Virus Clearance by Salt-Tolerant Anion Exchange Chromatography in Flow-Through Mode

Verena Vollmar[1], Sabine Faust[1], Anja Trapp[1], Egbert Müller[2], Benjamin Zeitler[3], Alexander Faude[1]

[1]Rentschler Biopharma SE, Laupheim, Germany, verena.vollmar@rentschler-biopharma.com

[2]Tosoh Bioscience GmbH, Darmstadt, Germany

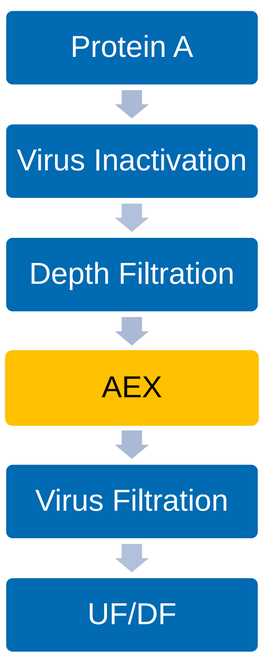
[3]Labor Dr. Merk & Kollegen GmbH, Ochsenhausen, Germany

Introduction

In order to ensure viral safety of biopharmaceutical products, the capacity of downstream processes to remove or inactivate viruses is determined by virus clearance studies. Anion exchange (AEX) chromatography in flow-through mode is commonly claimed in monoclonal antibody (mAb) purification processes for virus removal by ionic adsorption[1,2]. Aiming for intensified processes, salt-tolerant or multimodal AEX resins are more and more used as polishing steps providing an optimized selectivity for impurity removal.

TOYOPEARL® NH_2-750F (Tosoh Bioscience LLC) is a salt-tolerant AEX resin designed for mAb aggregate removal in flow-through mode. Polymeric beads have been functionalized with a primary amine-containing ligand providing different selectivity compared to conventional quaternary amine (Q)-based AEX resins. In contrast to commonly used pH range for virus removal of ≥ pH 6, optimal pH range for aggregate clearance depends on the mAb molecule and can be even lower. Thus, virus removal capacity of TOYOPEARL® NH_2-750F was determined at mild acidic pH and compared to the multimodal Capto™ adhere (GE Healthcare).

mAb Purification



Protein A affinity chromatography is commonly used for mAb capture followed by low pH virus inactivation. Subsequently, depth filtration is performed to remove precipitates. Aimed at the removal of aggregates, host cell proteins, leached protein A as well as viruses, multimodal AEX chromatography in flow-through mode can be applied as polishing step. By selection of an appropriate pH value, non-binding conditions for the mAb are achieved while impurities adsorb to the resin. The purification process is completed by virus filtration and ultra- and diafiltration (UF/DF).

Figure 1: Process scheme of a two-column mAb purification approach.

Virus Clearance Study Design

In order to prove robust virus removal at mild acidic pH, four relevant model viruses representing a broad range of physico-chemical properties (Table 1) were used:

Table 1: List of tested model viruses.

Virus	Size (nm)	Enveloped	Genome	Resistance
Murine Leukemia Virus (MuLV)	80 - 110	Yes	ssRNA	Low
Pseudorabies Virus (PRV)	120 - 200	Yes	dsDNA	Medium
Mammalian Reovius Type 3 (MRV-3)	60 – 80	No	dsRNA	Medium
Minute Virus of Mice (MVM)	18 - 24	No	ssDNA	Very high

The TOYOPEARL® NH_2-750F and Capto™ adhere resins had been packed to 20 cm bed height with a diameter of 0.5 cm. Chromatography runs were performed in flow-through mode at pH 5.25 - 6.00 (Table 2). To serve as starting material, a depth filtered mAb solution was titrated to a certain pH value and spiked with 1.0 % (v/v) pre-filtered virus. The column load was set to 200 $g_{Protein}L_{Resin}^{-1}$.

Relevant fractions were analyzed by $TCID_{50}$ endpoint dilution titration (EPT). To improve the detection limit due to low virus concentrations, large volume plating (LVP) infectivity assay was performed on the flow-through/wash fractions. In addition, virus stability over the experimental period was analyzed exemplary in load hold controls included in selected runs. All virus related work was performed by Labor Dr. Merk & Kollegen GmbH.

Table 2: Plan of chromatography conditions tested.

Chromatography Resin	MuLV	MRV-3	PRV	MVM
Toyopearl® NH_2-750F	pH 5.50* pH 6.00*	pH 5.50* pH 6.00*	pH 5.50* pH 6.00*	pH 5.25 pH 5.50* pH 5.75 pH 6.00*
Capto™ adhere	pH 5.50	pH 5.50	pH 5.50	pH 5.50

* Duplicate runs

Virus Removal by TOYOPEARL® NH_2-750F at Mild Acidic Conditions

At first, runs on TOYOPEARL® NH_2-750F at pH 5.5 were performed. Except for MVM, no residual infectious virus could be found in the flow-through/wash fractions (Figure 2), resulting in high removal capacity for MuLV, PRV and MRV-3. For comparison of the logarithmic reduction values (LRV) between the different model virus, it has to be taken into account that the total virus load to the column differed by type.

