Research Article

Cation exchange membrane chromatography: An efficient alternative to multi-column for avoiding the impact of loading density variation on performance

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Abstract

Background: Resin-based cation exchange (CEX) column chromatography is widely used for charge variant separation/reduction. However, in a CEX process where a wash step is introduced to reduce weakly bound acidic charge variants, its performance is greatly affected by the loading density, resulting in poor robustness. We previously demonstrated that multi-column chromatography could resovle this problem, with the key strategy involving converting 3–4 large cycles into a greater number of small cycles. Recently, membrane chromatography has emerged as a promising alternative to column chromatography. CEX membrane, which can be operated under high flow rate, naturally supports the conversion of a large cycle into numerous small cycles. **Objective:** This study aimed to demonstrate that CEX membrane chromatography offers a superior option for addressing the low robustness in the chromatography's wash step. **Methods:** CEX membrane chromatography was applied to reduce acidic charge variants, and its effectiveness was evaluated using capillary isoelectric focusing analysis of the purified samples. **Results:** Under appropriate conditions, CEX membrane chromatography consistently lowered acidic charge variants to the required level. **Conclusion:** Compared to the multi-column approach, CEX membrane chromatography allows for a more straightforward implementation, has higher productivity, and achieves greater cost efficiency. Therefore, it serves as a better alternative to address the low robustness issue.

Keywords: Acidic charge variant, Bind–elute mode, Cation exchange membrane, Robustness, Wash, Weakly bound byproduct

1. Introduction

Recombinant antibodies can undergo a wide array of chemical modifications (*e.g.*, amidation, deamidation, oxidation, sialylation, N-terminal glutamine cyclization, C-terminal lysine cleavage, *etc.*), leading to the formation of charge variants.[1,2] As some charge variants show reduced potency, unintended side-effects, or altered clearance rate,[3-5] the content of these variants needs to be well managed in some instances.[6,7] Ion exchange chromatography is a suitable choice for separating/reducing charge variants. In practice, cation exchange (CEX) chromatography is employed more often than anion exchange chromatography since most monoclonal antibodies have basic properties (isoelectric point [pI] > 7).[8-12] Under typical conditions applied for CEX chromatography, acidic and basic variants, which are weakly and strongly charged, are eluted before and after the main species, respectively. In a previous study, where CEX chromatography was used to reduce the amount of acidic charge variants, we found that loading density significantly impacted the performance of the wash step developed for partially removing these variants, making the process less robust.[13] To address this issue, we proposed and demonstrated that multi-column chromatography provided a suitable solution.[13] The rationale behind this strategy is that, in the multi-column setting, smaller columns are used, which requires an increased number of runs to process the same amount of protein. For all runs but the last one, the column can be loaded at a defined

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density under which the wash condition is developed (hence, charge variant reduction and step yield are well-balanced). Although for the last run, its loading can deviate from the defined amount, leading to compromised quality or yield, it exerts a minimal impact on the overall quality and yield, as its product only accounts for a small portion.

Recently, membrane chromatography has emerged as a promising alternative to resin-based column chromatography.[14-18] The most remarkable advantage of membrane chromatography lies in its high productivity.[19] In comparison to resin beads (pore size: 60–120 nm), membrane adsorbers have a larger pore size (0.6–3 μm) and hence, greater permeability. While in resin bead mass transfer relies on slow diffusion, in membrane transport of biomolecules to their binding sites occurs by fast convection, allowing high binding capacity to be achieved at a fast flow rate/short residence time. The high flow rate enabled by membrane chromatography can significantly increase productivity. Membranes for different chromatographic modes (*e.g*., affinity and ion exchange) are currently available to support capture, intermediate, and polishing purification.[14-19] CEX membranes that are commercially available include Natrix CH from Merck Millipore, Mustang S from Pall, and Sartobind S from Sartorius.[20-22] Among them, Natrix CH offers the highest binding capacity. Specifically, the typical dynamic binding capacities of monoclonal antibodies for Natrix CH, Mustang S, and Sartobind S at 10% breakthrough are 80 mg/mL, 30 mg/mL, and 26 mg/mL, respectively.[20-23] CEX membrane has been shown to be effective in separating antibody charge variants.[24-26]

We previously developed a multi-column approach to address the low robustness of a CEX process that relies on a pre-elution wash for acidic charge variant reduction.[13] The key to this approach is converting 3–4 large cycles into an increased number of small cycles. CEX membrane chromatography, with a cycle time shorter than that of CEX column chromatography, naturally supports splitting a large cycle into dozens of small cycles and can therefore serve the same purpose as the multi-column approach. In the current study, using Natrix CH, we demonstrated that CEX membrane chromatography could effectively and robustly reduce acidic charge variants to the required level with comparable yield. In comparison to the multi-column approach, CEX membrane has several advantages, including a more straightforward implementation (unlike multi-column chromatography, which requires special equipment such as AKTA periodic counter-current chromatography or BioSimulated Moving Bed, CEX membrane chromatography can be conducted using a regular chromatography system), higher productivity, and lower cost. Thus, CEX membrane chromatography offers a better solution to overcome the low robustness issue.

