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Comparison of various columns used in anion exchange chromatography method for mAb purification

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Abstract

The impact of resins with varying ligands and pH levels on human IgG4 protein was analysed using anion exchange chromatography. Initially, a resin screening study involving five different positively charged ligands from four different brands was conducted on largely purified monoclonal antibodies, following Protein A capture. Subsequently, the influence of pH levels (6, 7, and 8.1) on an IgG4 protein with an isoelectric point of 6.9 was assessed using a single resin. Throughout the resin screening, all protein quality analyses were performed to identify the resin with the most compatible ligand. The study on pH effects revealed that when the pH exceeded 6.9, various protein fragments were removed, directly affecting the protein charge variant. When the protein pH was at or below the isoelectric point, the anion exchange chromatography flow-through method achieved a maximum protein recovery of 92-98%.

Introduction

The biopharmaceutical industry has progressed remarkably since the first FDA approval of a recombinant protein, recombinant insulin, in the early 1980s. Currently, more than half of approved recombinant proteins are derived from mammalian cell lines such as Chinese hamster ovary (CHO), baby hamster kidney (BHK), mouse myeloma (NS0 and SP2/0), and human cell lines (HEK293) (Kunert & Reinhart, 2016). These systems are favoured due to their inherent ability to perform post-translational modifications similar to those in humans. CHO cells are widely utilized because they support the expression of complex glycoproteins with desirable quality attributes, are resistant to human viral pathogens, and efficiently secrete proteins into the culture medium, facilitating downstream processing (Li et al., 2021).

Monoclonal antibodies (mAbs) represent the largest class of biotherapeutic proteins and play a pivotal role in the advancement of the biopharmaceutical field. Unlike polyclonal antibodies, mAbs recognize a single epitope and belong to a single immunoglobulin class, making them highly specific tools for disease diagnosis and therapy. Extensive structural and functional studies have established antibodies as central mediators of humoral immunity. The critical role of humoral immunity in host defence underscores the necessity of deeper investigation into antibody subclasses.

Humans produce five major immunoglobulin isotypes: IgM, IgD, IgG, IgA, and IgE. Among these, IgG is the predominant isotype in serum and extracellular fluids. It is a tetrameric molecule composed of two

identical γ heavy chains and two identical light chains, and it mediates long-term immunity by neutralizing pathogens and promoting opsonization via complement activation (Schroeder & Cavacini, 2010). Each IgG contains a conserved N-glycosylation site at Asn297 on the Fc region, contributing to effector function and stability. IgG molecules are further classified into four subclasses—IgG1, IgG2, IgG3, and IgG4—based on their abundance and functional properties, including antigen binding, complement activation, and half-life (Sadeghalvad & Rezaei, 2021).

The downstream processing of mAbs aims to recover the antibody with minimal loss while ensuring high purity. During purification, host cell-derived impurities (e.g., host cell proteins, DNA, endotoxins, and medium additives) and product-related variants (e.g., aggregates and fragments) must be removed (Zhu et al., 2017). Viral clearance is also critical and typically achieved through dedicated filtration steps (Han et al., 2011).

Charge heterogeneity is a well-documented phenomenon in mAb production, arising from post-translational modifications such as deamidation, glycation, or C-terminal lysine clipping. These result in acidic or basic variants that differ from the main species and may impact antibody structure, stability, or function, potentially leading to immunogenicity (Du et al., 2012). Therefore, analytical monitoring and control of charge variants are essential for product consistency.

One of the most crucial quality attributes of mAbs is monomeric purity, which can be compromised by aggregate formation. Aggregates—formed due to stress factors such as extreme pH, temperature shifts, or shear—are clusters of misfolded antibodies that can trigger adverse immune responses (Zhang et al., 2019). Consequently, effective purification strategies are required to minimize these species (Sánchez-Trasviña et al., 2021).

Antibody purification is typically initiated with a capture step, commonly using Protein A affinity chromatography, which enables high selectivity and yields while removing bulk impurities (Cataldo et al., 2020; Matte, 2020; Ramos-de-la-Peña et al., 2019). This is followed by polishing steps employing ion exchange, hydrophobic interaction, or mixed-mode chromatography to refine product purity (Müller-Späth et al., 2010; Nadar et al., 2022). Virus inactivation—generally performed post-capture—along with virus filtration and buffer exchange complete the process (Gomis-Fons et al., 2020).

Among polishing techniques, anion exchange (AEX) chromatography is commonly employed due to its ability to resolve charge variants and remove process-related impurities. AEX utilizes positively charged resins that interact with negatively charged proteins. Since most antibodies have high isoelectric points (pI), AEX is often operated in flow-through mode by setting the pH below the pI to prevent antibody binding (Chen et al., 2022). However, at higher pH values, binding to AEX resin increases, which enables the separation of mAbs based on pKa differences. This approach can be used to distinguish antibodies with subtle charge heterogeneities, although high pH may induce deamidation or proteolysis (Jackobek et al., 2020). In this study, we aim to optimize AEX chromatography conditions as a polishing step for an IgG4 antibody produced in CHO cell culture.

Materials and Methods

All experiments and studies were carried out with the materials and equipment available in the Abdi İbrahim pharmaceutical company. After the production of target mAb produced by CHO cell cultured in a bioreactor in the Upstream Laboratory, the purification of mAb via Protein A chromatography, optimization and development of the polishing step AEX chromatography were performed in the Downstream Laboratory. Finally, the protein samples, which were obtained from optimization process, were analysed in the Analytical Laboratory.

Monoclonal antibody production

The mAbs used for the experiment were humanized IgG4 proteins obtained after 14 days of production in recombinant CHO cells in a 10L BioFlo 320 (Eppendorf, Hamburg, Germany) bioreactor. Clarification of IgG4 proteins in the harvesting process after bioreactor production was performed with a 0.08 m^2 Sartoclear depth filter (Sartorius, Göttingen, Germany). With this process, large cell fragments were removed from the mAbs based on exclusion to ease the downstream chromatographic purification. The mAb protein that was used in this research has 150 kDa size and 6.9 pI.

