THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF CHEMICAL ENGINEERING

# Effects of Solution Ionic Strength and Prefilter Characteristics on the Sterile Filtration of Glycoconjugate Vaccines

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## ABSTRACT

Sterile filtration is an important step in the production of nearly all biopharmaceuticals, ensuring that the final drug product is completely free of any bacteria. However, membrane fouling can limit the lifetime and effectiveness of the sterile filters, particularly during the filtration of large biotherapeutics like many vaccine products. The aim of this study was to evaluate the effects of ionic strength and prefiltration on the sterile filtration fouling behavior of glycoconjugate vaccines produced by coupling the outer capsular polysaccharide from a bacterium to an immunogenic protein. Two glycoconjugate serotypes were filtered through 0.22 µm pore size sterilizing grade membranes. Constant pressure filtration was carried out at varying buffer solution ionic strength, and the effectiveness of different prefilter characteristics was examined. It was found that Serotype 2 fouled more quickly than Serotype 4, potentially due to greater numbers of large particles in the glycoconjugate generated from Serotype 2. The solution ionic strength had no apparent influence on the fouling behavior. Prefiltration was equally effective at reducing sterile filter fouling when using integral, batch, or inline prefilter configurations. The filter capacities after prefiltration were increased by more than 60%, and in some cases the presence of the prefilter completely eliminated fouling of the sterile filter. For Serotype 2, a prefilter with a 5 µm pore size provided the greatest increase in filter capacity. These results provide important insights into the fouling behavior during sterile filtration of glycoconjugate vaccines and suggest potential strategies to extend the lifetime and improve the performance of sterile filter membranes.

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# Chapter 1

## Introduction

From the very first vaccine in 1796[1] to the rapid development of COVID-19 vaccines two hundred years later, vaccines have played an instrumental role in preventing serious illness and the death of countless individuals. As knowledge in the field of microbiology has grown, so has the ability of researchers to develop vaccines that are safer and more effective than their earlier counterparts, almost all of which were either inactivated or attenuated viruses. One example of this is the design of polysaccharide-based vaccines for protection against bacterial diseases, such as pneumococcal and meningococcal infections. In recent decades, polysaccharide vaccines have been produced based on the polysaccharide capsule of disease-causing bacteria. This innovation led to a decline in the prevalence of these diseases, but there was often insufficient immunological response in young children and infants. This is due to the inability of polysaccharide vaccines to elicit a T-cell response, without which it is difficult to generate long-term immunologic memory.[2]

In the 1980's glycoconjugate vaccines were developed that contain a bacterial polysaccharide covalently conjugated to a highly immunogenic carrier protein.[3] These glycoconjugate vaccines do produce a T-cell response, strengthening the immune response during both initial vaccination and after a secondary booster. Figure 1 shows the structure and response of a typical glycoconjugate vaccine used against bacterial diseases in comparison to that of the polysaccharide alone. Glycoconjugate vaccines were first available in the United States in 1987 with the development of the *Haemophilus influenzae* type *b* (*Hib)* vaccine by

Connaught Laboratory (now Sanofi Pasteur)[4], followed by pneumococcal and meningococcal conjugate vaccines in 2000 and 2005, respsectively.[3]

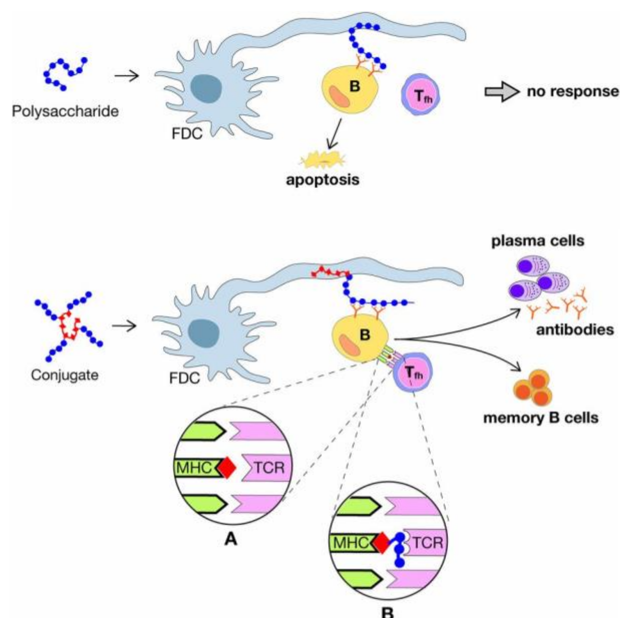


**Figure 1:** Illustrated response of B and T cells to vaccines formulated from polysaccharides alone (top) and glycoconjugates (bottom). [5] As shown, the glycoconjugate is able to stimulate a T cell response, since it can bind with a complex (MHC) on the surface of the B cell that engages T cell receptors (TCR).

Vaccines must undergo multiple purification steps throughout the manufacturing process to ensure product quality. Sterile filtration through 0.2 µm pore size (sterilizing grade) membranes is a critical component of the vaccine purification process, ensuring that the final drug product (which is collected in the filtrate stream that passes through the membrane) is completely free of any infectious bacteria. Membrane processes can also be used for removal of cell debris and other large aggregates,[6] and ultrafiltration membranes can be used to remove any unconjugated polysaccharide from the glycoconjugate vaccine. This is an especially important step since excess free polysaccharide in the vaccine can decrease the immunological response in many patients.[7]

One of the main challenges in the sterile filtration step is membrane fouling due to the obstruction of membrane pores by components in the solution being filtered. As a membrane fouls, the ability of the membrane to provide high yields of the purified product decreases and the required transmembrane pressure needed to obtain a given filtration velocity increases.[8] As a result, many researchers have studied the underlying fouling phenomena and have tried to develop methods of decreasing the rate and extent of membrane fouling to extend the membrane's lifetime. A wide variety of factors, including solution conditions, membrane type, and filtration system configuration, have been found to influence the extent of membrane fouling, with differing results depending on the materials being filtered.

Previous studies have shown that buffer solution ionic strength influences the transmission of charged pneumococcal vaccine serotypes through ultrafiltration membranes with different pore size.[9] The effective radius of the negatively charged serotypes decreased with increasing ionic strength due to the reduction in intramolecular repulsive electrostatic interactions between the charged groups on the polysaccharide chains. This led to an increase in transmission of the charged serotypes through the membrane at higher ionic strength. Neutral serotypes, however, exhibited very similar filtration behavior over a range of ionic strengths due to the absence of any significant electrostatic interactions. It is unclear how to extrapolate these results to the behavior of glycoconjugates through much larger pore size sterile filtration membranes.

Another method that has been used to minimize membrane fouling is the addition of a prefiltration step. The presence of a prefilter upstream of the sterile filter can prevent larger molecules or aggregates from reaching the sterile filter and causing premature fouling. Kelly, Opong, and Zydney found that the flux decline during sterile filtration of bovine serum albumin

(BSA) protein through 0.16 µm pore size membranes was significantly reduced (or almost completely eliminated in some cases) by using smaller molecular weight cut-off membranes to prefilter the BSA solutions.[12] More recently, Cutler et al. investigated the effect of prefiltration of various proteins through different microfiltration membranes.[13] Their results indicated that the ideal prefiltration membrane is dependent on the type of protein filtered. There is currently no published data on the effect of prefiltration on fouling during sterile filtration of vaccine glycoconjugates.

