Optimizing Virus Filter Performance with Prefiltration

by Todd Ireland, Glen Bolton, and Megumi Noguchi

Demonstrating viral clearance during biomanufacturing requires considering a number of factors including ease of validation, scalability, and robustness. One factor to consider during the design of a virus clearance step is how to achieve robust virus removal at a reasonable cost. Use of parvovirus-retentive filters, the method of choice for downstream viral clearance, can be expensive. Adding appropriate prefiltration, such as adsorptive depth filters, can dramatically improve the economics and capacity of such a clearance step. By using a simple experimental approach, you can determine the optimal ratio of prefilter to virus filter area and lower filtration costs. Dealing with extractables and implementing virus spiking studies also requires careful consideration.

Protecting Filters

Viresolve NFP (normal flow parvovirus) membranes from Millipore are high-flux composite membranes that use a size-exclusion mechanism to achieve a greater than four-log removal of parvoviruses. They remove larger viruses with equal or better efficiency (**1**). In some applications, small changes in feed quality can affect the filterability of a protein solution. Such variability can be induced by many factors including changes in protein concentration, storage time and conditions, and freeze-thaw cycles. Aggregates in a protein solution also can decrease the capacity of a filter (**2**). To reduce the impact of aggregates and other contaminants and protect the NFP filter, we investigated a range of prefilter options. One key finding was that size-based prefilters were generally not as effective as adsorptive-based prefilters in protecting NFP filters (**3**).

We found that charge-modified depth filters such as the Viresolve prefilter (VPF, Millipore) were most effective at protecting NFPs. VPF is a depth filter comprising diatomaceous earth (DE), cellulose fibers, and a negatively charged resin binder. Further characterization work shows that this filter works by an adsorptive mechanism (**3**). Table 1 summarizes these conclusions.

A polyclonal IgG in PBS buffer was used as a model protein for this testing. The first set of experiments determined the effect of prefilter loading on the NFP capacity. Capacity decreased as the prefilter loading increased, suggesting that the prefilter has a finite capacity for the fouling species that it removes. Once that capacity is reached, those impurities break through and begin to affect NFP performance. For the model stream used in this study, a prefilter loading of 100–200 L/m^2 is recommended.

A second set of experiments shows the effect of prefiltration flux or residence time on the performance of a downstream NFP filter. As flux increased and residence time decreased, the ability of the prefilter to protect NFP was reduced. That is typical of an adsorptive media. A third set of experiments showed the effect of changes in solution conditions on protection of the NFP filter (**3**). Both ionic strength (5–150 mM NaCl) and pH (5–8.5) were adjusted. Increased ionic strength improved protection, whereas the pH effect was less dramatic. For this model stream, high salt conditions at pH 7–8 demonstrated optimal protection.

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Because its removal mechanism is not completely understood, the effectiveness of the VPF may vary depending on the nature of the fouling species. The cellulose backbone and resin binder possess ionic characteristics that may contribute charge-specific binding capacity. Diatomaceous earth has been shown to exhibit hydrophobic characteristics (**4**). In this application,

the increased performance at higher ionic strength suggests a hydrophobic mechanism.

Prefilter Sizing

A simple experimental approach was developed to determine optimum sizing for the Viresolve prefilter. It uses the area ratio of the VPF to the NFP as a parameter to be defined during process development.

The test method involves four steps:

1. Evaluate Viresolve prefilter (VPF) feasibility by comparing NFP baseline performance (without the prefilter) and capacity with the prefilter in-line. This is accomplished through constant pressure V_{max} determinations. NFP sizing is based on V_{75}, which is 50% of the V_{max} value.
2. Evaluate area ratio of the VPF to NFP (A_{VPF}/A_{NFP}) to understand how much prefilter is required to protect the final filter.
3. Conduct simulation trial(s) to verify performance on a small scale.
4. Conduct a pilot-scale confirmation run.

The constant pressure test for measuring V_{max} uses the following relation (5):

$$1 \ldots \quad \frac{t}{V} = \frac{t}{V_{max}} + \frac{1}{Q_i}$$

where

t = time

V = volume processed

Q_i = initial flow rate

V_{75} is calculated as equal to $0.5 \times V_{max}$ and is also defined as the volumetric throughput where $Q = Q_i \times 0.25$.

