Virus Reduction and Impurity Removal Potential of Nuvia aPrime 4A Resin

William H. Rushton,[1] Xuemei He,[1] Irene Chen,[1] Sharon Bola,[1] Akunna Iheanacho,[2] Coral Fulton,[2]
Jenifer Dean,[2] William Kerns,[2] Kaye Peden,[2] Katelyn Pritchard,[2] Erin Reynolds,[2] and Sreyry Schaerdel[2]
[1]Bio-Rad Laboratories, Inc., 6000 James Watson Drive, Hercules, CA 94547
[2]Texcell North America, Inc., 4991 New Design Road, Frederick, MD 21703

Abstract

We utilized a design of experiments (DOE) approach to investigate the effect of buffer pH and conductivity on the clearance of the minute virus of mice (MVM) as well as the removal of other product- or process-related impurities by the mixed-mode chromatography Nuvia aPrime 4A Resin. Results from this study have offered insights on the interactions between the resin and MVM particles, as well as the design space for the removal of this virus. Although there is no single solution for every product or purification process, practical considerations for developing a mixed-mode chromatography process with effective impurity clearance will be discussed in this application note.

Introduction

Incorporating Viral Safety During Downstream Processing

For all biotechnology products derived from cell lines of human or animal origin, there is a risk of viral contamination from the source material or from adventitious agents during the production process. Adventitious agents, including viruses, can enter a production process from different routes. Various strategies have been employed to reduce the risk of contamination during the purification process. Virus reduction, or viral clearance, involves the removal or inactivation of viral contaminants and is one of the strategies employed to enhance product safety.

The main objectives of viral clearance studies are to evaluate process steps that can be considered effective in removing or inactivating viruses and to quantitatively estimate the overall level of virus reduction obtained by the manufacturing process. Regulatory authorities require that manufacturers of biotherapeutics perform a risk assessment and evaluate orthogonal process steps dedicated to virus inactivation and/or virus removal.

The downstream processing of a monoclonal antibody (mAb) relies heavily on chromatographic separation. A variety of ion-exchange, affinity, size exclusion, hydrophobic interaction, and mixed-mode chromatography resins are available from many commercial vendors and are designed to remove product- and process-related impurities. Product-related impurities for mAbs include aggregates or fragments, charge variants, and posttranslational modification variants. Process-related impurities include host cell proteins (HCPs), nucleic acids from host cells (hcDNA/RNA), endotoxins, viruses, and if affinity capture is used, fragments of recombinant Protein A. Because obtaining adequate viral clearance while expediting process development is still a concern among process engineers and developers, Bio-Rad Laboratories, Inc., in collaboration with Texcell North America, Inc., evaluated the virus reduction potential of the mixed-mode chromatography Nuvia aPrime 4A Resin.

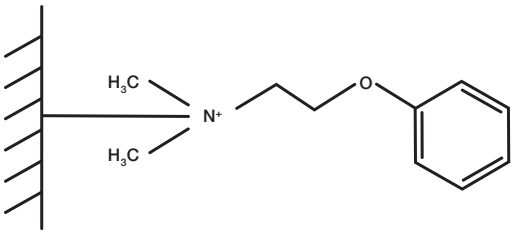

Fig. 1. Mixed-mode ligand for Nuvia aPrime 4A Resin.

Characteristics of Nuvia aPrime 4A Resin

Nuvia aPrime 4A is a hydrophobic anion exchange resin. The resin is designed with a positively charged hydrophobic functional ligand (Figure 1). This functional ligand can interact with biomolecules through electrostatic and hydrophobic interactions.

The resin particles have open pores to allow efficient mass transfer and are mechanically strong. These features make Nuvia aPrime 4A Resin suitable for downstream processing, as the purification step can be operated at fast flow rates. All technical properties of this resin are listed in Table 1.

Table 1. Properties of Nuvia aPrime 4A Resin.