The aim of this study was to gain a deeper understanding of the factors controlling the extent of membrane fouling during sterile filtration of glycoconjugate vaccines, with a particular focus on the effects of buffer ionic strength and the use of different prefilters. Experiments were performed with the Durapore® polyvinylidene fluoride (PVDF) 0.22 µm pore size sterilizing grade membrane using glycoconjugates provided by Pfizer. The results obtained in this investigation provide important insights that can be used to reduce membrane fouling and increase the performance of the sterile filtration step used during commercial vaccine production.

# Chapter 2

# Materials and Methods

## Solution Preparation

Buffered salt solutions were prepared for use in all experiments. Solid sodium chloride (Sigma Aldrich) and Tris-HCl buffer (Invitrogen) were dissolved in deionized (DI) water to create solutions of desired ionic strength. The pH of each solution was measured using an Oakton pH 700 benchtop pH meter and adjusted to a pH of 7 using either 6 M HCl or 1 M NaOH as needed. After the pH adjustment, buffer solutions were prefiltered through 0.2 µm Supor® 200 membranes (Pall Corporation) or Millipore PVDF filters to remove any undissolved particulates. Solutions of 10, 100, and 500 mM ionic strength (containing 1, 10, and 50 mM Tris, respectively) were prepared to examine glycoconjugate filtration at varying buffer conditions. Prefiltration experiments were performed with the glycoconjugate in the buffer in which they were obtained from Pfizer, and 100 mM NaCl/10 mM Tris buffer was used to wash the membranes prior to filtration.

## Glycoconjugate Serotypes

Glycoconjugate serotypes used in these experiments were provided by Pfizer Inc. (Dublin, Ireland). Each serotype consisted of a specific bacterial polysaccharide covalently conjugated to a tetanus toxoid carrier protein. Different serotypes were used for each set of experiments; these were designated Serotype 2 and Serotype 4 for consistency with other experimental studies in the Zydney laboratory. Glycoconjugate Serotype 4 was used for all

experiments at different ionic strength. Figures 2 and 3 show the structures of glycoconjugate serotypes typical of those used in this work; the details of the specific serotypes are proprietary.

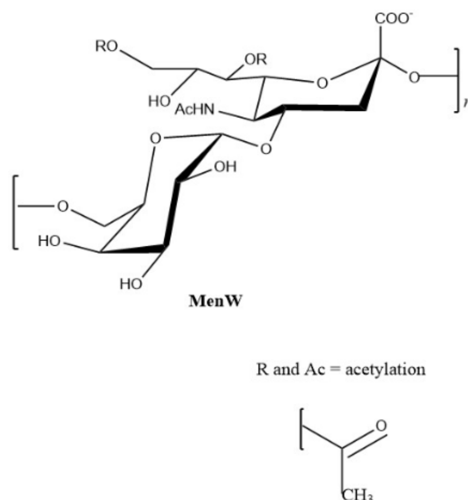


**Figure 2:** Structure of monosaccharide unit for meningococcal polysaccharide type W. From reference 10.

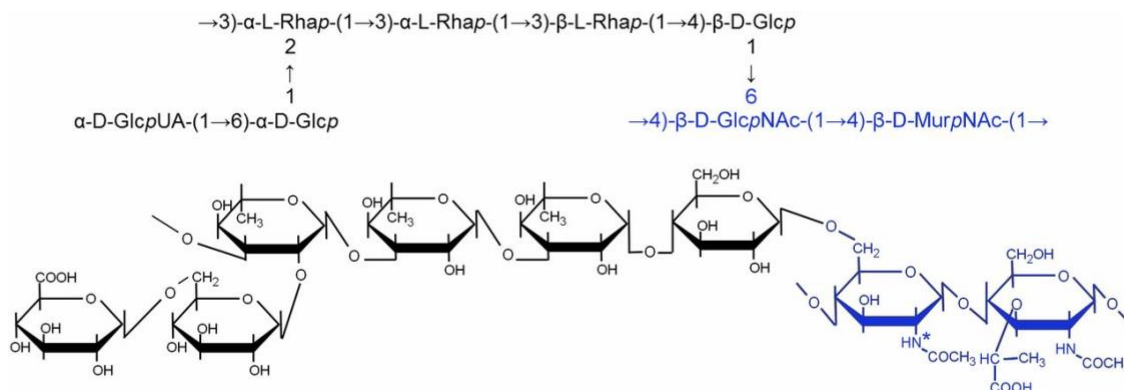


**Figure 3:** Structure of a typical capsular polysaccharide for *Streptococcus pneumoniae* shown in black. Blue structure simulates cell wall linkage. From reference 15.

The glycoconjugate size distribution was examined by dynamic light scattering (DLS) using a Malvern ZETASIZER Nano ZS90 (Malvern, UK). 60 μL samples of the glycoconjugate solution were placed in a Malvern quartz cuvette (ZMV1002, Malvern, UK) for analysis.

# Sterile Filtration

## Constant Pressure Filtration

A schematic of the experimental set-up for constant transmembrane pressure sterile filtration is shown in Figure 4. Either DI water, buffer, or the specific glycoconjugate solution was loaded into a pressurized reservoir, which was connected via flexible tubing to a stirred cell (Amicon) containing the filtration membrane at the base of the cell. Experiments were performed at room temperature with Durapore® polyvinylidene fluoride (PVDF) sterilizing-grade membranes with a pore size of 0.22 μm (MilliporeSigma, Bedford, MA). The membranes were first rinsed with deionized water for 30 minutes followed by the appropriate ionic strength buffer for an additional ten minutes. The permeability of the membrane (in units of LMH/psi) was determined by measuring the flux of buffer though the membrane over a range of transmembrane pressures. The permeability was evaluated from the slope of a graph of buffer flux (LMH) versus transmembrane pressure (psi). Around 50 mL of the glycoconjugate solution was then loaded into the feed reservoir and the solution was filtered for approximately one hour at a constant pressure of 2 psi. The filtrate flux was measured every five minutes by timed collection and the total volume of filtered solution was recorded. Following an hour of filtration, any remaining glycoconjugate was removed from the system and the reservoir was emptied and refilled with buffer. The membrane was rinsed with buffer for 10 minutes, and the permeability of the fouled membrane was measured to provide an indication of the extent of irreversible fouling.

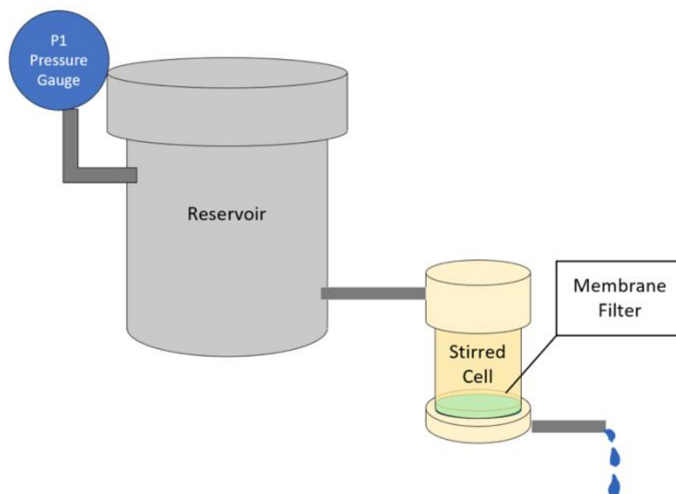


**Figure 4.** Experimental setup for constant pressure filtration.

### Constant Flux Prefiltration

Prefiltration experiments were performed at constant flux using 0.45, 0.65, or 5.0 µm pore size Millipore PVDF membranes as the prefilter. Initial data were obtained using three different flow configurations: integral, batch, and inline prefiltration.[14] For the "integral filtration" configuration, the prefilter and sterile filter were placed directly on top of each other in