2. Materials and methods

2.1. Materials

L-histidine, L-histidine monohydrochloride, sodium acetate trihydrate, sodium chloride, and sodium hydroxide were purchased from Merck (Germany). Acetic acid was purchased from J.T. Baker (United States of America [USA]). Sodium phosphate monobasic and sodium phosphate dibasic were bought from Sigma (Germany). Natrix CH membrane was from Merck (USA). POROS XS resin was procured from Thermo Fisher Scientific (USA). A protein bridged ethylene hybrid size exclusion chromatography column (4.6 × 150 mm) was purchased from Waters (USA). A fluorocarbon (Fc)-coated capillary isoelectric focusing (cIEF) cartridge was from Protein Simple (USA). Precast SurePAGE 4–12% gradient Bis–Tris gels were purchased from GenScript (China). The protein markers used were home-made. A 20X 2-(N-morpholino)ethanesulfonic acid running buffer and 4X lithium dodecyl sulfate sample buffer were purchased from Thermo Fisher Scientific (USA). The monoclonal antibody and bispecific antibody (bsAb) used in the current study were both expressed in stably transfected CHO-K1 cells cultured in Hypro 100 culture medium supplemented with Cell Boost 7a and 7b (HyClone). The cells were cultured for 14 days before harvest.

2.2. Equipment

An AKTA Pure 150 system, equipped with Unicorn software version 7.8 (Cytiva, Sweden), was used for column and membrane chromatography. pH and conductivity were measured using a SevenExcellence S470 pH/conductivity meter (Mettler-Toledo, USA). Protein concentration was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). An ACQUITY UPLC H-Class PLUS Bio System (Waters, USA) was employed for size-exclusion chromatography-ultra-high performance liquid chromatography (SEC-UPLC). cIEF analysis was performed by using an Imaged cIEF Analyzer from Protein Simple (USA). Cell cultivation was carried out using a bioreactor system from Applikon Biotechnology (Netherlands). The eStain LG protein staining system from GenScript (China) was utilized for staining and destaining of protein gels.

2.3. CEX membrane chromatography

The Natrix CH used has a membrane volume (MV) of 1 mL. To evaluate the impact of loading density on resolution, three runs were conducted under different loading densities (10, 20, and 50 mg/mL). Under each density, the membrane was washed with 50 mM histidine hydrochloride (His–HCl; pH 5.5) buffer for 20 MVs after loading. Subsequently, protein was eluted under linear salt gradient (buffer A: 50

mM His–HCl, pH 5.5; buffer B: 500 mM His–HCl, pH 5.5; 0–60% B over 120 MVs).

To identify the best wash condition, four initial runs were carried out under a loading density of 40 mg/mL with different wash strengths. Specifically, for these four runs, the membrane was first washed with 50 mM His–HCl (pH 5.5) for 20 MVs after loading. For runs 1–4, the membrane was next washed with 15%, 16%, 17%, and 18% buffer B (500 mM His–HCl, pH 5.5), respectively, for 25 MVs. After that, for all four runs, the membrane was subsequently washed with 50 mM His–HCl (pH 5.5) for 20 MVs. Finally, the protein was eluted with 27% buffer B.

On discovering that the yield at 40 mg/mL was much lower than that of column chromatography, three runs were conducted under a reduced loading density of 20 mg/mL with varying wash strengths. For these three runs, the membrane was first washed with 50 mM His–HCl (pH 5.5) for 20 MVs after loading. For runs 1–3, the membrane was washed with 17%, 18%, and 19% buffer B (500 mM His–HCl, pH 5.5), respectively, for 25 MVs. After that, for all three runs, the membrane was subsequently washed with 50 mM His–HCl (pH 5.5) for 20 MVs. Finally, the protein was eluted with 27% buffer B. The final protocol adopted the 18% buffer B wash with a slightly increased volume (*i.e*., 30 MV).

For the bsAb, a run was first conducted under a linear salt gradient elution. After loading, the membrane was washed with 50 mM sodium acetate–acetic acid (NaAc–HAc), pH 5.5 (buffer A) for 20 MVs. Then, the protein was eluted by linearly increasing to 30% buffer B (50 mM NaAc–HAc, 1 M NaCl, pH 5.5) over 120 MVs. Another run was conducted under defined wash and stepwise elution. After loading, the membrane was consecutively washed with 50 mM NaAc–HAc, pH 5.5 (for 20 MVs) and 6% buffer B (for 10 MVs). The protein was lastly eluted with 11% buffer B.

For all runs, the flow rate was set at 10 mL/min with a corresponding residence time of 6 s.

2.4. CEX column chromatography

The CEX column with a 0.5 cm diameter was packed with POROS XS resin to a bed height of 10.8 cm, and the column volume (CV) was approximately 2.1 mL. For the charge variant reduction, the column was loaded at 10 mg/mL. After loading, the column was washed with 50 mM His–HCl (pH 5.5) for six CVs. Next, a linear salt gradient (buffer A: 50 mM His–HCl, pH 5.5; buffer B: 500 mM His–HCl, pH 5.5; 0–60% B over 20 CVs) was applied for elution. For the bsAb purification, the column was loaded at 40 mg/mL. After loading, the column was washed with 50 mM NaAc–HAc, pH 5.5 (buffer A) for five CVs. Protein was eluted under a linear salt gradient by reaching 30% buffer B (50 mM NaAc–HAc, 1 M NaCl, pH 5.5) over 20 CVs. For all runs, the flow rate was set at 0.42 mL/min with a corresponding residence time of 5 min.

