow-through using our platform buffer conditions (pH ≥ 7.0, conductivity < 4 mS/cm). When buffer conductivity was increased either by adding NaCl to the current buffer system or the buffer was switched to citrate, step recovery was improved to greater than 90%, however, suboptimal HCP and viral clearance was observed (internal unpublished data).

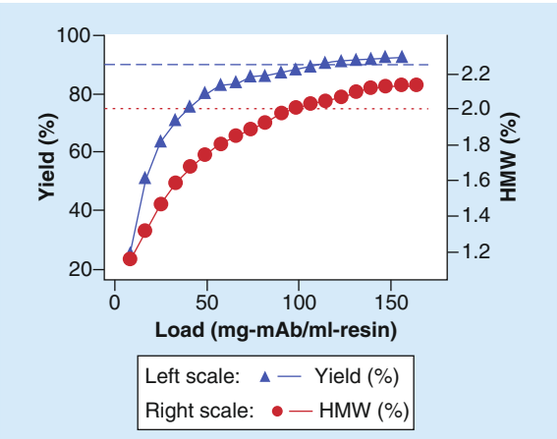

Figure 3. Process performance for mAb-A from an XQ column run at pH 8.5 showing process yield (blue) and residual high molecular weight (HMW) species (red).

Condition screening & optimization using 96-well filter plates

Although the pI of mAb-A fell outside the neutral or acidic range it was included in the study as it exhibited QFF chromatography behavior consistent with mAbs having pI in the neutral or acidic range. Here, mAb-A was used for high-throughput condition screening over a broad range of pH and NaCl conditions to investigate the low flow-through process yield (74.8%) observed in a column run. The results are summarized in contour plots (Figure 1).

As shown in Figure 1A, when QFF resin in batch filter plates was operated at pH ≥ 7.0 and NaCl < 20 mM NaCl (equivalent to 4 mS/cm approximately), a significant percentage of antibody product bound to the resin, resulting in low flow-through process yield, ranging from 40 to 80%. Under the same pH condition, as the NaCl concentration was increased, higher flow-through process yield was observed. This was in good agreement with the small-scale column model experiment. A similar trend was observed with the XQ resin (Figure 1B).

The residual HMW level in the flow-through product pool was affected by equilibration buffer pH and conductivity in a more complex manner (Figure 2). As shown in the QFF contour plot (Figure 2A), efficient HMW removal was achieved at pH values > 8.0. Given that the product co-bound to the resin along with HMW in these conditions, the low process yield observed was expected (in Figure 1A). Conditions were not identified by HTS that resulted in acceptable process yield (> 90 %), residual HMW (< 1.5 %) and HCP (< 20 ng/mg, not shown in the figure). In contrast, experimental runs with the XQ resin revealed conditions, leading to acceptable HMW and process yield (Figures 1B & 2B).

To test whether the results of the HTS could be replicated in column mode chromatography, a run was performed using an XQ column, pre-equilibrated with 20

mM Tris, pH 8.5, with a conductivity of 3.12 mS/cm. As expected, a low flow-through process yield (40 %, Figure 3) was observed when 5 mg mAb/ml resin was loaded (Figure 3 vs Figure 1, red area). The HMW level in the flow-through pool was 1.2% (Figure 3), which was again in a good agreement with HTPD results. As an increasing quantity of mAb-A was loaded onto the column, process yield increased, eventually reaching 90% at load of 100 mg/ml resin. Similarly, the HMW level increased with the antibody load. At load of 150 mg-mAb/ml-resin, the residual HMW in the flow-through pool was 2.14% (Figure 3). HCP in the column flow-through was 20 ng/mg while host DNA and leached ProA were below assay quantification limits (Table 3). As expected, the column strip pool contained high levels of HMW, HCP, DNA and leached ProA, indicating efficient capture of these impurities by the resin. The experiment confirmed HTPD might predict antibody column performance only at an equivalent load, 5 mg/ml. Based on this initial finding, we decided to develop the operating conditions for flow-through XQ chromatography in column runs through a response surface DOE.