Prefilter feasibility is determined through a simple method of linear extrapolation from two data points as shown in Figure 1. The left-most data point, which lies on the Y axis, represents the NFP filter capacity without prefiltration. For this point, with a V_{75} of about 180 L/m², the area ratio is zero because no prefilter is used. The second data point, for V_{75} = 850 L/m², was generated by running a VPF (5 cm²) in-line with the NFP (3.5 cm²). This resulted in an area ratio of 1.4. The data points are connected by a straight line to generate the graph of expected V_{75} values for any area ratio. The optimum area ratio is chosen as that which results in NFP throughput (V_{75}) of 500 L/m². From the data in Figure 1, throughput of 500 L/m² requires an area ratio of 0.7. A throughput value of 500 L/m² is driven by the virus validation studies. It is primarily related to the impact of the viral preparations on the filter performance, which is discussed below. Figure 1 also shows the results of testing intermediate values of the area ratios and confirms the linearity of our approach in this application.

Figure 1: Estimation of the prefilter area ratio required for a sizing of 500 L/m²

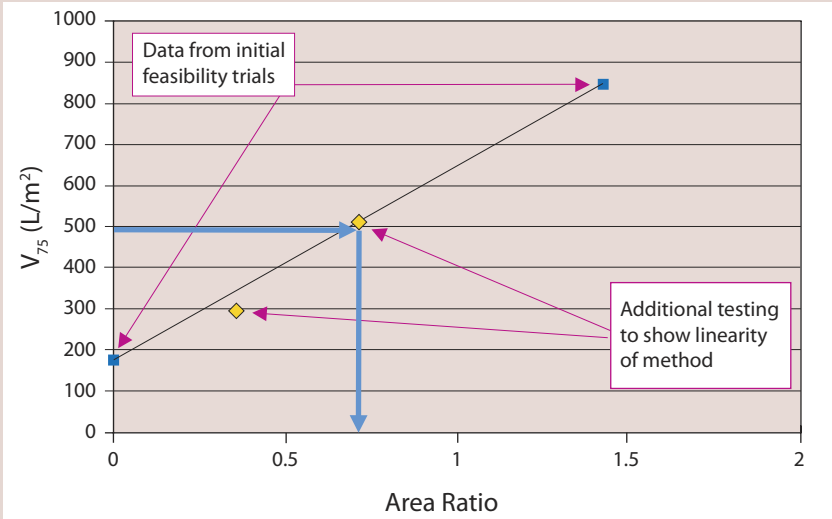
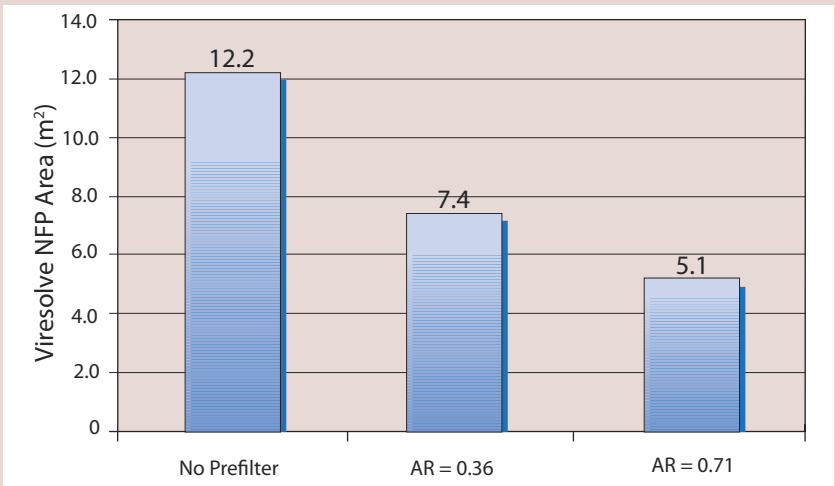


