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The Effects of a Prefilter on the Sterile Filtration of Glycoconjugate Vaccines

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ABSTRACT

Glycoconjugate vaccines that are produced from the capsular polysaccharides of pathogenic bacteria can provide strong immune protection against a wide range of bacterial disease. One of the critical steps in the production of these vaccines is the use of sterile filtration to ensure sterility of the final vaccine product. The objective of this research was to determine how the introduction of a prefilter in the sterile filtration process will affect the capacity of the sterile filter used to process these glycoconjugate vaccines. It is hypothesized that the addition of the prefilter will reduce the fouling of the sterile filter and therefore increase the membrane capacity by removing trace quantities of large species present in the feed. To test this hypothesis, a series of sterile filtration experiments were performed using 0.22 μm Durapore sterilizing grade filters both with and without different prefilters. Data were obtained using two different glycoconjugate serotypes (containing capsular polysaccharides from different bacterial strains) provided by Pfizer. It was found that when the Serotype 3 was processed through a 0.45 μm prefilter prior to the sterile filter, the sterile filter capacity increased from 28 to 80 L/m^2 representing nearly a 200% increase in capacity. Corresponding results for Serotype 2 showed an even greater effect, with almost no increase in pressure seen for the sterile filter after prefiltration through the 0.45, 0.65, and 5 μm pore size prefilters. Dynamic light scattering data clearly demonstrated that the prefilters removed a large component present in the glycoconjugate feed that was approximately 5 μm in effective size. These results provide important insights into the potential for improving the performance of the sterile filtration process by employing an appropriate prefilter, although additional studies will be required to optimize the combined prefiltration / sterile filtration process.

TABLE OF CONTENTS

LIST OF FIGURES iii

LIST OF TABLES iv

ACKNOWLEDGEMENTS v

Chapter 1 Introduction 1

1.1 History of Vaccines 1
1.2 Glycoconjugate Vaccines 1
1.3 Sterile Filtration 3
1.4 Research Scope 4

Chapter 2 Materials and Methods 6

2.1 Glycoconjugates and Filters 6
2.2 Sample Preparation and Experimental Setup 7

Chapter 3 Results and Discussion 11

3.1 Reproducibility 11
3.2 DLS Measurements 12
3.3 Fouling Behavior of Serotype 3 – Inline Prefiltration 13
3.4 Fouling Behavior of Serotype 2 – Batch Filtration 16

Chapter 4 Conclusions 18

Chapter 5 Future Studies 20

BIBLIOGRAPHY 22

LIST OF FIGURES

Figure 1: 3D rendering of glycoconjugate vaccine[5].....

Figure 2: SEM cross section of 0.22 µm PVDF filter highlighting the symmetrical pore size.[9] 7

Figure 3: Regular single membrane sterile filtration experiment setup. 8

Figure 4: Batch filtration experimental setup.....9

Figure 5: Experimental setup for inline filtration 9

Figure 6: TMP profiles for repeat experiments showing Serotype 2 filtration through a 0.22 µm sterile filter .....

Figure 7: Serotype 3 size distribution before and after prefiltration through the 5 µm PVDF membrane.....12

Figure 8: Serotype 2 size distribution before and after prefiltration through the 5 µm PVDF membrane.....13

Figure 9: TMP as a function of Loading for Serotype 3 through the 0.45 an d 5 µm prefilters and a separate 0.22 µm sterile filter at a flux of 5 µm/s.....14

Figure 10: TMP profiles for Serotype 3 through the 0.22 µm sterile filter with and without inline prefiltration through different pore size prefilters. Flux is set at 5 µm/s.....15

Figure 11: Fouling behavior for the different batch prefilters for Serotype 2. The flux was set at 5 µm/s.....16

Figure 12: Sterile filtration data with TMP as a function of Loading for Serotype 2. The flux set at 5 µm/s.....17

LIST OF TABLES

Table 1: Membrane capacity during sterile filtration of Serotype 3 with different prefilters ..16

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Chapter 1

Introduction

1.1 History of Vaccines

Throughout history, humans have been trying to stay ahead of diseases to reduce sickness and save lives whether it be by the bodies' natural defense mechanisms or through vaccines. The first vaccines were a form of live attenuated viruses which involved doctors inoculating patients with largely inactive or less severe forms of the virus that caused the disease[1]. These inoculations induced an immune response that led to production of antibodies that would protect the patient if they encountered the same pathogenic organism in the future. However, this method often proved to be unreliable and dangerous. Science has come a long way with vaccines being easier to produce, more effective when administered, and safer for the patient receiving them. Many new types of vaccines have been developed in recent years including killed/inactivated viruses, protein subunits, mRNA, polysaccharide, and glycoconjugate vaccines[1].

Polysaccharide vaccines employ the capsular polysaccharide from the pathogenic bacteria's extracellular matrix and allow the body to create antibodies that bind strongly to the specific sugars present on the surface of the bacteria. However, using the polysaccharide only induces a T cell-independent response, which means that the antibody is produced in the absence of T-cells.[2] A T cell-independent response prevents sustained antibody production in the body, especially in small children and the elderly, and is therefore not an ideal vaccine candidate.

