Practical Guide: Selecting the Optimal Resins for Aggregate Removal

Payal Khandelwal, PhD
Bio-Rad Laboratories, Inc., 6000 James Watson Drive, Hercules, CA 94547

Purification Solutions Bulletin 6808

Comprehensive Solutions for Aggregate Issues

The success of any biologic drug, for example, monoclonal antibodies (mAbs), recombinant proteins, or biosimilars, depends greatly on downstream purification. Among the challenges process scientists often face during this process is the formation and/or removal of aggregates of monomers. Although the aggregates are physically and chemically similar to monomers, their presence in the final purified product, especially a therapeutic mAb, is undesirable for many reasons. First, aggregates also often contain other impurities, like host cell proteins (HCPs) and DNA, leading to the formation of complex contaminants. This can increase the risk of anaphylaxis or immunogenic response in patients. Second, aggregates of therapeutic mAbs often demonstrate different bioactivity/potency profiles, storage stability, and pharmacodynamic/pharmacokinetic properties than their monomeric counterparts (Lang et al. 2011). For these reasons, aggregate removal has become a major focus of downstream processing.

Aggregate formation can be mediated by several processes: (1) high-titer fermentation can lead to the mispairing of disulfide bonds during biosynthesis and the unfolding or denaturation of drug molecules at cell growth temperatures (≥25°C) (Rathore et al. 2013); (2) Protein A affinity chromatography, which is used to clear bulk impurities present in the feedstock, requires strong acidic elution conditions that can trigger structural changes and promote oligomerization of pH-sensitive molecules; (3) as a viral inactivation measure, the Protein A eluate is often maintained at low pH for 30–60 minutes, which can exacerbate aggregate formation; and (4) aggregates can also form during and after purification if the downstream processing conditions/methods are not ideal.

Bio-Rad has provided a progressive selection of chromatography resins for process-scale purification of biologics for more than 50 years. As the biopharmaceutical industry has grown, the presence of aggregates has become the subject of intense and increasing scrutiny. In this guide we provide a brief snapshot of the different resins that can be used for aggregate removal/minimization in process-scale purification workflows.

Ion Exchange (IEX) Chromatography Resins

Since aggregate molecules are chemically multiples of the monomer, they have proportionally greater surface charge. This makes ion exchange resins ideal for their purification. Bio-Rad offers new small 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™ S Resin

This is a high-capacity, strong cation exchange (CEX) resin with excellent dynamic binding characteristics and low backpressures at high flow rates. The potential of Nuvia S for the clearance of aggregates was shown using a monoclonal antibody, mAb G, which tends to aggregate during acidic elution from a Protein A affinity chromatography column (Figure 1). Nuvia S successfully reduced the aggregate content from 13.8% in the loaded material to <1% in the purified eluate while maintaining 93% mAb recovery (bulletin 5984). The high flow rates offered by this resin provide the additional advantage of minimizing the antibody's exposure to proteases and nucleases present in the cell culture feedstream, thereby minimizing antibody degradation.

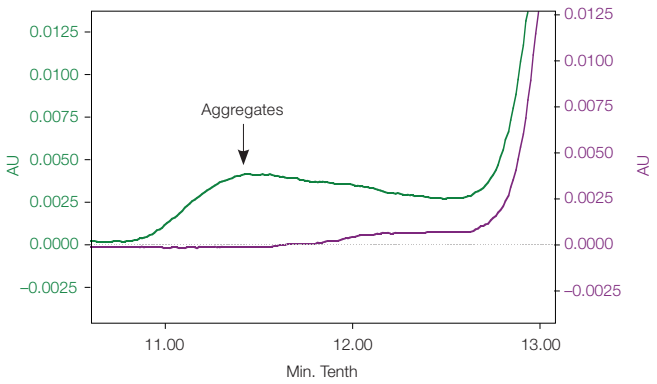

Fig. 1. HPLC-SEC analysis of aggregate removal from mAb G using Nuvia S Resin. Load (—); Fractions 3–8 (—).

