Brief Report

Enhanced aggregate separation with Sartobind® Rapid A Protein A membrane in the purification of aggregation-prone antibodies

Gaoya Yuan, Meng Qu, and Yifeng Li*

Downstream Process Development (DSPD), WuXi Biologics, Shanghai 200131, China

Abstract

Background: Aggregates are common byproducts associated with the production of recombinant antibodies, and their removal poses considerable challenges to the downstream purification. When a Protein A column is used for product capture, large aggregates do not bind due to the size-exclusion effect, whereas small aggregates (*e.g.*, dimers) co-bind with monomers. Although small aggregates bind marginally stronger than the monomer, the difference is usually too small to effect an effective separation of these two species. Thus, Protein A column chromatography generally lacks the ability to separate monomers from co-binding small aggregates. Recently, Protein A membrane has emerged as a promising alternative to resin-based Protein A columns. **Objective:** This study aimed to evaluate the potential of Sartobind® Rapid A Protein A membrane's monomer-aggregate separation. **Methods:** A Protein A column and a Sartobind® Rapid A membrane were used to separately process five culture harvests containing a high percentage of aggregates, and their performances were compared. Aggregate clearance was monitored by analysing relevant elution fractions using size-exclusion chromatography-high-performance liquid chromatography. **Results:** Sartobind® Rapid A membrane showed stronger aggregate separation capability than the resin-based Protein A column and effectively removed most aggregates in all feed materials. **Conclusion:** Sartobind® Rapid A membrane outperforms resin-based Protein A columns for antibodies and Fc-fusion proteins with aggregate-rich harvests. By removing most of the aggregates at the capture stage, Sartobind® Rapid A membrane significantly alleviates the purification burden on polishing steps.

Keywords: Aggregate, Antibody, Bispecific antibody, Protein A column, Protein A membrane, Sartobind® Rapid A

1. Introduction

Aggregates are common byproducts associated with the production of recombinant antibodies and Fc-fusion proteins.[1-3] Their removal poses considerable challenges to the downstream purification. This issue becomes more serious for complex molecules such as bispecific antibodies (bsAbs), whose production is usually accompanied by high amounts of aggregates.[4-7] For example, for a symmetric bsAb adopting the appended immunoglobulin G format, the aggregate content exceeds 15% even after optimization on molecular design.[8] Protein A resins are the most extensively used affinity media for product capture in antibody and Fc-fusion protein purification.[9-11] We previously demonstrated that large antibody aggregates do not bind to resin-based Protein A column.[12,13] The reason is that they are too large to enter the pores of resin beads and therefore cannot access the Protein A ligands inside. Small aggregates (*e.g.*, dimers), on the other hand, can go through the pores and co-bind with monomers. Although small aggregates bind slightly stronger than the monomer, the difference tends to be too small to enable an effective separation of these two species.[14] Thus, Protein A column chromatography generally lacks the capability of separating co-binding small aggregates from monomers under typical pH gradient elution conditions.[15] Consequently, small aggregate removal mainly relies on post-capture polishing steps.[16-22] However, in cases where small aggregate contents are high, relying entirely on the polishing steps for aggregate removal results in poor robustness throughout the entire downstream process. In such cases, it is highly desirable for

***Corresponding author:**
Yifeng Li (li_yifeng@wuxibiologics.com)

Submitted: 28 August 2025; Revision received: 10 October 2025; Accepted: 13 October 2025; Published: 10 November 2025

How to cite this article: Yuan G, Qu M, Li Y. Enhanced aggregate separation with Sartobind® Rapid A Protein A membrane in the purification of aggregation-prone antibodies. *J Biol Methods.* 2026;13(1):e99010089. DOI: 10.14440/jbm.0279

the Protein A medium to achieve an improved resolution and at least partially remove the co-binding aggregates.

Recently, Protein A membrane has emerged as a promising alternative to resin-based Protein A columns.[23-27] Compared to a resin bead, a membrane adsorber yields a higher productivity, which is a remarkable advantage.[23-26] The fundamental reason lies in the fact that, in resin beads, mass transfer is mediated by slow diffusion, whereas in membrane adsorbers, it is mediated by quick convection. Additional advantages of membrane chromatography include low pressure drop, small facility footprint, good scalability, and disposability. A prior observation on Fibro PrismA Protein A membrane supported the conclusion that size-exclusion effects prevent large antibody aggregates from binding to the Protein A column. Large aggregates recovered from the Protein A column flow-through can be captured using this Protein A membrane, which has larger pore sizes.[28] Thus, when Protein A membrane is used for product capture, both large and small aggregates can co-bind with monomers.

Since aggregates are byproducts that need to be removed, the Protein A column has the advantage of excluding large aggregates from binding. However, as aforementioned, the Protein A column is less effective at removing small aggregates that co-bind with monomers.[14,15] In the current study, we demonstrated that although Sartobind® Rapid A Protein A membrane may allow both large and small aggregates to bind, it provides a better resolution than resin-based columns and can effectively separate monomers from co-binding aggregates. Thus, Sartobind® Rapid A membrane is a better choice than resin-based Protein A columns for antibodies and Fc-fusion proteins with aggregate-rich harvests. In addition, we previously found that Sartobind® Rapid A membrane can also effectively remove half-antibody, a common byproduct associated with the production of asymmetric bsAbs.[29] By removing both low-molecular-weight byproducts and aggregates during product capture, the Sartobind® Rapid A membrane significantly eases the purification burden on polishing steps and potentially allows for a two-chromatography-step process.

