Nuvia™ S Media: A High-Capacity Cation Exchanger for Process Purification of Monoclonal Antibodies

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Introduction

Nuvia S is a high-capacity strong cation exchanger (CEX) designed for optimal bioprocessing performance. It is built on the macroporous rigid UNOsphere™ base matrix, through the incorporation of polymeric surface extenders with optimized density of sulfonate groups. Such an approach gives Nuvia S excellent dynamic binding characteristics for target biomacromolecules and low backpressure at the high flow rates required for modern downstream processes.

This application note describes the effects of flow rate, load pH, and conductivity on the binding of polyclonal human immunoglobulin G (huIgG) and monoclonal antibodies (mAbs) by this new chromatographic media. Preliminary studies on the removal of product-related impurities using Nuvia S were also conducted. Our data indicate that this cation exchanger maintains very high binding capacity over a broad range of linear flow rates and variations in load composition, providing the robustness needed for initial capturing of mAbs from cell culture harvest. The resolution power of Nuvia S also makes it an outstanding choice for intermediate polishing operations.

Material and Methods

Determination of Binding Capacity of Nuvia S for Human Immunoglobulins

Nuvia S was packed in a 1.1 x 20 cm column and equilibrated with 40 mM sodium acetate and 30 mM sodium chloride (pH 5.0). HuIgG (Sigma-Aldrich) was dissolved in the same buffer to a final concentration of 4.9 mg/ml, filtered through a 0.2 µm membrane, and then loaded onto the column at linear velocities of 150, 300, or 600 cm/hr, respectively. The dynamic binding capacities of huIgG at 10% breakthrough were determined by in-line UV absorption at 280 nm on a BioLogic DuoFlow™ system (Bio-Rad Laboratories, Inc.) (Figure 1A). For comparative study, Nuvia S and commercially available cation exchangers were packed into 10 ml columns (1.1 x 9.5 ± 0.3 cm). Their binding capacities for huIgG at linear velocities of 300 and 600 cm/hr were also determined (Figure 1B).

Binding of Human Immunoglobulin G by Nuvia S as a Function of Load pH and Conductivity

Sodium chloride was added to 20 mM sodium acetate (pH 4.5) to prepare binding buffers with conductivities of 5, 10, or 15 mS/cm at room temperature, as determined by a CDM210 conductivity meter (Radiometer Analytical). Nuvia S was packed into columns (0.7 x 5.5 cm) and equilibrated with the binding buffers to be studied. HuIgG was dissolved in a matching buffer to 5 mg/ml, filtered through a 0.2 µm membrane, and then loaded at 300 cm/hr. Dynamic binding capacities at 10% breakthrough were determined by in-line UV absorption at 280 nm on a BioLogic DuoFlow system (Figure 2).

Capture of mAbG from cell culture media by Nuvia S and Effect of Conductivity on its Dynamic Binding Capacity

Cell culture feed stock containing mAbG was reconstituted by diafiltering purified mAbG against 10 volumes of fresh cell culture media (HAM's nutrient mixture F-12, Sigma-Aldrich), using a Biomax 50K Pellicon XL ultrafiltration membrane (Millipore Corporation) on a Labscale TFF system (Millipore). The retentate was clarified with a 0.2 µm filter, and the final concentration of mAbG was 4 mg/ml. This reconstituted cell culture feed stock was conditioned by mixing with DI water, 1 M acetic acid, and 1 M sodium acetate (pH 5.0) to a final pH of 5.0 and conductivity of 7.08 mS/cm or 9.34 mS/cm. A 1 ml column (0.7 x 2.7 cm) packed with Nuvia S was equilibrated with 10 CV of 40 mM sodium acetate (pH 5.0). This preconditioned feed stock was loaded onto the column at 300 cm/hr, and the dynamic binding capacity of mAbG at 10% breakthrough was determined by in-line UV absorption at 280 nm on a BioLogic DuoFlow system.