Nuvia™ HR-S Resin

This is a high-resolution CEX resin. It has a hydrophilic polymer matrix with an open pore structure designed for fast and efficient mass transfer and superior flow properties at high flow rates. It provides excellent scalability for large-scale downstream manufacturing. It is ideal for the separation of high molecular weight impurities like mAb aggregates and closely related biomolecules (bulletin 6439). It also effectively reduced the aggregate content from 10% in the load to 0.46–0.85% in the eluate, with a monomer recovery of 66–81% (Figure 2).

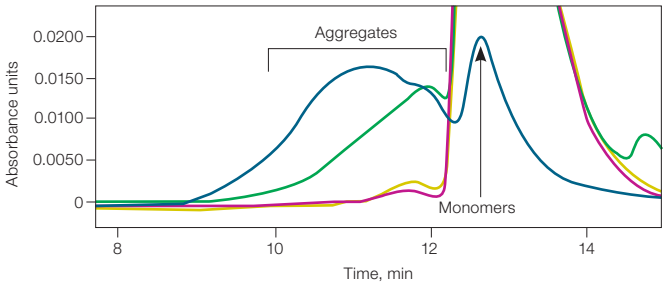

Fig. 2. SEC profiles of the load (—), selected pools (Fr 17–20) (—) and (Fr 17–19) (—), and aggregated pool (Fr 21–22) (—).

Nuvia HR-S is also superior in minimizing aggregate content with high recovery rates relative to other small particle CEX resins. In a direct comparison, Nuvia HR-S yielded <0.3% remnant aggregate content with >80% recovery versus ~70% recovery with Resin 1 (Figure 3).

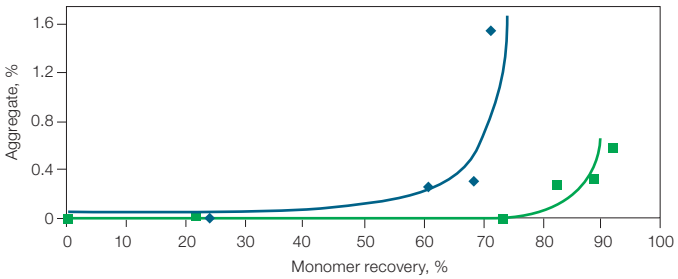

Fig. 3. Performance of Nuvia HR-S (■) vs. Resin 1 (◆).

Hydrophobic Interaction Chromatography (HIC) Resins

As some aggregates show increased surface hydrophobicity over monomers, HIC can also be used for aggregate removal. Changes in pH can alter the hydrophobicity of proteins and promote their binding to/release from HIC resins. Similarly, changes in salt concentrations can vary the strength of protein interactions with the resins, thereby modifying their purification. High salt concentrations often help proteins bind to HIC resins. Hence, under a reverse salt gradient, aggregates remain bound to the resin while monomers are eluted. Bio-Rad provides two types of HIC resins: Macro-Prep® t-Butyl, which is mildly hydrophobic and can be used to purify proteins with few or weak hydrophobic regions, and Macro-Prep® Methyl, which is weakly hydrophobic and can be used to purify proteins with strong hydrophobic regions.

Mixed-Mode Chromatography Resins

Techniques that use IEX and HIC resins may also induce the formation of additional aggregates or multimers due to increased protein concentrations or the salt concentration and/or pH requirements for elution. Mixed-mode chromatography resins can overcome these purification challenges.

Mixed chromatographic modes allow separation based on both charge and either hydrophobicity or metal affinity in a single step. Bio-Rad's mixed-mode resins offer unique separation properties, unparalleled selectivity, and outstanding resolution and can be used for efficient aggregate removal.

