Improving Aggregate Removal from a Monoclonal Antibody Feedstream Using High-Resolution Cation Exchange Chromatography

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Tech Note

Protein Purification

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Abstract

Nuvia™ HR-S Resin, a hydrophilic open-pore, high-resolution cation exchange (CEX) resin (media), was shown to reduce aggregate content to <0.3% with >80% monomer recovery from a monoclonal antibody feedstream containing 8.9% aggregates. In a side-by-side comparison with a high-resolution agarose-based resin, Nuvia HR-S showed comparable aggregate removal but at significantly higher recovery levels. Furthermore, aggregate content and overall recovery were shown to be functions of the conductivity of the elution buffer at the end of collection.

Introduction

Ever increasing performance demands in protein purification require more selective chromatography methods to effectively remove aggregates and other impurities. At high titer levels, aggregates often present a unique purification challenge. One emerging approach to address this challenge is the use of new, smaller-particle chromatography resins that are optimized for high resolution and capacity. Such resins can be particularly productive in challenging situations and during final polishing steps (He et al. 2010). Nuvia HR-S is the latest member of this new class of high-resolution resins; it features a hydrophilic polymer matrix with an open-pore structure designed for fast and efficient mass transfer and superior flow properties at high flow rates. Here, we compare the performance of Nuvia HR-S to that of a high-resolution agarose-based resin. We also describe the relationship between elution buffer conductivity, monomer recovery, and aggregate content.

Materials and Methods

Test solution

The test solution contained a mixture of aggregates and monomers obtained from a purified monoclonal antibody solution (mAb G). Aggregates were generated as follows: 500 µl 5 M NaCl and 1 ml 1 M glycine were added to 100 ml of previously prepared mAb G solution in 5 mM Na phosphate, pH 7.0. The pH was adjusted to 2.95 with 900 µl 1 M HCl. This solution was held at 37°C for 20 min. The pH was then raised to ~5.0 and sterile-filtered through a 0.22 µm membrane filter.

Column

Nuvia HR-S and an agarose-based resin (Resin 1) were packed in a Bio-Scale™ MT2 Chromatography Column (Bio-Rad Laboratories, Inc.) to give a final dimension of 7 x 56 mm (2.1 ml volume).

Chromatography system and protocol

Chromatography steps were carried out using the BioLogic Duoflow™ Chromatography System (Bio-Rad Laboratories, Inc.), which is capable of monitoring multiple variables, including absorbance (A_{280}/A_{260}), conductivity, pressure, and pH.

Aggregate removal conditions on the BioLogic Duoflow Chromatography System:

Buffer A1: 40 mM Na acetate, pH 5

Solution A2: 1 M NaOH

Buffer B1: 40 mM Na acetate with 1 M NaCl, pH 5

Flow rate: 0.96 ml/min

Isocratic hold: 100% A1 (3 column volumes, CV)

Inject 20 ml aggregate and monomer mixture

Gradient: 0–100% B1 (20 CV)

Isocratic hold: 100% B1 (2 CV)

Isocratic hold: 100% A2 (5 CV)

Recovery calculation

Monomer recovery calculation was based on total absorbance in the pool and the load and was normalized to the monomer percentage in both.

$$\text{Monomer recovery} = \frac{\text{Total } A_{280} \text{ in pool x \% monomer in pool}}{\text{Total } A_{280} \text{ in load x \% monomer in load}}$$

Size exclusion chromatography (SEC)

Analytical SEC was performed with an Agilent Bio SEC-5 Column (Agilent Technologies, Inc.) at a flow rate of 0.35 ml/min on the BioLogic Duoflow Chromatography System. Sterile-filtered PBS with 0.02% Na azide was used as the equilibration buffer. Ten microliters of sample were injected into the BioLogic Duoflow Chromatography System. Aggregates were defined as the front eluting peak on the SEC profile.

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Results and Discussion

Preparation of aggregate and monomer test solution

The in vitro generation of aggregates was designed to obtain a final composition of ~10% aggregates. Several initial attempts were made with varying ratios of monomeric to aggregated antibody. A final ratio of 2.5 volumes of monomeric antibody solution to 1 volume of aggregated antibody solution was chosen to yield a composition with the following properties:

A_{280} = 6.3 or 4.56 mg/ml
pH = 4.91
Conductivity = 2.6 mS/cm
Aggregate = 8.9%

A plot of log MW of six protein standards vs. their retention times yielded a straight line (R^2 = 0.96). Using this plot and the retention times of the aggregates, the apparent MW of the aggregate was calculated to be 386 kD.

