A Non-Affinity Chromatography Resin Alternative for Capture Purification of Antibodies

Payal Khandelwal and Mark Snyder
Bio-Rad Laboratories, Inc., 6000 Alfred Noble Drive, Hercules, CA 94547

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Abstract

Protein A affinity purification has multiple limitations in terms of productivity at manufacturing scale and the costs associated with making commercial antibodies, especially biosimilars. Here, we propose a viable alternative, an ion exchange (IEX) resin, Nuvia S, for the capture purification of such biomolecules. We present instances where its use has resulted in a productive and economical purification workflow.

Introduction

Antibody purification workflows typically involve three steps consisting of capture, intermediate, and polish chromatography. The capture purification step is primarily tasked with eliminating bulk impurities from the feedstream and increasing the concentration of the target biomolecule in the resulting eluate. Protein A affinity chromatography, which is commonly used for capture purification, generally meets these requirements. It provides high purity in a single step and is highly selective since only the Fc region of the antibody can bind to the Protein A ligand.

However, other requirements must be met if the antibody is to be used for therapeutic purposes. For biosimilars, one crucial requirement is cost efficiency. Affinity-based resins are expensive and not cost-effective, especially for biosimilars. In addition, Protein A does not efficiently capture molecules like IgMs or certain IgG subclasses, bispecific monoclonal antibodies, or fusion constructs. In addition, Protein A possesses low base stability, low binding capacity, and leaches ligand into the process stream.

To overcome these limitations, we propose the use of Nuvia S Cation Exchange Resin for the capture step. Cation exchange (CEX) chromatography is shown to provide high specificity and scalability, is significantly less expensive than Protein A, and can be used at high flow rates to facilitate rapid processing and high productivity. This article shows the value of using Nuvia S for capture purification of antibodies with some examples.

Materials and Methods

Chromatography

CEX and Protein A resins were purchased and processed per manufacturer's instructions. Chromatography runs were performed on either a BioLogic DuoFlow System or an NGC Discover 10 Pro Chromatography System.

Biosimilar Purification

An adalimumab biosimilar produced in Chinese hamster ovary (CHO) cells, purchased from Syd Labs, was used for the comparative workflow experiment. Complete experimental details are described in bulletin 7130. Cost comparisions were carried out based on the resin costs for 2018 and the buffers and steps required for 100 purification cycles to produce ~1 g of the biosimilar.

IgM Purification

Three IgMs expressed in HEK cells via transient expression were capture purified with Nuvia S Resin and further purified with CHT Ceramic Hydroxyapatite Media, Type II (CHT-II-40). Complete experimental details are described in bulletin 6966.

Analytical Assays

Host cell protein (HCP) content was measured using an HCP ELISA Assay Kit from Cygnus Technologies. DNA clearance was studied using the Quant-iT PicoGreen dsDNA Assay Kit from Invitrogen Corporation.

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Results

Improved Process Economy

An adalimumab (HUMIRA) biosimilar was captured on Nuvia S and a Protein A resin in parallel in a workflow involving three purification steps. The cost comparison of the complete workflow, including the amounts of resin and buffer and the conditioning steps required to purify the biosimilar to >99% purity, showed that the use of Nuvia S for the capture step significantly reduced the final cost of goods (Figure 1). Side-by-side comparison of host cell protein (HCP) removal showed comparable purity of the final target fraction (Table 1). Additionally the biosimilar purified with Nuvia S capture was functionally active, as measured by its TNF-alpha binding (data not shown). Refer to bulletin 7130 for details on this case study.

