THE PENNSYLVANIA STATE UNIVERSITY
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# EFFECTS OF SOLUTION CONDITIONS ON VIRUS RETENTION IN A COMMERCIAL VIRUS REMOVAL FILTER

MATTHEW MICKLIN
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Reviewed and approved* by the following:

Andrew Zydney
Distinguished Professor of Chemical Engineering
Thesis Supervisor and Honors Adviser

Wayne Curtis
Professor of Chemical Engineering
Faculty Reader

* Signatures are on file in the Schreyer Honors College.

# ABSTRACT

Virus filtration is an essential part of the downstream purification process in the production of biotherapeutics and is used to remove small parvoviruses from the product solution. Virus filtration is typically thought to be strictly a size-based exclusion process; the possibility of adsorptive effects on virus retention has been given little attention. The objective of this study was to examine the effects of solution pH and ionic strength on virus retention using the Viresolve® NFP membrane. Bacteriophage ϕX-174 was used as a model parvovirus. The solution conditions were controlled by using appropriate buffers (acetate, phosphate, and carbonate) with different concentrations to prepare the virus feed solution. Virus retention was greatest at low pH and low ionic strength, conditions where there is a significant electrostatic attraction between the positively charged virus and the negatively charged membrane. In addition, these conditions showed very robust virus retention; there was no transient increase in virus transmission in response to a pressure disruption, a phenomenon which is known to occur at neutral pH due to diffusion of previously captured virus out of the membrane pores. Virus retention was also a function of protein fouling, likely due to changes in the underlying pore size distribution. These results provide new insights into the factors governing virus filtration for the preparation of safe biotherapeutics.

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# Chapter 1

## Introduction

Advances in the production of biopharmaceuticals are critical to maintain the high quality needed for these complex therapeutics, most of which are delivered intravenously or by subcutaneous injection to avoid degradation in the digestive track. Because biopharmaceuticals are much larger in size than the more commonplace small-molecule drugs (roughly 150,000 Da vs <1,000 Da), they cannot be synthesized via simple organic chemistry. Instead, they are produced biologically and require significantly more complex processing to remove impurities and contaminants. Many expensive unit operations are typically used to accomplish this task, each focused on the removal of specific impurities.

Although the biopharmaceutical production process can vary significantly between different classes of products, they almost always follow the same general outline of seed train expansion, cell culture for production, harvest, purification, and formulation. The seed train expansion process begins in the laboratory, where a certain host cell line is selected for production. Chinese hamster ovary (CHO) cells are most commonly used for production of complex proteins like monoclonal antibodies, although bacterial cells have also been used for proteins that require minimal post-translational modification. A piece of recombinant DNA that codes for the desired protein is cloned into the cells. The genetically-modified cells are then grown in a series of vessels that increase in size, typically following the pattern of small vial, shaker flask, wave bag, and ending in large bioreactors. Once the cells have finished growing in the largest bioreactor, they begin producing and secreting the desired protein product into the

growth media. The harvest process is typically performed by centrifuging the mixture to separate the secreted protein product from the large host cells. Depth filtration is then used to further remove any cellular debris. The downstream purification process typically varies the most between different products, and it commonly involves a series of chromatography and filtration processes that remove impurities and contaminants to yield a highly purified final product. The downstream purification process must meet all of the regulatory requirements for product release, typically set by the FDA, including the removal of both adventitious and endogenous viruses.

Obtaining very high levels of viral clearance is a significant challenge in the downstream purification of protein-based therapeutics. Viruses can be introduced in the nutrient-enriched media, in the buffers used in chromatographic processes, through contact with equipment, and from the air within the processing facility. The presence of even a single virus particle in the final drug product could be detrimental to the consumer's health and safety. In order to prevent this occurrence, multiple unit operations focused primarily on viral clearance are implemented in the downstream purification process. The two most common processes are a viral inactivation step, which usually involves incubation of the product at low pH to induce denaturation of the virus, and a viral filtration step, which is implemented to physically remove any virus from the protein solution. Chromatography can also provide significant levels of virus removal due to differences in binding to the chromatographic resin. Virus filtration membranes have pores around 20 nm in size, which is required to remove the smallest parvovirus. These pores are just large enough to provide good passage of the therapeutic protein, which is on the order of 8 nm in size for a monoclonal antibody.

There are several companies that currently produce virus removal filters used by pharmaceutical companies in their downstream processing. This includes the Planova series membranes (produced by Asahi Kasei Bioprocess), Ultipor (produced by Pall) and the Viresolve series membranes (produced by Millipore). The Planova filters are hollow fiber membranes made of either cellulose or polyvinylidene fluoride.[1] In contrast, the Ultipor and Viresolve filters are flat sheet membranes used with dead-end (normal) flow filtration. The Ultipor DV20 has a fairly homogenous pore structure in which the pore size is relatively uniform throughout the depth of the filter.[2] The Viresolve NFP and VPro filters are highly asymmetric, with a tight skin layer on top of a more open pore structure.[3] These membranes are sold in multi-layer configurations to obtain the required high degree of virus clearance, and they are used with the more open pore side facing the feed solution.