2.5. SEC-UPLC

SEC-UPLC was performed on an ACQUITY UPLC H-Class PLUS Bio System armed with an ACQUITY UPLC protein bridged ethylene hybrid size exclusion chromatography column (4.6 × 150 mm). A total of 10 µg sample was injected per run. The mobile phase consisted of 50 mM sodium phosphate and 300 mM sodium chloride at pH 6.8. Isocratic elution was carried out over a period of 8 min at a flow rate of 0.4 mL/min. Protein elution was monitored using ultraviolet absorbance at 280 nm.

2.6. cIEF

A protein simple iCE3 system with an Fc-coated cIEF cartridge was used for this analysis. The master mix contained the following components: 0.5 µL of pI 8.18 marker, 0.5 µL of pI 10.10 marker, 1.0 µL of Pharmalyte 3–10, 3.0 µL of Pharmalyte 8–10.5, 35.0 µL of 1% methylcellulose, 37.5 µL of 8 M urea, 1.0 µL of 200 mM arginine, and 1.5 µL of ultrapure water. The solution injection was composed of 20 µL of diluted sample at 1.0 mg/mL and 80 µL of master mix. Focusing was performed at 1,500 V for a minute, followed by 3,000 V for an additional 8 min.

2.7. Non-reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Non-reducing SDS-PAGE was performed using precast SurePAGE 4–12% gradient Bis–Tris gels from GenScript. Sample loading buffer (4X lithium dodecyl sulfate) and gel running buffer (20X 2-[N-morpholino]ethanesulfonic acid) were from Thermo Fisher Scientific. Samples were heated at 70°C for 5 min. All samples were loaded at an equal protein amount (~0.5 µg/well). Electrophoresis was carried out at 80 V for 120 min. Gels were stained and destained using the eStain LG protein staining system from GenScript.

3. Results and discussion

3.1. The impact of loading density on the resolution of bind–elute mode CEX membrane chromatography

In a previous study, we successfully reduced the content of acidic charge variants in a purification intermediate from approximately 29% to below 24%, as required, using multi-column CEX chromatography; the method showed improved process robustness compared to the single-column approach.[13] The current study aimed to explore the feasibility of using the CEX membrane to achieve the same goal. In general, the resolution of the CEX

column. This is because, in a membrane device, flow paths can vary significantly in length, leading to dispersion effects, such as peak broadening and poor resolution.[27,28] For the case under study, the CEX column was previously loaded at a density of 40 mg/mL, which proved to be the highest possible loading density that ensures sufficient resolution between acidic charge variants and the main species.[13] In the current study, we first conducted three runs using the Natrix CH CEX membrane under different loading densities (10, 20, and 50 mg/mL) to evaluate their impacts on resolution (the membrane was loaded with the same purification intermediate). An overlay of the corresponding chromatograms is shown in Figure 1A. According to the chromatograms, there is no noticeable difference in resolution under these three loading densities. In addition, we found that, for CEX column chromatography, there was no appreciable resolution between the charge variants and the main species even at a relatively low loading density (i.e., 10 mg/mL) (Figure 1B). This observation suggests that the separation between acidic charge variants and the main species is subtle and challenging. For both the CEX column and the membrane, although acidic charge variants are likely being further enriched in the peak front under low loading density than under high loading density, the improved resolution cannot be reflected in the chromatogram.

3.2. Effective reduction of acidic charge variants by bind–elute mode CEX membrane chromatography

For the CEX membrane, no noticeable difference was found in resolution under different loading densities. We first selected the same loading density as that previously used for the CEX column (*i.e.*, 40 mg/mL) for further evaluation. According to the previous data, under this loading density, 130 mM histidine is an appropriate wash condition, effectively balancing acidic charge variant reduction and step yield (*i.e.*, >6% acidic charge variant reduction and >70% step yield). Thus, to simplify the