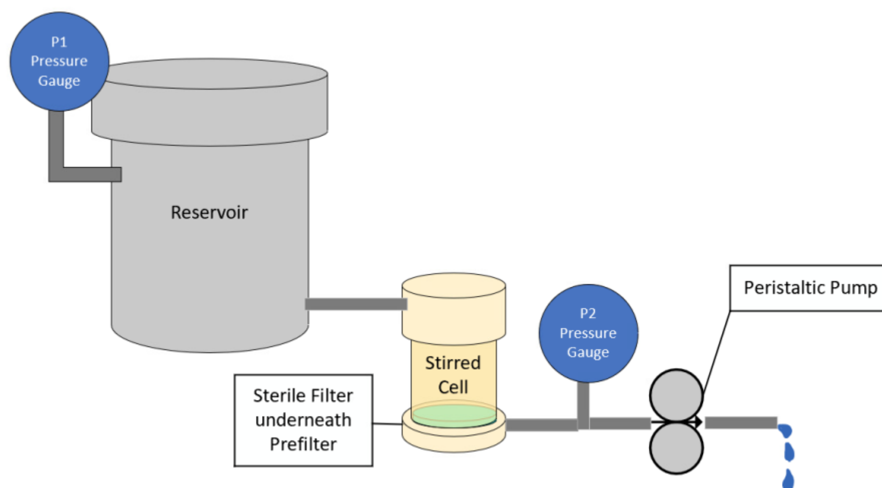


**Figure 5:** Integral prefilter configuration.

the base of the stirred cell (Figure 5). Constant flux was maintained by placing a Rainin Dynamax RP-1 peristaltic pump on the permeate exit line, with the feed reservoir pressurized to approximately 20 psi. Pressure gauges were placed immediately upstream (P1) and downstream (P2) of the stirred cell to evaluate the transmembrane pressure difference (TMP) across the layered configuration of the two membranes. TMP was evaluated as the difference between P1 and P2.

Before the filtration experiment, both the 0.22 μm sterilizing grade filter and the prefilter were separately washed by connecting a stirred cell directly to a feed reservoir containing 10 mM Tris buffer. The filters were each rinsed with buffer for five minutes, and the permeabilities were then measured using the same procedure as for the constant pressure experiments. The membranes were then placed together in the same stirred cell with the prefilter directly on top of the sterile filter. The glycoconjugate solution was then loaded into the reservoir, the reservoir was pressurized to 20 psi, and the pump was set to provide a filtrate flux of 5 μm/s (corresponding to 18 $L/m^2/h$ or 18 LMH). The pressure displayed on each pressure gauge (P1 and P2) was recorded every 5 or 10 minutes until a TMP of approximately 10 psi was reached, after which the filtration was ended.

A similar configuration was used for the batch filtration method (Figure 6). In this configuration, only the prefilter was placed in the base of the stirred cell, and all else remained the same as in the integral filtration. After the TMP across the prefilter reached 10 psi, the collected permeate solution was filtered as a batch through the sterile filter (using the same experimental configuration) at a constant flux until a TMP of 10 psi was reached. One potential disadvantage of batch prefiltration is that aggregates could reform during collection of the filtrate and/or preparation of the sterile filtration apparatus.

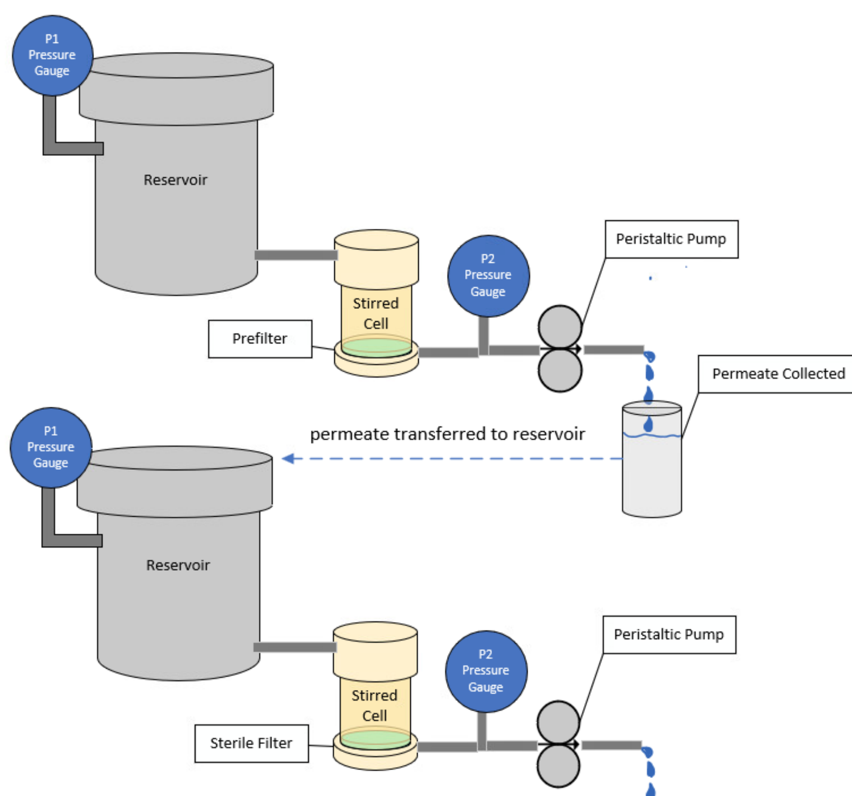


**Figure 6:** Batch prefilter configuration.

Experiments using an inline prefilter were conducted using the experimental set up shown in Figure 7. In this case, the reservoir was directly connected to a stirred cell containing the prefilter. The exit from this stirred cell was then connected to a 0.22 µm sterile filter in a separate stirred cell followed by the peristaltic pump to control the filtrate flux. Pressure gauges were placed on the reservoir (P1), between the prefilter and sterile filter (P2), and after the sterile filter (P3) to measure the transmembrane pressures (TMP) across both membranes. TMP1 was calculated as the difference between P1 and P2, and TMP2 was the difference between P2 and P3. Membrane washing and permeability measurements were carried out using the same methods as for integral and batch filtration. During filtration, P1, P2, and P3 were all recorded in 5 or 10 minute intervals until either TMP1 or TMP2 reached approximately 10 psi.