2. Materials and methods

2.1. Materials

Ethanol, sodium acetate trihydrate, sodium chloride, sodium hydroxide, and tris(hydroxymethyl)aminomethane were purchased from Merck (Germany). Acetic acid (HAc) was bought from J.T. Baker (United States of America [USA]). Sodium dihydrogen phosphate dihydrate and disodium hydrogen phosphate dihydrate were procured from Sigma-Aldrich (USA). MabSelect SuRe LX Protein A resin and Sartobind® Rapid A Protein A membrane were obtained from Cytiva (USA) and Sartorius (Germany), respectively. BioCore SEC-300 stainless steel column (5 μm, 7.8 × 300 mm) was from NanoChrom (China). The five molecules (two monoclonal antibodies [mAbs] and three bsAbs) used in this study were expressed in stably transfected CHO-K1 cells and cultured for 14 days before harvest.

2.2. Equipment

An AKTA pure 150 system, equipped with Unicorn software version 7.8 (Cytiva, USA), was used for Protein A column and membrane chromatography. pH and conductivity were measured using a SevenExcellence S470 pH/conductivity meter (Mettler-Toledo, USA). Protein concentration was determined by employing a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Size-exclusion chromatography-high-performance liquid chromatography (SEC-HPLC) analysis was performed on an Agilent 1260 liquid chromatography instrument from Agilent Technologies (USA). The bioreactor system from Applikon Biotechnology (Netherlands) was used for cell cultivation.

2.3. Protein A column chromatography

The Protein A column packed with MabSelect SuRe LX resin had a volume of approximately 2.0 mL (0.5 cm in diameter and 10 cm in height). After equilibration with 50 mM Tris, HAc, and 150 mM sodium chloride (NaCl; pH 7.4), the column was loaded at 30 mg/mL for mAb A, mAb B, bsAb A, and bsAb B and 20 mg/mL for bsAb C. On loading, the column was sequentially washed with the equilibration buffer (its composition is listed above); 50 mM Tris, HAc, 0.5 M NaCl (pH 7.4); 50 mM sodium acetate (NaAc)-HAc, and 50 mM NaCl (pH 5.5); each for five column volumes (CVs). For elution, a linear pH gradient from the last wash buffer to 50 mM HAc and 50 mM NaCl (pH 3.0) was carried out over 20 CV. The flow rate was 0.39 mL/min, which corresponds to a residence time of 5 min.

2.4. Protein A membrane chromatography

The Protein A membrane used in this experiment was Sartobind® Rapid A, which has a volume of 1.2 mL. Following equilibration with 50 mM Tris, HAc, and 150 mM NaCl (pH 7.4), the membrane was loaded at 30 mg/mL for mAb A, mAb B, bsAb A, and bsAb B and 20 mg/mL for bsAb C. For runs conducted under a linear pH gradient elution, after loading, the membrane was sequentially washed with the equilibration buffer (its composition is listed above); 50 mM Tris, HAc, 0.5 M NaCl (pH 7.4); 50 mM NaAc-HAc, and 50 mM NaCl (pH 5.5); each for 20 membrane volumes (MVs). For bsAb C, the second wash with 0.5 M NaCl buffer was skipped. The membrane was eluted by reaching 50 mM HAc and 50 mM NaCl (pH 3.0) over 120 MV. For the run

conducted under stepwise pH gradient elution for mAb A, after loading, the membrane was subjected to the same three sequential washes as described above (each for 20 MV). The membrane was eluted with 50% of 50 mM HAc and 50 mM NaCl (pH 3.0) for 30 MV. For all runs, the flow rate was 5 MV/min (residence time: 12 s) for loading and elution, and 10 MV/min for all other steps.

2.5. SEC-HPLC

SEC-HPLC was performed with a BioCore SEC-300 stainless steel column (7.8 × 300 mm) to assess monomer/aggregate content. A total of 100 µg of sample was injected per run. The mobile phase was a 50 mM sodium phosphate buffer containing 300 mM sodium chloride (pH 6.8). Isocratic elution was performed for 20 and 30 min for the Protein A eluate and clarified culture harvest, respectively. For all runs, the flow rate was 1.0 mL/min. Absorbance was monitored at 280 nm.

3. Results and discussion

3.1. Limited aggregate separation by Protein A column under typical linear pH gradient elution

For bsAbs, high aggregate content (*i.e.*, >20%) is common. Even for regular mAbs, sometimes the aggregate content in their culture harvest can exceed 10%. Recently, while purifying an aggregation-prone mAb (mAb A), we made an observation consistent with previous ones: A Protein A column under typical linear pH gradient elution cannot separate monomers from co-binding aggregates. As the SEC-HPLC profile shown in Figure 1A suggests, the clarified culture harvest contained approximately 16.8% aggregates, which came in different sizes. According to our previous findings, large aggregates were unable to bind due to the size-exclusion effect, whereas small aggregates co-bind with monomers.[12,13,28] When this culture harvest was processed using a Protein A column under