Protein A chromatography purification

The clarified cell culture fluid was purified with bind-eluate method by the Protein A chromatography, which is the first of the downstream chromatographic purification steps. MabSelect PrismA (Cytiva, Marlborough, USA) resin which is driven from Alkaline-stabilized protein A (*Escherichia coli*) and ÄKTA avant 150 (Cytiva, Marlborough, USA) device were used in Protein A chromatography. The MabSelect PrismA resin was packed into the HiScale 50/40 column (Cytiva, Marlborough, USA) containing 294 mL of resin at 15 cm column height. The Protein A purification method includes column equilibration, three steps of column wash, elution, and column sanitization. The mobile phases with their respective pH and volume are given in Table 1.

Virus inactivation step

The pH of the eluate obtained from Protein A purification was first reduced to pH 3.5 with 2 M acetic acid. After 1h incubation at room temperature (20°C)

with 100 rpm, the pH was adjusted to 8.1 with 2 M Tris base (Sigma-Aldrich, Burlington, USA).

Table 1. Table showing the properties and the volumes of mobile phases used in Protein A chromatography

Protein A Chromatography	Mobile Phase	pH	Volume (CV*)
Equilibration	50 mM Tris-HCl, 150 mM NaCl	7.4	5 CV
Wash 1	50 mM Tris-HCl, 150 mM NaCl	7.4	5 CV
Wash 2	50 mM Tris-HCl, 1 M NaCl	7.4	5 CV
Wash 3	50 mM Sodium Acetate	5.5	5 CV
Elution	50 mM Sodium Acetate	3.3	6 CV
Sanitization	0.1 M NaOH	-	5 CV

*CV is column volume of HiScale 50/40 column, which was 294 mL

Resin screening in AEX chromatography

As a first step of AEX polisher step, resin screening was performed. Five different resins were used for resin screening. Table 2 shows five different resins that were used for resin screening and their respective strong anion exchanger ligand and pore size information. Each resin has prepacked in 1 mL column supplied by the manufacturer. Each resin containing columns were attached to the ÄKTA avant 150 device at five different column attachment positions. After the virus inactivation process, some of sample portioned to resin screening experiment and it was adjusted to 3 mS/m by adding water for injection (WFI).

Table 2. Table showing the brand names, ligand types, and particle sizes of five different resins used in resin screening in the AEX polishing step of protein A chromatography

Resin Name (Brand)	Ligand	Particle Size
Capto Q (Cytiva)	Quaternary amine	~90 µm
Poros HQ (Thermo Scientific)	Quaternized Polyethyleneimine	~50 µm
Nuvia Q (Bio-Rad)	Trimetilamin	~85µm
Eshmuno Q (Merck)	Polyvinyl Ether	~85µm
Pros XQ (Thermo Scientific)	Proprietary quaternary amine	~50 µm

The AEX chromatography method includes column equilibration, column wash, strip, and column sanitization steps. The mobile phases with their respective pH and volume are given in Table 3. The dynamic binding capacity of the working column was over 150 g/L, 15 mL of protein sample with 10.35 g/L concentration was loaded to the columns. As the residence time (RT) of the protein in the column was desired as 3 RT, the flow rate was determined as 0.33 mL/min.

Table 3. Table showing the types, pH values and volumes of mobile phases used during the steps in AEX chromatography

AEX Chromatography Steps	Mobile Phase	pH	Volume (CV*)
Equilibration	50 mM Tris	8.1	5 CV
Column Wash	50 mM Tris	8.1	5 CV
Strip	50 mM Tris, 2M NaCl	7.7	5 CV
Sanitization	0.5 M NaOH	-	5 CV

*CV is volume of prepacked column, which was 1 mL

pH optimization of protein in AEX chromatography

After the AEX resin screening process, three independent protein samples were taken from the same protein A chromatography and virus inactivation processes. The pH of the two samples was adjusted to pH 7 and pH 6 with 2 M acetic acid (Table 4 and 5, respectively) while other sample was kept constant at pH 8.1 (Table 6). For protein samples at pH 6, 7, and 8, 50 mM Tris buffers with corresponding pH values were used as equilibration and column washing mobile phases, identical to those used in resin screening.

Table 4. Table showing the types, pH values and volumes of mobile phases used during the steps in AEX chromatography experiment numbered 1

AEX Chromatography Steps	Mobile Phase	pH	Volume (CV*)
Equilibration	50 mM Tris	6	5 CV
Column Wash	50 mM Tris	6	5 CV
Strip	50 mM Tris, 2M NaCl	7.7	5 CV
Sanitization	0.5 M NaOH	-	5 CV

* CV is volume of prepacked column, which was 1 mL.

Table 5. Table showing the types, pH values and volumes of mobile phases used during the steps in AEX chromatography experiment numbered 2

AEX Chromatography Steps	Mobile Phase	pH	Volume (CV*)
Equilibration	50 mM Tris	7	5 CV
Column Wash	50 mM Tris	7	5 CV
Strip	50 mM Tris, 2M NaCl	7.7	5 CV
Sanitization	0.5 M NaOH	-	5 CV

* CV is volume of prepacked column, which was 1 mL.

Table 6. Table showing the types, pH values and volumes of mobile phases used during the steps in AEX chromatography experiment numbered 3

AEX Chromatography Steps	Mobile Phase	pH	Volume (CV*)
Equilibration	50 mM Tris	8.1	5 CV
Column Wash	50 mM Tris	8.1	5 CV
Strip	50 mM Tris, 2M NaCl	7.7	5 CV
Sanitization	0.5 M NaOH	-	5 CV

* CV is volume of prepacked column, which was 1 mL.

The study was carried out with 1 mL prepacked column from Nuvia Q column (Bio-Rad, Hercules, USA). The dynamic binding capacity of the working column was over 150 g/L. The column loading volume of samples were 17.6 mL for protein sample at pH 6, 17.16 mL for protein sample at pH 7, and 16.93 mL for protein sample at pH 8.1 with the concentration of 8.18 g/L, 8.32 g/L, and 8.28 g/L, respectively. Since the residence time (RT) of the protein in the column was desired to be 3 RT, the flow rate was worked as 0.33 mL/min.