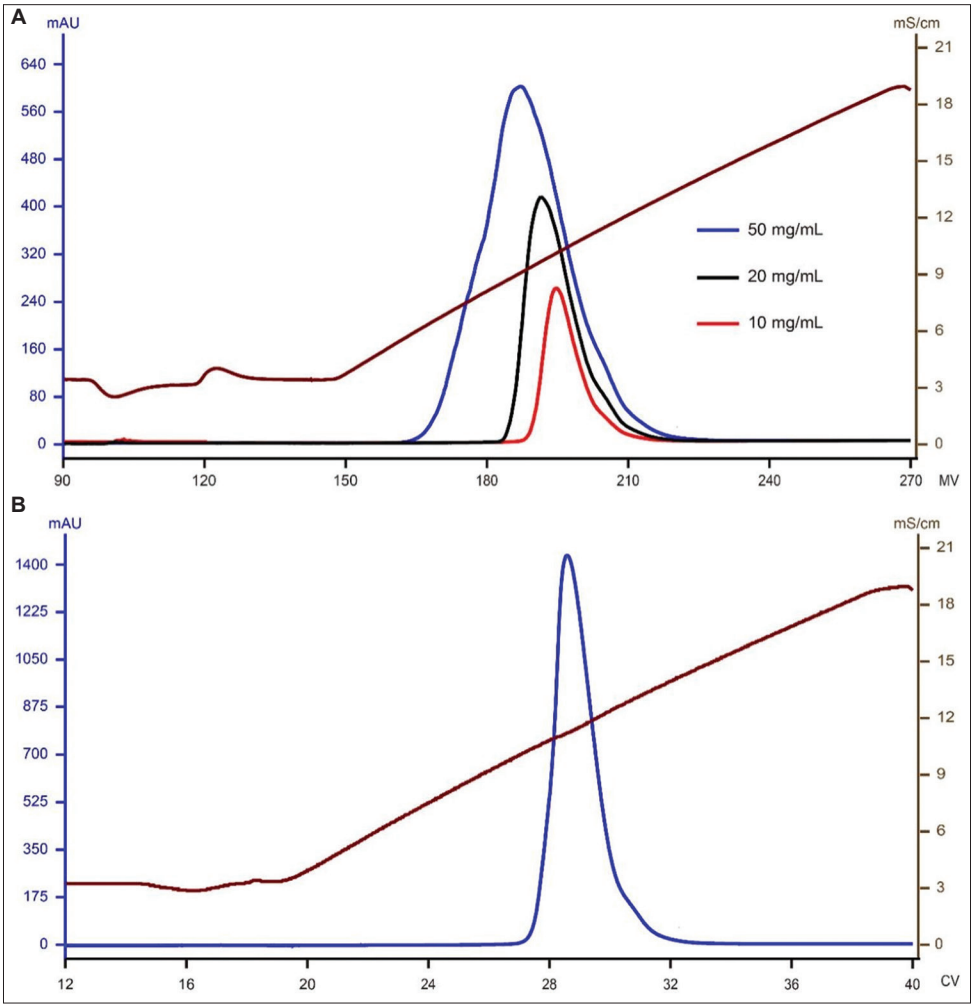

Figure 1. CEX chromatograms. (A) Overlay of CEX (membrane) chromatograms of runs conducted under three different loading densities. The absorbance curves corresponding to different loading densities (*i.e.*, 10, 20, and 50 mg/mL) are indicated. (B) CEX (column) chromatogram of a run conducted under the loading density of 10 mg/mL. For all runs, the load material was a post-Protein A purification intermediate containing approximately 29% of acidic charge variants. The bound antibody was eluted under a linear salt gradient.
Abbreviation: CEX: Cation exchange.

process development for CEX membrane chromatography, we tested four different histidine concentrations around 130 mM for the wash. The yield and quality data of these runs are summarized in Table 1. An overlay of the corresponding chromatograms is shown in Figure 2A. According to the data, eluates from runs 3 and 4 (the corresponding histidine

Table 1. Relevant information of the four cationic exchange membrane chromatographic runs under the loading density of 40 mg/mL

Run no.	Wash 2 (buffer B, %)	Histidine concentration (mM)	Sample	Mass (%)	Acidic/main/basic (%)	Acidic/main/basic (%)
NA	NA	NA	Load	NA	2.0/98.0/ND	29.3/63.2/7.4
1	15	117.5	Wash	11.1	NM	NM
			Elution	82.5	1.1/98.9/ND	26.9/64.1/9.0
2	16	122.0	Wash	25.3	NM	NM
			Elution	72.0	1.4/98.6/ND	25.3/64.6/10.0
3	17	126.5	Wash	38.4	NM	NM
			Elution	60.8	1.5/98.5/ND	23.0/65.7/11.3
4	18	131.0	Wash	48.5	NM	NM
			Elution	47.3	1.8/98.2/ND	21.0/65.7/13.3

Abbreviations: HMWs: High-molecular-weight species; LMWs: Low-molecular-weight species; NA: Not applicable; NM: Not measured; ND: Not detected.