DOE condition optimization using XQ in column mode

Three XQ operating parameters, equilibration buffer/load pH, conductivity and residence time, were investigated in a response surface DOE study. The response parameters with respect to XQ performance included

Table 2. Summary of mAb-T POROS XQ column study.

Run	pH	Cond (mS/cm)	Residence time (min)	HMW (%)	HCP (ng/mg)	Yield (%)	V/V (FT/load)
1	6.5	9.0	10	2.06	13	100.0	1.3
2	7.5	6.0	10	0.64	10	86.6	1.9
3	7.0	10.5	7	1.68	12	83.0	1.4
4	6.5	6.0	4	1.44	10	82.2	1.4
5	7.5	6.0	10	0.75	12	95.1	2.2
6	6.5	6.0	10	1.55	10	93.3	1.4
7	8.0	7.5	7	0.64	12	23.6	1.3
8	7.0	7.5	7	1.49	9	102.0	1.7
9	6.5	6.0	10	1.54	10	98.0	1.4
10	7.0	7.5	7	1.59	10	102.0	2.0
11	7.5	9.0	4	1.33	13	87.2	2.4
12	7.0	7.5	13	1.24	10	89.7	2.0
13	6.0	7.5	7	2.04	11	97.7	1.7
14	7.5	6.0	4	1.03	11	82.8	1.9
15	7.0	7.5	7	1.34	10	95.9	2.3
16	7.0	7.5	7	1.36	9	77.3	1.7
17	7.0	4.5	7	0.89	10	85.9	1.8
18	7.0	7.5	7	1.22	8	102.0	2.5
19	7.5	6.0	4	0.56	10	90.9	2.7
20	7.0	7.5	7	1.27	8	89.7	2.3
21	7.5	9.0	10	1.14	10	84.7	2.1
22	7.0	7.5	1	1.51	11	94.0	1.9
23	6.5	9.0	10	2.00	11	83.7	1.2
24	6.5	9.0	4	1.79	10	89.7	1.6
25	6.5	6.0	4	1.51	9	82.2	1.4
26	7.5	9.0	10	1.19	11	87.8	2.0
27	6.5	9.0	4	1.83	11	83.3	1.3
28	7.5	9.0	4	1.24	12	86.5	2.1

HCP: Host cell proteins; HMW: High molecular weight species; V/V: Volume ratio.

Table 3. Summary of XQ purification process performance.

Antibody	Equilibration buffer	Process loading (mg/ml)	Yield (%)	HMW (%)	HCP (ng/mg)	DNA (pg/mg)	ProA (ng/mg)
mAb-A	20 mM Tris, pH 8.5 3.21 mS/cm	150	93	2.14	20	BQ	0.2
mAb-FL	50 mM Tris, pH 7.5 9.18 mS/cm	314	98	0.66	11	BQ	0.4
mAb-FY	50 mM Tris, pH7.5 10.10 mS/cm	114	91	1.31	26	BQ	BQ
mAb-R	20 mM NaPi, pH 6.8 7.50 mS/cm	100	89	1.36	7	ND	ND
mAb-T	20 mM NaPi, pH 7.2 6.00 mS/cm	100	92	0.58	15	BQ	ND
mAb-T (Pilot)	20 mM NaPi, pH 6.8 7.10 mS/cm	100	93	0.80	7	BQ	0.1
mAb-P	50 mM Tris, pH7.5 9.02 mS/cm	300	97	1.13	26	BQ	ND
mAb-C	50 mM Tris, pH7.5 10.10 mS/cm	100	90	0.54	4	BQ	BQ

BQ: Below quantification limit; HCP: Host cell proteins; HMW: High molecular weight species; ND: Not determined.

process yield, residual impurity levels (HMW, HCP, DNA and leached ProA) and volume ratio of flow-through to feed. Experimental results for mAb-T are summarized in Table 2, and Figures 4 and 5. As shown in Figure 4, operating pH had a significant effect on both process yield and residual HMW, while conductivity