Conductivity optimization of protein in AEX chromatography

With the completion of the pH study, the conductivity experiment, which is the final optimization study, was started. The sample obtained after Protein A and virus inactivation and whose conductivity were not adjusted with WFI was divided into four equal parts and used it to conductivity optimization experiment. Before the study, the samples were adjusted to 3.6, 5, 6, 9.6 mS/cm conductivity values by adding WFI and were

made suitable for the study. The buffer sets used in the experiment were used as shown in Table 7.

Table 7. Table showing the types, pH values and volumes of mobile phases used during the steps in AEX chromatography in conductivity experiment

AEX Chromatography Steps	Mobile Phase	pH	Volume (CV*)
Equilibration	50 mM Tris	8.1	5 CV
Column Wash	50 mM Tris	8.1	5 CV
Strip	50 mM Tris, 2M NaCl	7.7	5 CV
Sanitization	0.5 M NaOH	-	5 CV

* CV is volume of prepacked column, which was 1 mL

The study was carried out with 1 mL prepacked column from Nuvia Q column. The dynamic binding capacity of the working column was over 150 g/L. The column loading volume of samples were 60 mL for protein sample at conductivity 3.6 mS/cm, 61 mL for protein sample at conductivity 5 mS/cm, 62 mL for protein sample at conductivity 6 mS/cm and 73 mL for protein sample at conductivity 9.6 mS/cm with the concentration of 2.51 g/L, 2.46 g/L, 2.43 g/L, and 2.06 g/L, respectively. The mobile phases for the conductivity experiment are given in Table 7. Since the RT of the protein in the column was desired to be 3 RT, the flow rate was worked as 0.33 mL/min.

Concentration and recovery analysis via protein A HPLC

Concentration analysis was performed in the analytical laboratory by connecting the MAbPac Protein A column (Thermo Scientific, Waltham, USA) to the Prominence i-Plus (Shimadzu, Kyoto, Japan) High Performance Liquid Chromatography (HPLC) device. At the first stage, a calibration curve was drawn in the range of 5-0.1 g/L with the original mAb protein at different concentrations by serial dilution. Then, since the concentration of the product after AEX chromatography would be above this value range, it was diluted 10 times before analysis. Finally, the column was equilibrated with 7.5 pH 150 mM NaCl-50 mM Sodium phosphate buffer before processing and the product was injected into the column for analysis and eluted with 2.5 pH 150 mM NaCl-50 mM Sodium phosphate mobile phase.

Monomeric purity analysis via size exclusion chromatography HPLC

Monomeric purity analysis was performed by Size Exclusion Chromatography (SEC) in the Analytical Laboratory by connecting the TSK gel SW column (Tosoh, Tokyo, Japan) to the Prominence i-Plus HPLC device (Shimadzu, Kyoto, Japan). The concentrations of the original product and post-AEX mAb protein were diluted to 2 g/L before injecting into the column, then the column was equilibrated with 6.8 pH 0.2 M sodium phosphate buffer. The original product was first injected into the column and accepted as reference. Finally, flow-through AEX proteins were injected into the column and their separation time from the column was analysed.

Charge variant analysis via ion exchange chromatography HPLC

Charge variant analysis was performed by Ion Exchange Chromatography (IEC) in the Analytical Laboratory by connecting the ProPac WCX-10 HPLC column (Thermo Scientific, Waltham, USA) to the Prominence i-Plus HPLC device (Shimadzu, Kyoto, Japan). Prior to the study, the concentration of the original product and the protein after AEX chromatography was adjusted to 5 g/L. The column was equilibrated with 20 mM mass mobile phase before the products were injected. First, the column was calibrated with three injections of the original product, and then all samples were injected into the column. The proteins bound to the column were eluted in a linear gradient with 20 mM mass-1M NaCl mobile phase.

Residual DNA analysis with qPCR

Residual DNA analysis was performed in the Analytical Laboratory with resDNASEQ Quantitative CHO DNA Kits in a QuantStudio 5 (Thermo Scientific, Waltham, USA) PCR device. First, different dilutions were prepared in the concentration range of 300-0.003 pg/uL to create a reference curve. Flow-through AEX protein was diluted 10-fold to be fit into prepared reference range. As the first step, a lysis plate was created for DNA isolation, for this, Proteinase K buffer was added to the proteins, followed by the lysis mixture composition. This process was applied to both reference products and sample proteins. Finally, the prepared mixtures were placed in a 96-well PCR plate and analysed. The process was carried out in the PCR device for 40 cycles and denaturation at 95 °C for 15 s and, annealing at 60 °C for 1 min in each cycle.

Results and Discussion

Optimization of AEX chromatography polishing step

When the AEX chromatogram images of five different resin screening trials were evaluated, similar chromatogram peaks were observed. The only noticeable difference was observed in the study with Poros HQ resin, where an extra peak appeared during the column wash step. This additional peak indicates that the resin ligand was able to retain weakly negatively charged species that were not fully removed during the wash step. Since a mobile phase with a different pH or conductivity value was not employed during the column wash, these weakly negative impurities were eventually eluted passively without requiring external force. This can be clearly seen in Figure 1. Apart from this, as expected in the chromatogram images, positively charged mAb proteins were collected without binding to the column, as seen in the sample application part and peak in the strip step where the negatively charged impurities attached to the resin were removed from the column with high salt concentration. Studies have demonstrated that AEX flow-through chromatography is particularly effective in removing impurities like DNA

and HCPs. For example, the use of hybrid AEX technologies has shown a significant reduction in DNA and HCP levels, which supports the utility of AEX methods in improving product quality and protecting subsequent purification steps (Castro-Forero et al., 2015; Koehler et al., 2019).