Figure 2: Impact of prefiltration (prefilter area ratio = AR) on NFP performance



Process Economics

Based on process development results and process simulation runs, we recommend an initial process design. For our example monoclonal antibody (MAb) application, the process intermediate consists of a 2000-L pool at a protein concentration of 5 g/L. The optimum process using the VPF consists of 5 m² of VPF followed by a 4.3-m² NFP filter. Figure 2 demonstrates the dramatic impact of prefiltration on the use of NFP filters in purification of a MAb solution. In the absence of a prefilter, this process requires 12.2 m² of NFP to filter 2000 L of process fluid. An area ratio of 0.36 reduced NFP requirements to

Table 1: Summary of VPF characterization studies

Observations	Conclusion
Ability of prefilter to protect NFP is a function of • prefilter loading, • prefilter flux, and • buffer conditions (ionic strength, pH)	Prefilter works by an adsorptive mechanism

Figure 3: Effect of prefilter on NFP filtration costs

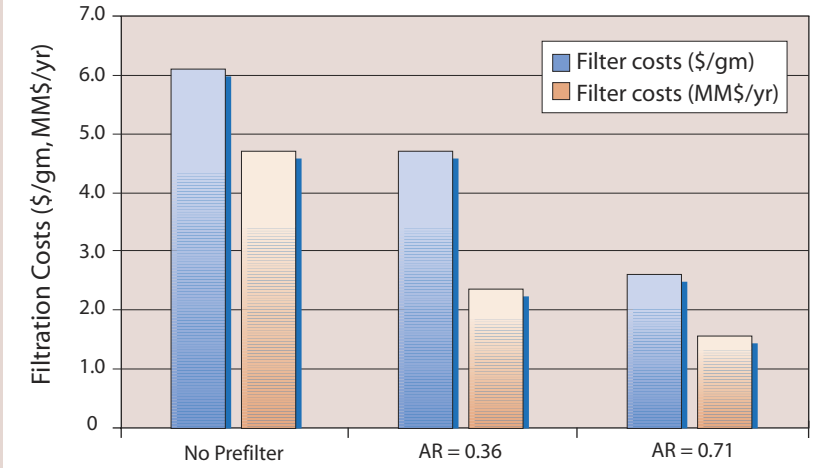
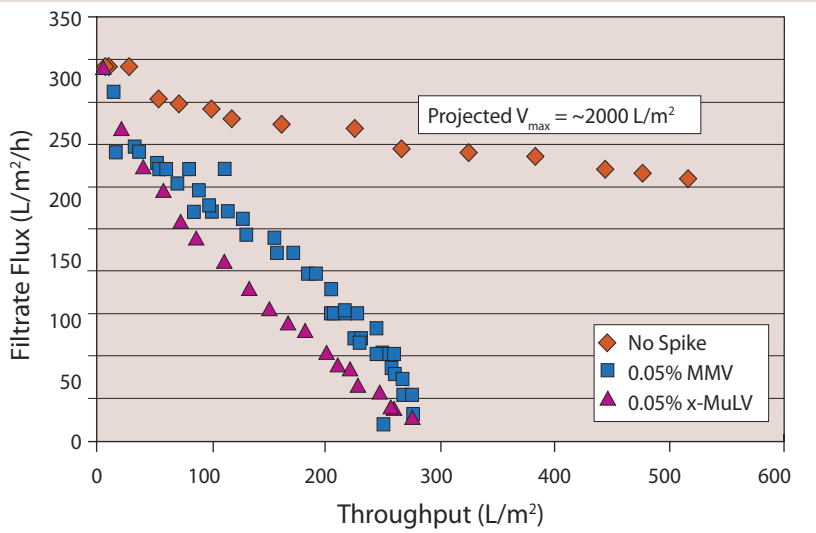


Figure 4: Effect of virus spike on NFP process flux; MAb concentration = ~5 g/L; buffer = 50 mM Tris HCL + 50 mM NaCl; pH = 7.5; Δp = 30 psi



7.4 m^2, whereas an area ratio of 0.71 further reduced NFP requirements by more than 50% to 5.1 m^2.