Initial separation of aggregate from monomer using Nuvia HR-S

During initial development, a protocol that deviated from that in the Materials and Methods section was used. It involved a flow rate of 2 ml/min and a gradient of 0–1 M NaCl in the running buffer. As shown in Figure 1, there was considerable overlap of the monomer and aggregate peaks (fractions (Fr) 17–22). Depending on the fraction pooling method, monomer recovery of 66–81% was achieved with an aggregate content of 0.46–0.85%. This observation is consistent with the premise that greater recovery results in higher aggregate content. The quality of the recovered pools (Figure 2) is shown in the overlay of the enlarged front shoulders of each SEC profile. The results demonstrated the feasibility of using Nuvia HR-S to separate aggregates from the monomer and showed that the extent of residual aggregates depended on the fractions included in the pool. Additionally, the overlap of monomer and aggregates in the Nuvia HR-S elution profile suggested that a lower flow rate and a shallower gradient (0.1–0.4 M NaCl) could be beneficial.

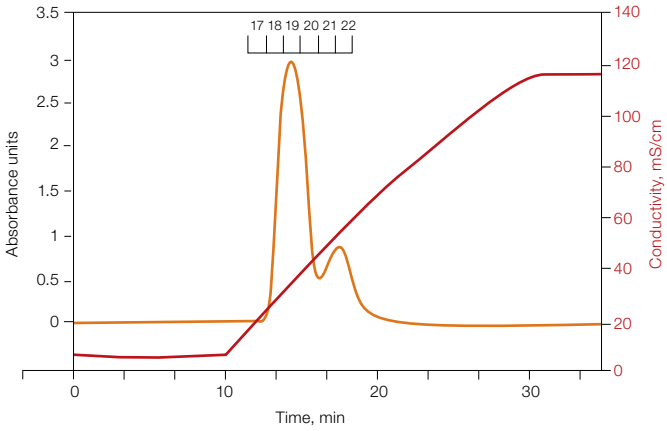


Fig. 1. Separation of aggregates from monomer by Nuvia HR-S before optimization.

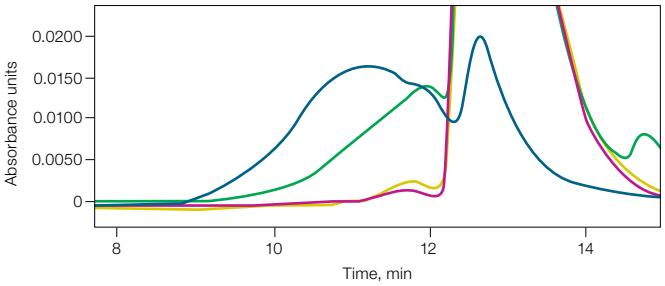


Fig. 2. SEC profiles of the load (—), selected pools (Fr 17+18+19+20) (—) and (Fr 17+18+19) (—), and aggregated pool (Fr 21+22) (—).

Comparison of Nuvia HR-S with a commercial small particle size CEX resin

A loading level of 46 mg IgG/ml resin was employed to challenge the binding capacity of Nuvia HR-S and Resin 1, and aggregate clearance by each resin was evaluated. A comparison of the elution profiles obtained from each chromatography run using the conditions described in the Materials and Methods section is shown in Figure 3. As indicated by the absorbance profile, the chromatography conditions resulted in a low yield for Resin 1 due to incomplete binding during the loading phase. In contrast, Nuvia HR-S was able to quantitatively retain the antibody before elution.

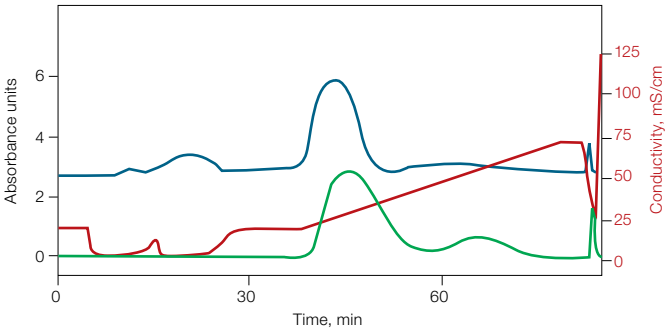


Fig. 3. Elution profiles of Nuvia HR-S (—) vs. Resin 1 (—) after optimization.

Rather than subjectively determining the pertinent fractions, a strategy was designed to measure the aggregate content and the monomer recovery as fractions were progressively pooled during elution. As shown in Figure 4, both resins show an increase in aggregates as the pooling was extended to increase monomer recovery. While Nuvia HR-S was able to deliver a final aggregate content of <0.3% and a recovery of >80%, Resin 1 recovered less than 70% of total monomer at the same target aggregate content. This is due to the lower binding capacity of Resin 1, as confirmed by the increase in its absorbance during loading (Figure 3).