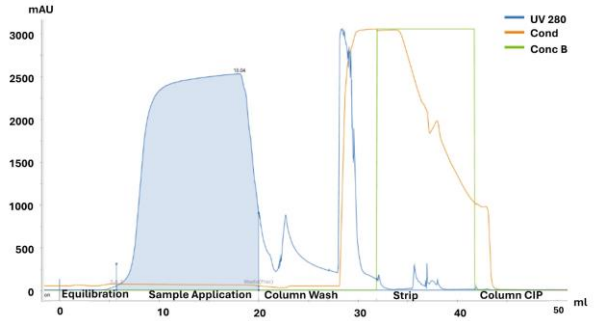


Figure 1. Flow-through AEX profile of Poros HQ resin. The chromatogram illustrates each step of the purification process, including equilibration, sample application, column wash, strip, and column cleaning-in-place (CIP). UV absorbance at 280 nm (blue line) indicates the presence of proteins in the flow-through and eluted fractions. Conductivity (orange line) and buffer B concentration (green line) were monitored to reflect buffer conditions and gradient transitions throughout the run.

Analysis of the conductivity and buffer B concentration profiles further supports the interpretation of impurity behavior in the Poros HQ chromatogram. During the column wash step, the conductivity (orange trace) and buffer B concentration (green trace) remained constant and low, indicating the absence of any applied salt gradient or elution force. Despite this, an additional UV peak was observed, suggesting the passive release of loosely bound, weakly acidic impurities. This observation confirms that these species were not strongly retained and eluted without the need for ionic strength modulation. Upon transition to the strip phase, a sharp increase in both conductivity and buffer B concentration was introduced, correlating with the elution of more tightly bound impurities. The observed strip peak corresponds to these negatively charged contaminants, which were effectively removed from the resin surface under high-salt conditions. This behavior highlights the importance of conductivity and buffer modulation in manipulating electrostatic interactions during AEX chromatography and further validates the selectivity of the Poros HQ resin for process optimization.

In the second study, the effect of the pH value of the protein on the surface charge and the quality differences that would occur in the mAb protein were examined. The pI of the target mAb protein is 6.9, but as seen in Figure 2, the product at 8.1 pH value was collected in the sample application step without binding to the resin, which shows that the product is still in a positive charge. In some cases, even if the total charge becomes negative, the surface charges can remain positive, and this might have happened in the sample with pH 8.1. In the chromatography images of the protein samples at three different pH, the protein sample at pH 6 showed no peaks in the column washing and strip step. This indicates the amount of impurity is very low and the entire mAb protein has a positive charge. The protein sample at pH 7 had small peaks in strip step whereas the protein sample at pH 8.1 showed a large strip peak. The reason for these peaks may be due to impurity or weak mAb proteins with different surface charges at different pH values. In other words, with AEX chromatography, it is possible to avoid impurities and increase the quality of the product (Sánchez-Trasviña, et al., 2021).

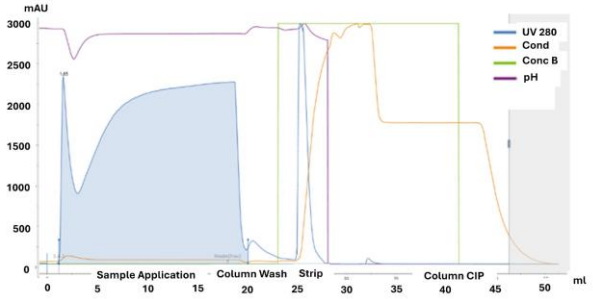


Figure 2. Flow-through AEX profile of Poros HQ resin at pH 8.1. The chromatogram demonstrates the separation behavior under pH 8.1 conditions, including the sample application, column wash, strip, and column cleaning-in-place (CIP) steps. Protein absorbance was monitored at 280 nm (blue line). Conductivity (orange line), buffer B concentration (green line), and pH (purple line) were simultaneously recorded to track buffer composition and environmental changes during the purification process.

Furthermore, analysis of the conductivity and buffer B concentration profiles supports the interpretation of binding behavior under these conditions. During the sample application step at pH 8.1, the conductivity remains low and stable, indicating a low ionic strength environment that favours binding of negatively charged species to the positively charged resin. However, since the mAb protein is still positively charged at this pH, it does not interact with the resin and flows through. In the strip phase, a sharp increase in conductivity and buffer B concentration is observed, corresponding with the elution of retained impurities or weakly interacting species. This correlation confirms that the elution was driven by an increase in salt concentration, which disrupts electrostatic interactions, and further implies that the strip peaks seen at higher pH levels are likely composed of species with varying surface charge properties. These observations highlight the importance of controlling conductivity and salt gradients in AEX chromatography to fine-tune separation performance and maximize product purity.

In the last study, the effect of different conductivity values on AEX purification was examined. When looking at the chromatograms in Figure 1 and 2 (the orange line with Cond. abbreviation), no difference is observed during sample loading in flow through mode. The only difference in the chromatograms is seen in the extra impurity peaks that come from the washing step applied to collect the product remaining in the

column in flow through mode after sample application. This difference is not seen at the low conductivity values of 3.6, 5, and 6 mS/cm, but only at the 9.6 mS/cm conductivity value. Considering the studies on this subject, it is known that low conductivity value is more effective in removing host cell DNA and host cell protein in AEX purification (Jeon et al, 2022; Kurák & Polakovič, 2022). It is very likely that the difference in the impurity peak observed on the chromatogram images is due to the host cell DNA or host cell protein binding to the column during sample application, separating from the target protein, and then moving away from the column in the washing step.

Product concentration analysis and recovery measurement

The recovery rates of the target protein after processing were compared as the first step in the evaluation of the resin screening study with five different resins. Table 8 shows the protein amounts of the loaded protein samples before and after the AEX chromatography, and the percentage of recovery calculated by dividing the amount of protein leaving the column by the protein sample entering and multiplying by 100. As the recovery rates of different resins are compared, and the best recovery was found in the Poros XQ column (Thermo Scientific, Waltham, USA). It would be wrong to choose the best column according to the product recovery alone without evaluating the protein quality analysis. Therefore, protein quality was evaluated, and all data should be taken into consideration for a decision.