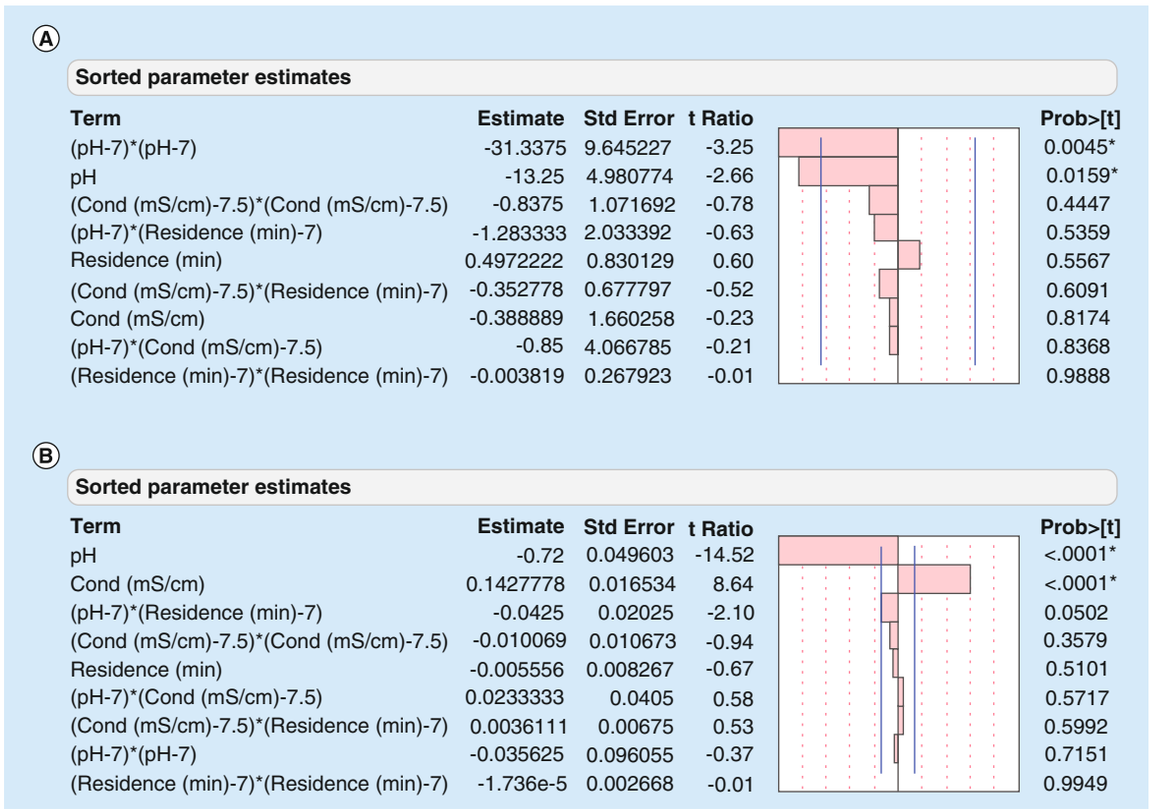

Figure 4. Linear squares fit of mAb-T from the XQ column DOE study for process yield (A) and residual high molecular weight species (B).

only impacted the residual HMW significantly. There was an interaction observed between residence time and pH, which had only marginal impact (-0.0425%) on HMW level and was therefore excluded in further analysis. In addition, residual DNA and leached ProA levels from these runs were below assay quantification limits, and residual HCP was approximately 10 ng/mg, therefore, our analysis focused on residual HMW and process yield.

As shown in the contour profiles (Figure 5), under the same pH condition, residual HMW in the flow-through increased with equilibration buffer conductivity from 4.5 to 10.5 mS/cm. In contrast, the HMW level decreased as load pH increased from 6.0 to 7.5 for a given conductivity. This was not unexpected as more HMW was expected to bind onto the resin under higher pH and lower conductivity conditions. An interesting contour profile of process yield was observed. With a load of 100 mg-mAb/ml-resin, the highest yield was observed in the center of the pH and conductivity operating range (pH 6.8, 7.1 mS/cm). The overlapping blue and red contours in Figure 5 indicate a 'sweet spot', optimal conditions, defined by >90% process yield and <1.5% HMW.