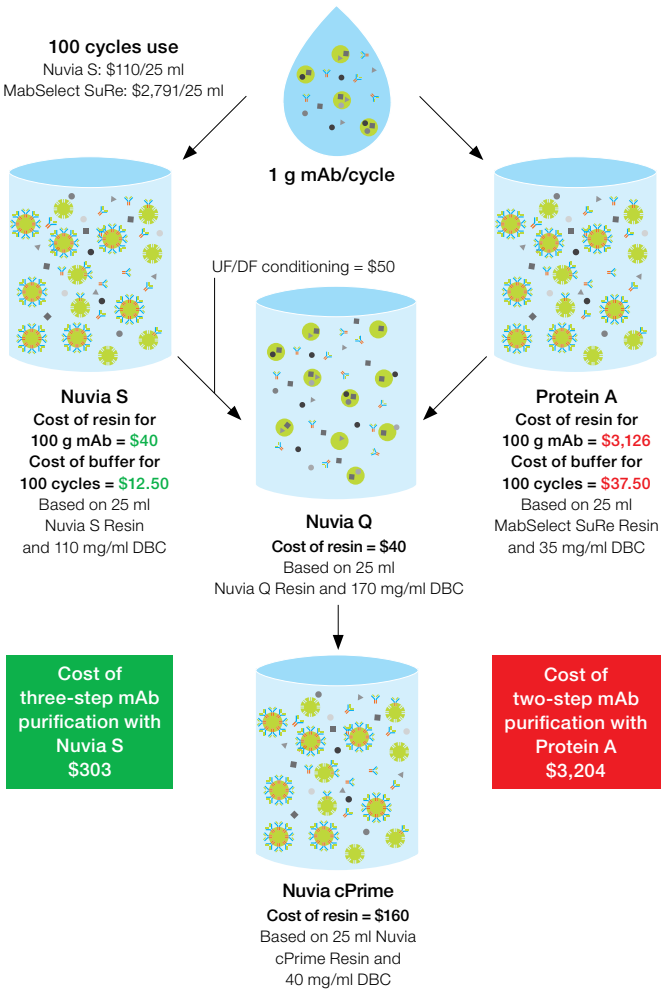

Fig. 1. Cost cutting by replacing the Protein A resin with Nuvia S Resin to improve process economics during biosimilar purification. Pricing mentioned is valid for 2018.

Table 1. Comparison of HCP clearance on 96-well filter plates between Nuvia S Resin and a Protein A resin.

	HCP, ppm Nuvia S Capture	HCP, ppm Protein A Capture
CHO cell supernatant	72,000	90,300
Capture purification	6,800	980
Nuvia Q intermediate polish purification	250	20
Nuvia cPrime final polish purification	5	20

Easy Purification of IgMs

The biological properties of immunoglobulin M (IgM) antibodies make them effective vehicles for in vitro diagnostics and therapeutics, especially in cancer, infectious diseases, and stem cell therapies. Although IgMs are composed of pentameric complexes of Ig monomers, purification strategies used for IgG cannot be translated directly for IgM purification because of the multiple differences between IgG and IgM. Protein A resins generally show poor binding to many IgM antibodies. IgMs are also generally unstable in the acidic conditions required for elution from Protein A. Typically, IgMs are more charged than IgGs and hence are retained more strongly on ion exchangers. Therefore, IEX resins such as Nuvia S can serve as efficient resins for the capture step of such. To demonstrate this utility, three IgMs with isoelectric points (pI) from 7.0 to 7.5 were captured on Nuvia S Columns and further purified on a mixed-mode resin, CHT-II-40. The resultant IgMs showed a significant level of purity (Figure 2).

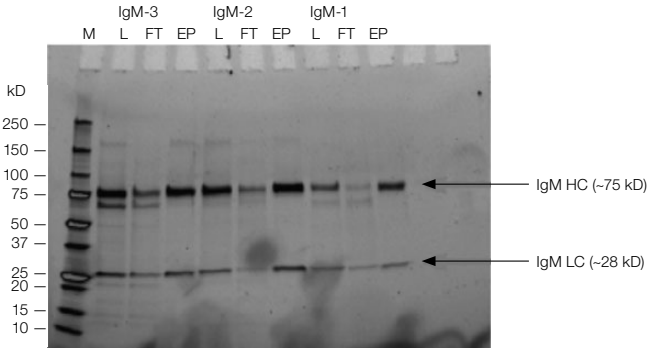

Fig. 2. Analysis of the neutral/basic IgMs purified on Nuvia S and CHT-II-40 Columns. Fractions collected during the chromatography run using the optimized protocol were analyzed by SDS-PAGE. Equal volumes (22.5 µl) of each fraction were loaded on the gel. M, prestained protein standards; L, load; FT, flowthrough; EP, eluted protein; HC, heavy chain; LC, light chain.