Since the mode of the AEX chromatography is flow-through, it was expected to show higher binding recovery compared to bind-elute methodology (Pergande & Cologna, 2017). The increase in pH above pI value of protein decreases the recovery of chromatographic method while increases the removal of weak variants of the protein sample, such as proteins with different post-translational modifications (Becerra & Buyel, 2022; Zhu et al., 2017). As the pH condition of resin screening experiment was higher than the pI value of protein, the recovery was around 70%. The reduction of pH to 6 and 7 have resulted in increase in higher recovery value, as indicated in Table 8. The recovery results of protein purification with different pH conditions in Nuvia Q column prove that some of the target proteins turn negative with values above the pI point and therefore bind to the column. This was expected due to the absence of a strip peak in the chromatogram images. The lowest recovery was found in experimental setup with Poros HQ resin. Unlikely, Poros HQ resin was found to be relatively stable in high pH conditions and provide high recovery rates.

Monomeric purity analysis

AEX chromatography is one of the most preferred monomeric impurity removal (Parra & Gebski, 2011). This is clearly seen in the test results. As shown in Table 9, the monomeric purity of the Protein A elution product increased in all studies except Capto Q resin after AEX purification. The aggregate amount was found below 1% in all resin studies. Purity levels of 99% indicate that the protein sample did not contain any aggregates and that there was no need for an extra purification step that may be required to remove away.

Capto Q resin is widely used in purification via AEX chromatography. In the current research, Capto Q resin was tested to remove monomeric impurity, but it showed a monomeric purity level of around 99.25%. This was the lowest monomeric purity level among the resins tested. However, successful results have been obtained with Capto Q resin. In a patent study conducted in 2014, the depth filtrate was purified using Capto Q resin to remove impurities and obtain a product of the desired purity, and very high purity rates were obtained (Parra & Gebski, 2011; Yüce et al., 2021). It shows that specific proteins do not always produce the same successful results in the same resin.

Table 8. IgG titer results and the calculated recovery values of five different resins, three different pH, and conductivity value

Resin (Brand)	Loaded protein sample volume (mL)	Loaded protein sample conc. (mg/mL)	Loaded protein sample amount (mg)	Flow through protein volume (mL)	Flow through protein conc. (mg/mL)	Flow through protein amount (mg)	Rec. (%)
Capto Q (Cytiva)	18.72	10.35	193.75	18.72	7.86	147.139	76%
Poros HQ (Thermo Scientific)	14.287	10.35	147.87	14.287	7.385	105.509	71%
Nuvia Q (Bio-Rad)	15.58	10.35	161.25	15.58	7.945	123.783	77%
Eshmuno Q (Merck)	15.58	10.35	161.25	15.58	7.545	117.551	73%
Pros XQ (Thermo Scientific)	15.825	10.35	163.79	15.825	8.335	131.901	81%
Nuvia Q (Bio-Rad) pH: 6	17.6	8.18	143.97	17.6	7.995	140.712	98%
Nuvia Q (Bio-Rad) pH: 7	17.16	8.32	142.77	17.16	7.6615	131.471	92%
Nuvia Q (Bio-Rad) pH: 8	16.93	8.28	140.18	16.93	6.5187	110.362	79%
Nuvia Q (Bio-Rad) Cond: 3.6 mS/cm	60	2.51	150.6	60	2.29	137.4	91%
Nuvia Q (Bio-Rad) Cond: 5 mS/cm	61	2.46	150.06	61	2.32	141.52	94%
Nuvia Q (Bio-Rad) Cond: 6 mS/cm	62	2.43	150.66	62	2.35	145.7	96%
Nuvia Q (Bio-Rad) Cond: 9.6 mS/cm	73	2.06	150.38	73	1.87	136.51	90%

Table 9. Different brand resins' monomeric purity results of flow-through AEX chromatography studies

Resin (Brand)	Monomeric Purity (%)
Protein A Eluate	99.43
Capto Q (Cytiva)	99.25
Poros HQ (Thermo Scientific)	99.78
Nuvia Q (Bio-Rad)	99.55
Eshmuno Q (Merck)	99.46
Pros XQ (Thermo Scientific)	99.56
Nuvia Q (Bio-Rad) pH:6	99.69
Nuvia Q (Bio-Rad) pH:7	99.69
Nuvia Q (Bio-Rad) pH:8	99.7
Nuvia Q (Bio-Rad) Cond: 3.6 mS/cm	99.64
Nuvia Q (Bio-Rad) Cond: 5 mS/cm	99.14
Nuvia Q (Bio-Rad) Cond: 6 mS/cm	99.35
Nuvia Q (Bio-Rad) Cond: 9.6 mS/cm	98.89

Poros HQ resin has been shown to provide the best removal of monomeric impurities. Previously, Poros HQ resin was found to have high binding capacity against impurities, which improved cleanliness under different process conditions (Matos et al., 2016). Poros HQ resin also gave successful results in achieving high monomeric purity after high recovery percentage.

According to the monomeric impurity results of the protein sample with different pH, the pH value did not show any significant change in monomeric purity. Therefore, it can be concluded that pH change does not trigger aggregate formation. However, serious peak size differences are clearly observed in the strip step in different pH experiments. It is seen in the SEC result that this is not due to monomeric impurity. A different impurity is removed in this step. When the studies were examined, it was known that lower monomeric purity and lower host cell DNA removal rate would be obtained due to the ionic charge that would occur at high conductivity values (Trnovec et al., 2020).

In the study, a lower monomeric purity value was obtained at high conductivity value (9.6 mS/cm) than at low conductivity value (3 mS/cm). This is because the salt in the sample creates an ionic charge and causes the protein to bind to impurities (Trnovec et al., 2020). Since this ionic force is lower at low conductivity value, lower binding and higher monomeric purity are obtained.

Charge variant analysis

One of the most important protein quality parameters in mAb production is to obtain the charge variants of the product at the desired parameters. In particular, the acidic variant of the protein may increase during stability studies, so it is an important factor to decrease the level as close as possible to the lower limit. Considering the different studies performed, different variants of proteins with specific pI values may have different pH values (Matos et al., 2016). In particular, the different pH conditions were expected to bring protein to the desired acidic variant level. The aim was to reduce the weak and acidic variant as much as possible.