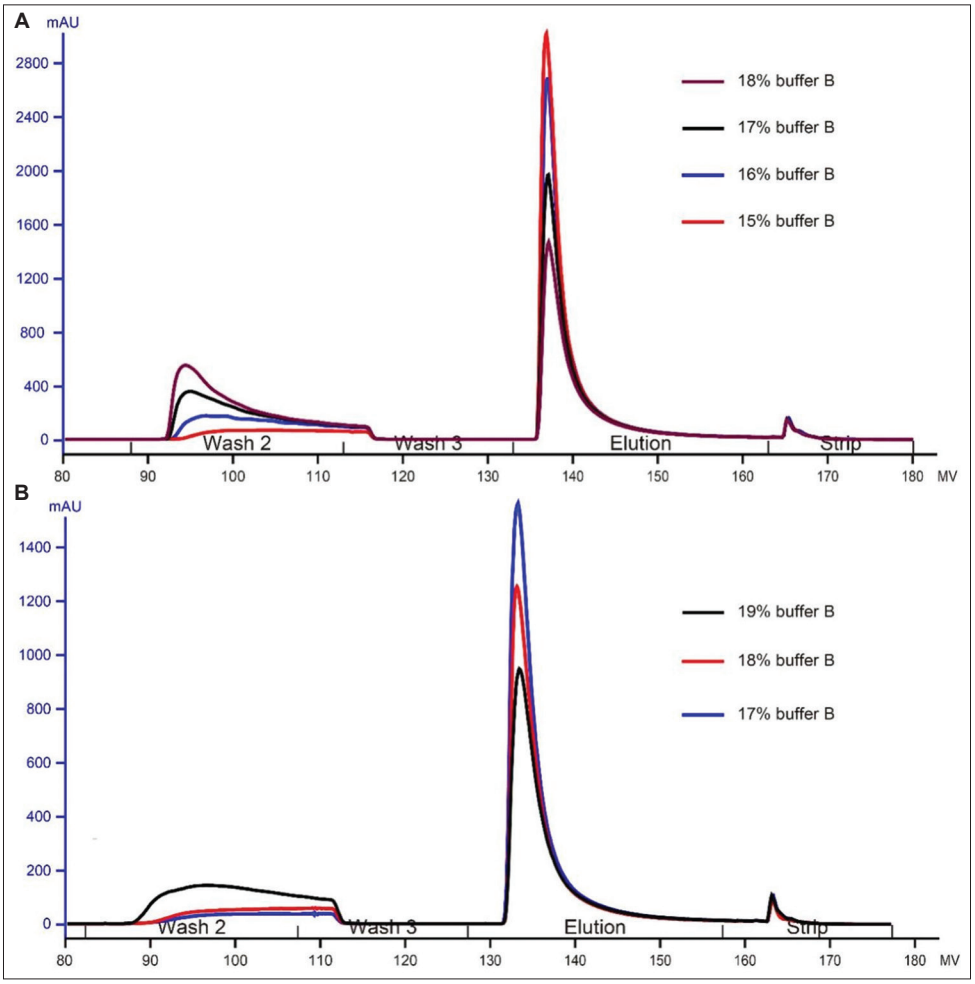

Figure 2. Overlay of CEX (membrane) chromatograms of runs subjected to a wash step with different strengths. (A) At a loading density of 40 mg/mL, the absorbance curves corresponding to different wash buffer compositions (*i.e.*, 15%, 16%, 17%, and 18% of buffer B) are indicated. (B) At a loading density of 20 mg/mL, the absorbance curves corresponding to different wash buffer compositions (*i.e.*, 17%, 18%, and 19% of buffer B) are indicated. For all runs, the load material was a post-Protein A purification intermediate containing approximately 29% of acidic charge variants. The bound antibody was eluted under a stepwise salt gradient.
Abbreviation: CEX: Cation exchange.

concentrations used for wash were 126.5 and 131 mM, respectively) met the requirement for acidic charge variant control, but their yields (60.8% and 47.3%, respectively) were substantially lower than that of the CEX column (>70%). To improve yield, we further tested 124.3 mM histidine for wash, which is the intermediate concentration between those used for runs 2 and 3. Under this condition (chromatogram not shown), the content of the acidic charge variant was reduced to 23.8%, which scarcely satisfied the requirement, and the step yield was 62.1%, only marginally improved.

Despite the effectiveness of the CEX membrane chromatography in reducing acidic charge variants to the required level at the loading density of 40 mg/mL, the step yield is lower than that of its column counterpart. A similar observation on the yield difference between the CEX membrane and column chromatography was made by other researchers.[25] This is likely because, as mentioned in the previous section, the CEX membrane has a lower resolution than the CEX column. Therefore, we performed a similar wash condition screening study at a lower loading density (*i.e.*, 20 mg/mL), hoping that better resolution could be achieved under this condition. The yield and quality data for this part of the study are summarized in Table 2. An overlay of the corresponding chromatograms is shown in Figure 2B. Under the intermediate histidine concentration (*i.e.*, 131.0 mM), the level of acidic charge variants was still slightly higher than required. For washing, both histidine concentration and volume impact the reduction of acidic charge variants, with histidine concentration playing a significant role. Thus, to further reduce acidic charge variants, we increased the wash volume from 25 MVs to 30 MVs while maintaining the same histidine concentration, as further increasing the histidine concentration may lead to a significant drop in yield. This change allowed the acidic charge variants to be reduced to 22.8% with a step yield of 69.6%. Thus, as expected, under reduced loading density, sufficient acidic charge variant reduction was attained with a significantly improved step yield, which was comparable to that of the column chromatography. The yield improvement is likely to have benefited from enhanced resolution under this condition. Finally, eight additional cycles were conducted under the same conditions (*i.e.*, 131.0 mM histidine and 30 MVs for wash), and the overlay of these chromatograms (nine in total) is shown in Figure 3. Analysis of the pool of the nine eluates indicated that similar yield and quality (69.7%

Table 2. Relevant information of the three cation exchange membrane chromatographic runs under the loading density of 20 mg/mL