Property	Description
Functional group	Aromatic hydrophobic anion exchanger
Base matrix composition	Macroporous highly crosslinked polymer
Median particle size	50 ± 10 µm
Ligand density	100 ± 20 µeq/ml
Dynamic binding capacity (DBC)*	≥50 mg/ml at 300 cm/hr
Recommended linear flow rate	50–300 cm/hr
Pressure-flow performance**	Under 3 bar at flow rate of 300 cm/hr
Compression factor	1.1–1.25
pH stability***	Short-term: 2–14
Shipping solution	20% ethanol + 1 M NaCl
Regeneration	1 M NaOH
Sanitization	1 M NaOH
Storage conditions	20% ethanol
Storage temperature	Room temperature
Chemical stability†	1 M NaOH, 1 M HCl, 25% acetic acid, 8 M urea, 6 M guanidine HCl, 3 M NaCl, 1% Triton X-100, 20–70% ethanol, 30% isopropanol
Shelf life††	5 years

* 10% breakthrough capacity determined with 1.0 mg/ml of an acidic mAb (pI ~6.9) in 20 mM $NaPO_4$, pH 7.8.
** 20 x 20 cm packed bed (1.25 compression factor).
*** No significant change in ligand density after 200 hr contact at 22ºC.
† No significant change in ligand density after 1 week contact at 22ºC.
†† Stored at room temperature in 20% ethanol + 1 M NaCl.

Nuvia aPrime 4A chromatography can be operated in both bind and elute mode and flow-through mode. Basic proteins that have a high pI would carry a positive charge at neutral pH, which will cause repulsion with the resin/ligand, and these proteins will be present in the flow-through fractions. Conversely, acidic species will have a negative charge at neutral pH and will bind to the ligand. Target proteins with a low pI, HCPs, hcDNA/RNA, or endotoxins will bind to the resin through hydrophobic and electrostatic interactions. Depending upon the pI and surface hydrophobicity of the target molecule, buffer pH and conductivity can be manipulated to achieve the desired protein selectivity and recovery.

Experimental Approach

A DOE approach was taken for a basic mAb (pI 9.0) to investigate the effect of buffer pH, NaCl concentration, residence time, and feedstream load level to remove impurities, such as MVM, HCPs, dsDNA, endotoxin, and mAb aaggregates, using Nuvia aPrime 4A Resin. Initial experiments were performed with Mini Bio-Spin Columns, each containing 100 µl of Nuvia aPrime 4A Resin, to optimize process conditions. A two-level full factorial screening design suggested by JMP Software was employed. A total of 18 different conditions with buffers in the pH range of 6.0–8.0 and NaCl concentrations up to 150 mM were evaluated (Table 2). Before sample application, spin columns containing resin were equilibrated with the appropriate buffer. Each feedstream was spiked with MVM and applied to the spin column. The load level of the feedstream ranged from 30–70 mg/ml and was incubated with resin to mimic a residence time of either 3 or 7 minutes. At the end of the incubation period, the flowthrough was collected, and samples were assayed for virus titer using infectivity assays. The results are shown in the contour plot from the DOE studies (Figure 2).

Table 2. DOE setup for flow-through mode.

Experiment Number	Buffer pH	Buffer [NaCl], mM	Residence Time, min	Column Load, mg/ml
1	6	150	3	70
2	8	150	7	30
3	6	150	7	70
4	6	0	7	30
5	8	0	7	70
6	8	0	3	30
7	7	75	5	50
8	6	150	3	30
9	6	0	3	30
10	8	0	3	70
11	8	150	7	70
12	8	150	3	70
13	6	0	3	70
14	8	150	3	30
15	6	0	7	70
16	8	0	7	30
17	7	75	5	50
18	6	150	7	30

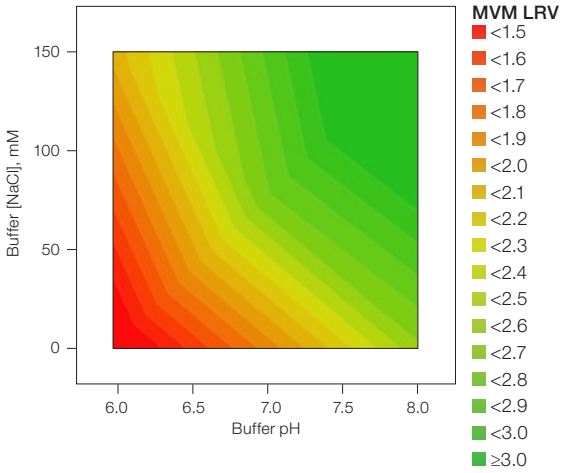

Fig. 2. Contour plot from DOE studies. MVM LRV, minute virus of mice log reduction value.