Although virus filters are thought to provide a simple size-based virus retention, the actual behavior of virus filters is considerably more complex. Jackson et al.[4] showed that there was a significant decline in virus retention during the course of a virus filtration process using the Ultipor DV20 membrane. In this case, the degree of virus retention decreased by more than a factor of 100 after the filtration of only 50 L of solution per $m^2$ of membrane area. Jackson et al. attributed this decline in virus retention to the accumulation of retained virus within the fluid contained in the pores of the Ultipor DV20 membrane.

Subsequent work by Woods and Zydney[5] demonstrated that a short (transient) disruption in the applied pressure could cause a significant reduction in virus retention for the Ultipor DV20 membrane. This procedure simulates the events of an unexpected process shutdown or the use of a buffer flush to recover residual product from within the membrane module after completion of the virus filtration. A 5-10 min pressure disruption caused as much as a 100-fold reduction in

virus retention, which was then restored as the filtration continued. This effect was attributed to the ability of the previously captured virus to diffuse out of the membrane pores upon removal of the applied pressure; these viruses could then migrate laterally within the filter and eventually pass through the membrane and into the filtrate when the pressure was restored. This behavior was confirmed by confocal microscopy experiments, which showed multiple bands of captured virus within the filter (using viruses that were labeled with fluorescent dye).

The objective of this study was to extend the work done by Woods and Zydney to the highly asymmetric Viresolve® NFP membranes and to examine the effects of solution conditions such as pH and ionic strength on virus retention during both constant-pressure and pressure-release filtration procedures. Virus capture for membranes that were fouled by protein was also investigated. Data were obtained with the bacteriophage ϕX-174, the first DNA-based virus to ever have its genome sequenced, as a model parvovirus. These results provide important new insights into the factors controlling virus retention with commercially available virus filtration membranes.

# Chapter 2

# Materials and Methods

## *Membranes*

Viresolve® NFP membranes were provided by EMD Millipore (Bedford, MA) in a single layer format. The membranes were manually cut into small, circular disks, 47 mm in diameter, using a stainless steel cutting device.

## *Bacteriophage*

All experiments were performed using bacteriophage ϕX-174 (ATCC-13706-B1™), obtained from American Type Culture Collection (ATCC, Manassas, VA), as a model parvovirus. ϕX-174 is approximately 24 – 26 nm in size, which is similar to the size of commercially relevant parvovirus. Luria-Bertain (LB) media was prepared by mixing 10 g/L Bactro™ Tryptone (BD-211705), 5 g/L Bacto Yeast extract (BD-212750), and 10 g/L NaCl in deionized water. The pH was adjusted to 7.5 using NaOH, and the media was sterilized in an autoclave and stored until further use. The ϕX-174 was propagated in the host *E. coli CGSC* obtained from the *E. coli* Genetic Stock Center at Yale University (New Haven, CT). The *E. coli* were grown in LB media at 35 °C, and the bacteriophage were added once the *E. coli* had reached an optical density between 0.3-0.4. The mixture was incubated for 5 hours, and the resulting suspension was centrifuged at 3500 rpm and 4°C to remove lysate/debris. The supernatant containing the ϕX-174 was decanted and stored at 4°C until further use.

***Buffer Preparation***

Acetate, phosphate buffered saline (PBS), and carbonate buffers were prepared with pH values of roughly 5, 7, and 10, respectively. The relative concentrations of acid and base needed for the acetate and carbonate buffers were determined using the Henderson-Hasselbach equation:

$$pH = pKa + log\left(\frac{[A^-]}{[HA]}\right)$$

where [HA] is the concentration of acid, $[A^-]$ is the concentration of conjugate base, and the $pK_a$ provides a measure of the acid's ability to dissociate in water. pKa values for the acetate and carbonate buffers (determined from their acid component) are 4.75 and 10.32, respectively. PBS was bought as a commercial product and thus these calculations were not performed for this buffer. The ionic strength of each buffer was calculated using the following equation:

$$I = \left(\frac{1}{2}\right)\sum_{i}^{n} C_i Z_i^2$$

where $C_i$ is the molar concentration of ion "i" and $Z_i$ is the charge (or valence) of ion "i".

***Virus Filtration***

Virus filtration experiments were conducted using single layers of the Viresolve® NFP membranes, which were housed directly in a 47 mm stainless steel filter holder (EMD Millipore Corp., Bedford, MA). The membranes were placed with the skin-side down, which allows for the support region on the upstream side of the membrane to act as a "depth filter" that protects the skin-side from fouling. The bacteriophage suspension was added to the desired buffer (acetate,

phosphate, or carbonate) and ultrasonicated for 45 minutes. Ultrasonication disrupts any virus aggregates ensuring a uniform dispersion of individual virus particles. The mixture was then pre-filtered through a 0.2 μm cellulose acetate syringe filter into a 1 L reservoir, which was connected to the filter holder by plastic tubing. The filtration began by air-pressurizing the reservoir to 30 psi, controlled using an Aschcroft pressure regulator. The filtrate was collected in 1 mL fractions using microcentrifuge tubes. For constant pressure experiments, ~12 mL of phage solution was filtered through the membrane using a constant applied pressure of 30 psi. For the pressure release experiments, the reservoir was initially filled with ~24 mL of the phage suspension. The first 12 mL of the phage suspension were filtered through the membrane at a constant pressure of 30 psi. At this point, the pressure was released and kept at 0 psi for about 10 minutes, at which point the system was re-pressurized to 30 psi and the remaining 12 mL were filtered. The filtrate flux (volumetric flow rate per unit membrane area) was measured throughout the experiment by timed collection.

