Viral clearance capability of cation exchange and recommendations

POROS cation exchange chromatography resins

Introduction

To assure product safety, regulatory agencies require a viral clearance assessment of the purification process for all biopharmaceutical products. Chromatography steps, commonly used in downstream bioproduction, can contribute to a process' ability to remove virus. Therefore, an assessment of the viral clearance capabilities of each step is valuable to address this regulatory requirement. This publication demonstrates the viral clearance capabilities of the various steps of a model cation exchange (CEX) chromatography unit operation and also discusses recommendations and considerations for designing viral clearance processes.

Virus selection, scale-down model, and experimental design

A viral clearance study should mimic the intrinsic and extrinsic viral risks associated with the product and include a selection of model viruses that vary in size, shape, genome type, and physicochemical resistance. Clearance of parvovirus and retrovirus is commonly tested prior to phase 1 for biological products derived from mammalian cell lines, including monoclonal antibody and recombinant protein products. The two most commonly used viruses that present with a range of viral characteristics were chosen for this study: minute virus of mice (MVM) and xenotropic murine leukemia virus (xMuLV). MVM is a nonenveloped, single-stranded DNA parvovirus that ranges in size from 18 nm to 26 nm. XMuLV is a highly charged, enveloped, single-stranded RNA retrovirus that ranges in size from 80 nm to 120 nm.

Salt tolerance of viral binding and/or eluting at the CEX chromatography step was evaluated using Thermo Scientific™ POROS™ XS Strong Cation Exchange Resin as an example to show viral clearance capability under higher-conductivity conditions. A similiar approach can be used to determine the viral clearance capabilities of Thermo Scientific™ POROS™ 50 HS Strong Cation Exchange Resin.

In addition, the CEX step was run at three different pH conditions: 4.5, 5.0, and 6.0—with the wash and elution solution pH matched to the load pH. Human IgG (MilliporeSigma Cat. No. G4386, 155–160 kDa, pI ~6.9) was used as the column load material for the model CEX process. After the post-load wash, the column was washed with higher-conductivity solutions in a stepwise manner.

The salt concentrations of the wash solutions evaluated are summarized in Table 1. The column format was 0.46 cm (D) x 20 cm (L), 3.3 mL. The study was conducted at 300 cm/hr at room termperature. The column was loaded with 80–100 mg IgG per mL of resin with a 5% virus spike. For each wash step, the entire pool was collected and evaluated for viral content. The viral log reduction or clearance was then calculated.

Table 1. Viral clearance on POROS XS Strong Cation Exchange Resin in bind-and-elute mode.

	Process conditions		
Load pH	6.0	5.0	4.5
Load salt concentration (mM)	25	25	100
Load capacity (mg/mL resin)	80	80	100
	$\log_{10}$ (xMuLV clearance)		
FT/wash	3.5	4.0	2.1
20 mM MES, 50 mM NaCl	ND	3.0	ND
20 mM MES, 100 mM NaCl	1.8	1.8	ND
20 mM MES, 200 mM NaCl	1.1	1.9	ND
20 mM MES, 300 mM NaCl	1.0	2.0	3.1
20 mM MES, 400 mM NaCl	ND	1.9	ND
20 mM MES, 500 mM NaCl	1.0	1.5	1.7
2 M NaCl	ND	1.1	ND

Column format: 0.46 cm (D) x 20 cm (L), 3.3 mL; flow rate: 300 cm/hr; load: 5 mg/mL human polyclonal IgG, 5% virus spike. FT = flow through, ND = not determined.

Viral clearance on CEX chromatography resin

In general, CEX chromatography does not provide robust viral clearance, as it is affected by specific process conditions and virus characteristics. Despite this, there are many industrial processes incorporating a Thermo Scientific™ POROS™ CEX chromatography resin that exhibit good viral clearance. Table 1 summarizes viral clearance on POROS XS resin for xMuLV. Under the study conditions, the salt concentration in the load appeared to have a greater impact on virus flow-through (lower clearance in FT/wash) than pH in the range tested. The load condition that had the best viral clearance was pH 4.5 with 100 mM NaCl when eluting with 300 mM NaCl. These conditions yielded 3.1 log reduction value (LRV) with a polyclonal IgG molecule that has a pI (6.9) that is lower than typical. Therefore, POROS CEX resins can deliver good viral clearance depending on the process conditions.

There are two main strategies for maximizing viral clearance on CEX resins:

1. Create conditions that drive the flow-through of virus and the binding of the target molecule.

2. Create conditions that drive the binding of both the target molecule and the virus, then switch to conditions that differentially elute the target molecule and the virus (typically, the retained virus is eluted during column cleaning).

Strategies for optimizing viral flow-through are noted below. These operating conditions may also promote the partitioning of other process impurities, such as aggregate, host cell protein, and leached protein A, into the flow-through, ultimately driving higher target molecule purity.

- Optimize the operating pH to drive flow-through of the virus and binding of the target molecule while considering binding capacity and yield. Viral particles typically have a low isoelectric point. Therefore, an operating pH that is 1 to 3 units below the pI of the target molecule is recommended. For example, most monoclonal antibodies have a pI between 8 and 9, so the best operating pH to facilitate flow-through of the virus would be between pH 5.5 and 7.5.

- Increase the conductivity of the load to 5–15 mS/cm to drive flow-through of the virus but binding of the target molecule. Increased conductivity can promote protein stabilization and improve impurity removal. The conductivity can be increased using sodium chloride or a higher buffer concentration, depending on the buffer system. The pH and conductivity of the column load, equilibration, and wash buffers should be matched.

The best bind-and-elute approach to retain the virus centers on loading at lower conductivity and low pH. Elution should be optimized to the lowest salt concentration possible, so as to elute the target molecule and retain the virus. Typically, less than 300 mM sodium chloride is optimal for target molecule elution and virus retention. Another approach in bind-and-elute mode is to load at low pH, then wash with a higher-pH buffer or a higher-salt wash to try to wash the virus off the column but retain the target molecule.

Conclusion

With POROS CEX chromatography resins, robust viral clearance can be attained. Understanding how process conditions can impact virus partitioning or removal in a CEX chromatography operation will provide increased flexibility when designing a purification scheme and help maximize viral clearance. Viral clearance can be achieved under higher-conductivity conditions. Improved salt tolerance, as seen on POROS XS resin, decreases the need for dilution of the feed stream or inclusion of a diafiltration step prior to loading on the column, potentially resulting in a more efficient and cost-effective process.

Speak to a technical specialist about how these resins can help you improve your current process at **thermofisher.com/purification-contact**

References

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Ordering information

Product	Quantity	Cat. No.
POROS XS Strong Cation Exchange Resin	25 mL	4404339
	50 mL	4404338
	250 mL	4404337
	1 L	4404336
	5 L	4404335
	10 L	4404334
POROS 50 HS Strong Cation Exchange Resin	50 mL	1335906
	250 mL	1335911
	1 L	1335907
	5 L	1335909
	10 L	1335908

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