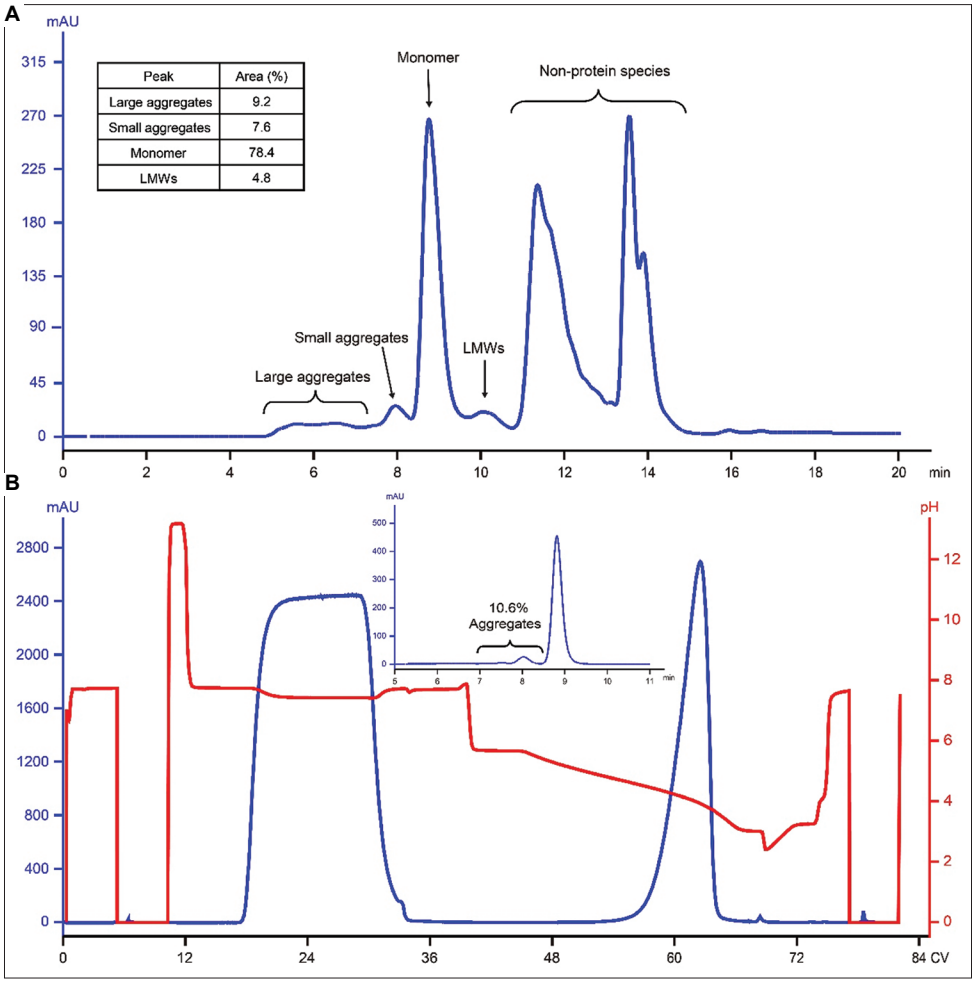

Figure 1. Analytical and processing data of an mAb-containing culture harvest. (A) SEC-HPLC profile of mAb A-containing culture harvest. Aggregates (large and small), monomers, and low-molecular-weight species are labeled, and their corresponding percentages are indicated. Peaks (absorbances) due to non-protein species in the culture media are marked out. (B) Protein A column chromatogram of a run conducted under a linear pH gradient elution. The column was loaded with clarified culture harvest containing an aggregation-prone mAb (mAb A), whose SEC-HPLC profile is shown in (A). Inset: SEC-HPLC profile of Protein A column eluate. The percentage of aggregates is indicated.
Abbreviations: mAb: Monoclonal antibody; SEC-HPLC: Size-exclusion chromatography-high-performance liquid chromatography.

a linear pH gradient elution, the chromatogram showed a single elution peak (Figure 1B). This suggests that, under these conditions, the Protein A column provided poor resolution, rendering the separation of monomers from aggregates unlikely. Unsuccessful aggregate separation was confirmed by SEC-HPLC of the elution peak content, which indicated that the eluate contained 10.6% aggregates (Figure 1B). The 10.6% aggregates mainly contained small aggregates (after removal of most large aggregates, the relative percentage of small aggregates increased) and tiny amounts of large aggregates that bind to the ligands on the resin surface. The data suggested that the Protein A column failed to remove co-binding aggregates, which was consistent with expectations.

3.2. Excellent aggregate separation capability of Sartobind® Rapid A Protein A membrane

Given that the Protein A membrane has a higher productivity than the Protein A column, we evaluated the feasibility of replacing the Protein A column with Sartorius' Sartobind® Rapid A Protein A membrane for product capture. During the evaluation, when the same mAb A-containing culture harvest was processed, the Sartobind® Rapid A membrane showed a stronger aggregate separation capability than the Protein A column under a comparable linear pH gradient elution. As shown in Figure 2A, the Sartobind® Rapid A membrane demonstrated improved resolution, as indicated by the presence of two well-resolved elution peaks. SEC-HPLC of the main elution peak content (fraction 1) suggests that aggregates are essentially absent (0.1%) from this portion (Figure 2A). This represents a significant improvement in aggregate removal compared to the corresponding aggregate content found in Protein A column eluate (*i.e.*, 10.6%). For the main peak (fraction 1), its yield was 74.3%. As a relatively complete separation between monomers and aggregates was accomplished under the linear pH gradient elution, a stepwise elution protocol was subsequently developed with ease. The