***Protein Fouling Experiments***

To examine the effects of protein fouling on virus retention, the membrane was initially challenged with a solution of Immunoglobulin G (IgG) until the filtrate flux had declined by a target amount. A 0.1 g/L IgG solution was used to achieve a flux decline of 50%; a 1 g/L IgG solution was used to achieve a final flux of 25% of the initial value. At this point the remaining protein solution was removed from the system through a side-vent in the filter holder. The reservoir was then filled with the ϕX-174 phage suspension and a standard constant-pressure

virus filtration experiment was performed. All protein fouling/virus filtration experiments were performed at pH 10.

### *Pfu Assay*

Initial and final bacteriophage concentrations were determined by a plaque forming unit (pfu) assay. Hard agar plates were prepared by adding 10 g/L Difco Agar (BD-214530) to the LB media, which was then poured into petri dishes. Soft agar was prepared by adding 4 g/L Difco Agar to the LB media, which was then poured into 50 mL centrifuge tubes. The filtrate fractions (containing any transmitted phage) were serially diluted by 10-fold with DI water. 200 μL of *E. coli* was added to 100 μL of the diluted phage suspension. The soft agar was melted in a microwave and 900 μL of the liquid soft agar was added to each fraction. The resulting mixture was poured over a hard agar plate and left to solidify. As the *E. coli* cells become infected by the bacteriophage, they lyse and spread the viral infection to adjacent cells, which will subsequently lyse as well. This forms a plaque within the soft agar, which can be seen by the naked eye. The number of plaque-forming units (pfu) was used to evaluate the concentration of virus, typically reported as pfu/mL. Only samples with a plaque count in the range of 10-200 were counted; it was not possible to determine the number of separate plaques for samples with higher phage concentrations and there was too large an uncertainty with samples having lower phage concentrations. An image of an agar plate with plaques is shown in Figure 1.[6]

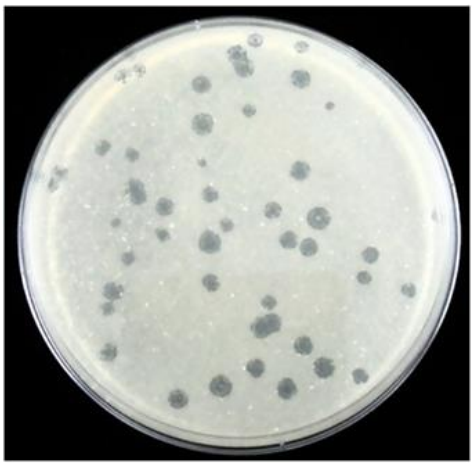

**Figure 1: Plaque Forming Assay**

***Zeta Potential Measurements***

The membrane zeta potential was evaluated from the measured streaming potential as described by Burns and Zydney.[7] Buffered salt solutions were prepared using potassium chloride, sodium carbonate, sodium bicarbonate, acetic acid, sodium acetate trihydrate, citric acid monohydrate, and/or disodium hydrogen phosphate. The ionic strength of the solutions was adjusted using KCl and the pH was adjusted using HCl and KOH as needed. The buffer conductivity was measured using a Thermo Orion Conductivity meter (Thermo Scientific, Waltham, MA). The buffers were prefiltered through 0.2 µm Supor-200 membranes to remove any large particles or undissolved solids prior to being used. Ag/AgCl electrodes were prepared using 1 mm diameter silver wires. The wires were straightened, lightly sanded, placed in nitric acid for 10 seconds, and rinsed with distilled water. The silver wire and a steel wire, used as a reducing electrode, were placed in separate beakers each containing 1 M KCl. A Kimwipe was used as a salt-bridge between the beakers. The two wires were connected to a DC power source with the current maintained at 20 mA for 20 minutes. A uniform Ag/AgCl coating was formed over the silver wire. The streaming

potential was measured using a device consisting of two Plexiglas chambers. A 25 mm membrane disk was placed between the chambers and secured with an O-ring. The Ag/AgCl electrodes were inserted into the chambers with the tips about 1 mm away from the membrane. The chambers were filled with the desired buffer solution and the feed port of one chamber was connected to a pressurized feed reservoir filled with additional buffer. The electrodes were connected to a Keithley 2000 multimeter to measure the streaming potential ($E_z$) as a function of transmembrane pressure ($\Delta P$) between 14 and 35 kPa, set using an Ashcroft digital pressure regulator. The apparent zeta potential, $\zeta_{app}$, was calculated from the slope ($dE_z/d\Delta P$) using the Hemholtz-Smoluchowski equation:

$$\zeta_{app} = \frac{\mu \Lambda_0}{\varepsilon_0 \varepsilon_T}$$

where $\mu$ and $\Lambda_0$ are the viscosity (kg/(m*s)) and conductivity (S/m) of the buffer solution, respectively, $\varepsilon_0$ is the permittivity of a vacuum ($(A^2s^4)/(m^3kg)$), and $\varepsilon_T$ is the dielectric constant. The experiments were run in triplicate at each pH.