Run no.	Wash 2 (buffer B, %)	Histidine concentration (mM)	Sample	Mass (%)	Acidic/main/basic (%)	Acidic/main/basic (%)
NA	NA	NA	Load	NA	2.0/98.0/ND	29.3/63.2/7.4
1	17	126.5	Wash	9.7	NM	NM
			Elution	81.7	0.7/99.3/ND	28.1/62.1/9.8
2	18	131.0	Wash	16.5	NM	NM
			Elution	74.2	0.7/99.3/ND	25.6/64.9/9.5
3	19	135.5	Wash	36.1	NM	NM
			Elution	55.2	1.0/99.0/ND	21.6/65.7/12.7

Abbreviations: HMWs: High-molecular-weight species; LMWs: Low-molecular-weight species; NA: Not applicable; NM: Not measured; ND: Not detected.

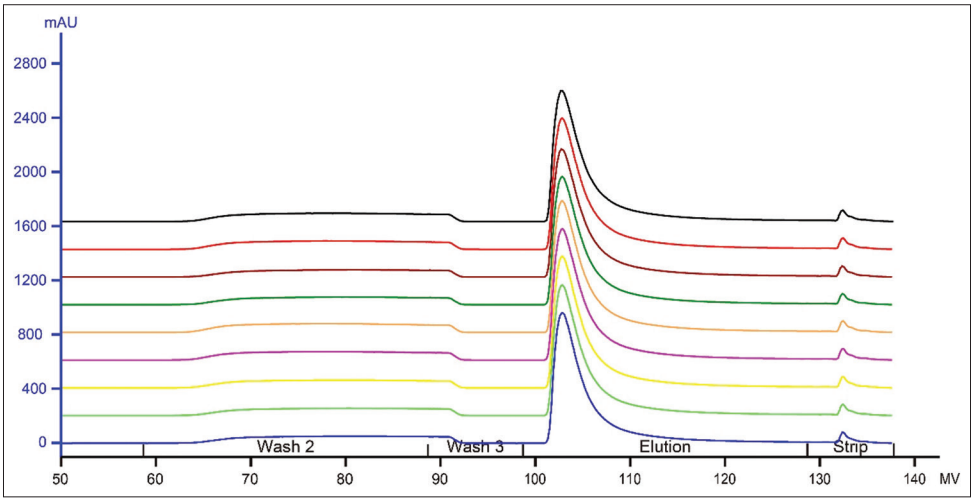

Figure 3. Overlay of cation exchange membrane chromatograms from nine cycles. For all runs, the membrane was loaded at 20 mg/mL with the same post-Protein A purification intermediate, and they were conducted following the same protocol.