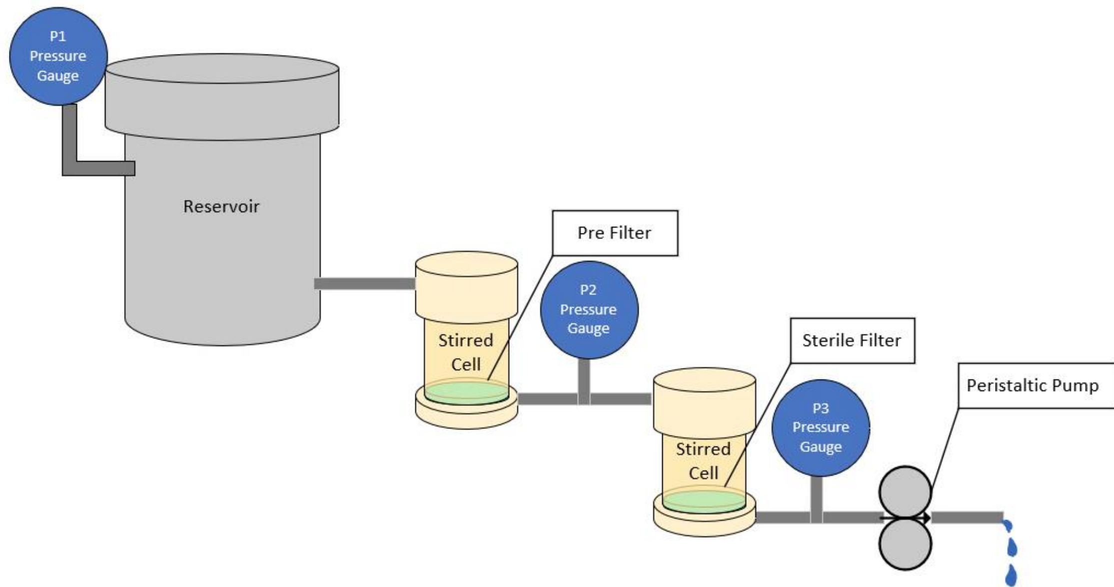


**Figure 7:** Inline prefilter configuration.

# Chapter 3

# Results

## Glycoconjugate Characterization

The size distributions for Serotypes 2 and 4 were determined using dynamic light scattering (DLS), with typical results shown in Figures 8 and 9 and Table 1. Serotype 4 was slightly larger than Serotype 2, although the differences were not statistically significant. For context, similar glycoconjugates, labeled Serotypes 1 and 3, were found to have average sizes of 80 and 150 nm, respectively.[11] Although Serotypes 2 and 4 have average sizes below 150 nm, the distribution clearly shows species as large as 400 nm, which is well above the 220 nm pore size reported for the PVDF membranes.

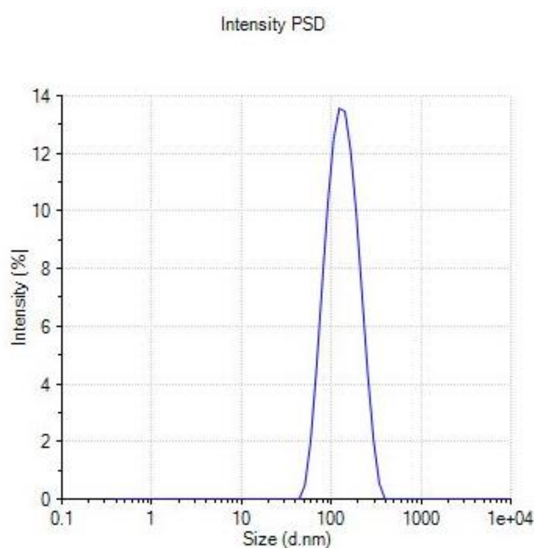


**Figure 8:** Size distribution of Serotype 2, with an average size of 107 nm.

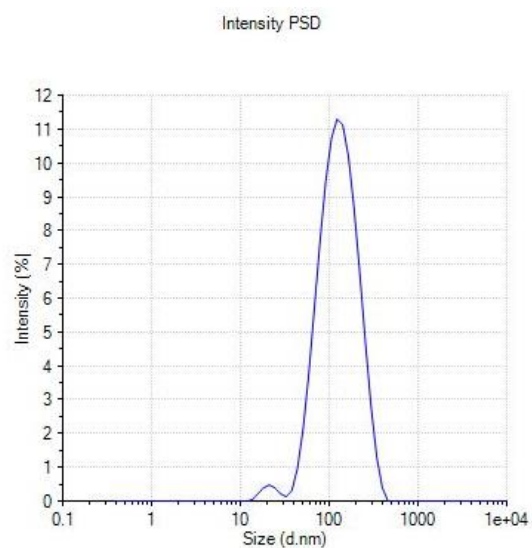


**Figure 9:** Size distribution of Serotype 4, with an average size of 116 nm.

**Table 1:** Average size and polydispersity index (PDI) of glycoconjugate serotypes.

| | Average Size (nm) | Peak (nm) | PDI |
|---|---|---|---|
| **Serotype 2** | 107 | 140 | 0.23 |
| **Serotype 4** | 116 | 141 | 0.18 |

## Varying Buffer Solution Ionic Strength

Typical experimental data for the filtration of glycoconjugate Serotype 4 at a constant pressure of 2 psi through the 0.22 µm Durapore membrane at different ionic strengths are shown in Figure 10. In each case, the initial filtrate flux of the glycoconjugate was approximately 40% less than the buffer flux evaluated at the same transmembrane pressure immediately before the

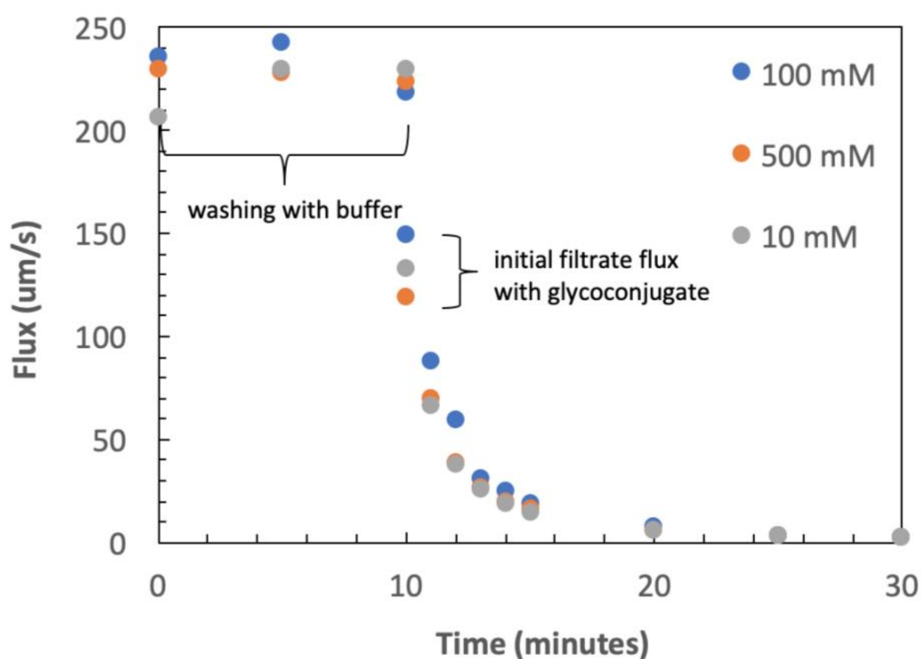


**Figure 10:** Constant pressure filtration at varying buffer ionic strength.

start of the glycoconjugate filtration. The filtration flux for all three ionic strengths decreased sharply with time, decaying by more than 90% compared to the initial filtrate flux after less than 10 minutes of filtration. The flux profiles were nearly identical for all three conditions examined in Figure 10, with no apparent dependence of the flux on solution ionic strength.

The permeability of the Durapore® membranes were measured before and after each filtration experiment, with results summarized in Table 2. The initial permeability ranged from 410 to 470 LMH/psi, with no apparent dependence on the solution ionic strength. Similarly, the permeability at the end of the experiment ranged from 1.5 to 2.0 LMH/psi, with no apparent dependence on either the initial permeability or the solution ionic strength. In each case, the permeability decreased by more than 99.5% due to irreversible membrane fouling, which likely reflects the blockage of a significant fraction of the pores in the PVDF membrane by the larger components present in the glycoconjugate solutions. The lack of any dependence on the solution conditions suggests that the concentration and size of these larger components is unaffected by the solution ionic strength, at least over the range of conditions examined in Figure 10.