As shown in Figure 3, the Poros HQ column in the resin screening study had the lowest acidic value and was in the conformity value range. For the pH conditions of protein sample was below the pI point or at neutral, the protein sample remained completely positive and there was no change in the acidic variant. According to the comprehensive studies examined, it was seen that the variants of the protein began to turn negative above the pI value. By using this change in the study, only acidic variants were removed from the protein before the mAb protein turned completely negative (Matos et al., 2016). According to our results at pH values 6 and 7, a value of 17% was obtained in the acidic variant, while at pH 8.1 above the pI value, a value of 15% of the acidic variant was obtained, resulting in removal of the acidity. For this reason, our findings are parallel to Matos's results.

Surface potentials are known to vary as a function of the ionic strength of the surrounding solution. Increasing the ionic strength screens the surface charge, which reduces the magnitude of the surface potential and the distance over which it affects penetrating ions (Green & Andersen, 1991). When Figure 3 is examined, protein charge variant changes at different conductivity values are seen. Since the protein could not change its surface tension when the conductivity charge of the medium was 3.5 and 6 mS/cm, there was no change in its net charge, and the charge variant results were similar. However, at a conductivity value of 9.6 mS/cm, the ionic strength affected the surface charge of the protein and caused acidic variants to bind to the column.

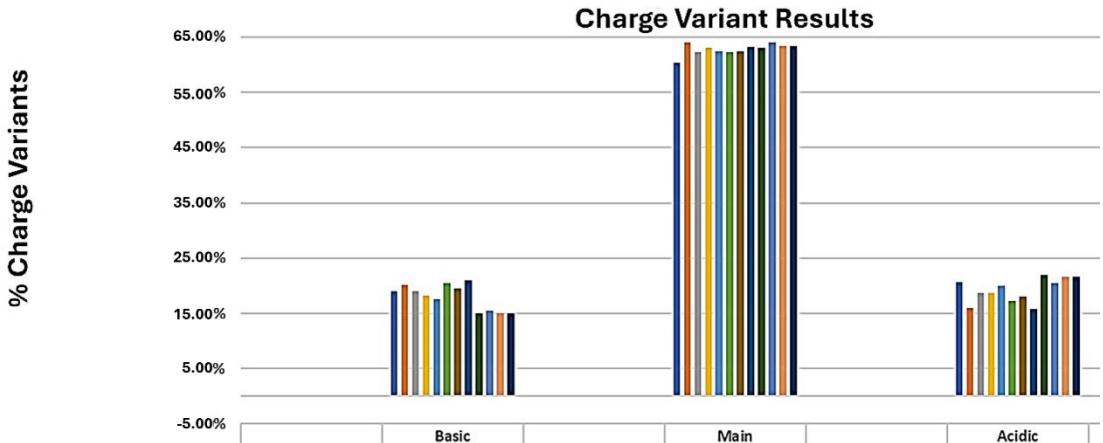
Host cell DNA and protein analysis

One of the most important data obtained after AEX purification was host cell DNA, as the main purpose of the polishing step is known to eliminate the host cell DNA from the protein sample (Stone et al., 2018).

As shown in Table 10, host cell DNA was mostly removed from the target protein in five different resin studies, three different pH, and four different conductivity value studies. The biggest reason for this finding is the negative charge of DNA in all conditions, while AEX resin ligand is always positively charged. According to the pH value of the protein sample, it is possible to evaluate whether the product will bind to the column only. Although there was no significant difference, in previous examinations, Capto Q resin was found to be quite successful in removing host cell DNA (Stone et al., 2018), and as the result of the study is examined, a DNA result below 0 (ng/mg) is seen and this confirms these examinations.

Table 10. Different brand resins' residual DNA results of flow-through AEX chromatography studies

Resin (Brand)	Residual DNA (ng/mg)
Capto Q (Cytiva)	<0.0000
Poros HQ (Thermo)	0.00001
Nuvia Q (Bio-Rad)	0.00544
Eshmuno Q (Merck)	0.00508
Pros XQ (Thermo)	0.00432
Nuvia Q (Bio-Rad) pH:6	0.00453
Nuvia Q (Bio-Rad) pH:7	0.00573
Nuvia Q (Bio-Rad) pH:8	0.00978
Nuvia Q (Bio-Rad) Cond: 3.6 mS/cm	0.009
Nuvia Q (Bio-Rad) Cond: 5 mS/cm	0.009
Nuvia Q (Bio-Rad) Cond: 6 mS/cm	0.009
Nuvia Q (Bio-Rad) Cond: 9.6 mS/cm	0.016



	Basic	Main	Acidic
Capto Q (Cytiva)	19.03%	60.34%	20.64%
Poros HQ (Thermo Scientific)	20.08%	64.01%	15.92%
Nuvia Q (Bio-Rad)	19.05%	62.21%	18.75%
Eshmuno Q (Merck)	18.16%	63.12%	18.73%
Pros XQ (Thermo Scientific)	17.50%	62.48%	20.03%
Nuvia Q (Bio-Rad) ph:6	20.47%	62.29%	17.24%
Nuvia Q (Bio-Rad) ph:7	19. 52%	62.49%	17.99%
Nuvia Q (Bio-Rad) ph:8	20.97%	63.16%	15.88%
Nuvia Q (Bio-Rad) Conductivity: 3.6 mS/cm	15.07%	63.03%	21.90%
Nuvia Q (Bio-Rad) Conductivity: 5 mS/cm	15.47%	64.02%	20.50%
Nuvia Q (Bio-Rad) Conductivity: 6 mS/cm	15.07%	63.38%	21.55%
Nuvia Q (Bio-Rad) Conductivity: 9.6 mS/cm	14.98%	63.42%	21.59%

Figure 3. Charge variant distribution of the target molecule in flow-through anion exchange chromatography (AEX) using various resins and buffer conditions. The bar chart presents the percentage distribution of basic, main, and acidic charge variants for the purified molecule across multiple AEX resins and conditions. Resins include Capto Q (Cytiva), Poros HQ, and Pros XQ (Thermo Scientific, Waltham, USA), Eshmuno Q (Merck, Rahway, USA), and Nuvia Q (Bio-Rad, Hercules, USA). Additionally, the impact of pH (6, 7, 8) and conductivity (3.6–9.6 mS/cm) conditions on charge variant profiles was assessed using Nuvia Q.