A column run was performed at pH 7.2 and 6.0 mS/cm with a load of 100 mg-mAb/ml-resin to confirm the DOE finding. The results are summarized in Table 3. As expected, acceptable process yield (92%) and residual HMW level (0.58%) were achieved. In addition, residual HCP (15 ng/mg), and DNA (below assay quantification limit) met our process criteria.

A pilot run was performed with mAb-T at a 240-l cell culture scale. 20 mM sodium phosphate, pH 6.8 was used as the equilibration buffer. Sodium chloride was added to increase the buffer conductivity to 7.1 mS/cm (Table 3). A flow-through process yield of 93% was achieved while HMW was reduced to 0.80%. Residual HCP in the product pool was 7 ng/mg, while leached ProA was 0.1 ng/mg, below the specified limit. Residual DNA was removed to below assay quantification limit. An acceptable process performance was therefore achieved at pilot scale, indicating that the process developed for mAb-T is scalable.

Additional case studies

Five additional acidic or neutral mAbs including mAb-C, mAb-FL, mAb-FY, mAb-P and mAb-R were tested using XQ as a polishing step following the conditions in Table 3. The results are summarized in the same table. Acceptable process performance was achieved for all tested mAbs. mAb-C, a canine antibody with lowest pI among those tested (pI 6.4), was used to demonstrate XQ process performance in two side by side experimental runs. In Run 1, a buffer at pH 7.5, 4.69 mS/cm, was