***Fluorescent Labeling***

Fluorescent dyes Cy5 and SYBR Gold were purchased from Sigma-Aldrich and Life Technologies, respectively. Cy5 labeling of bacteriophage was done according to the protocol described by Dishari et al.[8] The bacteriophage solution was first concentrated to 4 x $10^{10}$ pfu/mL by centrifugation at 3800 rpm using a spin-concentrator with an Ultracel 100 kDa membrane (EMD Millipore, Billerica, MA). The phage were then buffer exchanged into 0.1 M $NaHCO_3$ buffer (around 15 wash cycles) using the spin-concentrator to remove any free (unreacted) dye. The SYBR Gold labeling protocol was adapted from that presented by Choi et al.[9] About 10 mL

of the bacteriophage suspension (concentration of $10^{10}$ pfu/mL) was buffer exchanged and concentrated to $10^{11}$ pfu/mL in 1X TAE buffer (MediaTech, Manassas, VA) using a spin concentrator. 1 µL of SYBR Gold ($10^4$ X) dissolved in DMSO was added to the phage suspension. The solution was vortexed and allowed to react at room temperature in the absence of light. The labeled phage were stored at 4 ºC.

***Confocal Laser Scanning Microscopy***

Confocal images were obtained after the virus filtration experiment to directly visualize the location of captured virus within the filter. The membrane was first challenged with 12 mL of the Cy5-labeld phage at 30 psig at which point the pressure was released for about 10 minutes. Contrary to a typical pressure-release experiment, the feed reservoir was then emptied and refilled with 12 mL of the SYBR Gold-labeled phage. The system was then re-pressurized to 30 psig and the filtration continued until the 12 mL was filtered. Confocal images were obtained using an Olympus Fluoview™ 1000 confocal laser scanning microscope. Membranes were cut into small pieces (1.5 cm x 1 cm) and mounted on separate glass slides. The membranes were wet with a small drop of 10% glycerol, covered with a glass coverslip, and sealed using nail-polish. The membrane was placed over a 100x oil objective lens (Numerical Aperture, NA=1.25). The Cy5 and SYBR Gold dyes were excited with 488 nm and 610 nm wavelength lasers, respectively, and imaged at emission wavelengths of 510 and 670 nm, with the zoom magnification of the lens set at 2.0. Optical cross-sectioning was done at 0.3-µm intervals with multiple images in the x-y plane stacked to construct an image along the z-axis through the depth

of the membrane. Viresolve NFP® membranes were scanned from both sides. The two acquired images were combined to generate a scan through the full depth of the membrane.

# Chapter 3

## Results and Analysis

Data for the virus filtration experiments are presented as the log-mean reduction value (LRV) vs cumulative volumetric throughput (in L per $m^2$ membrane area). The LRV provides a direct measure of virus retention:

$$LRV = -log_{10}\left(\frac{C_{filtrate}}{C_{feed}}\right)$$

where $C_{filtrate}$ is the concentration of virus in the collected filtrate fraction and $C_{feed}$ is the concentration of virus in the initial virus solution fed to the filtration system. The logarithmic concentration ratio allows results that differ by orders of magnitude to be presented on the same plot. For example, if one filtration experiment removes 10x as much virus as another experiment, this will correspond to an increase in LRV by plus one. Typical virus filtration membranes are designed to have an LRV of at least four, corresponding to a 10,000-fold reduction in virus concentration.

All virus filtration experiments were performed using a target initial feed concentration of approximately 2 x $10^7$ pfu/mL. Any small variations in feed concentration were accounted for by use of the concentration ratio in the evaluation of the LRV. Filtration experiments were performed over relatively short filtration times, using small process volumes (~12 mL for the constant pressure experiments, which corresponds to a volumetric throughput of 9 L/$m^2$); thus there was relatively little fouling and the flux remained relatively constant varying from ~280 L/($m^2$*h) at the beginning of the experiment to ~240 L/($m^2$*h) at the end. There was no evidence for any difference in flux with the different solution conditions (pH or ionic strength).

Typical data for virus retention during constant pressure filtration experiments performed at different pH in both a 13 and 40 mM ionic strength solution are presented in Figure 2.