Removing Degradation Fragments of mAbR with Nuvia S

The chromatographic separation of degradation fragments from intact mAbR was conducted on a BioLogic DuoFlow system (Figure 3). A 1 ml column (0.56 x 4 cm) packed with Nuvia S was equilibrated with 15 CV of 20 mM sodium acetate and 20 mM sodium chloride (pH 4.7, Buffer A). A Chinese hamster ovary (CHO) cell culture harvest containing mAbR was diluted with DI water to reduce its conductivity to 7.91 mS/cm. This crude material was adjusted to pH 4.7 with 1 M phosphoric acid, clarified by filtration through a

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0.2 μm filter, and then loaded onto the pre-equilibrated column at 300 cm/hr. The post-loading wash was performed with 15 CV of Buffer A, followed by 15 CV of 20 mM sodium acetate and 76 mM sodium chloride (pH 4.7, Buffer B). The bound mAbR was eluted in 15 CV of 20 mM sodium acetate and 188 mM sodium chloride (pH 4.8, Buffer C). The used column was stripped with 5 CV of 1 N NaOH. Protein fractions were analyzed by SDS-PAGE under reducing conditions, using a Criterion™ Tris-HCl 4–20% gradient gel (Bio-Rad) followed by staining with Bio-Safe™ Coomassie stain (Bio-Rad). Impurities in fractions collected during the Buffer B wash were identified by mass spectroscopy using an Autoflex II MALDI-TOF mass spectrometer (Bruker Corporation). These column fractions were pooled and analyzed with a size-exclusion high performance liquid chromatography (SEC-HPLC) column on a BioLogic DuoFlow system. The integrity of mAbR recovered in the eluate pool was also examined in the same manner.

Removing High Molecular Weight Aggregates of mAbG with Nuvia S

The chromatographic separation of high molecular weight aggregates from monomeric mAbG was performed on a BioLogic DuoFlow system (Figure 4). A 1 ml column (0.56 x 4 cm) packed with Nuvia S was equilibrated with Buffer A. A mAbG stock solution was diluted with DI water to reduce its conductivity to 4.4 mS/cm, followed by pH adjustment to 4.7 with 1 M acetic acid. This solution was then loaded onto the column at 300 cm/hr. After washing with 5 CV of 20 mM sodium acetate and 160 mM sodium chloride (pH 4.7, Buffer D), mAbG was eluted in 20 mM sodium acetate and 245 mM sodium chloride (pH 4.9, Buffer E). Three CV of 1 N NaOH were used to strip the column following purification. Contents of high molecular weight aggregates in the load and eluate pool from Nuvia S were analyzed using the SEC-HPLC method described above.

Results and Discussion

Nuvia S, like all UNOsphere-based chromatography media, is characterized by its excellent flow properties and fast mass transfer. Surface extenders grafted into the pore space of the Nuvia S matrix make the sulfonic acid ligands more available to electrostatic interaction with opposite charges on the incoming antibody molecules. As shown in Figure 1, Nuvia S exhibited efficient binding for huIgG in the linear velocity range of 150 to 600 cm/hr, significantly outperforming all commercial CEX media tested. The huIgG binding capacity on Nuvia S was found to be 114 mg/ml even at a high flow rate of 600 cm/hr. Such performance allows Nuvia S to deliver the speed and throughput needed in process manufacturing of mAbs. For initial capture, operation at high flow-rates offers an additional advantage of minimizing the target antibody's exposure to proteases and nucleases present in the cell culture feedstream.
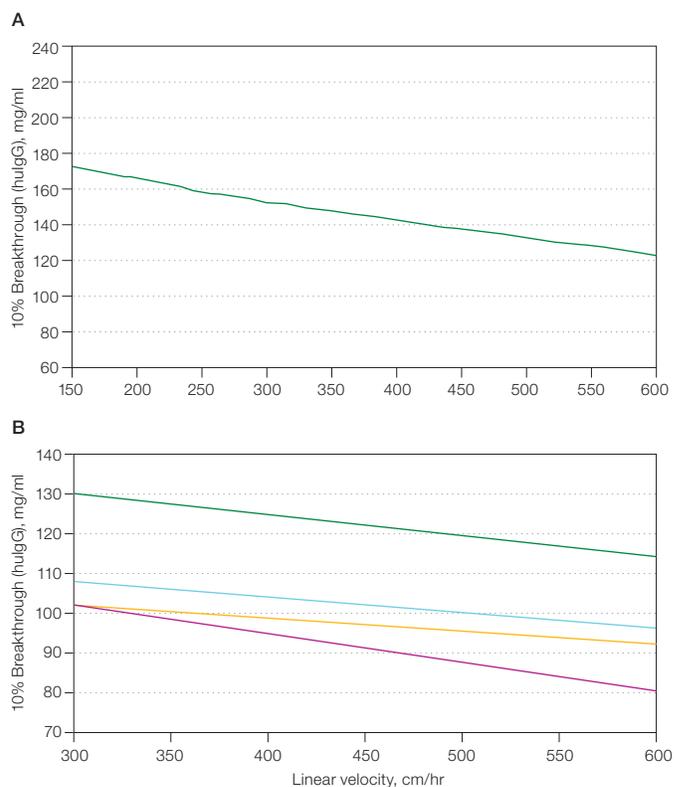
Fig. 1. Binding of huIgG by Nuvia S media at various linear velocities. A, binding on a Nuvia S column (1.1 x 20 cm). **B,** comparison of Nuvia S with commercially available CEX media. (—), Nuvia S; (—), CEX 1; (—), CEX 2; (—), CEX 3. Column size, 1.1 x 9.5 ± 0.3 cm.