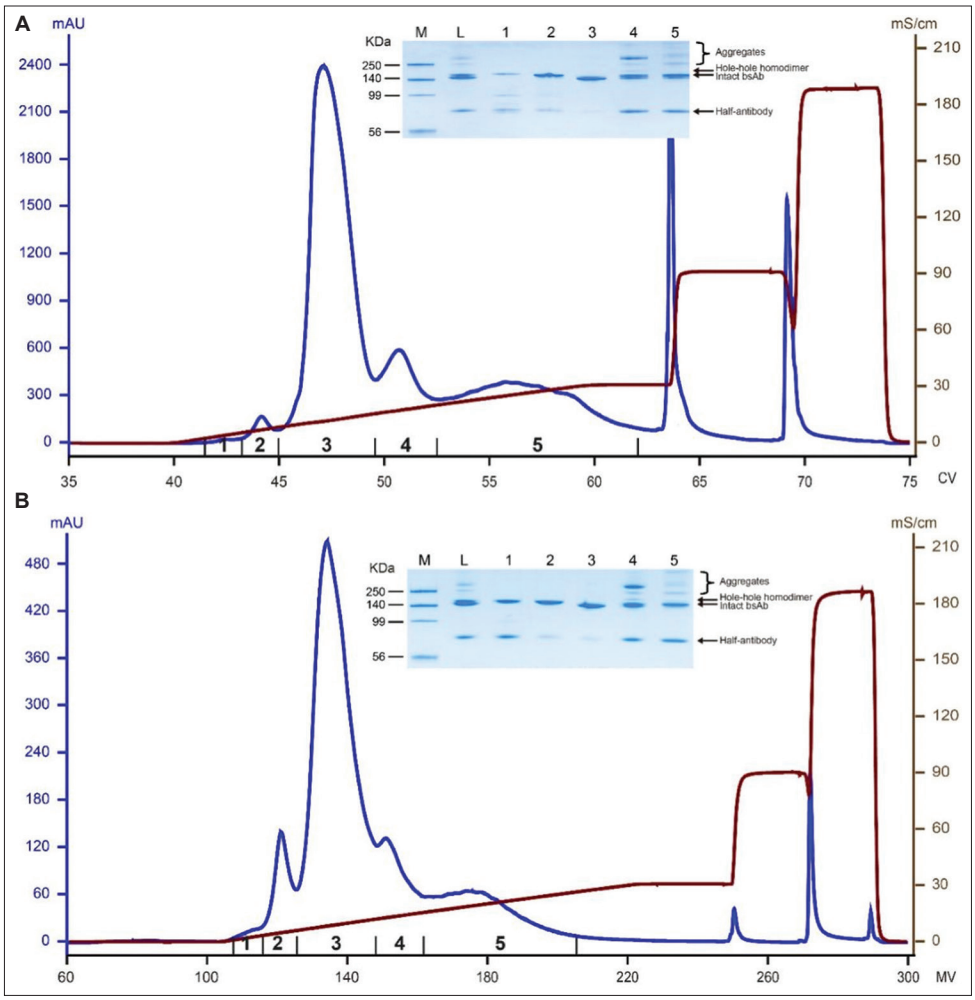

Figure 4. CEX chromatograms of runs conducted to purify an asymmetric bsAb. (A) Chromatogram of a run conducted with a CEX column. (B) Chromatogram of a run conducted with CEX membrane. Inset in A and B SDS-PAGE analysis of relevant fractions. M indicates protein markers; L denotes load; and lanes 1–5 represent the elution fractions 1–5. The load material was a purification intermediate, post-Protein A capture, which contained hole half-antibody, hole-hole homodimer, knob half-antibody, and aggregates, in addition to the target bsAb. For the CEX column and membrane, runs were conducted under the same loading density (*i.e*., 40 mg/mL) and identical linear salt gradient elution (*i.e*., 0–300 mM NaCl).
Abbreviations: bsAb: Bispecific antibody; CEX: Cation exchange; SDS-PAGE: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

and 23.0% acidic content, respectively) were accomplished, suggesting that the performances of these nine runs were highly consistent.

3.3. A theoretical comparison between CEX column (single and multiple) and CEX membrane chromatography in productivity and media usage

To further demonstrate the advantages of CEX membrane chromatography, we compared single-column CEX, multi-column CEX, and single-membrane CEX in terms of productivity and media usage, assuming that 500 L of purification intermediate with a concentration of 16 mg/mL needs to be processed. The comparison data are summarized in Table 3. For the multi-column mode, although its processing time was longer than that of the other two modes, it can be

Table 3. Comparison of single-column CEX, multi-column CEX, and single-membrane CEX in terms of productivity and media usage

Parameters	Single-column	Multi-column	Single-membrane
Volume (L)	33.4[a]	12.6 (4.2×3)[b]	4.0
Load density (g/L)	40	40	20
Residence time (s)	300	300	6
Cycle time (min)	257	55[c]	13.6
Cycle number[d]	6	48	100
Processing time (h)	25.7	44.0	22.7
Productivity (g/L/h)	9.3	14.5	88.2

Note: [a]For the single column mode, the column used has a dimension of 45 cm (D)×21 cm (H). [b]For the multi-column mode, three columns of the same size are used. For each column, its dimension is 20 cm (D)×13.3 cm (H). [c]For the multi-column mode, at a given time, the three columns are at different phases: load, wash, or elution. The longest phase time is used as the cycle time. [d]Assuming that 500 L of purification intermediate with a concentration of 16 mg/mL needs to be processed.
Abbreviation: CEX: Cation exchange.

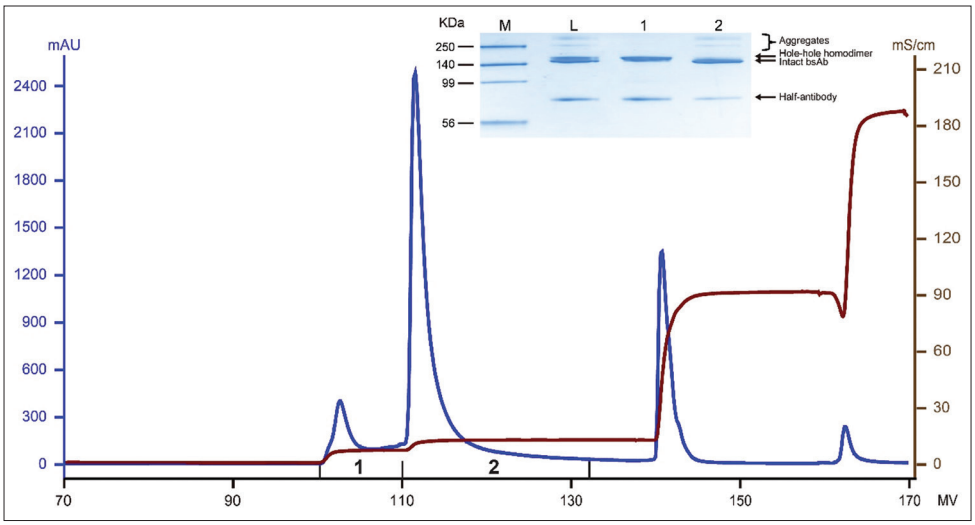

Figure 5. CEX membrane chromatogram of a run conducted under stepwise salt gradient elution. Inset, SDS-PAGE analysis of relevant fractions. M indicates protein markers; L denotes the load; and lanes 1 and 2 represent elution fractions 1 and 2. The load material used was the same bsAb purification intermediate. A pre-elution wash was developed to remove weakly bound hole half-antibody and hole-hole homodimer.
Abbreviations: bsAb: Bispecific antibody; CEX: Cation exchange; SDS-PAGE: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

conducted continuously. Thus, for all three modes, processing can be completed within 2 days. As the data suggested, membrane chromatography required the least volume of media (*i.e*., 4.0 L). In addition, the CEX membrane showed the highest productivity, which is approximately 10- and six-fold of that of the single-column and multi-column modes, respectively.