**Table 2.** Membrane permeability and fouling with Serotype 4.

| | **10 mM** | **100 mM** | **500 mM** |
|---|---|---|---|
| **Initial Membrane Permeability (LMH/psi)** | 430 | 410 | 470 |
| **Final Membrane Permeability (LMH/psi)** | 2.0 | 1.8 | 1.5 |
| **Permeability Reduction** | 99.5% | 99.6% | 99.7% |

## Constant Flux Prefiltration

### *Comparison of Prefiltration Methods*

Based on the results presented in the previous section, it was decided to examine the potential of using a prefilter to reduce the concentration of any larger aggregates that might be present in the glycoconjugate solutions. Initial experiments examined the behavior of the integral, batch, and inline prefiltration configurations with 0.45 µm pore size prefilters using Serotype 2. Data from all three experiments are shown in Figure 11, with the transmembrane pressure shown on the y-axis and the volumetric throughput shown on the x-axis, with the latter calculated by multiplying the constant filtrate flux (in LMH) by the filtration time. The curve for integral prefiltration represents the TMP across both the prefilter and sterile membrane filter since both membranes are placed in the base of the stirred cell during integral prefiltration. Data for the inline and batch prefiltration experiments show the TMP across only the 0.45 µm prefilter.

As seen in Figure 11, the TMP curves for the three prefiltration configurations looked very similar, with the TMP rising slowly at the start of the filtration and then increasing very rapidly as the membrane becomes more heavily fouled. The calculated value of the filter capacity—defined as the volumetric throughput at which the TMP first exceeded 10psi—was 70 ± 4 L/m$^2$ for the batch and integral prefilter configurations, while the results for the inline prefiltration had a slightly smaller capacity of 55 ± 2 L/m$^2$. The very similar results using the different prefilter configurations suggest that the TMP increase is dominated by fouling of the prefilter. Results for the separate TMP across both the prefilter and sterile filter from additional inline prefiltration experiments are shown subsequently.

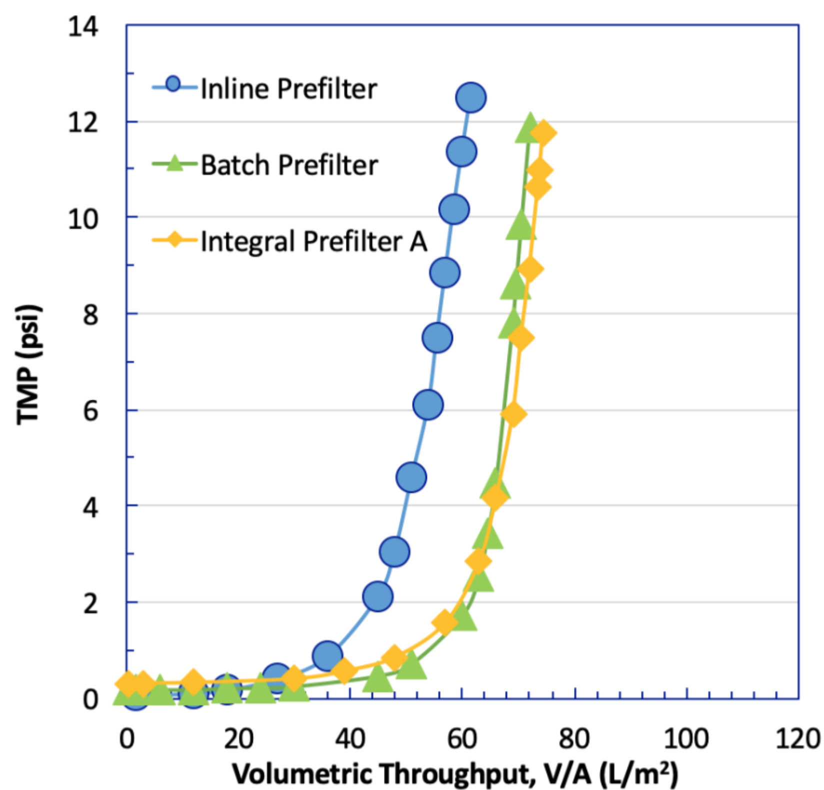


**Figure 11:** Data from constant flux prefiltration experiments of Serotype 2 with inline, batch, and integral prefilter configurations. From Zhuoshi Du of Pennsylvania State University, Department of Chemical Engineering.

***Inline Prefiltration with Serotype 2***

Figure 12 shows typical results obtained during constant flux filtration experiments performed with Serotype 2, either with (right panel) or without (left panel) a 5 µm pore size Millipore PVDF membrane as a prefilter. In both cases, data were obtained at a constant filtrate flux of 18 LMH**.** The y-axis shows the calculated transmembrane pressure across both the sterilizing grade filter and the 5 µm pore size prefilter, while the x-axis shows the volumetric throughput. The experiment without the prefilter showed a rapid increase in TMP, with the TMP exceeding 10 psi after less than 40 L/m$^2$ of filtration (corresponding to less than 150 min).

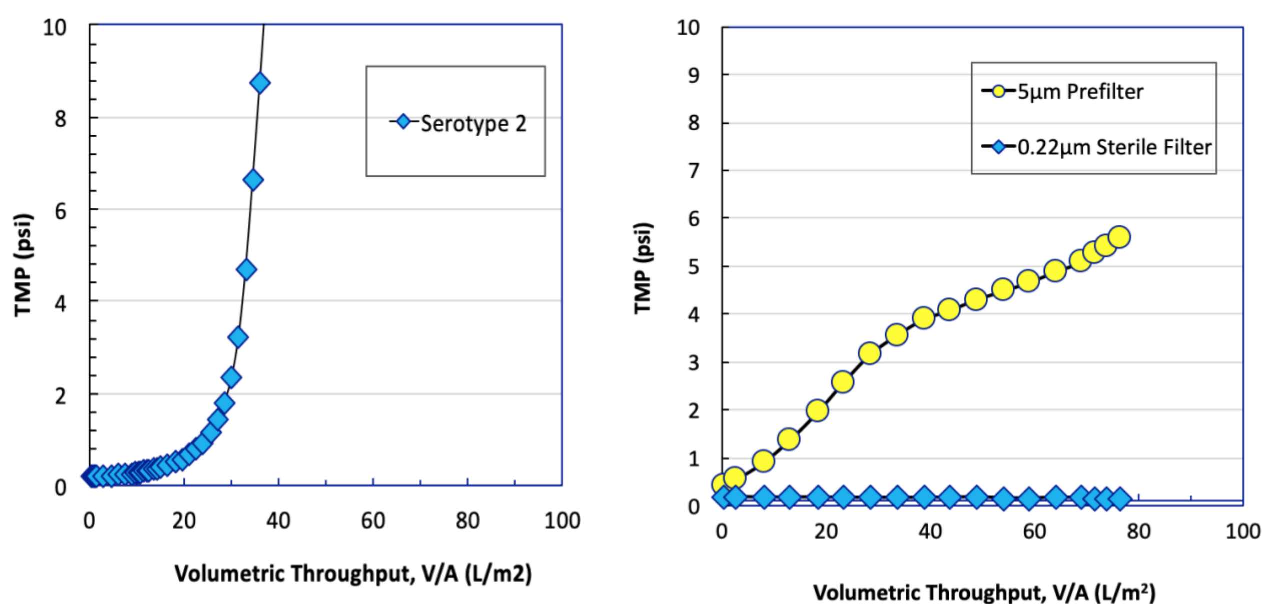


**Figure 12:** TMP versus volumetric throughput for a sterile filter operating alone (left) and across both the 5µm prefilter and sterile filter when used in the inline configuration (right).