The pI value of a monoclonal antibody as well as the pH and conductivity of the product pool determine the charge state of both antibody molecules and ligands on CEX media, and hence the strength of electrostatic interaction between them. The effect of load conductivity was studied at three different pH values. At pH 4.5, the maximal binding capacity of huIgG on Nuvia S was achieved at 10 mS/cm instead of 5 mS/cm, which is contradictory to the intuitive belief that binding to CEX media is promoted by lowering buffer conductivity. This phenomenon has been well documented and thoroughly studied (Harinarayan et al. 2006, Stone and Carta 2007, Stone et al. 2009, Tao and Carta 2008, Yuan et al. 2000). In our experiments, increasing operation pH to 5.0 led to higher binding capacity of huIgG at 5 mS/cm. However, further elevation of conductivity or pH weakened the interaction between huIgG and charged functionalities on the media, resulting in a dramatic decrease in binding capacity at 15 mS/cm or pH 5.5 (Figure 2).

In light of the interdependence of load pH and conductivity, dynamic binding capacity of a mAb should be determined with multiple pH-conductivity combinations during design of experiments (DoE) process development and optimization.
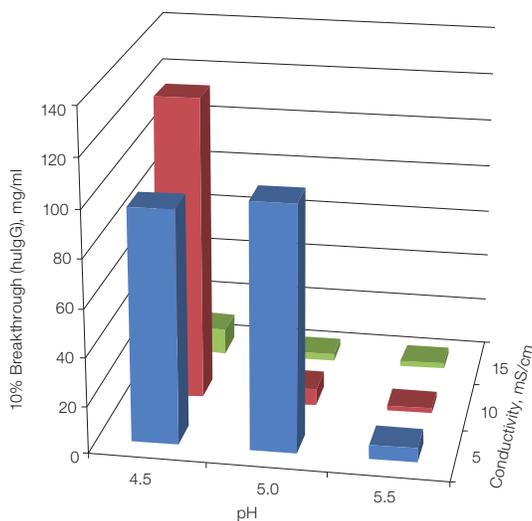
Fig. 2. Dynamic binding capacity of huIgG by Nuvia S media as a function of load, pH, and conductivity. Nuvia S was packed into 0.7 x 5.5 cm columns and equilibrated with the appropriate binding buffer. HuIgG was loaded in the same buffer at a flow rate of 300 cm/hr.

A typical mAb harvest from mammalian expression culture contains a substantial amount of sodium chloride, requiring preconditioning or in-line dilution to reduce its conductivity before loading onto a cation exchange column. Sensitivity to conductivity may be different for individual mAbs. At pH 5.0, the binding of a basic monoclonal antibody mAbG by Nuvia S was tested at 7.1 and 9.3 mS/cm, which corresponds to 50–75 mM of sodium chloride in solution.

The 10% breakthrough binding capacity decreased only slightly, from 130 to 115 mg/ml, when conductivity was changed from 7.1 to 9.3 mS/cm, providing consistent capture of mAbG with Nuvia S over a relatively wide conductivity window.