When different studies with different pH values were examined, it was found that higher pH value was more effective in removing host cell DNA. However, the results of the study show that lower pH values for the protein used were more effective in removing host cell DNA. The reason for this difference is that at high pH values, the protein may also gain a surface charge due to the pI point difference, and the host cell DNA and protein may be bound to each other, which seems to be a common result (Stone et al., 2018).

When Table 10 is examined, it is seen that there is a more obvious separation of host cell DNA at different conductivity values. Studies on this subject have shown that better host cell DNA removal is achieved with low electrostatic attraction. The study yielded results that support these studies (Stone et al., 2018). A better host cell DNA separation was obtained at conductivity values of 3, 5, and 6 mS/cm compared to the conductivity value of 9.6 mS/cm. The reason for this is that the host cell DNA can bind to the protein instead of the column due to the electrostatic attraction that will occur at high conductivity values. This situation can be prevented with low conductivity.

Polishing step is an important method for removing impurities from the cell line such as host cell DNA, monomeric impurities, and cell culture media additives. Considering the removal of all these impurities, cost and time saving, AEX chromatography is one of the best mAb protein polishing methods. In this report, we're investigated the effect on the protein recovery, monomeric purity, host cell DNA removal, and charge variant of the mAb polishing purification step by working with different pH and conductivity values and resin screening using resins with different ligands.

Even though each AEX resin has a positive charge, it is not possible to get the same result in mAb proteins as each resin is positively charged with different ligands. Therefore, it was aimed to find the best results using resins with different ligands. Most analysis results were observed to be better for Poros HQ resin in terms of certain performance metrics. However, when factors such as cost-efficiency, ease of use, and recovery rates were considered, Nuvia Q resin was determined to be more suitable for the process. For these reasons, Nuvia Q resin was studied in the majority of this study. Nuvia Q resin demonstrated a favorable balance between high recovery rates and desirable protein quality. Specifically, a recovery rate of 77% and an acidic variant level of 18.75% were achieved, which are critical parameters for downstream processing. Additionally, the cost-effectiveness of Nuvia Q resin makes it a practical choice for large-scale operations without compromising the removal of impurities or protein integrity. This resin also maintained a consistent performance across different conditions, ensuring reliable and reproducible results, which is essential for both process optimization and compliance with regulatory standards.

Conclusion

Different pH values were examined on the mAb protein have pI value of 6.9, and the recovery analyses were found similar under pH conditions equal or below the pI value. However, as the pI point was exceeded, weakly acidic variants of mAb proteins turned into negative charges was concluded. Under pH condition of 8.1, the recovery was found to be decreased while the acidic variant value decreased. Under normal conditions, as the pI value is exceeded, the protein turns negative. However, various studies have shown that protein surface charges can remain positive. When working with the mAb protein, it is important to think broadly and study different conditions. It is possible to bring the charge variant to the desired level with different polishing steps but achieving these results with such a low recovery loss shows the success of the study. For the protein we studied, it was found that it was more advantageous to work with a pH of 6 when high protein recovery value and high host cell DNA removal were targeted in the process, but if the acidic variant removal was targeted in the process, it was found to be more advantageous to work with a pH value of 8.1.

When different conductivity values were examined, it was seen that lower monomeric purity and lower host cell DNA removal data were obtained due to the increase in electrostatic charge at higher conductivity values. At all conductivity values studied below 9.6 mS/cm, impurities were more easily bound to the column and removed from the protein due to the provision of ion balance.

These findings hold significant implications for both production and commercial applications of mAb proteins. The ability to optimize recovery, purity, and impurity removal by fine-tuning pH, conductivity, and resin selection not only enhances the efficiency of the downstream process but also contributes to cost-effectiveness and scalability in industrial settings. Achieving high recovery with minimal acidic variant formation is particularly critical for meeting regulatory requirements and ensuring consistent product quality. Moreover, the insights gained from this study can guide the development of robust, reproducible, and economically viable purification strategies, which are essential for competitive positioning in the biopharmaceutical market.

Author Contributions

FO: Designed, Performed, Analyzed, Writing, Review and Editing.

MEU: Designed, Analyzed, Review and Editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

References

Becerra, A., & Buyel, J. (2022). Optimizing downstream purification of high-quality plasmid DNA with POROS™ Chromatography *Resins. Cell & Gene Therapy Insight*, *8*(1), 87. https://doi.org/10.18609/cgti.2022.029

Castro-Forero, A. A., Jokondo, Z., Voloshin, A., Hester, J. F. (2015). Anion-exchange chromatographic clarification: Bringing simplification, robustness, and savings to MAb purification. *BioProcess Int*., *13*(6), 52-57.

Cataldo, A. L., Burgstaller, D., Hribar, G., Jungbauer, A., & Satzer, P. (2020). Economics and ecology: Modelling of continuous primary recovery and capture scenarios for recombinant antibody production. *J Biotechnol*, *308*, 87-95. https://doi.org/10.1016/j.jbiotec.2019.12.001

Chen, S.-T., Xu, W., Cai, K., Ferreira, G., Wickramasinghe, R., & Qian, X. (2022). Factors affecting robustness of anion exchange chromatography: Selective retention of minute virus of mice using membrane media. *J Chromatogr B Analyt Technol Biomed Life Sci.* https://doi.org/10.1016/j.jchromb.2022.123449

Du, Y., Walsh, A., Ehrick, R., Xu, W., May, K., & Liu, H. (2012). Chromatographic analysis of the acidic and basic species of recombinant monoclonal antibodies. *MAbs*, 4(5), 578-585. https://doi.org/10.4161/mabs.21328

Gomis-Fons, J., Andersson, N., & Nilsson, B. (2020). Optimization study on periodic counter-current chromatography integrated in a monoclonal antibody downstream process. *J Chromatogr A.* https://doi.org/10.1016/j.chroma.2020.461055

Green, W., & Andersen, O. (1991). Surface Charges And Ion Channel Functıon. *Annual Review of Physiology*, 53, pp. 341-359. https://doi.org/10.1146/annurev.ph.53.030191.002013

Han, X., Hewig, A., & Vedantham, G. (2011). Recovery and Purification of Antibody. *Antibody Expression and Production Cell Engineering*, 305-40. https://doi.org/10.1007/978-94-007-1257-7_14