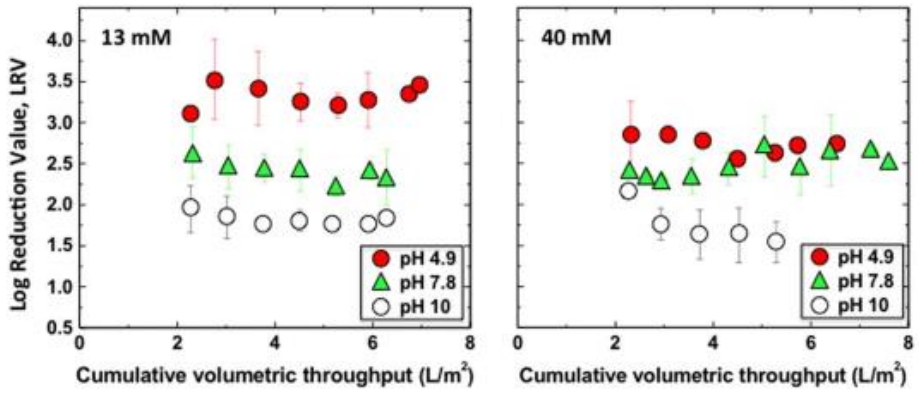


**Figure 2: Virus Retention in Constant Pressure Filtration Experiments**

The error bars represent the standard deviation for two replicated trials performed at each condition, with the actual data label placed at the average of the two. In several cases the error bars were smaller than the size of the symbol and are not shown for clarity. Virus retention was greatest in the pH 4.9 solution, particularly at low ionic strength. For example, the LRV in the 13 mM solution at pH 4.9 is approximately 1 unit higher (10x higher virus retention) than that at pH 7.8 and 1.5 units higher than that at pH 10. This effect is much less pronounced at the higher ionic strength of 40 mM, with very similar level of virus retention at pH 4.9 and 7.8. Note that all of the data in Figure 2 were with single layers of the Viresolve NFP filter. The commercial filter has 3 membranes in series and would thus be expected to have an LRV of 6 to as high as 10 under the conditions examined in these experiments.[3]

Corresponding data for the pressure release experiments in a 190 and 13 mM ionic strength solutions are presented in Figure 3.

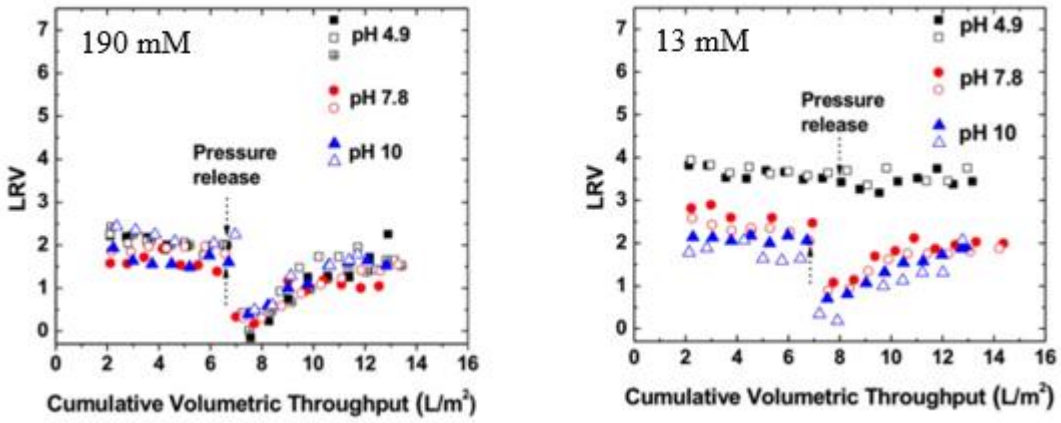


**Figure 3: Virus Retention in Pressure Release Filtration Experiments**

The open and filled symbols are results from repeat experiments, with the data showing a high degree of repeatability. The results at high ionic strength show a significant reduction in virus retention (LRV) immediately after the occurrence of the pressure disruption, with the LRV returning to its initial steady-state level after filtration of several $L/m^2$. However, at low ionic strength, this effect is not observed for the experiment performed at pH 4.9 using the acetate buffer. In addition, the LRV throughout the filtration at pH 4.9 is significantly greater than that at pH 7.8 and 10, an effect which is also observed in the constant pressure experiments (Figure 2). Thus, the pressure-release effect described by Woods and Zydney[5] is only observed under certain pH and ionic strength.

In order to understand the effects of solution conditions on virus retention, zeta potential measurements were performed to estimate the surface charge on the membrane in the different buffer solutions. Typical data are presented in Figure 4:

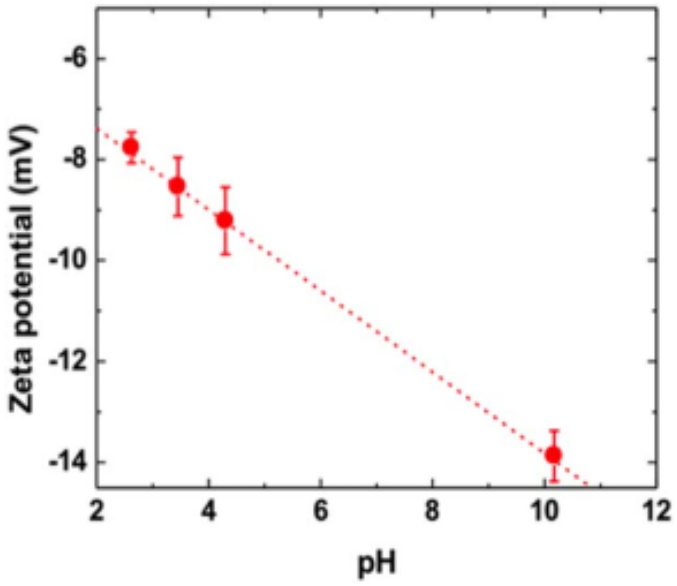


**Figure 4: Zeta Potential Measurements (Experiments performed by Ki-Joo Sung)**

In each case, the measured values of the streaming potential varied linearly with the applied transmembrane pressure ($r > 0.98$), which allows the zeta-potential to be calculated directly from the slope using the Helmholtz-Smoluchowski equation. The error bars indicate the standard deviation of three repeat measurements obtained at each pH value. The zeta-potential values were negative at all pH values studied, corresponding to a net negative charge on the membrane, with $\zeta$ = -7.7 mV at pH 2.7 and -14 mV at pH 10. The increase in negative charge at high pH is consistent with the generation of negative hydroxyl ($-OH^-$) groups under these conditions.

The bacteriophage $\phi$X-174 is reported to have an isoelectric point (pH of zero net charge) of pH 6.6.[10] This means that the virus is negatively-charged at pH 7.8 and 10, but becomes positively charged at pH 4.9. Thus, the loss in virus retention in response to the pressure disruption is only observed under conditions where there are repulsive electrostatic interactions between the negatively-charged virus and negatively-charged membrane or at high ionic strength

where the electrostatic interactions become greatly reduced. In contrast, virus filtration at low pH and low ionic strength, conditions which should cause a strong attractive electrostatic interaction between the negatively-charged membrane and positively-charged virus, give rise to higher levels of virus retention (Figure 2) and no change in LRV after the pressure release.

The interactions between the virus and membrane were examined in more detail using confocal microscopy to directly visualize virus capture within the membrane. Most importantly, by proper choice of labeling, it is possible to distinguishing between the virus that were part of the challenge before the pressure release (in this case labeled with the red Cy5 dye) and the virus that were in the challenge after the pressure release (labeled with green SYBR Gold dye). A typical set of confocal images are presented in Figure 5.

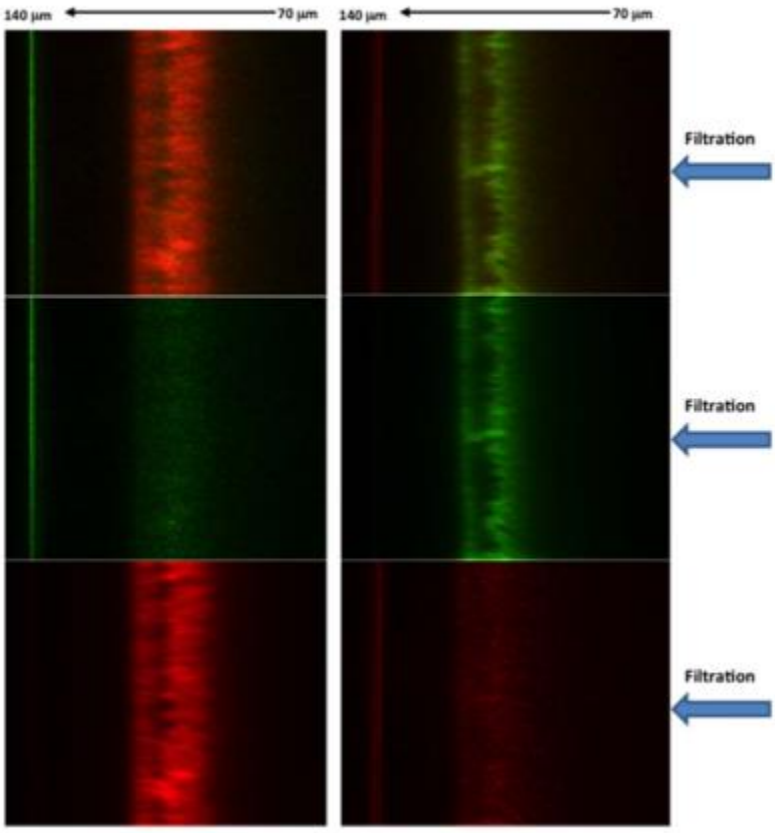


**Figure 5: Confocal Microscopy Results (Experiments performed by Dr. Shudipto Dishari)**

The left panel shows results of an experiment run at pH 4.9 while the right panel shows results for an experiment run at pH 10, both at 13 mM ionic strength. The top, middle, and bottom sections are confocal images of the same membrane but with different excitation/emission wavelengths to separately see the differently labeled phage. Thus, the bottom panel shows the red phage challenged before pressure release, the middle panel shows the green SYBR Gold phage challenged after pressure release, and the top panel shows an overlay of both images (all done automatically using the software available with the confocal microscope). In all cases, the filtration occurs from the right side of the image, as shown by the arrows, with the faint bands at the far left side of each image representing the exit of the membrane. The images at pH 10 show that most of the phage is captured in a diffuse band approximately 20 μm in from the exit of the filter. Similar results were described by Dishari et al.[10] for a series of constant pressure experiments (without any pressure release). However, there is also a very thin band of red-labeled phage right near the exit of the filter; this band showed no measurable amount of the green virus (filtered after the pressure release). This suggests that some of the previously captured (red) virus were able to travel farther into, and in some cases all the way through, the membrane after the pressure release, consistent with the sharp decrease in virus retention observed in Figure 3.