Chromatographic purification of mAbR with a Nuvia S column is shown in Figure 3. Initial elution studies using a linear gradient revealed that some contaminants were weakly bound by this media. They could be selectively desorbed from the column by low ionic strength buffers (data not shown). A step elution protocol was developed on the basis of this finding (Figure 3A). The predominant impurity, removed by a wash step with Buffer B (Figure 3B), was identified by MALDI-TOF mass spectrometry as a degradation fragment from mAbR's L chain. The intact mAbR was recovered in a salt-step elution by Buffer C (Figure 3B), free of fragments or aggregates as revealed by SEC-HPLC analysis (Figure 3C).

The potential use of Nuvia S for the clearance of higher-order mAb structures has been explored using mAbG, a monoclonal antibody that tends to aggregate during acidic elution from a Protein A affinity chromatographic column. The loaded material in this study contained 13.8% of mAbG aggregates. A salt-step elution was designed to recover monomeric mAbG, while retaining aggregates on the column until stripping with 1 N NaOH (Figure 4). The overall yield of monomeric mAbG in such chromatographic preparation was 93%, and the aggregate contamination level was effectively reduced to <1% (Figure 4 inset).
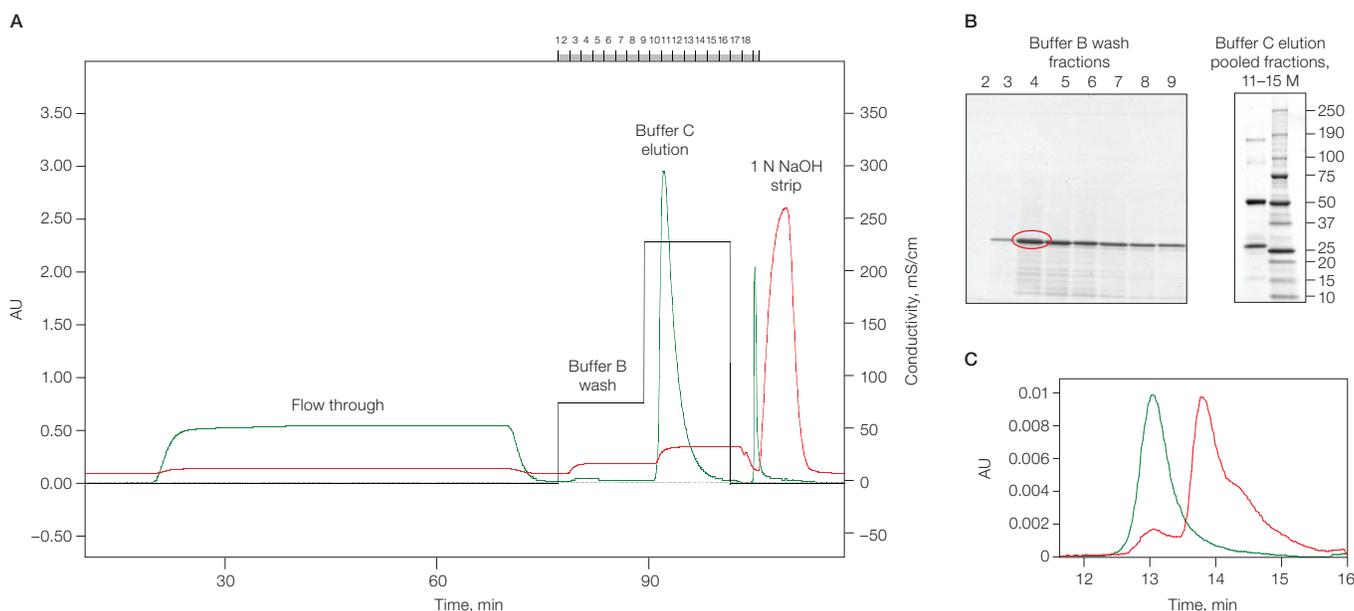