In contrast, the sterile filter that was used in the inline prefiltration experiment showed essentially no measurable fouling, with the TMP varying from 0.16 to 0.18 psi over nearly 80 L/m$^2$ of filtration. These data indicate that the 5 µm pore size prefilter is able to effectively protect the sterilizing grade filter from fouling, presumably by removing the key foulant species

present in the feed solution of Serotype 2. However, it was not possible to detect any changes in either mean size or pore size distribution between the feed and prefiltered solution using dynamic light scattering; the DLS intensity plots for these solutions were identical within the accuracy of the DLS measurements. The 5 µm pore size prefilter did show a significant increase in transmembrane pressure during the filtration experiment, with the TMP increasing from approximately 0.5 psi to more than 5 psi after 70 $L/m^2$ of filtration. This TMP increase was somewhat surprising since DLS results did not show evidence of any aggregated material as large as the 5 µm pores of the prefilter. The experiment was ended because the glycoconjugate material in the reservoir was exhausted after 75 $L/m^2$. Even further capacity could have been obtained since the TMP across the 5 µm prefilter reached only 5 psi, and these membranes can easily be operated up to a TMP of at least 20 psi.

Corresponding prefiltration results with 0.45 or 0.65 µm pore size prefilters are shown in Figure 13 alongside results with the 5 µm prefilter. In each case, data are shown for only the TMP across the prefilter, since the 0.22 µm sterilizing grade filter showed almost no measurable

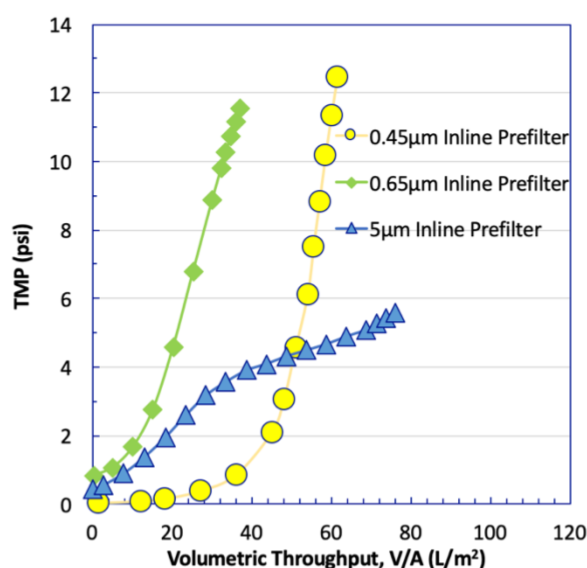


**Figure 13:** TMP across inline prefilters at varying pore sizes.

fouling during any of the filtration experiments with a change in TMP of less than 0.09 psi. The capacity of the prefilter, however, varied significantly among the experiments, with values ranging from 33 L/m$^2$ for the 0.65 µm prefilter to more than 80 L/m$^2$ for the 5 µm prefilter. The highest capacity for the largest pore size prefilter was expected, but the values for the 0.45 and 0.65 µm prefilters do not follow the expected trend. The difference in filtration behavior for these experiments, however, may simply reflect the inherent run-to-run variability among experiments.

***Inline Prefiltration of Serotype 4***

A series of prefiltration experiments were also performed with Serotype 4. Figure 14 compares the fouling behavior of the sterilizing grade filter (without the presence of a prefilter) for the two serotypes. Both serotypes show qualitatively similar behavior, with a somewhat

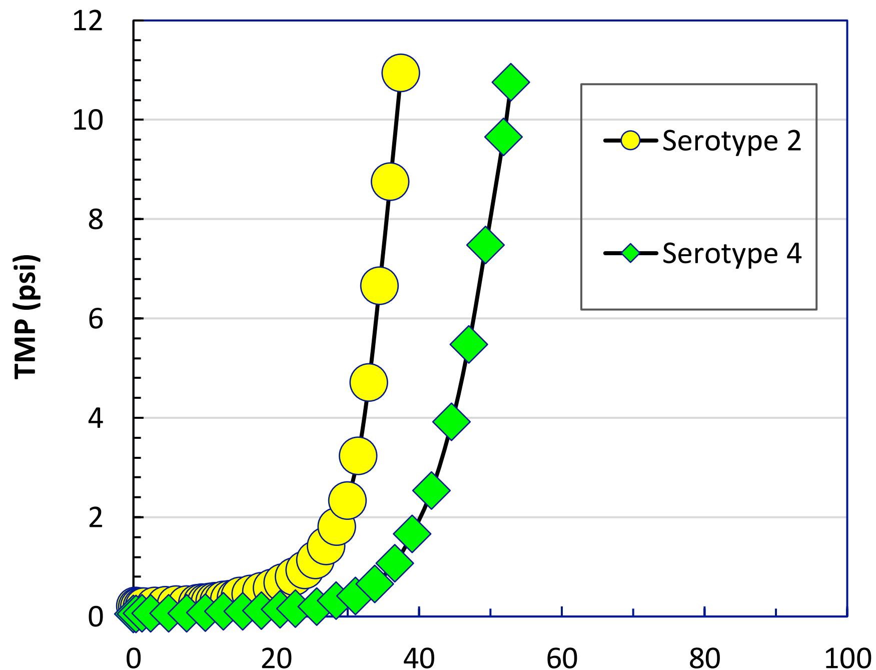


**Figure 14:** Comparison of sterile filter fouling with Serotypes 2 and 4.

slower rate of increase in the TMP for Serotype 4. The capacity of the filter for Serotype 4 was 52 L/m$^2$, which is approximately 40% larger than that for Serotype 2.

Figure 15 shows TMP data for an experiment using an 0.45 µm inline prefilter, with results shown for the TMP across both the 0.45 µm prefilter and the 0.22 µm sterilizing grade filter. The 0.22 µm filter showed no measurable increase in TMP over more than 90 L/m$^2$ of filtration. In contrast to the data for Serotype 2, the TMP across the 0.45 µm prefilter when using Serotype 4 increased to only 0.23 psi before the reservoir was emptied; the TMP for Serotype 2 exceeded 10 psi after a comparable volumetric throughput. No further experiments were conducted with Serotype 4 due to time constraints and availability of material.

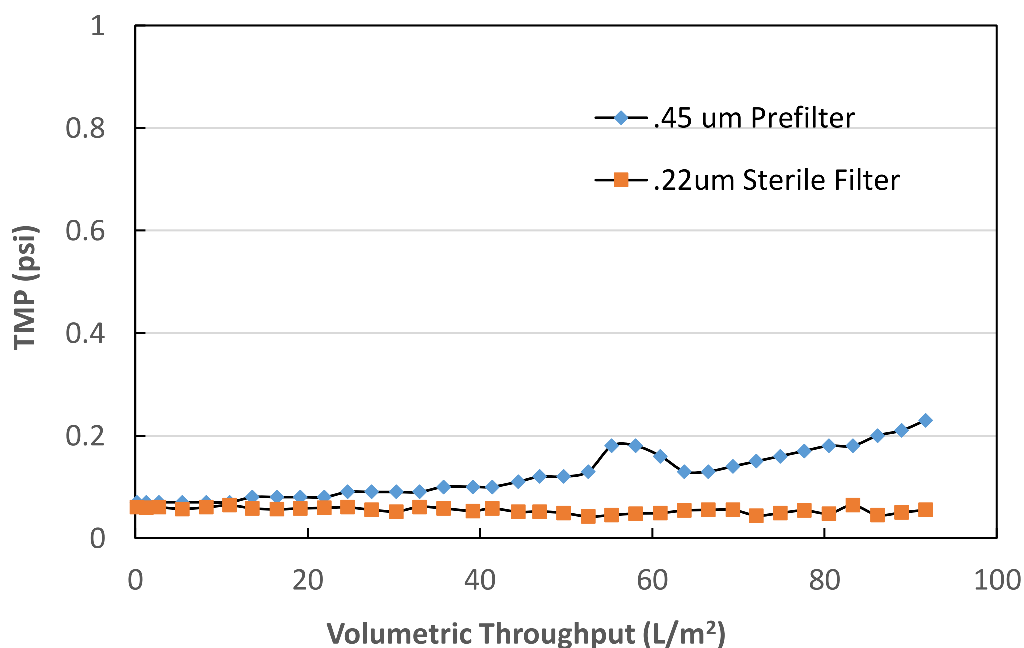


**Figure 15:** Inline prefiltration with a 0.45 µm prefilter and 0.22 µm sterile filter.

# Chapter 4

## Discussion

Sterile filtration of glycoconjugate vaccines is a necessary step of production to remove bacteria and other unwanted particulate material from the final product. A large challenge with sterile filtration of large glycoconjugate vaccines is fouling of the membranes used in the process. This type of fouling leads to very low capacity during the sterile filtration step, requiring the use of multiple membrane filters. The work described in this thesis aimed to examine specifically the effects of solution ionic strength and prefiltration on the fouling behavior.