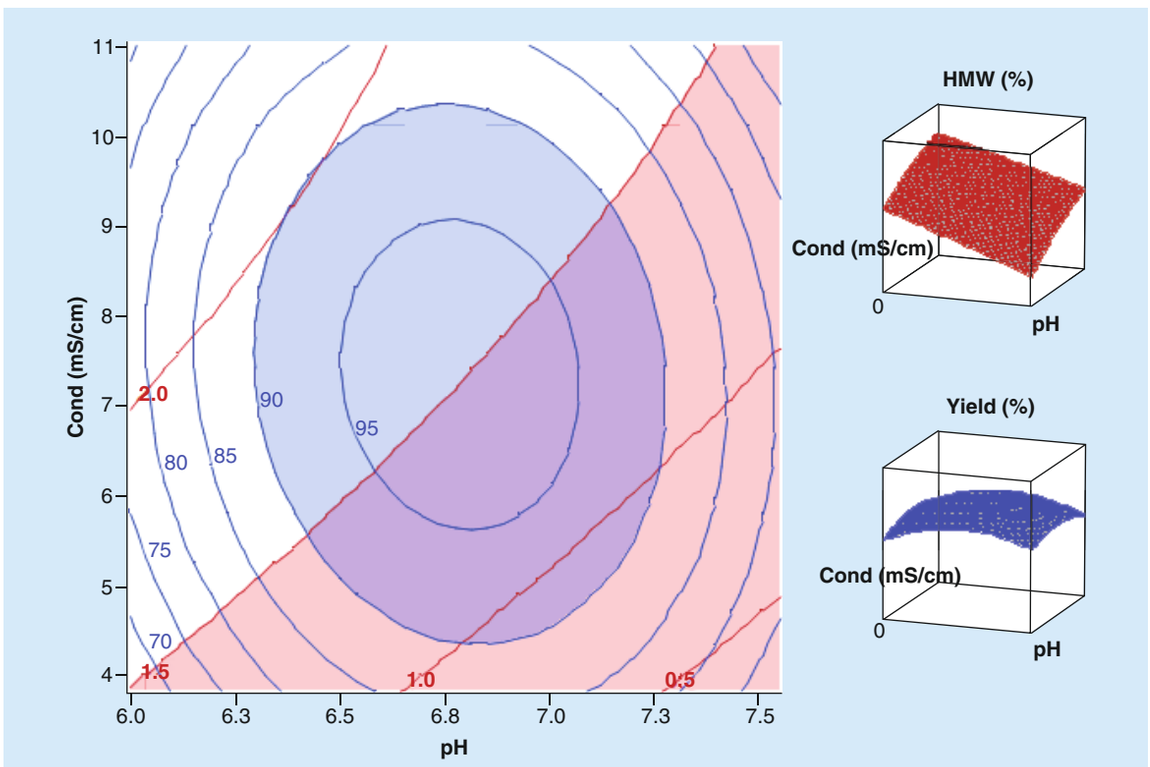

Figure 5. Contour profiles of residual high molecular weight (HMW) species (red) and yield (blue) from DOE runs using XQ resin. Residual HMW ≤ 1.5% was highlighted in red and yield ≥ 90% in blue.