3.4. Separation of other types of weakly bound byproducts by bind–elute mode CEX membrane chromatography

Charge variants represent one of the most difficult-to-separate byproducts. As shown in Figure 1B, even CEX column chromatography provides no appreciable resolution at a low loading density. Nevertheless, the CEX membrane can effectively reduce acidic charge variants to the required level under appropriate conditions, although its resolution is typically inferior to that of the CEX column. For byproducts that are weakly bound and less challenging to separate than acidic charge variants, the CEX membrane achieves a separation performance comparable to that of a CEX column. As a demonstration, a bsAb purification case is presented, where the weakly bound byproducts—half-antibody and hole–hole homodimer—serve as representative examples. In this case, the target molecule is an asymmetric bsAb, which utilizes knobs-into-holes technology to promote heavy chain heterodimerization. Major byproducts include hole half-antibody, hole-hole homodimer, knob half-antibody, and aggregates. As the hole half-antibody and the hole-hole homodimer have a pI lower than that of the target bsAb, they bind more weakly to the CEX membrane than the product. As shown in Figure 4A, the hole half-antibody and hole-hole homodimer (enriched in elution fractions 1 and 2) were well separated from the target bsAb by the CEX column under a linear salt gradient elution. The hole-hole homodimer migrated slightly slower than the target bsAb (fraction 3) on gel (Figure 4A). The knob half-antibody, which has a higher pI than the target bsAb, was enriched in the late elution fractions (4 and 5) with aggregates (Figure 4A). Under the same loading density and identical elution conditions, the CEX membrane provided equally good separation (Figure 4B). Based on the result of the linear gradient elution, a protocol for stepwise elution was developed for the CEX membrane, in which weakly bound byproducts were removed by a pre-elution wash (Figure 5). Effective removal of weakly bound hole half-antibody and hole-hole homodimer by the wash step was confirmed by the SDS-PAGE results (Figure 5). Thus, in addition to the acidic charge variants, the CEX membrane can effectively remove other types of weakly bound byproducts through an appropriate wash step.

4. Conclusion

Membrane chromatography is not an entirely new technology, but previously its use was mainly limited to the polishing step under flow-through mode (*e.g*., anion exchange) due to the generally low binding capacity of membrane adsorbers.[29] In recent years, the binding capacities of both Protein A and CEX membranes have been greatly improved,[30,31] paving the way for membrane chromatography to be used in these two steps, which are typically conducted under a bind–elute mode. In comparison to traditional column chromatography, the significant advantage of membrane chromatography is its high

productivity, which allows a smaller volume of media to be used and therefore reduces costs. For example, to process a 2,000 L batch of clarified harvest at manufacturing scale, the amount of Protein A media needed is up to 40-fold less if a Protein A membrane is used to replace Protein A resin for product capture.[32] Additional advantages of membranes over resins include low pressure drop, small facility footprint, high scalability, and disposability (hence eliminating the need for packing, unpacking, cleaning, validation, and storage).[16-18] Recently, an integrated full-membrane platform has been developed for antibody purification.[33]

Load-dependence is a common problem associated with the bind–elute mode chromatography, where a pre-elution wash is applied to remove weakly bound byproducts.[13,34] Previously, using CEX chromatography to reduce acidic charge variants, we demonstrated that a multi-column approach could resolve this problem.[13] In the current study, we showed that CEX membrane chromatography offered a solution superior to the multi-column approach for addressing this limitation. In comparison to the previous solution, CEX membrane chromatography not only allows for equally robust acidic charge variant reduction but also offers the following advantages: more straightforward implementation, higher productivity, and greater cost-efficiency. Using a bsAb purification case, we demonstrated that CEX membrane chromatography could also be used to remove other types of weakly bound byproducts through an appropriate wash step. Similarly, Protein A membrane can improve the performance of a Protein A process where a pre-elution wash is applied to remove weakly bound half-antibody byproduct.[35] In general, membrane chromatography can improve the robustness of bind–elute mode chromatography by avoiding the impact of loading density variation on performance (for membrane chromatography, as the cycle time is very short, changes in the amount of protein that needs to be processed can be managed by adjusting the cycle number instead of the loading density). This new advantage, along with several other well-recognized ones, will certainly promote a broader application of membrane chromatography in the biopharmaceutical industry.

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Conflict of interest

The authors are employees of WuXi Biologics (Shanghai) Co., Ltd. The authors declare that this affiliation did not influence the study design, data interpretation, or manuscript preparation. No other conflicts of interest, financial or otherwise, are declared.

Author contributions

Conceptualization: Yifeng Li
Data curation: All authors
Formal analysis: All authors
Investigation: All authors
Writing–original draft: Yifeng Li
Writing–review & editing: All authors

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability statement

The data and supporting information are available either within the article or on request.

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