To confirm the results of the spin column DOE studies, follow-up experiments were performed with packed Foresight Columns (inner diameter: 0.8 × 10 cm, 5 ml). pH and NaCl concentration [NaCl] (or conductivity) were evaluated. Each buffer condition evaluated is listed in Table 3. Column loads were spiked with either MVM (experiments 1–4) or xenotropic murine leukemia virus (XMuLV) (experiment 5). MVM is a nonenveloped, single-stranded DNA parvovirus that ranges in size from 18 to 26 nm. XMuLV is a highly charged, enveloped, single-stranded RNA retrovirus that ranges in size from 80 to 120 nm.

Table 3. Experimental conditions: scale-up studies with Bio-Rad Foresight Columns.

Experiment Number	Buffer Condition	Spiking Virus	Test Article
1	10 mM sodium phosphate, 150 mM NaCl, pH 8	MVM	mAbX
2	10 mM sodium phosphate, 200 mM NaCl, pH 8	MVM	
3	10 mM Bis-Tris, 150 mM NaCl, pH 8	MVM	
4	10 mM sodium phosphate, pH 6	MVM	
5	10 mM sodium phosphate, 150 mM NaCl, pH 8	XMuLV	

mAbX, monoclonal antibody X; MVM, minute virus of mice; XMuLV, xenotropic murine leukemia virus.

Before sample application, Foresight Columns were equilibrated with the appropriate buffer. A residence time of 5 min was utilized with a load ratio of 50–55 mg/ml of resin. Each run was performed in flow-through mode and all chromatography was performed using an AKTA Pure Fast Protein Liquid Chromatography (FPLC) System.

Results and Discussion

A DOE approach was used to establish effective clearance conditions for MVM and product- and process-related impurity clearance with the mixed-mode chromatography resin, Nuvia aPrime 4A Resin. As MVM contains capsid proteins with a pI of 6.1–6.2, there is a net negative charge on the surface of the protein at near neutral pH (6–8). The contour plot illustrates that the upper right quadrant with high pH and high NaCl concentration leads to greater MVM log reduction value (LRV). These results demonstrate that MVM is more effectively retained by Nuvia aPrime 4A Resin at higher pH and NaCl concentrations. Buffer pH and conductivity have the potential to impact electrostatic as well as hydrophobic interactions. At pH 8, the virus particles interact with the quaternary amines of the Nuvia aPrime 4A ligand electrostatically. The hydrophobic interaction is enhanced by the higher NaCl concentration in the buffer. Residence time did not seem to have much effect on MVM removal while the load ratio did show a slight positive effect on MVM clearance (Figure 3).

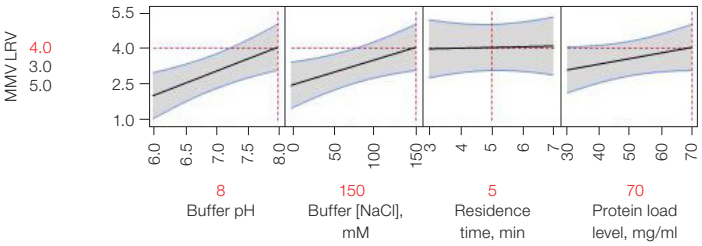

Fig. 3. Impact of pH, NaCl concentration, residence time, and load ratio on MVM clearance. MVM LRV, minute virus of mice log reduction value.

To verify the results of the DOE, further studies were executed using packed scale-up columns. A summary of the viral clearance results for column loads spiked with MVM is included in Table 4. In brief, all buffer conditions resulted in greater than 4.2 logs of clearance with the exception of one buffer condition. This buffer condition (10 mM sodium phosphate, pH 6.0) achieved only 2.23 logs of clearance and was included as a negative control to demonstrate confirmation of a predicted trend. A summary of the viral clearance results for the column load spiked with XMuLV is included in Table 5. For XMuLV, only one buffer condition was tested (10 mM sodium phosphate, 150 mM NaCl, pH 8.0) and greater than 5.07 logs of clearance was achieved. The results of the runs with Foresight Columns are in good agreement with the spin column DOE predictions. The mixed-mode chromatography step with Nuvia aPrime 4A Resin was effective in removing both MVM and XMuLV.