Jackobek, R., Herrick-Wagman, S., Zhu, L., Francis, C., Solbrand, A., Eriksson, C., D'Antona, A. M. (2020). Automated pH and conductivity conditioning using feedback control to support a two-step continuous purification process. *Journal of Chromatography* A, 1630. https://doi.org/10.1016/j.chroma.2020.461537

Jeon, H. J., Choi, B. K., Hwang, S. I., Kim, S. H., Kim, G. J., Park, J. C., Yang, Z. Y., & Hwang, K. Y. (2022). Optimization for Simultaneous Removal of Product/Process-Related Impurities of Peptide Fc-Fusion Protein Using Cation Exchange Chromatography. *Processes*, *10*(11), 2359. https://doi.org/10.3390/pr10112359

Koehler, K.C., Jokondo, Z., Narayan, J., Voloshin, A.M., Castro-Forero, A.A. (2019). Enhancing Protein A performance in mAb processing: A method to reduce and rapidly evaluate host cell DNA levels during primary clarification. *Biotechnol Progress*., 35: e2882. https://doi.org/10.1002/btpr.2882

Kunert, R., & Reinhart, D. (2016). Advances in recombinant antibody manufacturing. *Appl Microbiol Biotechnol*, 3451-61.

https://doi.org/10.1007/s00253-016-7388-9

Kurák, T., & Polakovič, M. (2022). Adsorption Performance of a Multimodal Anion-Exchange Chromatography Membrane: Effect of Liquid Phase Composition and Separation Mode. *Membranes*, *12*(21), 1173. https://doi.org/10.3390/membranes12121173

Li, W., Fan, Z., Lin, Y., & Wang, T.-Y. (2021). Serum-Free Medium for Recombinant Protein Expression in Chinese Hamster Ovary Cells. *Front Bioeng Biotechnol*, *9*(64). https://doi.org/10.3389/fbioe.2021.646363

Matos, T., Mohamed, E., & Queiroz, J. (2016). Capto™ Resins for Chromatography of DNA: A Minor Difference in Ligand Composition Greatly Influences the Separation of Guanidyl-Containing Fragments. *Chromatographia*, *79*(19-20), 1277-1282. https://doi.org/10.1007/s10337-016-3148-3

Matte, A. (2020). Recent Advances and Future Directions in Downstream Processing of Therapeutic Antibodies. *Int J Mol Sci*, *23*(15). https://doi.org/10.3390/ijms23158663

Müller-Späth, T., Aumann, L., Ströhlein, G., Kornmann, H., Valax, P., Delegrange, L., Morbidelli, M. (2010). Two step capture and purification of IgG2 using multicolumn countercurrent solvent gradient purification (MCSGP). *Biotechnol Bioeng*, *107*(6), 974-984. https://doi.org/10.1002/bit.22887

Nadar, S., Somasundaram, B., Charry, M., Billakanti, J., Shave, E., Baker, K., & Lua, L. (2022). Design and optimization of membrane chromatography for monoclonal antibody charge variant separation. *Biotechnology Progress*, *38*(6). https://doi.org/10.1002/btpr.3288

Parra, S. C., & Gebski, C. (2011). Benefits of a Revised Approach to Anion Exchange Flow-Through Polish Chromatography. *BioPharm International*, *11*(3).

Pergande, M., & Cologna, S. (2017). Isoelectric Point Separations of Peptides and Proteins. *Proteomes*, *5*(1), 4. https://doi.org/10.3390/proteomes5010004

Ramos-de-la-Peña, A., González-Valdez, J., & Aguilar, O. (2019). Protein A chromatography: Challenges and progress in the purification of monoclonal antibodies. *J Sep Sc,*, *42*(9), 1816-1827. https://doi.org/10.1002/jssc.201800963

Sadeghalvad, M., & Rezaei, N. (2021). Introduction on Monoclonal Antibodies. *IntechOpen*. https://doi.org/10.5772/intechopen.98378

Sánchez-Trasviña, C., Flores-Gatica, M., Enriquez-Ochoa, D., Rito-Palomares, M., Mayolo-Deloisa, K. (2021). Purification of Modified Therapeutic Proteins Available on the Market: An Analysis of Chromatography-Based Strategies. *Front. Bioeng. Biotechnol*., 9, 1-25. https://doi.org/10.3389/fbioe.2021.717326

Schroeder, H., & Cavacini, L. (2010). Structure and function of immunoglobulins. *J Allergy Clin Immunol*, *125*(2), 41-52. https://doi.org/10.1016/j.jaci.2009.09.046

Stone, M., Borman, J., Ferreira, G., & Robbins, D. (2018). Effects of pH, conductivity, host cell protein, and DNA size distribution on DNA clearance in anion exchange chromatography media. *Biotechnol Prog*., *34*, pp. 141–149. https://doi.org/10.1002/btpr.2556

Trnovec, H., Doles, T., Hribar, G., Furlan, N., & Podgornik, A. (2020). Characterization of membrane adsorbers used for impurity removal during the continuous purification of monoclonal antibodies. *J Chromatogr A*., 1609. https://doi.org/10.1016/j.chroma.2019.460518

Yüce, M., Sert, F., Torabfam, M., Parlar, A., Gürel, B., Çakır, N., Çapan, Y. (2021). Fractionated charge variants of biosimilars: A review of separation methods, structural and functional analysis. *Anal Chim Acta*. https://doi.org/10.1016/j.aca.2020.12.064

Zhang, X., Chen, T., & Li, Y. (2019). A parallel demonstration of different resins' antibody aggregate removing capability by a case study. *Protein Expression and Purification*, 153, 59-69. https://doi.org/10.1016/j.pep.2018.08.011

Zhu, M. M., Mollet, M., Hubert, R. S., Kyung, Y. S., & Zhang, G. G. (2017). Industrial Production of Therapeutic Proteins: Cell Lines, Cell Culture, and Purification. *Handbook of Industrial Chemistry and Biotechnology*. (ss. 1639-1669). USA. https://doi.org/10.1007/978-3-319-52287-6_29