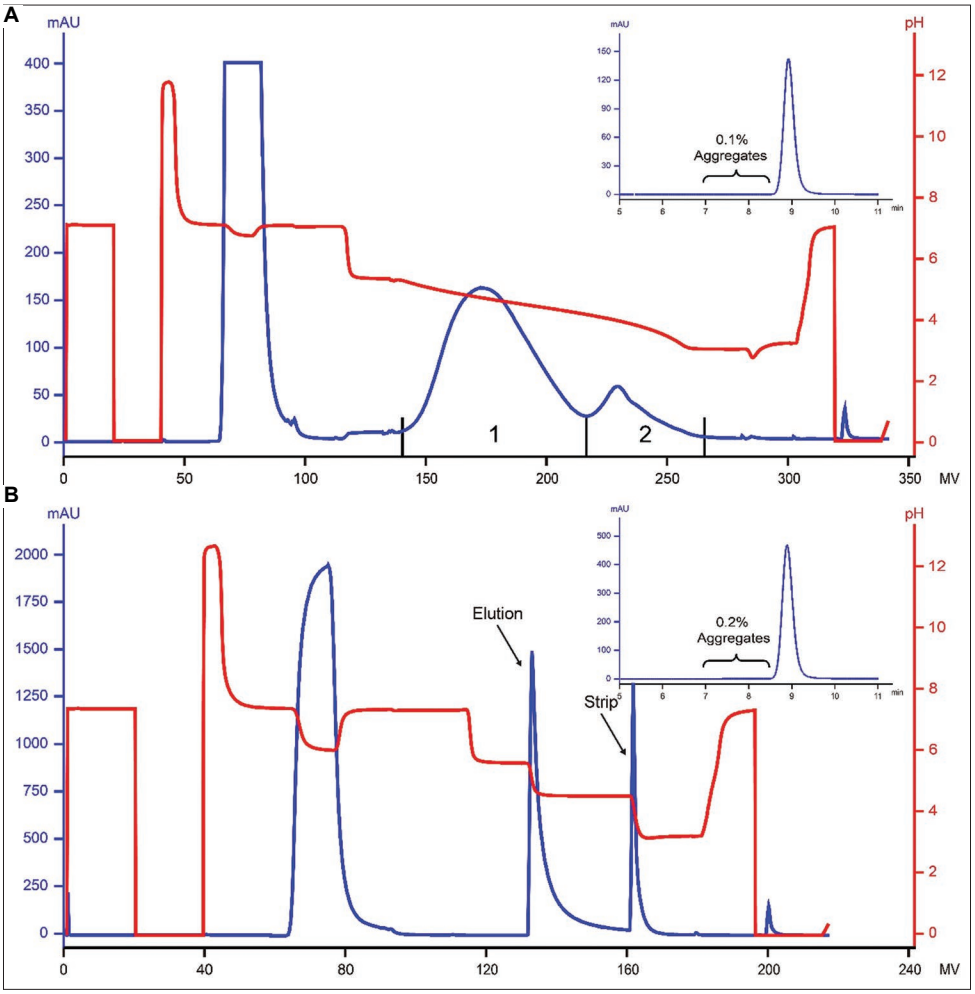

Figure 2. Protein A membrane chromatograms. Runs were conducted under a (A) linear and (B) stepwise pH gradient elution. The Protein A membrane was loaded with the same mAb A-containing culture harvest as that used for the Protein A column. Under the linear pH gradient, the chromatogram was different from that of the Protein A column and contained two well-resolved peaks (fractions 1 and 2). Inset, SEC-HPLC profiles of the corresponding Protein A membrane eluates (fraction 1 under the linear pH gradient). The percentage of aggregates is indicated.
Abbreviations: mAb: Monoclonal antibody; SEC-HPLC: Size-exclusion chromatography-high-performance liquid chromatography.

chromatogram of a run conducted under stepwise pH gradient elution is shown in Figure 2B. Under this condition, the aggregate content was reduced to 0.2% (Figure 2B), and the step yield of this run was 77.9%.

3.3. Confirmation of Sartobind® Rapid A membrane's superiority in aggregate separation in four additional cases

The Sartobind® Rapid A Protein A membrane demonstrated a stronger aggregate separation capability than the Protein A column. To confirm that this is a general property of Sartobind® Rapid A membrane rather than an exception, we further compared Protein A columns with Sartobind® Rapid A membranes on aggregate separation in four additional scenarios (mAb B and bsAbs A-C) whose culture harvests contained high amounts of aggregates: 20.9%, 19.9%, 22.9%, and 23.7%, respectively, according to the SEC-HPLC results (Figure 3). For each molecule, the same culture harvest was processed using the Protein A column and the Sartobind® Rapid A membrane under a linear pH gradient elution in parallel. For mAb B and bsAbs A-C, their corresponding Protein A (column and membrane) chromatograms are shown in Figure 4A-D, respectively. In all cases, the Sartobind® Rapid A membrane attained better monomer–aggregate separation than the Protein A column, whose eluates still contained a high percentage of aggregates (Figure 4A-D). The improved resolution of the Sartobind® Rapid A membrane is directly visible in the chromatograms, which all contain two peaks (Figure 4A-D). For cases 1–4, aggregates in the membrane's main elution peak (fraction 1) were reduced to 1.4%, 1.0%, 2.3%, and 1.1%, respectively (Figure 4A-D). Thus, Sartobind® Rapid A membrane achieved virtually complete clearance of aggregates, which is remarkable. According to the data (Figure 4A-D), small ones are predominant in the remaining aggregates. This is not surprising. As large aggregates are more different from monomers than small aggregates, they get better separated. For these four cases, Sartobind® Rapid A membrane main peak yields were 70.8%, 71.8%, 69.8%, and 71.9%, respectively (in all cases, low-molecular-weight byproducts did not bind and therefore they exerted no impact on titer or yield). The SEC-HPLC data of column and membrane eluates for the previous mAb case and these four additional cases are also summarized in Table 1. As can be seen from the data, in all cases, the secondary elution peak of membrane chromatography mainly contained aggregates (64.6–76.9%) and a small portion of monomers (23.1–34.9%).

3.4. Impact of sodium chloride concentration in mobile phase on aggregate separation using Sartobind® Rapid A membrane

A significant difference between the Protein A column and Protein A membrane is their operating flow rates (residence