In the case of constant pressure filtration, the sterilizing grade membranes experienced irreversible fouling, as evidenced by a permeability reduction of over 99% of the initial permeability value after only 20 minutes of filtration. A comparison of results at different ionic strengths shows a dramatic decline in filtrate flux within the first 10 minutes of filtration for all experiments, as well as a very similar extent of fouling, as indicated by the irreversible decline in the permeability. These results demonstrate that the buffer ionic strength has no significant effect on membrane fouling during sterile filtration through the 0.22 μm PVDF membrane, in contrast to results obtained during ultrafiltration of unconjugated polysaccharide serotypes through much smaller pore size membranes.[9] These differences can likely be attributed to the differing fouling mechanisms of the serotypes and membranes used in these studies. In Ledingham's investigation, the pores of the ultrafiltration membranes were smaller than the size of the free polysaccharides, so the fouling seen was largely due to the retention of the polysaccharides on the surface of the membrane. Thus, the increase in effective size of the negatively charged polysaccharides at low ionic strength caused an increase in retention and a corresponding increase in flux decline. In contrast, the pore size of the sterile filters used in this study (0.22

μm, or 220 nm) was larger than the average size of the glycoconjugates (107 and 116 nm) undergoing filtration. In this case, therefore, fouling was likely caused by larger components present in the glycoconjugate solution. Here, the size of these large species may be relatively unaffected by changes in buffer solution ionic strength, which could explain the very similar flux profiles at varying ionic strengths. The large reduction in sterile filter fouling seen in solutions prefiltered through larger pore size membranes confirms the role of large species in fouling these membranes. In addition, Ledingham examined fouling of the free polysaccharides, while the current study examined the fouling of the glycoconjugate formed by coupling the free polysaccharide to a small immunogenic protein. The covalent attachments involved in the formation of the conjugate likely stabilize the glycoconjugate, reducing any changes in effective size or conformation in response to changes in the solution ionic strength.

As previously mentioned, addition of a prefilter proved to be an effective method of slowing the fouling of the sterilizing grade filter, with the nature of the fouling dependent on the prefilter pore size and filtration system configuration. In integral prefiltration, the prefilter was placed directly on top of the sterile filter within the stirred cell. While this method may slow down fouling of the sterile filter, it is unlikely to be practical for large scale applications since the prefilter and sterilizing grade filters are sold as separate units, each housed within a separate membrane module. Batch and inline prefiltration can both be employed in manufacturing processes, and they also allow for direct measurement of the transmembrane pressures across both filters, unlike integral prefiltration. Batch prefiltration has the potential disadvantage that the aggregates could reform after the prefiltration, although no evidence was seen of this phenomenon in the experiments. The performance of the batch, inline, and integral prefilter configurations were essentially identical.

Our hypothesis is that during all prefiltration experiments, the prefilter was able to remove larger particles or aggregates that would rapidly foul the sterile filter. This led to increased volumetric throughput capacity of the sterile filter, effectively increasing its lifetime and usefulness for purification operations. The results from the three prefiltration configurations were very similar, suggesting that any aggregates were removed by the prefiltration. The capacity of the prefilter did differ significantly, however, with varying prefilter pore size. The largest pore size prefilter, with 5 μm pores, seems to be an ideal choice for extending the sterile filtration capacity of Serotype 2. It was able to filter more than twice the volume of glyconjugate solution compared to the smaller pore size prefilters, while still preventing any measurable fouling of the sterile filter. This suggests that the species primarily responsible for sterile filter fouling are large enough to be sufficiently trapped by the 5 μm pores. Such large aggregates were not visible in the DLS measurements, indicating that they must be present at very low total concentrations. The much greater extent of fouling seen with the 0.45 and 0.65 μm prefilters is likely due to the presence of a significant amount of material with size between 0.65 and 5 μm.

While all three prefilters experienced significant fouling with Serotype 2, Serotype 4 did not cause extensive fouling in the 0.45 μm prefilter. Since 0.45 μm is the smallest pore size used in these experiments, it can be hypothesized that the 0.65 and 5 μm filters would also not experience significant fouling with Serotype 4, although this does need to be confirmed experimentally. With this serotype, at a volumetric throughput of 60 L/m$^2$, the TMP across the prefilter was slightly less than 0.2 psi. With Serotype 2, however, the TMP at a throughput of 60 L/m$^2$ was approximately 50 times larger at 10 psi.

Although the differences in membrane fouling between the two serotypes might appear to be due to glycoconjugate size, DLS data showed very similar results with Serotype 4 actually having a slightly larger average size than Serotype 2. Using Nanoparticle Tracking Analysis (NTA), Emami et al. examined the number of larger particles over 200 nm in size for each of the serotypes examined in the Zydney lab.[11] The results showed a much higher count of large particles in an unfiltered sample of Serotype 2 compared to that for Serotype 4, which is consistent with the observed differences in fouling behavior seen in this thesis. Samples of both glycoconjugates after filtration through a 0.2 μm sterile filter still showed significant counts of particles greater than 200 nm based on the NTA, although again the particle count was higher for Serotype 2.

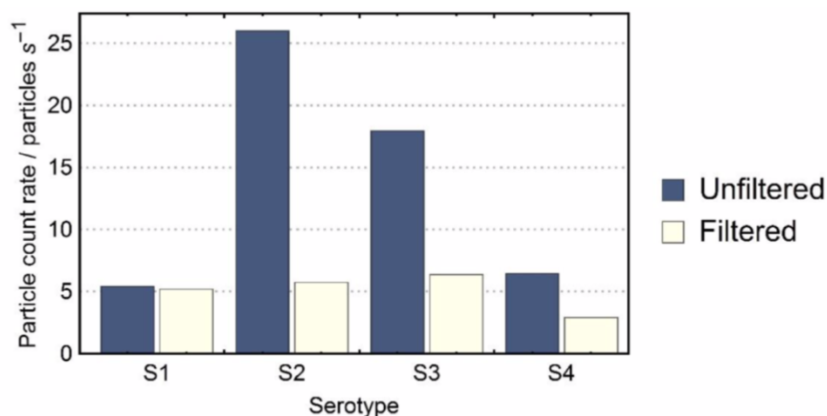


**Figure 16:** NTA results for all four serotypes showing large particle count/second for both feed (unfiltered) and permeate (filtered) solutions. From reference 12.