The results at pH 4.9 are considerably different (left-hand panels). First, there appears to be a larger quantity of red-labeled virus within the membrane, implying a greater amount of virus capture. This is consistent with the larger LRV observed during filtration at this low pH. Secondly, there is no band of red-labeled phage at the exit of the filter, in sharp contrast to what was observed at pH 10 (the origin of the slight green band near the filter exit is unknown). This

suggests that, under these low pH conditions, the previously trapped virus were unable to migrate farther into the membrane following the pressure release, consistent with the absence of any decrease in LRV after the process disruption.

In addition to virus retention, virus filters also need to provide good transmission (recovery) of the protein product. One of the potential challenges of performing virus filtration with protein solutions is the phenomenon of protein fouling. In order to examine the possible effects of fouling on virus retention, an experiment was performed in which the membrane was first purposely fouled by filtration of an IgG solution, after which the protein was removed and the membrane used to filter a virus suspension at constant applied pressure. The results for the virus retention are shown in Figure 6.

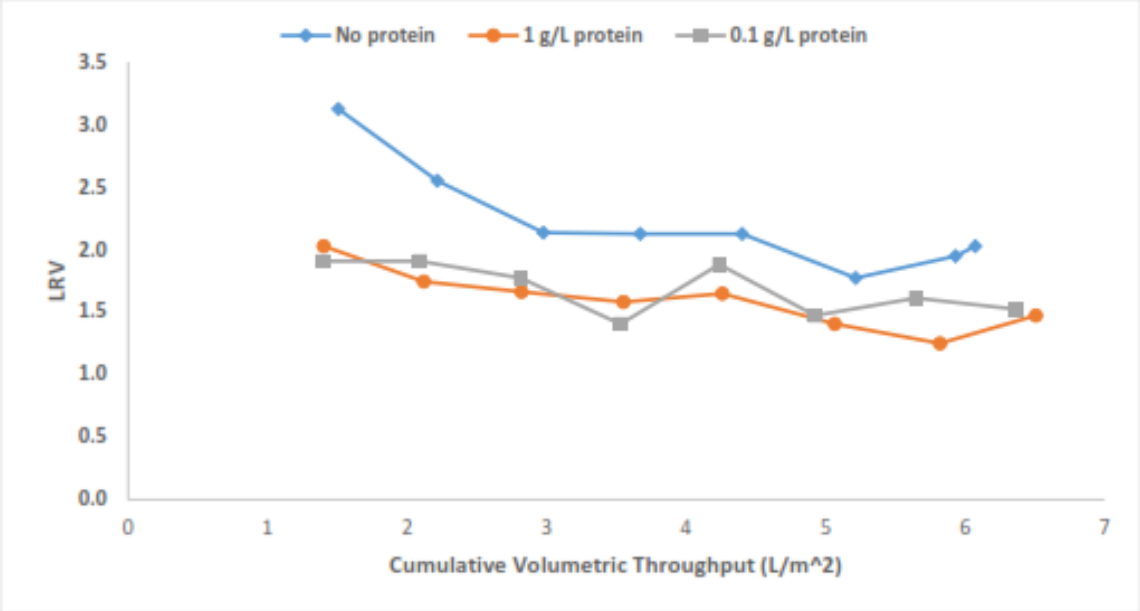


**Figure 6: Virus retention following protein fouling**

The highest degree of virus retention was obtained with the clean membrane, i.e., the membrane that was never exposed to the IgG. Virus retention by the membranes that were fouled with the IgG was approximately one-half to 1-log less than that for the clean membrane, with very similar results obtained with the membranes used to filter the 0.1 and 1.0 g/L IgG solutions (50% and 75% decrease in flux).

# Chapter 4

## Discussion

The results obtained in this study clearly demonstrate that virus retention with the Viresolve® NFP membrane is a function of both pH and ionic strength. The highest degree of virus retention was observed at low pH and low ionic strength, conditions in which there was an attractive electrostatic interaction between the negatively-charged membrane and the positively-charged virus. In addition, there was no loss in virus retention after a transient process disruption during filtration at pH 4.9, while there was more than a 1-log reduction in LRV after a process disruption during experiments at higher pH and/or higher ionic strength.

Virus filtration has typically been assumed to be governed purely by size-exclusion, with the virus particles trapped in the filter because they are larger than the pores within the membrane. However, the results obtained in this thesis clearly demonstrate the importance of electrostatic interactions in addition to size exclusion phenomena. The electrostatic attraction not only increases the likelihood of virus capture (i.e., the LRV), it also keeps previously captured virus from migrating deeper into the filter after a process disruption (or pressure release). This was confirmed by the confocal microscopy images which showed migration of previously captured phage deeper into the filter during an experiment at pH 10 but not at pH 4.9.