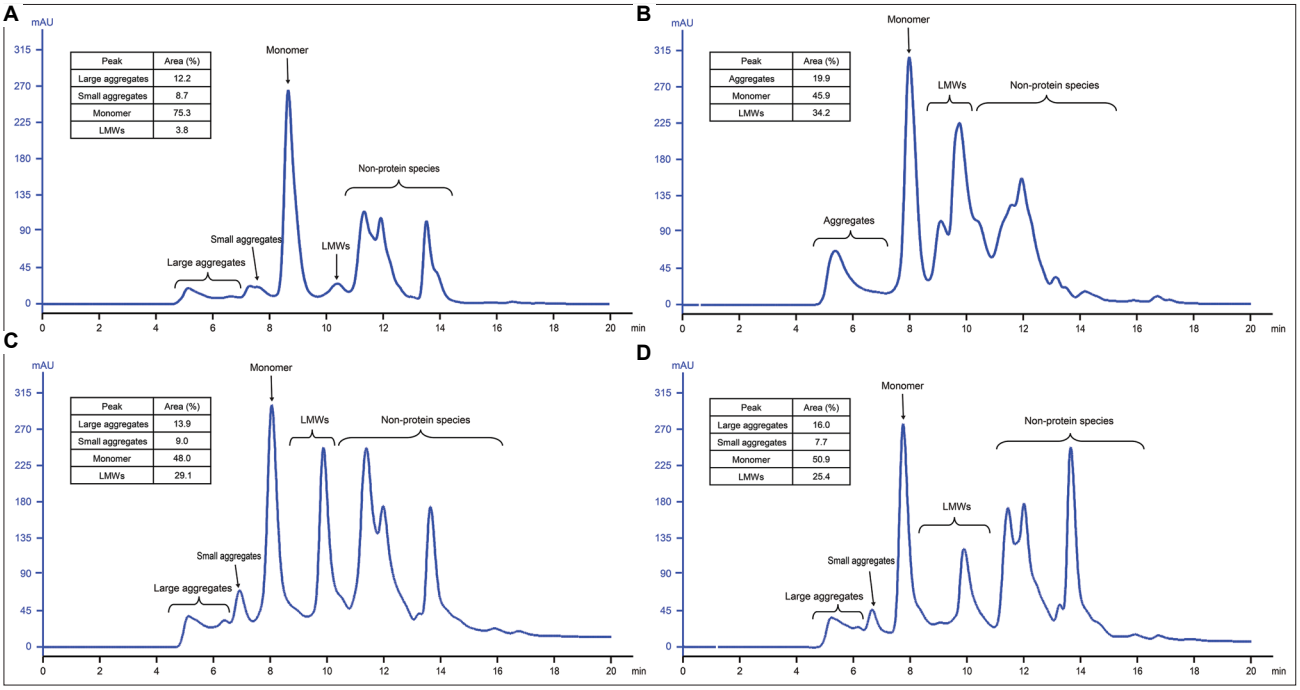

Figure 3. SEC-HPLC profiles of different culture harvests. (A-D) The culture harvests contained mAb B and bsAbs A-C, respectively. Aggregates (large and small), monomers, and LMWs are labeled, and their corresponding percentages are indicated. Peaks (absorbances) due to non-protein species in the culture media are marked out. For mAb B and bsAbs A-C, the percentages of aggregates (large and small) in the corresponding culture harvests are 20.9%, 19.9%, 22.9%, and 23.7%, respectively.

Abbreviations: LMWs: Low-molecular-weight species; mAb: Monoclonal antibody; SEC-HPLC: Size-exclusion chromatography-high-performance liquid chromatography.