Buffer pH and NaCl concentration also effect the elimination of other impurities during Nuvia aPrime 4A chromatography. Running a flow-through purification step at higher pH and NaCl concentrations may enhance the clearance of dsDNA and mAb aggregates while causing a minor compromise in endotoxin removal (Figure 4). These results demonstrate the overall impurity clearance efficiency of Nuvia aPrime 4A Resin as an intermediate polishing step for purification of a mAb. Investigational viral clearance studies can assist in defining the design space of a process step that targets product recovery and impurity removal.

Table 4. Removal of MVM by mixed-mode chromatography.

	Calculations for log Reduction Value			
Mixed-Mode Chromatography (Nuvia aPrime 4A Resin)	**Experiment 1**	**Experiment 2**	**Experiment 3**	**Experiment 4**
log_{10} total virus of hold control	7.25	7.53	7.67	7.43
Minus the log_{10} total virus of product	<3.04	<3.00	<2.97	5.20
log reduction value	>4.21	>4.53	>4.70	2.23

Table 5. Removal of XMuLV by mixed-mode chromatography.

	Calculations for log Reduction Value
Mixed-Mode Chromatography (Nuvia aPrime 4A Resin)	**Experiment 5**
log_{10} total virus of hold control	7.22
Minus the log_{10} total virus of product	<2.15
log reduction value	>5.07

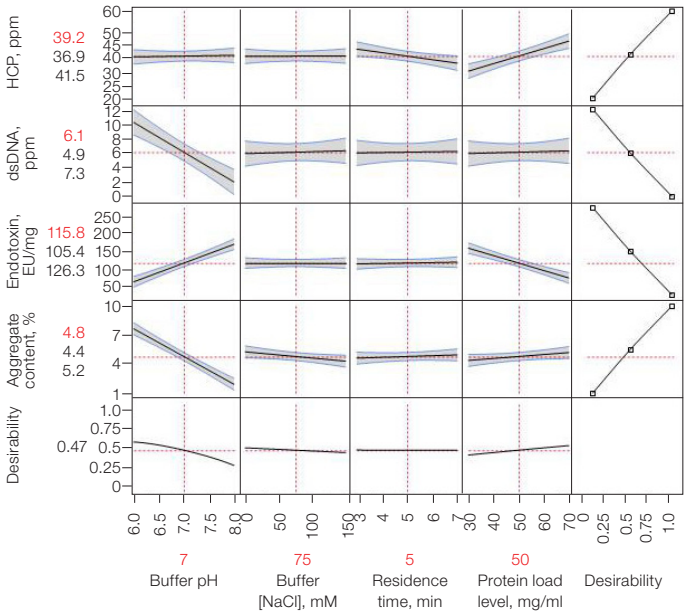

Fig. 4. Effects of buffer composition and operational parameters on impurity removal. HCP, host cell protein.

Conclusions

Regulatory authorities require viral clearance evaluations for manufacturing unit operations. Therefore, the virus removal or virus inactivation capacity of these operations should be determined during process development.

Studies using a DOE approach were performed to establish effective viral clearance conditions for a mAb using Nuvia aPrime 4A Resin. The viral clearance studies, as well as the overall impurity clearance achieved, demonstrate the efficiency of Nuvia aPrime 4A Resin as an intermediate polishing step.

Bibliography

Viral assay procedures
Blair GE (1985). Virology: A Practical Approach, Mahy BWJ, ed. (Oxford: IRL Press, Ltd.).

Guidance for testing worst-case conditions for virus inactivation steps
Chinniah S et al. (2016). Characterization of operating parameters for XMuLV inactivation by low pH treatment. Biotechnol Prog 32, 89–97.

European guidance for design of viral clearance studies
Committee for Proprietary Medicinal Products (1996). The European Agency for the Evaluation of Medicinal Products. Note for Guidance on Virus Validation Studies: The Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses. CPMP/BWP/268/95. https://www.ema.europa.eu/en/virus-validation-studies-design-contribution-interpretation-studies-validating-inactivation-removal, accessed August 31, 2021.

European guidance for virus safety testing and viral clearance
Committee for Proprietary Medicinal Products (2008). European Medicines Agency. Guideline on Virus Safety Evaluation of Biotechnological Investigational Medicinal Products. EMEA/CHMP/BWP/398498/2005. https://www.ema.europa.eu/en/virus-safety-evaluation-biotechnological-investigational-medicinal-products, accessed August 31, 2021.