The surface charge of both the membrane and the virus are functions of the solution conditions. The zeta potential experiments showed that the membrane has a significant negative charge at all pH values above 2.5, with the charge becoming more negative at high pH. The bacteriophage ϕX-174 has an external capsid composed of proteins, which have both positively-

charged (amine) and negatively-charged (carboxylic acid) groups. The protonation/deprotonation of these groups leads to a pH dependent surface charge. Michen et al.[10] found the isoelectric point of the bacteriophage ϕX-174, i.e., the pH at which the virus is effectively uncharged, to be approximately 6.6. Thus, the ϕX-174 and the membrane will be oppositely charged at all pH values between about 2.5 and 6.6, potentially leading to significant electrostatic attraction. This attraction can be screened at high salt concentrations, i.e., at high ionic strength. This screening occurs due to electrostatic attractions between the dissolved salt ions and the membrane and thus minimizes the electrostatic attractions between the virus and membrane.

Woods and Zydney[5] attributed the pressure-release effect to the diffusion of previously captured virus out of the pores when the filtration pressure is removed. These released virus can diffuse laterally throughout the membrane, potentially migrating to regions where the pore size is somewhat larger. Re-pressurization of the feed will cause these virus to migrate deeper into the membrane and in some cases moving all the way through the membrane and into the filtrate when the pressure is reapplied. The data presented in this thesis indicate that this migration does not occur at low pH/low ionic strength due to the electrostatic attraction between the oppositely-charged virus and membrane. In this case, the virus particles are held in place during the pressure release, eliminating the migration of virus with the porous structure of the filter.

The results from the protein fouling experiments indicate that virus retention is reduced when the membrane was fouled. Similar results were obtained by Bolton et al.[11] during filtration of therapeutic proteins spiked with different viruses through the Viresolve NFP membrane. Bolton et al. attributed this phenomenon to the preferential blockage of the smaller pores within the asymmetric membranes. Blockage of the small pores causes the fluid flow to be “shunted”

through the larger (non-virus retentive) pores of the membrane, thereby reducing the overall level of virus retention. This behavior is consistent with the experimental results obtained in this thesis, although additional experimental studies would need to be performed to confirm this behavior.

The results presented in this thesis demonstrate that virus filtration performance can be enhanced by using low pH and low ionic strength, conditions where adsorptive (electrostatic) interactions provide an additional mechanism of virus retention (along with size-exclusion). This effect could potentially be exploited by designing membranes with greater surface charge to increase the electrostatic attractions. However, these electrostatic interactions might also lead to greater levels of protein fouling, which has been shown to reduce the extent of virus retention. Future studies should be performed to examine the generality of these results using other membranes and viruses having different physical properties. These results could have important implications for the design of virus filtration processes for purification of biotherapeutics.

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# Academic Vita

Matthew Micklin
micklinm12@gmail.com

## Education

The Pennsylvania State University, University Park, PA
*B.S. in Chemical Engineering*, **Schreyer's Honors College**
*Minor in Economics*

## Leadership and Activities

**Undergraduate Research Lab (Spring 2014-present):** Research with Dr. Andrew Zydney on retention of bacteriophage viruses in commercial virus removal filters; studying effect of pH and ionic strength on virus retention; presented research at PSU Undergraduate Research Exhibition and AIChE regional conference

Co-author of publication in Biotechnology Progress: "Effects of solution conditions on Virus Retention by the Viresolve® NFP Filter"

**Vice President of American Institute of Chemical Engineers (Spring 2016):** Responsible for organizing our chapter's trip to the AIChE Mid-Atlantic Student Regional Conference

**Member of Penn State Blue Band Drumline (2012-present):** Cymbal section leader in 2013; practice about 20 hours a week and perform at all home football games, one Big Ten away game, and bowl games; also perform at high school band competitions and parades

**Mass Transfer Instructional Aide (Spring 2016):** Hold office hours and review sessions for students in the Mass Transfer course

## Work Experience

**Summer Intern: Regeneron Pharmaceuticals (Summer 2015):** Worked in the manufacturing support group of the Process Sciences department at the Industrial Operations and Product Supply branch; performed statistical analysis to investigate mathematical correlations between downstream process parameters and product quality in the large-scale production of monoclonal antibodies

**Cardiology Physicians (Summer 2011):** Member of office staff; converted paper medical charts to a new electronic database by extracting them and classifying them accordingly; helped organize patient charts

## Awards

**PSU Chemical Engineering Department: J.** Larry Duda Undergraduate Research Award

**Phi Kappa Phi:** Peter T. Luckie Award for Excellence in Research by a Junior

**Siemens Design Showcase:** Selected to present engineering design group project at Siemens symposium

**Honors Societies:** Phi Kappa Phi and National Society of Collegiate Scholars

## Publications

Dishari, SK., Micklin, MR., Sung, KJ., Zydney, AL., Venkiteshwaran A., Earley, JN. "Effects of solution conditions on virus retention by the Viresolve® NFP filter." *Biotechnology Progress* (2015). 31(5) 1280-1286.