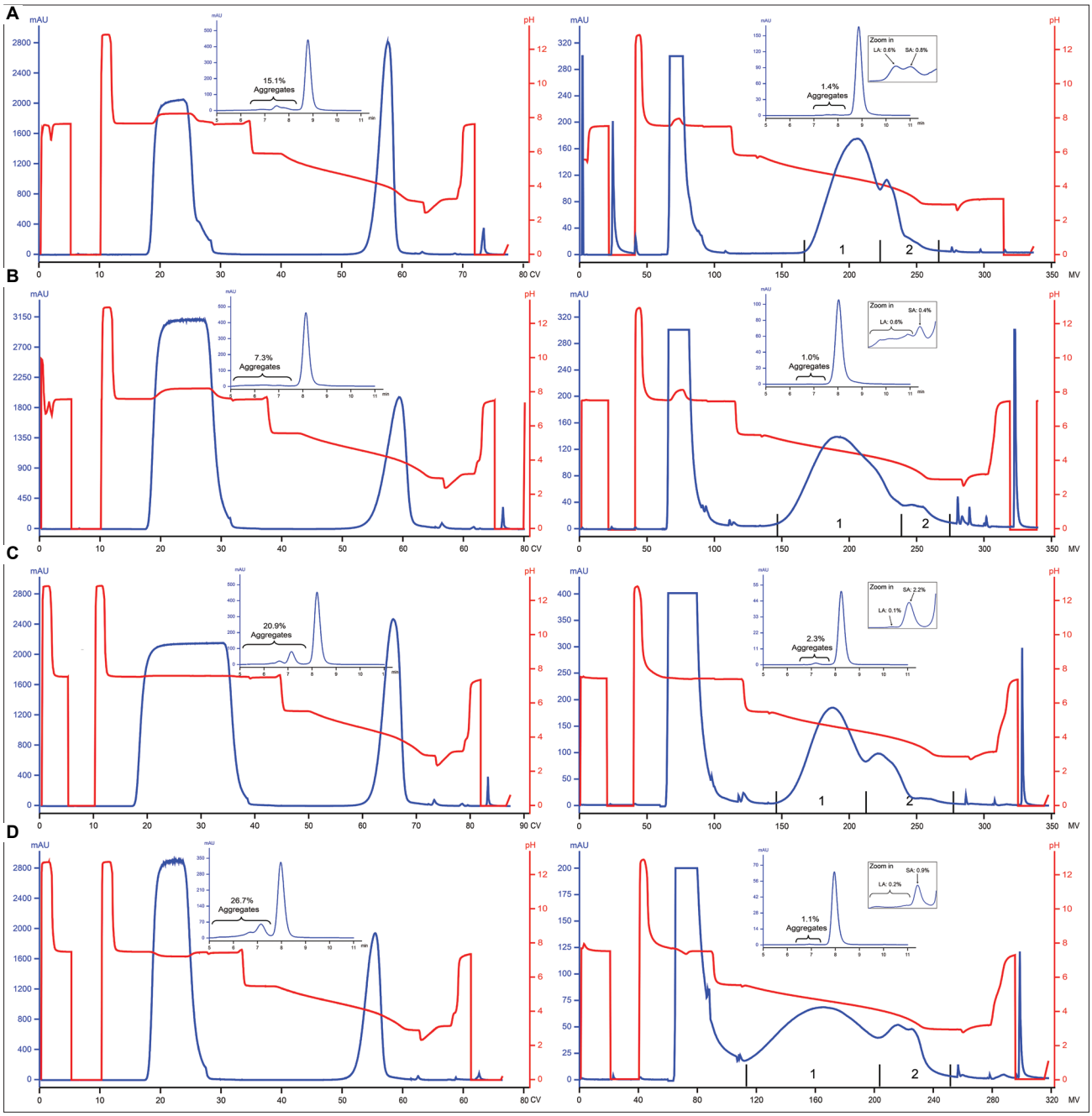

Figure 4. Chromatograms of Protein A runs conducted to process different culture harvests. (A-D) The column/membrane was loaded with culture harvests harboring mAb B and bsAbs A-C, respectively. Left and right, Protein A column and Protein A membrane chromatograms, respectively. For each molecule, the same feed material was processed using the Protein A column and the Protein A membrane. All runs were carried out under a linear pH gradient elution. Inset SEC-HPLC profiles of the corresponding Protein A eluates (fraction 1 in the case of Protein A membrane). The percentage of aggregates is indicated. For the data of the membrane eluate, a zoomed-in view of the high-molecular-weight region is provided.
Abbreviations: LA: Large aggregates; mAb: Monoclonal antibody; SA: Small aggregates; SEC-HPLC: Size-exclusion chromatography-high-performance liquid chromatography.

times for the former and the latter are measured in minutes and seconds, respectively).[23-26] To elucidate whether short residence time was the contributor to Sartobind® Rapid A membrane's strong aggregate separation capability, we carried out two additional runs at longer periods of residence time (*i.e.*, 60 and 120 s) using the mAb A-containing culture harvest as load material. We compared them with the one that was previously conducted at a shorter residence time (*i.e.*, 12 s). According to Figure 5, there was no significant difference among the chromatograms of these three runs, suggesting that short residence time was unlikely to be the reason for Sartobind® Rapid A membrane's superior aggregate separation capability.

We subsequently studied the impact of NaCl concentration in the mobile phase on monomer–aggregate resolution.

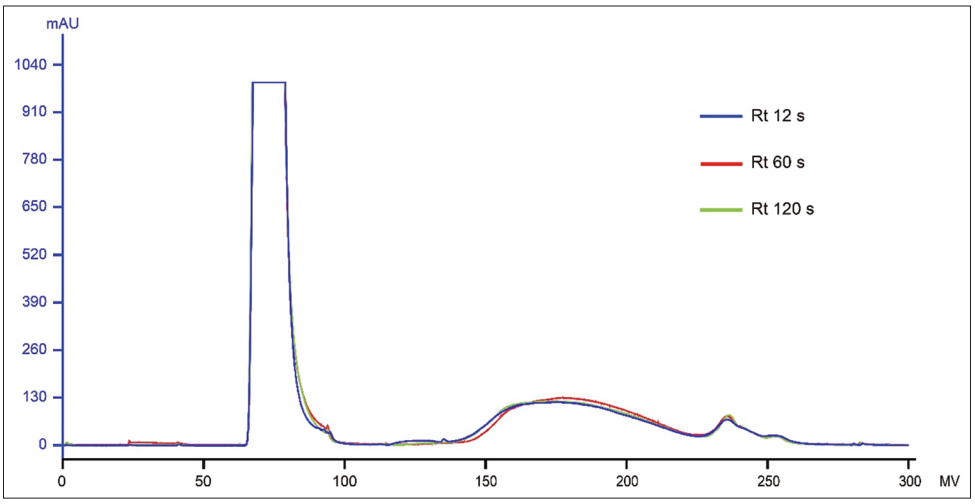

Figure 5. An overlay of Protein A membrane chromatograms of three runs conducted under different periods of residence time (12, 60, and 120 s). For all three runs, the monoclonal antibody A-containing culture harvest was used as the load material.
Abbreviation: Rt: Residence time.

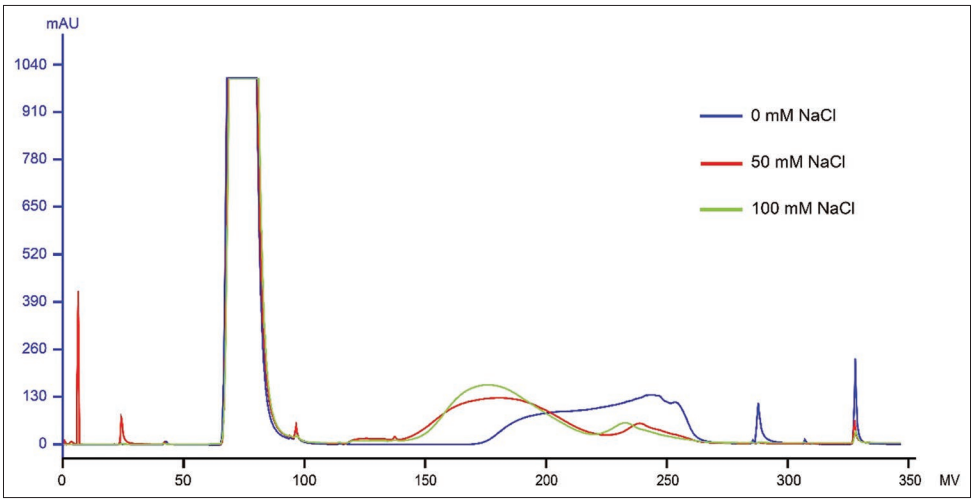

Figure 6. An overlay of Protein A membrane chromatograms of three runs whose mobile phase contained different concentrations of sodium chloride (NaCl; 0, 50, and 100 mM). For all three runs, the monoclonal antibody A-containing culture harvest was used as the load material.