This two-step purification process is designed to meet purity requirements for manufacture of diagnostic IgMs. The high capacity and selectivity of Nuvia S helped overcome the affinity-based challenges of low binding and/or recovery during IgM purification. However, therapeutic targets typically require significantly lower levels of process- and product-related impurities. For such purposes, this platform could be extended with a suitable third purification step. Further details of the two-step purification workflow with Nuvia S can be found in bulletin 6966.

Faster Processing Time

The speed at which capture is executed can be critical to the stability of target biomolecules that may be sensitive to proteases present in cell culture feedstreams. Nuvia S is built on a macroporous, rigid UNOsphere base matrix containing polymeric surface extenders with optimized density of sulfonate groups. The large pore size of UNOsphere matrices provides fast mass transfer. The combination of optimized charge density and large pore size gives Nuvia S a dynamic binding capacity (DBC) up to ~115 mg/ml even at the high flow rate of 600 cm/hr (Figure 3A). These properties also help Nuvia S outperform other CEX resins (Figure 3B) at different flow rates, making Nuvia S capable of high-throughput process manufacturing. Refer to bulletin 5984 for details on the experiment. Customer testing of Nuvia S Resin capture showed not only its high DBC but also its high salt tolerance and minimal feed conditioning requirement (Table 2).

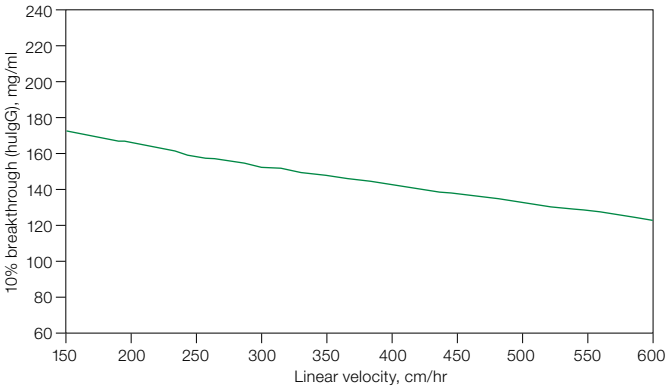

Fig. 3A. Binding of huIgG by Nuvia S Resin at varying linear velocities. Column size, 1.1 x 20 cm.

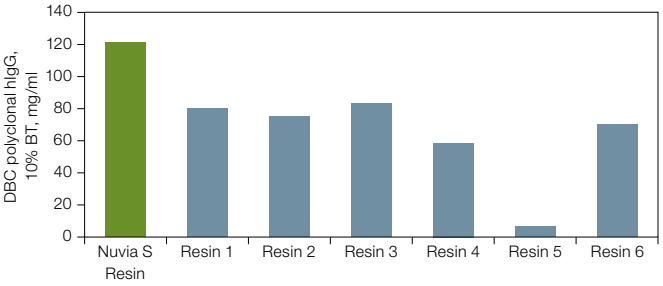

Fig. 3B. Binding of polyclonal human IgG by Nuvia S Resin. Comparison of Nuvia S and other commercially available CEX resins. Column size, 0.7 x 5.5 cm. The sample was loaded onto the column in 40 mM sodium acetate, pH 5.0 + 30 mM sodium chloride, washed, and then eluted with 40 mM sodium acetate, pH 5.0 + 1 M sodium chloride. DBC, dynamic binding capacity; BT, breakthrough.

Table 2. Evaluation of the DBC and high salt tolerance of Nuvia S.