# Chapter 5

## Conclusions and Recommendations

This work provides one of the first studies evaluating the effects of solution ionic strength and prefiltration on the fouling of PVDF membranes during sterile filtration of glycoconjugate vaccines. Both constant transmembrane pressure and constant flux filtration were examined. Buffer solution ionic strength does not significantly impact ultrafiltration of glycoconjugate Serotype 2, while addition of a prefiltration step shows significant potential to reduce sterile filter fouling for both Serotypes 2 and 4. If time, material, or financial limitations did not exist, including complications to laboratory access as a result of the COVID-19 pandemic, further research could be carried out in order to expand our understanding of glycoconjugate interactions and membrane fouling behavior.

In order to gain further insight into any electrostatic interactions between the glycoconjugates, it might be beneficial to obtain a quantitative measure of the charge of the different serotypes. This could be done by directly measuring the zeta potential of the glycoconjugates, e.g., by electrophoretic mobility experiments, and comparing the charge of these conjugated polysaccharides to free polysaccharides in different solution ionic strength buffers and at different solution pH.

There is essentially no published research on the effects of prefiltration on the fouling behavior of different glycoconjugates, although the Zydney laboratory is currently carrying out experiments that will expand on the prefiltration data presented in this thesis. It will be important to repeat the experiments conducted in this thesis, both to verify the results and to determine the reproducibility of the experimental observations. This is especially true for data on the fouling behavior of the different pore size prefilters. For example, prefiltration with Serotype 2 through a

0.45 µm prefilter unexpectedly had a greater capacity than prefiltration through a membrane with 0.65 µm pores; it is currently unclear if this is an actual trend or if it simply reflects the inherent run-to-run variability. Similarly, inline filtration of Serotype 4 was only carried out with a 0.45 µm pore size prefilter due to time and material limitations; experiments with the larger pore size prefilters are needed to obtain a more complete understanding of the filtration behavior of this serotype.

It would also be useful to extend these studies to include other glycoconjugate serotypes. Limited data for Serotypes 1 and 3 are presented in Appendix A based on experiments performed by Jordan Hillsley. If the greater number of particles larger than 200 nm in size is indeed responsible for the faster fouling of Serotype 2 than Serotype 4, then the fouling behavior of Serotypes 1 and 3 should reflect this as well. It would also be interesting to try to better understand the species in the glycoconjugate solutions that are causing membrane fouling, including the size and nature of these particles. This could potentially be done using Nanoparticle Tracking Analysis (NTA), or it might be possible to directly observe these species in scanning or transmission electron microscopy. Another approach would be to directly examine membranes that have been fouled by the glycoconjugate solution or to elute the foulants from the membrane and then characterize the eluted foulants by DLS, NTA, and/or electron microscopy. These results would provide important new insights into the fouling characteristics of these glycoconjugates during sterile filtration, which should help improve the manufacturing process for production of these life-saving vaccine treatments.

# Appendix A

## Sterile Filtration with Varying Glycoconjugate Serotypes

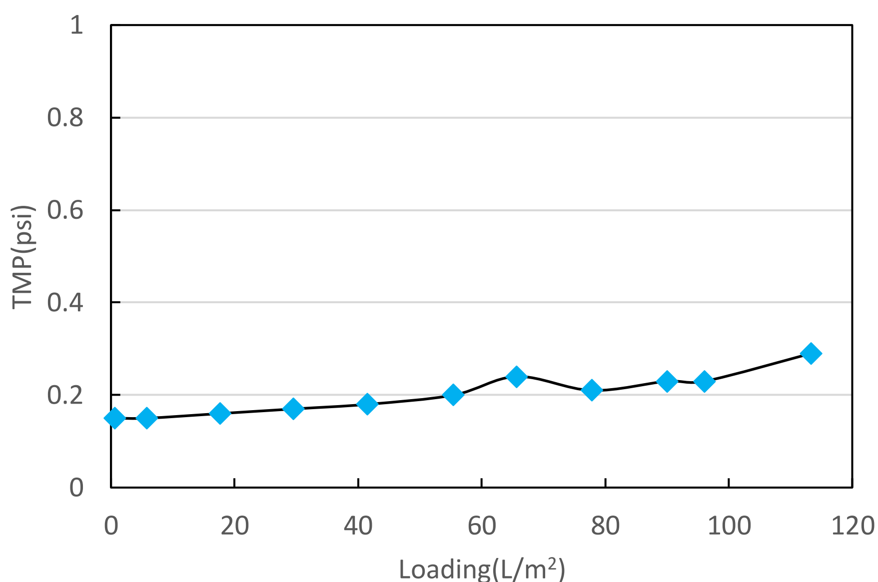


**Figure A 1:** TMP versus loading for sterile filtration of Serotype 1 at constant flux through a 0.22 µm PVDF membrane. From Jordan Hillsley of Pennsylvania State University, Department of Chemical Engineering.

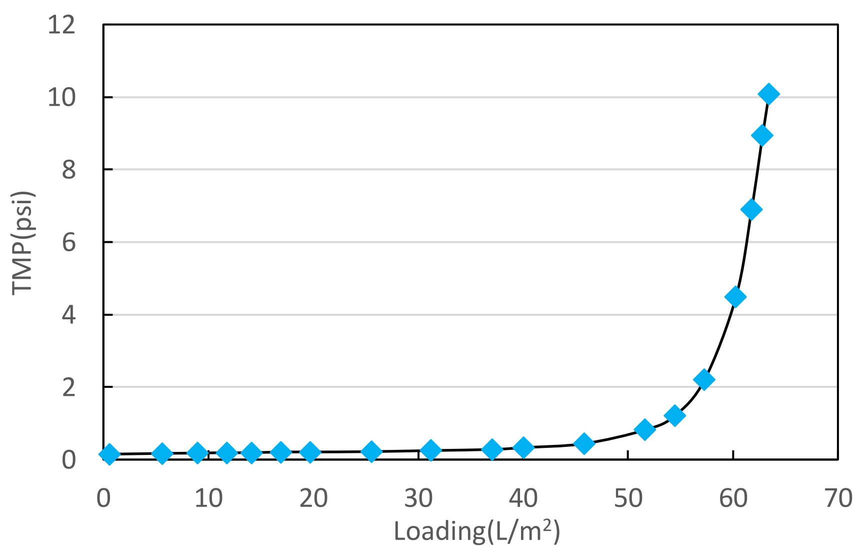


**Figure A 2:** TMP versus loading for sterile filtration of Serotype 3 at constant flux through a 0.22 µm PVDF membrane. From Jordan Hillsley of Pennsylvania State University, Department of Chemical Engineering.

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# ACADEMIC VITA

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- Designed an Excel tool to calculate pressure drop across a pipe system for chocolate or other specialty fluids
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**Process Engineering Co-Op**
Kimberly-Clark Corporation, Beech Island Mill — May-December 2019

- Led implementation of an alternative raw material, saving $100k annually
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- Redesigned an outdated calculator tool to make it more user friendly for operators

**Undergraduate Researcher**
PSU Department of Chemical Engineering; Zydney Research Lab — January 2017-present

- Conducted thesis research on factors influencing the membrane filtration of glycoconjugate vaccines
- Utilized common laboratory tools and practices: preparing buffers, testing pH, DLS, safe handling of biohazardous materials, etc.
- Collected and analyzed trends in experimental data, primarily with Excel

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Microsoft Office (Excel, PowerPoint, Word), PI Process Book, Mathematica, AutoCAD

## PUBLICATIONS

Emami P, Fallahianbijan F, **Dinse E**, Motevalian SP, Conde BC, Reilly K, Zydney AL. Modified intermediate pore blockage model describes fouling behavior during sterile filtration of glycoconjugate vaccines. *J. Membrane Sci.* 2020; 613: 118945

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