Fig. 3. Capture of mAbR from CHO cell culture using Nuvia S media. A, Chromatogram of mAbR purification by cation exchange chromatography on a Nuvia S column. (—), OD$_{280}$; (—), conductivity; (—), salt steps. **B**, SDS-PAGE of Buffer B wash fractions (2–9) and Buffer C elution fractions (11–15). M, Precision Plus Protein™ standard. The major protein impurity removed by wash with Buffer B (circled in red) was excised from gel and analyzed by MALDI-TOF mass spectroscopy. **C**, SEC-HPLC analysis of contaminants removed by Buffer B wash (fractions 2–9, —) and mAbR monomer eluted from the column by Buffer C (fractions 11–15, —).
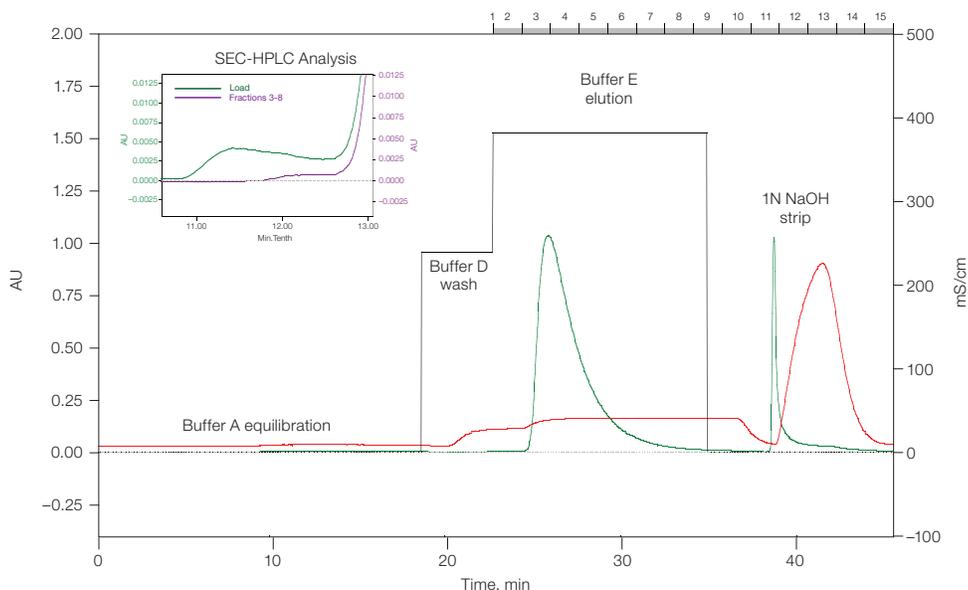
Fig. 4. Intermediate polishing of mAbG by Nuvia S media: Removal of mAbG aggregates. Both loaded material and pooled eluate fractions were analyzed by SEC-HPLC to assess the contamination level of mAbG aggregates. (**—**), OD$_{280}$; (**—**), conductivity; (**—**), salt steps.

Conclusion

Increasing process productivity and reducing operating costs continue to be the driving forces in process development. Recent advances in upstream processes have dramatically improved the productivity of cell culture fermentation. However, prolonged fermentation and high concentration of mAb molecules at harvest may also lead to product degradation and/or aggregation. These are the latest challenges for downstream processing of such drug materials. Binding capacity and purification performance are the most important considerations in selecting chromatographic media. Media with high capacity for target molecules and resolution power for impurity clearance are in demand. Our new high-capacity strong cation exchanger Nuvia S is suitable for both capturing and polishing steps in the manufacturing of monoclonal antibodies over a wide range of operating conditions. It offers the capacity and speed for processing large load volume with smaller columns and less buffer consumption. Additionally, Nuvia S is an exceptional tool for removing product-related contaminants such as mAb fragments and aggregates.

References

Harinarayan C et al. (2006). An exclusion mechanism in ion exchange chromatography. Biotechnol Bioeng 95, 775–787.

Stone MC and Carta G (2007). Protein adsorption and transport in agarose and dextran-grafted agarose media for ion exchange chromatography. J Chromatogr A 1146, 202–215.

Stone MC et al. (2009). Protein adsorption and transport in agarose and dextran-grafted agarose media for ion exchange chromatography: Effect of ionic strength and protein characteristics. J Chromatogr A 1216, 4465–4474.

Tao Y and Carta G (2008). Rapid monoclonal antibody adsorption on dextran-grafted agarose media for ion-exchange chromatography. J Chromatogr A 1211, 70–79.

Yuan Y et al. (2000). Size does matter: Electrostatically determined surface coverage trends in protein and colloid adsorption. Colloids and Surfaces A: Physicochemical and Engineering Aspects 165, 125–141.

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