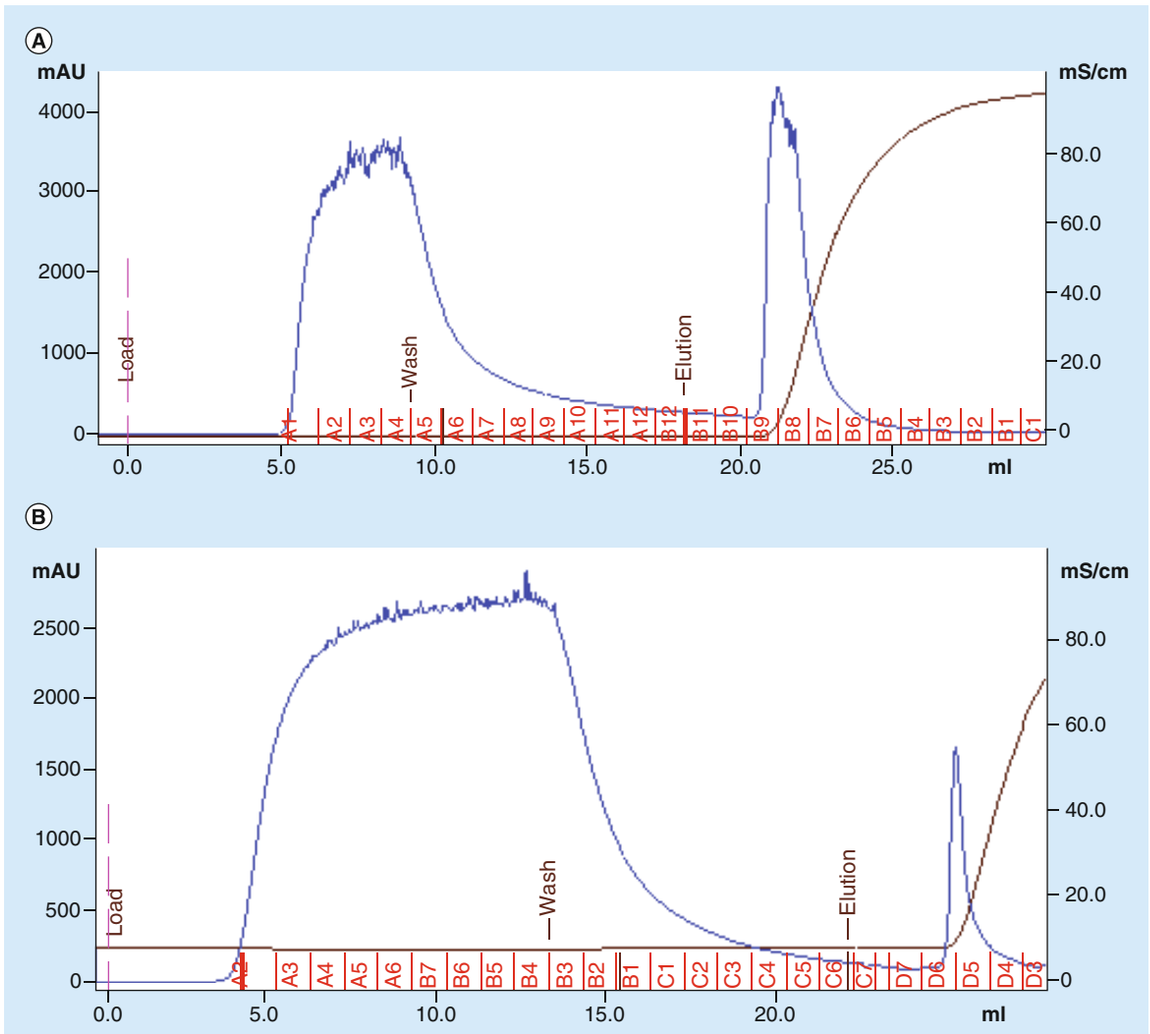

Figure 6. mAb-C purification chromatograms from XQ column runs at 4.69 mS/cm (A) and 10.10 mS/cm (B) display the effect of increasing conductivity. The column elution (or strip) peak at 4.69 mS/cm is larger due to an increase in product loss.

used as the equilibration buffer (Figure 6A). As expected, only 63% of antibody was recovered in the flow-through at a load of 100 mg/ml. HMW level in the flow-through pool was lower than 0.5 % (Figure 7A), and HCP was below 10 ng/mg (data not shown). Operating conditions in Run 2 were identical to Run 1 with the exception of conductivity which was increased to 10.1 mS/cm (Figure 6B). The process yield (90%) was significantly higher than that observed in Run 1 (Figure 7A & Table 3). The residual HMW in the flow-through was 1.0%, meeting the product quality criteria (Figure 7B). Under the tested conditions, HCP in flow-through pool was below 10 ng/mg while host DNA and leached ProA were below assay quantification limits. Materials bound to the resin were stripped off from the column at the end of the run and collected for characterization. As expected, the strip contained high levels of HMW, HCP and leached ProA (data not shown).

Discussion & future perspective

In mAb manufacturing processes, a flow-through mode AEX unit operation is generally preferred over bind-elute. Advantages can include higher process capacity, smaller column size, shorter cycle time, and in most instances lower manufacturing cost. In addition, smaller buffer volume is typically required, which may confer an advantage if there is a limit on buffer tank capacity.

When AEX is operated in the flow-through mode, impurities such as HCP, DNA, leached ProA, viruses, and endotoxins bind to the AEX resin (or membrane adsorber) while the antibody passes through. Acidic (pI < 7.0) and neutral (pI = 7.0–8.0) antibodies very often pose significant challenges to current two-column platforms using traditional Q chromatography as a product polishing step. Under normal operating pH conditions (pH 7.0–8.5), low buffer ionic strength is required, often resulting in a significant percentage of

product binding to the resin, leading to low process yields. The yield can be increased by lowering operating pH, which increases the risk of not achieving adequate clearance of HCP, and virus. In addition, low buffer conductivity can sometimes result in reduction of antibody solubility, leading to additional product loss during the process. Identifying conditions that achieve a balance between antibody solubility and Q column performance is an important consideration. The issue is compounded by the need to define a control strategy for HCP removal and viral clearance as part of a robust manufacturing process.

In contrast, salt tolerant XQ chromatography can be operated at relatively higher buffer conductivity under column conditions favoring antibody solubility while maintaining high column performance. As illustrated in this paper, XQ chromatography run in flow-through mode was used successfully as a polishing step in the purification of six acidic or neutral mAbs at buffer conductivities ranging from 6 to 10 mS/cm. We speculate that these product compatible buffer conditions led to improved mAb solubility, likely contributing to an acceptable process yield. For all antibodies tested in our study, acceptable clearance of host cell proteins, DNA and leached ProA was demonstrated. Additionally, residual HMW were reduced to <1.4%. Utiliza-