Calculation of virus titer based on plaque assay data
Darling AJ (2000). Design and interpretation of viral clearance studies for biopharmaceutical products. Methods in Biotechnology 9, 195–209.

Virus safety of plasma-derived medicinal products
European Medicines Agency (2011). Guideline on Plasma-Derived Medicinal Products. EMA/CHMP/BWP/706271/2010. https://www.ema.europa.eu/en/plasma-derived-medicinal-products, accessed August 31, 2021

TCID50 assay calculation by Spearman-Karber method
Hubert JJ (1984). Spearman-Karber method. In Bioassay, 2nd edition (Dubuque, Iowa: Hunt Publishing Company), 65–66.

Statistical analysis of viral assays for viral clearance
Li N and Yang H (2012). Statistical evaluations of viral clearance studies for biological products. Biologicals 40, 439–444.

Retrospective evaluation of viral inactivation and filtration data from multiple collaborators
Mattila J et al. (2016). Retrospective evaluation of low-pH viral inactivation and viral filtration data from a multiple company collaboration. PDA J Pharm Sci Technol 70, 293–299.

Validation of analytical methods
United States Pharmacopeia (1995). Validation of Compendial Methods. USP 23, item 1225, 1,982–1,994. Rockville, MD: United States Pharmacopeial Convention, Inc.

Guidance for viral clearance
United States Pharmacopeia (2016). Design, Evaluation, and Characterization of Viral Clearance Procedures. USP 39-NF 34, item 1059.1. Rockville, MD: United States Pharmacopeial Convention, Inc.

Q5A U.S. guidance for virus safety testing and viral clearance
U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER) (1998). Guidance for Industry: Q5A Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin. https://www.fda.gov/media/71394/download, accessed August 31, 2021.

21 CFR Part 58, GLP
U.S. Food and Drug Administration, Department of Health and Human Services (2011). Good Laboratory Practice for Nonclinical Laboratory Studies. Title 21 Code of Federal Register Part 58. govinfo.gov/app/details/CFR-2011-title21-vol1/CFR-2011-title21-vol1-part58/summary, accessed August 31, 2021.

Guidance for viral clearance specifically for human blood plasma products
World Health Organization (2004). WHO Guidelines on Viral Inactivation and Removal Procedures Intended to Assure the Viral Safety of Human Blood Plasma Products. WHO Technical Report, Series No. 924. who.int/publications/m/item/WHO-TRS924-Annex4, accessed August 31, 2021.

Visit bio-rad.com/aPrime for more information.

Nuvia aPrime 4A Resin is covered by U.S. Patent Number 9,669,402 and foreign counterparts.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Website *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *00 800 00 24 67 23* ***Belgium*** *00 800 00 24 67 23* ***Brazil*** *4003 0399*
Canada *1 905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *00 800 00 24 67 23* ***Denmark*** *00 800 00 24 67 23* ***Finland*** *00 800 00 24 67 23*
France *00 800 00 24 67 23* ***Germany*** *00 800 00 24 67 23* ***Hong Kong*** *852 2789 3300* ***Hungary*** *00 800 00 24 67 23* ***India*** *91 124 4029300* ***Israel*** *0 3 9636050*
Italy *00 800 00 24 67 23* ***Japan*** *81 3 6361 7000* ***Korea*** *82 2 3473 4460* ***Luxembourg*** *00 800 00 24 67 23* ***Mexico*** *52 555 488 7670*
The Netherlands *00 800 00 24 67 23* ***New Zealand*** *64 9 415 2280* ***Norway*** *00 800 00 24 67 23* ***Poland*** *00 800 00 24 67 23* ***Portugal*** *00 800 00 24 67 23*
Russian Federation *00 800 00 24 67 23* ***Singapore*** *65 6415 3188* ***South Africa*** *00 800 00 24 67 23* ***Spain*** *00 800 00 24 67 23* ***Sweden*** *00 800 00 24 67 23*
Switzerland *00 800 00 24 67 23* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 2 651 8311* ***United Arab Emirates*** *36 1 459 6150* ***United Kingdom*** *00 800 00 24 67 23*

Bulletin 3254 Ver A US/EG *21-0596 0921 Sig 0121*