Table 1. Size-exclusion chromatography-high-performance liquid chromatography data of the Protein A column and membrane eluates for five aggregation-prone molecules

Molecules	HMWs/monomer/LMWs (%)			
	Feed	Column eluate	Membrane peak 1	Membrane peak 2
mAb A	16.8[a]/78.4/4.8	10.6/89.4/0.1	0.1/99.9/ND	75.4/24.6/ND
mAb B	20.9/75.3/3.8	15.1/84.7/0.2	1.4/98.6/ND	68.8/31.2/ND
bsAb A	19.9/45.9/34.2	7.3/90.5/2.2	1.0/98.7/0.3	76.9/23.1/ND
bsAb B	22.9/48.0/29.1	20.9/79.0/0.1	2.3/97.7/0.1	70.0/29.9/0.1
bsAb C	23.7/50.9/25.4	26.7/73.1/0.1	1.1/98.5/0.4	64.6/34.9/0.5

Note. While the main reason for the high percentage of aggregates in the column eluate is the column's inability to separate aggregates, a minor reason is the nearly complete removal of low-molecular-weight byproducts, which increases the relative percentage of aggregates. [a]This is the percentage of total aggregates, including large ones and small ones.
Abbreviations: bsAb: Bispecific antibody; HMWs: High-molecular-weight species, LMWs: Low-molecular-weight species; mAb: Monoclonal antibody; ND: Not detected.

Specifically, two additional runs were conducted with mobile phases containing NaCl concentrations lower and higher than that of the previous run (0 and 100 mM NaCl vs. 50 mM NaCl), using the same mAb A-containing culture harvest as load material. We previously demonstrated that for the Protein A column, mobile phase salt concentration did not have a significant impact on the monomer-aggregate resolution.[15] For Sartobind® Rapid A membrane, the current data suggested that increasing mobile phase sodium chloride concentration from 0 to 50 mM greatly improved the monomer–aggregate resolution, and further increasing it to 100 mM made no additional improvement (Figure 6). At higher NaCl concentrations (*i.e.*, 50 and 100 mM), the retention time for aggregates remained essentially unchanged compared to a low salt concentration (*i.e.*, 0 mM). In contrast, the retention time for monomers was significantly shortened, leading to an improved monomer-aggregate separation. Thus, including an

appropriate amount of NaCl (*e.g*., 50–100 mM) in the mobile phase is critical for aggregate separation by Sartobind® Rapid A membrane.

4. Conclusion

Protein A resins are the most widely used affinity media for product capture in antibody and Fc-fusion protein purification. However, they generally lack the capability to separate monomers from co-binding aggregates. Recently, Protein A membrane has emerged as a promising alternative to resin-based Protein A columns. In the current study, we demonstrated that Sartobind® Rapid A Protein A membrane possesses stronger aggregate separation capability than the Protein A column. For the five case studies presented, Sartobind® Rapid A membrane reduced the aggregate content from approximately 16.8–23.7% (in the feed) to 0.1–2.3% (in the eluate). Protein A membrane, by removing practically all aggregates in the feed, exhibited excellent aggregate separation capability.

As there is no significant difference between the Protein A ligand used in resin and that used in membrane, we suspected that Sartobind® Rapid A's strong aggregate separation capability is likely attributed to the membrane material and/or the unique design of the membrane chamber. We found that salt concentration in the mobile phase was critical for the Sartobind® Rapid A membrane to achieve good monomer-aggregate separation. However, the fundamental reason for the Protein A membrane's greatly improved resolution warrants further investigation. The findings of the current study added an essential factor, namely high resolution, to the existing list of advantages that Protein A membrane offers, with high productivity and disposability being the most recognized ones. We previously also demonstrated that Sartobind® Rapid A membrane could effectively remove half-antibodies. For recombinant antibody and Fc-fusion protein purification, a typical downstream process contains three chromatographic steps: Protein A affinity capture and two polishing steps. As Sartobind® Rapid A membrane can effectively reduce both low-molecular-weight byproducts and aggregates in culture harvest to low levels during the capture stage, its use potentially enables a two-chromatography-step process. If this proved feasible, it would have a significant impact on the biopharmaceutical industry by significantly reducing the manufacturing costs of therapeutic antibodies and Fc-fusion proteins.

Acknowledgments

We are indebted to Dr Sherry Gu and Dr Hang Zhou for supporting this work. We thank our colleagues from Cell Culture Process Development for material generation. Our thanks also go to Mengying Tian for her assistance with SEC-HPLC analysis.

Funding

None.

Conflict of interest

Gaoya Yuan, Meng Qu, and Yifeng Li are employees at WuXi Biologics. The company played no role in the study design and the writing of this manuscript.

Author contributions

Conceptualization: Yifeng Li
Data curation: All authors
Formal analysis: All authors
Investigation: All authors
Methodology: Yifeng Li
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.

References

1. Vázquez-Rey M, Lang DA. Aggregates in monoclonal antibody manufacturing processes. *Biotechnol Bioeng*. 2011;108(7):1494-1508. doi: 10.1002/bit.23155
2. Li W, Prabakaran P, Chen W, Zhu Z, Feng Y, Dimitrov DS. Antibody aggregation: Insights from sequence and structure. *Antibodies (Basel)*. 2016;5(3):19. doi: 10.3390/antib5030019
3. van der Kant R, Karow-Zwick AR, Van Durme J, *et al*. Prediction and reduction of the aggregation of monoclonal antibodies. *J Mol Biol*. 2017;429(8):1244-1261. doi: 10.1016/j.jmb.2017.03.014
4. Li Y. A brief introduction of IgG-like bispecific antibody purification: Methods for removing product-related impurities. *Protein Expr Purif*. 2019;155:112-119. doi: 10.1016/j.pep.2018.11.011
5. Andrade C, Arnold L, Motabar D, *et al*. An integrated approach to aggregate control for therapeutic bispecific antibodies using an improved three column Mab platform-like purification process. *Biotechnol Prog*. 2019;35(1):e2720. doi: 10.1002/btpr.2720
6. Chen SW, Zhang W. Current trends and challenges in the

downstream purification of bispecific antibodies. *Antib Ther*. 2021;4(2):73-88.
doi: 10.1093/abt/tbab007