Evaluation	Monoclonal Antibody	DBC, mg/ml, at 10% BT	Experimental Details
Bio-Rad evaluation	mAbK	100	mAb capture, pH 5.0, 5.8 mS/cm; HCP clearance, 2.8 logs; DNA clearance, 1.7 logs
Customer 1 evaluation	mAb3	100–120	mAb capture, pH 4.5–5.0, 10–12 mS/cm
Customer 2 evaluation	mAb5	100	mAb capture; no dilution of crude culture filtrate

Single-Step Removal of Host Cell–Related Impurities

Protein A–based chromatography resins provide significant clearance of impurities. To demonstrate the ability of Nuvia S to serve the same function and to produce highly purified target biomolecules after a typical three-step purification process, five different clones of a mAb with isoelectric points from 6 to 9 were subjected to Nuvia S capture purification (Ng and Snyder 2012). HCP reduction of more than two orders of magnitude was obtained (Table 3). Significant removal of hcDNA was seen during the intermediate and final polish steps. SEC analysis of the purified sample showed the absence of soluble dimers or higher order aggregates, confirming the purity obtained with this process (data not shown).

Table 3. Concentrations of contaminants across three-step purification of monoclonal antibody.

Step	Host Cell Protein, ng/mg	DNA, ng/mg
Clarified TCF (diluted)	>50,000	7,500
Post-Nuvia S	469	6,803
Post-Nuvia Q	51	18
Post-CHT	11.3	0.3

Extensive Reusability and Consistent Performance

The use of the same resin for multiple cycles increases the cost efficiency of the purification process. Nuvia S Resin is more resistant to sodium hydroxide than Protein A resins. As an example of this, Ng and Snyder (2012) show overlays of chromatographic profiles produced by purifying hIgG on a Nuvia S Column for more than 50 cycles, which included a sanitization step of 1 N NaOH. The chromatography profile captured after every 5 cycles showed consistent UV absorbance data and no significant change in resin performance.

Accelerated storage studies with 1 N NaOH over a period of 840 hr (equivalent to 280 cycles with a 3 hr hold) showed no decrease in the DBC or recovery of hIgG (Figure 4), indicating that Nuvia S can withstand repeated exposure to commonly used sanitization and clean-in-place protocols.

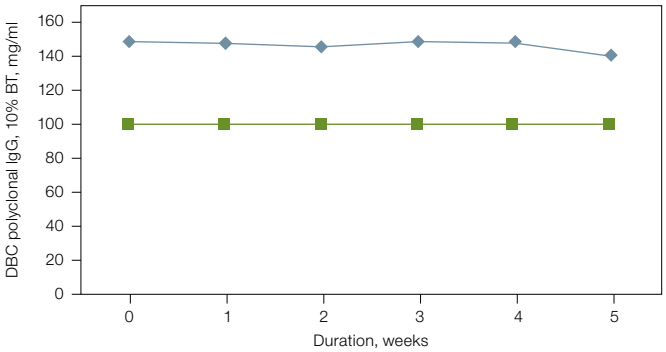

Fig. 4. Stability of Nuvia S. Results from an accelerated storage study (in 1 N NaOH) show no loss in dynamic binding capacity and recovery. hIgG binding capacity, 10% breakthrough (◆); recovery (%) (■); BT, breakthrough; DBC, dynamic binding capacity.

Conclusions

Although Protein A chromatography is frequently employed for capture purification of antibodies, its effectiveness and usefulness in contemporary manufacturing-scale purification is hampered by its high cost, base instability, and ligand leaching. Cation exchange resins, such as Nuvia S, which does not have these limitations, provide a highly practical alternative. Nuvia S provides specificity, scalability, and consistency at relatively low costs and can be operated with simple buffers at high working flow rates. In addition, Nuvia S elution can be effected using a change in pH in buffers with low ionic strength. This eliminates the requirement for a buffer exchange before the next chromatography step, which can also produce significant savings in equipment and labor at process scale. Find the complete technical features list for Nuvia S Resin in bulletin 5987.

References

Ng PK and Snyder MA (2012). pH-based cation exchange chromatography in the capture and elution of monoclonal antibodies. J Sep Sci 35, 29–35.

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