CHT™ Ceramic Hydroxyapatite Media

As mixed-mode media, CHT provides the advantage of dual CEX and metal affinity purification. It exhibits electrostatic, repulsive, and coordinate covalent bond formation when interacting with protein species. Its diverse selectivity allows for the highest clearance of aggregates and one-step removal of multiple product-related impurities (bulletin RP0033), including mAb aggregates and fragments, DNA, HCPs, virus, endotoxins, and Protein A. In comparison with other mixed-mode resins, CHT offers the best monomer recovery with minimal aggregate

levels in the purification of some mAbs, like mAb S (Figure 4) (bulletin 6749). It can effectively remove aggregates from a variety of sample feeds. In a simple and efficient single-step process, CHT was shown to remove essentially all of the aggregation and degradation products found in a human IgG_4 sample (bulletin 2940).

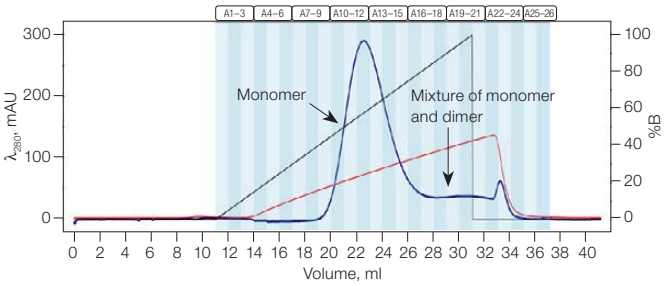

Fig. 4. Removal of mAb aggregates with CHT. OD 280 (—); conductivity (—), %B (—). The blue vertical lines represent where samples were collected.

Nuvia™ cPrime™ Resin

This resin combines hydrophobic and CEX interactions to provide highly robust recovery at high flow rates in commercial manufacturing settings. Its particle size is optimized to deliver exceptional flow properties, fast mass transfer, and stability. It is a salt-tolerant resin that can be effectively used for salt- and pH-sensitive mAb purifications with minimal feed conditioning. It has been shown to work well for clearing aggregates from a mAb preparation from CHO cells in a workflow including two other IEX resins, Nuvia S and Nuvia™ Q (bulletin 6241).

Bio-Rad offers a wide variety of chromatography resins that will remove aggregates and maintain product efficacy and safety. The information provided here can help you get started on your aggregate purification strategy. For technical/product support or to request a quote, email your regional Bio-Rad representative at process@bio-rad.com or contact our customer service at 1-800-4-BIORAD (1-800-424-6723).

References

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

Lang DA et al. (2011). Aggregates in monoclonal antibody manufacturing processes. Biotechnol Bioeng 108, 1,494–1,508.

Rathore AS et al. (2013). Aggregation of monoclonal antibody products: Formation and removal. Biopharm International 26, 40–45.

Explore our extensive selection of process-scale chromatography resins and their performance characteristics and applications (bulletin 6713). For process optimization of your aggregate removal, request a sample.

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *43 1 877 89 01 177* ***Belgium*** *32 (0)3 710 53 00* ***Brazil*** *55 11 3065 7550*
Canada *1 905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *420 241 430 532* ***Denmark*** *45 44 52 10 00* ***Finland*** *358 09 804 22 00*
France *33 01 47 95 69 65* ***Germany*** *49 89 31 884 0* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 459 6100* ***India*** *91 124 4029300*
Israel *972 03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *81 3 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *31 (0)318 540 666*
New Zealand *64 9 415 2280* ***Norway*** *47 23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04*
Singapore *65 6415 3188* ***South Africa*** *27 (0) 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *46 08 555 12700* ***Switzerland*** *41 026 674 55 05*
Taiwan *886 2 2578 7189* ***Thailand*** *66 662 651 8311* ***United Arab Emirates*** *971 4 8187300* ***United Kingdom*** *44 020 8328 2000*

Bulletin 6808 Ver A US/EG *16-0493 0516 Sig 1215*