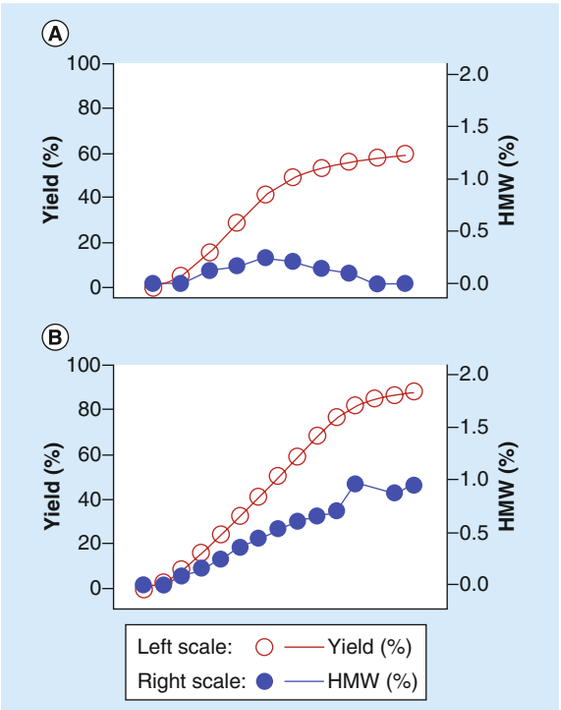

Figure 7. Summary of mAb-C process performance from POROS XQ column runs at 4.69 mS/cm (A) and 10.10 mS/cm (B), displaying the effect of increasing conductivity to process yield.

Executive summary

Background

- Traditional AEX chromatography is limited by the requirement for low loading buffer conductivity, often necessitating buffer exchange through tangential flow filtration (TFF) or dilution of the neutralized Protein A eluate for efficient impurity clearance.
- For acidic and neutral antibodies (isoelectric point (pI) ≤ 8.0), it is often challenging to achieve acceptable yield and adequate clearance for impurities.
- A scalable mAb polishing step using a salt tolerant chromatographic resin, XQ, has been developed.

Results

- Condition screening and optimization using 96-well filter plates
  - High-throughput condition screening in 96-well batch binding format was used to investigate the low process yield observed in traditional Q chromatography operated in flow-through mode for acidic or neutral antibodies and predict the process performance in column runs.
- DOE condition optimization using XQ in column mode
  - A DOE response surface study was used to optimize mAb operating conditions on XQ chromatography in scale-down column runs, which demonstrated acceptable process yield and clearance of process and product-related impurities including (HCP, DNA, leached ProA and HMW).
  - An acceptable process performance was therefore achieved at pilot scale, indicating that the process developed is scalable.
- Additional case studies
  - The great performance of XQ step for six acidic or neutral antibodies enables a ProA/XQ two-column platform for purification of acidic or neutral antibodies.

Discussion & future perspective

- The introduction of the XQ step enables a ProA/XQ two column platform for purification of acidic or neutral antibodies.
- XQ chromatography in flow-through mode is likely to provide a valuable alternative to mAbs with more basic properties.
- This study can be leveraged during future development to define critical process parameters, proven acceptable ranges, and a control strategy to support large-scale manufacturing.

tion of XQ chromatography enabled development of a two-column mAb purification platform, able to meet product purity targets for all antibodies evaluated in the study.

The unique salt tolerant nature of the XQ resin makes it compatible to most Protein A elution buffers, eliminating the need for pre-AEX buffer exchange or inline dilution. In addition, wider operating ranges can be defined relative to traditional Q chromatography, which is likely to result in greater process robustness and manufacturing flexibility. As such, XQ chromatography in flow-through mode is likely to provide a valuable alternative to mAbs with more basic properties.

Last, using a combination of multivariate, univariate, and modular process development methods in this study, an initial operating range for each of the parameters evaluated for the XQ chromatography step was defined. This study can be leveraged during future development to define critical process parameters, proven acceptable ranges and a control strategy to support large-scale manufacturing.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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