Due to low removal of the worst-case virus MVM at pH 5.5 (LRV_{MVM} ~ 2.1), next runs were performed at pH 6.0, leading to higher clearance of MVM (LRV_{MVM} ~ 6.7). Again, complete virus removal was observed for all other virus types.

In order to investigate the pH dependency of the MVM removal by TOYOPEARL® NH_2-750F resin, two additional runs were performed at pH 5.25 and 5.75, respectively. As expected, a low LRV_{MVM} of 1.4 was determined at pH 5.25. In contrast, at pH 5.75, an effective LRV_{MVM} of 4.1 was determined. All in all, it could be shown that the MVM removal improves with increasing pH value. For robust removal of MVM by this resin, a pH value ≥ 5.75 can be recommended.

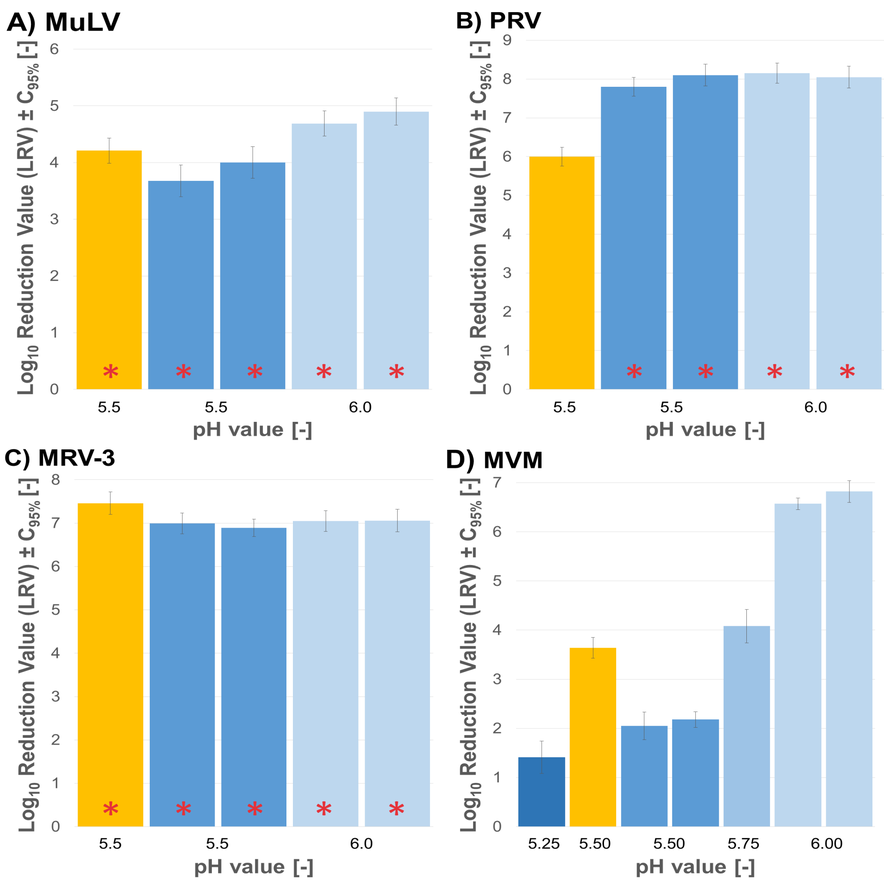


Figure 2: LRVs of AEX chromatography at different pH values on TOYOPEARL® NH_2-750F (blue) and Capto™ adhere (yellow), separated by virus type. Red signs (*) indicate that no residual virus was found in the flow-through/wash fraction.

For comparison, runs at pH 5.5 were performed with Capto™ adhere (Figure 2). High LRVs > 4 were reached for MuLV, PRV and MRV-3, even if residual PRV was found in the flow-through/wash fraction. Removal of MVM achieves an acceptable LRV_{MVM} of 3.6, which is comparable to MVM clearance of TOYOPEARL® NH_2-750F at pH 5.75.

Regarding regeneration, the phosphate buffer (0.4 M sodium phosphate, 1 M sodium chloride, pH 7.0) used was appropriate for TOYOPEARL® NH_2-750F, as most of the virus applied to the column could be recovered in the regeneration fraction (data not shown). In contrast to that, virus recovery by regeneration of Capto™ adhere was lower (LRV > 4). Reason might be a residual binding of viruses by hydrophobic interactions[3]. In case of Capto™ adhere, an appropriate buffer with high salt and acidic pH should be chosen for regeneration.

Conclusion

Using the salt-tolerant TOYOPEARL® NH_2-750F AEX chromatography resin, the examined model viruses can be successfully removed at mild acidic conditions (pH ≤ 6.0). In order to achieve effective clearance of the worst-case virus MVM, pH values ≥ 5.75 are recommended. The LRVs achieved are comparable to those by the multi-modal resin Capto™ adhere. Consequently, it is possible to achieve both, good virus and aggregate removal by this process step.

[1] Strauss *et al.* 2009. Biotechnol Bioeng 104(2): 371-380
[2] Miesegaes *et al.* 2014. Biotechnol Prog 30(1): 124-131
[3] Brown *et al.* 2017. Biotechnol Bioeng 114: 1487-1494

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