The processing cost savings are equally dramatic. In Figure 3, the lighter bar in each pair represents total filter costs for this process, in dollars per gram of purified protein. The darker bar represents filter costs per year (assuming 50 batches per year). Sizing the filter train to maximize NFP protection by applying an A_{VPF}/A_{NFP} area ratio of 0.71 yields savings of about $3 million per year for this unit operation.

Virus Spiking Considerations

Virus filters are ultimately sized based on a combination of the throughput and virus reduction that they can demonstrate in a virus validation experiment. When a VPF application is run in a validation study, the protein should be prefiltered first and then a virus spike added to the feed. That prevents putting a virus spike directly through the VPF, where some loss of virus titer may occur. Such virus loss would depend on solution conditions and be difficult to design into a spiking study. Filter performance in a spiking study is often quite different from the typical process performance, which may be due to impurities in the viral preps (**6**) as well as possible protein–virus interactions. Figure 4 illustrates this limitation (**7**). A MAb solution was filtered under conditions of no virus spike (top curve), a 0.05% spike of minute mouse virus (MMV), and a 0.05% spike of xenotrophic murine leukemia virus (X-MuLV). Flux curves for the various runs show the impact of those viral spikes.

Alternative approaches to conventional virus validation may allow a higher area ratio, but they may also require justification to the regulatory agencies (**8**). Targeting a flow-decay endpoint instead of a capacity endpoint may provide one solution. Another approach is to combine flow-decay methodology (validate to a percent of flow decay) with a run-plus-spike technique to validate to a predetermined capacity. Because such methods are not typically used for validating viral clearance, they should be discussed with regulators before implementation.

Prefilter Extractables

Depth-filter media are widely used in the biopharmaceutical industry. They are most commonly found in upstream applications such as bioreactor clarification. One concern in applying them in a downstream operation is about the extractables associated with them. Those extractables have been characterized to consist of extracts from the cellulose fibers, silica, and trace metals (**9**). The large majority of these species are removed during recommended preuse flushing. The small amount of extractables that remain are further reduced during a formulation UF/DF operation, which is the final step in typical MAb process template. A 10-inch Opticap capsule (Millipore) with a Viresolve prefilter was flushed with a minimum volume and then allowed to soak for 24 hours to generate artificially high levels of extracts. One liter of the process volume was run through a UF/DF process simulation (10× concentration followed by 10 volumes of diafiltration) using a Pellicon XL ultrafiltration cassette (Millipore) with an Ultracel 10-kDa regenerated cellulose membrane (Millipore). Table 2 summarizes the reduction of extractables during this diafiltration operation. Conductivity and TOC were reduced more than 50-fold during this process, and NVRs (nonvolatile residues) were reduced more than 100-fold to below the detection limits.

Improving Robustness

The Viresolve NFP viral clearance filter offers potentially very high

Table 2: Removal of VPF extractables by UF/DF

	Conductivity		TOC		Fibers		NVR	
	µS/cm	Reduction Factor	ppm	Reduction Factor	Number/m^2	Reduction Factor	mg/m^2	Reduction Factor
Primary extract pool	103	1	17.3	1	3306	1	731	1
NFP Filtrate	98	1	16.1	1	0	>281	675	1
UF/DF retentate pool	1.89	54	0.32	54	NA	NA	<6.2	<117
DI water	0.3	NA	0.16	NA	NA	NA	NA	NA

throughput and product flux for parvovirus clearance. Addition of a prefilter improves robustness of the virus clearance step and allows more applications to take advantage of these high throughputs and fluxes. We demonstrated that the Viresolve prefilter protects the NFP viral clearance filter and increases throughput.

Our simple experimental approach for determining the optimal area ratio of prefilter to final virus filter (A_{VPF}/A_{NFP}) can be applied to arrive at the most cost-effective prefilter and virus filter combination for virus removal. This method offers a substantial safety factor while realistically acknowledging validation limits encountered during viral clearance studies. Data from an actual MAb process shows that use of the VPF/NFP train can result in filtration cost savings of about 60%.

Acknowledgments

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