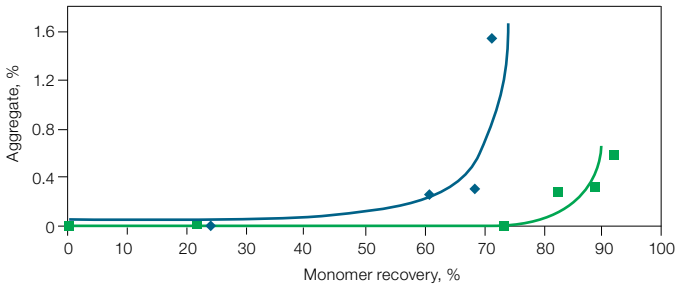


Fig. 4. Performance of Nuvia HR-S (■) vs. Resin 1 (◆).

Correlation between monomer recovery, aggregate content, and target conductivity

As depicted in Figure 4, aggregate recovery increases with increasing monomer recovery. In the Nuvia HR-S gradient elution, conductivity of the last fraction added to the pool was determined as the cutoff target of the pool. As shown, recovery is a function of the acceptable aggregate percentage and the target conductivity (Figure 5). Using the Fit Model function in JMP Software (SAS Institute Inc.), recovery could be correlated to those two measurements. A summary of actual and predicted data is shown in Figure 6.

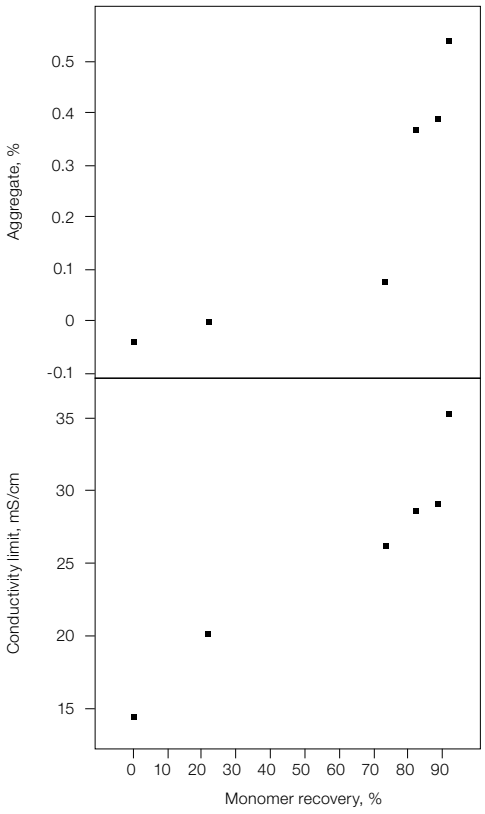
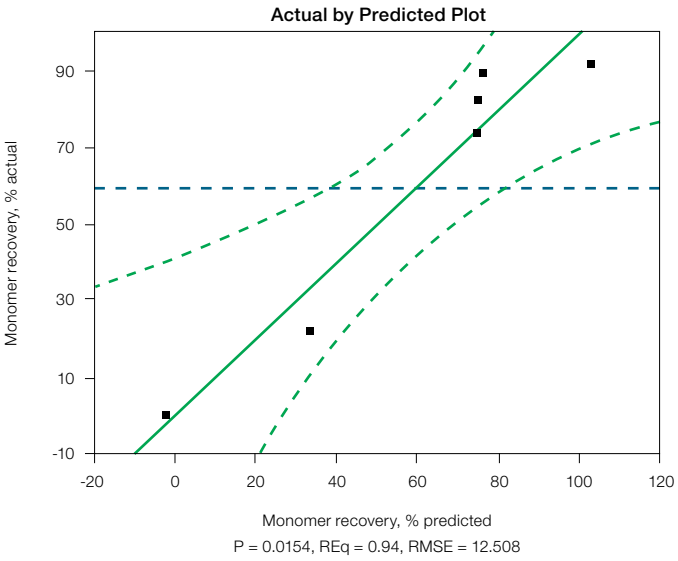


Fig. 5. Scatter plot matrix of aggregate content or conductivity vs. monomer recovery on Nuvia HR-S.



Summary of Fit

R^2	0.938098
R^2 adj	0.89683
Root mean square error	12.50828
Mean of response	59.78333
Observations (or sum wgts)	6

Fig. 6. Fit model of monomer recovery on Nuvia HR-S.

The model yielded excellent correlation (R^2 = 0.94). This finding confirms the need to determine conductivity for elution so that both recovery and aggregate levels are within acceptable limits.

Conclusion

The data presented here demonstrate that Nuvia HR-S High-Resolution Cation Exchange Resin effectively clears aggregates from an antibody feedstream with high initial aggregate content. In addition, Nuvia HR-S provides significantly higher percent recovery than a comparable agarose-based small-particle CEX resin. Finally, aggregate removal and final recovery are shown to be functions of buffer conductivity measured at the end of eluate collection.

References

He X et al. (2010) Nuvia S Media. BioProcess International 8, 59–61.

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