7. Ingavat N, Dzulkiflie N, Liew JM, *et al*. Investigation on environmental factors contributing to bispecific antibody stability and the reversal of self-associated aggregates. *Bioresour Bioprocess*. 2024;11(1):82.
doi: 10.1186/s40643-024-00796-y
8. Schanzer J, Jekle A, Nezu J, *et al*. Development of tetravalent, bispecific CCR5 antibodies with antiviral activity against CCR5 monoclonal antibody-resistant HIV-1 strains. *Antimicrob Agents Chemother*. 2011;55(5):2369-2378.
doi: 10.1128/AAC.00215-10
9. Hober S, Nord K, Linhult M. Protein A chromatography for antibody purification. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2007;848(1):40-47.
doi: 10.1016/j.jchromb.2006.09.030
10. Shukla AA, Hubbard B, Tressel T, Guhan S, Low D. Downstream processing of monoclonal antibodies – application of platform approaches. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2007;848(1):28-39.
doi: 10.1016/j.jchromb.2006.09.026
11. Bolton GR, Mehta KK. The role of more than 40 years of improvement in Protein A chromatography in the growth of the therapeutic antibody industry. *Biotechnol Prog*. 2016;32(5):1193-1202.
doi: 10.1002/btpr.2324
12. Yuan G, Qu M, Geng Q, Dong W, Zhang X, Li Y. Antibody aggregation can lead to reduced Protein A binding capacity and low step yield. *Protein Expr Purif*. 2023;210:106315.
doi: 10.1016/j.pep.2023.106315
13. Liu C, Tian M, Dong W, *et al*. SEC-HPLC analysis of column load and flow-through provides critical understanding of low Protein A step yield. *Protein Expr Purif*. 2024;216:106418.
doi: 10.1016/j.pep.2023.106418
14. Yu D, Song Y, Huang RY, *et al*. Molecular perspective of antibody aggregates and their adsorption on Protein A resin. *J Chromatogr A*. 2016;1457:66-75.
doi: 10.1016/j.chroma.2016.06.031
15. Zhang Y, Wang Y, Li Y. A method for improving Protein A chromatography's aggregate removal capability. *Protein Expr Purif*. 2019;158:65-73.
doi: 10.1016/j.pep.2019.02.017
16. Yigzaw Y, Hinckley P, Hewig A, Vedantham G. Ion exchange chromatography of proteins and clearance of aggregates. *Curr Pharm Biotechnol*. 2009;10(4):421-426.
doi: 10.2174/138920109788488842
17. Xu Z, Li J, Zhou JX. Process development for robust removal of aggregates using cation exchange chromatography in monoclonal antibody purification with implementation of quality by design. *Prep Biochem Biotechnol*. 2012;42(2):183-202.
doi: 10.1080/10826068.2012.654572
18. Lu Y, Williamson B, Gillespie R. Recent advancement in application of hydrophobic interaction chromatography for aggregate removal in industrial purification process. *Curr Pharm Biotechnol*. 2009;10(4):427-433.
doi: 10.2174/138920109788488897
19. Gao D, Wang LL, Lin DQ, Yao SJ. Evaluating antibody monomer separation from associated aggregates using mixed-mode chromatography. *J Chromatogr A*. 2013;1294:70-75.
doi: 10.1016/j.chroma.2013.04.018
20. Waller JA, Zheng J, Dyer R, *et al*. Ceramic hydroxyapatite chromatography plays a critical role in bispecific antibody purification process for impurity removal. *Antib Ther*. 2022;6(1):30-37.
doi: 10.1093/abt/tbac030
21. Ingavat N, Wang X, Liew JM, *et al*. Harnessing ceramic hydroxyapatite as an effective polishing strategy to remove product- and process-related impurities in bispecific antibody purification. *Bioresour Bioprocess*. 2023;10(1):93.
doi: 10.1186/s40643-023-00713-9
22. Rupčíková V, Molnár T, Kurák T, Polakovič M. Antibody aggregate removal by multimodal chromatography. *Molecules*. 2025;30(11):2363.
doi: 10.3390/molecules30112363
23. Busse RA, Gruenberg M, Kuchemueller KB, Toeppner K, Adametz P, Thom V. Sartobind Rapid A: Comparable high product quality as Protein A resins at high productivity. Application Note; 2022. Available from: https://www.sartorius.hr/media/3u2fwqe3/sartobind-rapid-a-comparable-high-product-quality-as-protein-a-resins-at-high-productivity.pdf [Last accessed on 2025 Oct 01].
24. Grünberg M, Kuchemüller KB, Töppner K, Busse RA. Scalable, robust and highly productive novel convecdiff membrane platform for mAb capture. *Membranes*. 2022;12(7):677.
doi: 10.3390/membranes12070677
25. Osuofa J, Husson SM. Comparative evaluation of commercial Protein A membranes for the Rapid purification of antibodies. *Membranes*. 2023;13(5):511.
doi: 10.3390/membranes13050511
26. Yang S, Braczkowski R, Chen SH, *et al*. Scalability of Sartobind Rapid A membrane for high productivity monoclonal antibody capture. *Membranes*. 2023;13(10):815.
doi: 10.3390/membranes13100815
27. Gehrmann N, Daxbacher A, Hahn R. Rapid purification of mAb using Protein A membranes yielding high HCP clearance. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2024;1232:123989.
doi: 10.1016/j.jchromb.2023.123989
28. Lu W, Zhang T, Wan Y, Li Y. Protein A membrane shows an advantage over Protein A resin in purifying antibodies/Fc-fusions forming noncovalent aggregates. *Chromatogr Sep Tech J.* 2024;4:127.
29. Yuan G, Qu M, Li Y. Effective and robust separation of half-antibody byproduct in bispecific antibody purification by Sartobind Rapid A Protein A membrane chromatography. *Protein Expr Purif*. 2026;237:106823.
doi: 10.1016/j.pep.2025.106823