1.2 Glycoconjugate Vaccines

One approach to generating a strong T cell-dependent response is to conjugate a highly immunogenic carrier protein to the polysaccharide to make what is known as a glycoconjugate vaccine;

these have been shown to invoke T-cells in the antibody synthesis process.[3] Typically, the carrier protein is a generic protein that triggers a strong immunogenic response in the body such as the tetanus toxoid protein, with the vaccine still generating a strong response to the polysaccharide (and not the tetanus protein).[4] The carrier protein allows the body's T-cells to interact with the vaccine and recognize the molecule as a potential threat. With the T cell-dependent response, the body should have sustained antibody production after vaccination, making it much more effective than just the polysaccharide vaccine.

To create a glycoconjugate vaccine, the polysaccharide is chemically conjugated to the protein via a covalent linkage, as shown in Figure 1. The carrier protein is represented by the gray helices and sheets, and the covalent linkage is shown by the light blue and yellow connection between the polysaccharide and the immunogenic carrier protein.

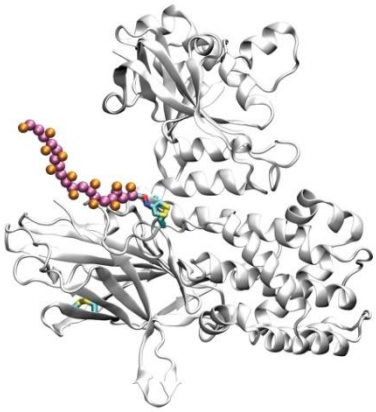

Figure 1: 3D rendering of glycoconjugate vaccine[5]

The second key component of the glycoconjugate vaccine is the polysaccharide chain, which is specific to the extracellular matrix of the target pathogenic bacteria. The combination of the carrier protein and the polysaccharide makes an effective vaccine against bacterial infection. The first glycoconjugate vaccine was produced in 1987 to prevent *Haemophilus influenzae* type b (Hib). The effectiveness of the Hib vaccine propelled glycoconjugates into the forefront of vaccine production.[3]

1.3 Sterile Filtration

The process of creating a biological pharmaceutical or therapeutic requires many complex steps. These are typically classified in two broad aspects: upstream processing and downstream processing. Upstream processing includes the fermentation tanks / bioreactors where the cells are grown to create the basic biologic component(s) of the drug. Downstream processing takes the cell suspension from the bioreactor and selectively removes the undesired material through a series of steps including membrane filtration and different types of chromatography, ultimately yielding a vaccine product that is safe to administer to patients. Without proper purification, undesired material, such as cell debris, host cell proteins, DNA, and bacteria, could remain in the therapeutic and ultimately cause harm to the patients. One of the key final steps in this process is the sterilization of the therapeutic, which is typically performed as part of the final fill-finish operations used to place the product in a vial for storage and delivery.

There are a number of methods available for sterilization including high temperature, appropriate chemicals, or filtration. When dealing with a glycoconjugate vaccine, temperature and chemical sterilization techniques can alter the 3-dimensional structure of the protein or cause chemical modification of the vaccine, which could reduce the effectiveness of the vaccine or lead to adverse side effects, either of which would make the vaccine unsafe to give to patients. To prevent any changes to the vaccine, sterile filtration becomes the most acceptable method for sterilization of these products. Sterile filtration is a technique where the solution is put through a membrane (often referred to as a sterilizing grade filter) with pores that are rated as being less than or equal to 0.22 µm in diameter. The average bacterium is about 1 to 2 µm in size, with the smallest bacterium getting down to 0.25 µm.[6] A 0.22 µm filter is specifically designed to retain all bacteria and any larger undesired particles on the basis of size while allowing the desired vaccine to flow through the pores so that it can be collected in the permeate. This ensures that the final vaccine is sterile without changing the vaccine's structure or properties.

Throughout the sterile filtration process, specific components of the vaccine can bind to or deposit on the membrane, ultimately reducing the filtrate flux (for operation at constant pressure) or increasing the

required pressure (for operating at constant flux). This is referred to as membrane fouling.[7] When fouling becomes too pronounced, the sterile filter is unable to be used further and therefore needs to be replaced, which increases the cost of manufacturing. Typically, fouling is caused by particles that are larger than the labeled pore size of the filtration membrane or that have specific interactions with the filter, e.g., attractive electrostatic interactions between oppositely charged species. The average size of glycoconjugate vaccines is only slightly less than the 0.22 μm sterile filter pore size; thus, fouling can potentially occur due to interactions between glycoconjugates or by deposition of larger components / aggregates in the feed that are generated during the chemical coupling of the polysaccharide to the carrier protein or due to other manufacturing operations or storage.

1.4 Research Scope

Membrane fouling is a highly undesirable aspect of sterile filtration. It can reduce the amount of solution that can flow through the membrane, requiring a larger transmembrane pressure (TMP) to drive the filtration process and ultimately requiring the replacement of the membrane. Many methods have been used to prevent fouling and increase the membrane capacity including proper selection of membrane materials, optimization of operating conditions, selection of the ionic strength of the solution, and pre-treatment of raw materials[7].

The focus of this research is on the pretreatment of the glycoconjugate vaccine before sterilizing the solution via the use of a prefilter. Prefilters provide a unique opportunity to reduce the number of large particles in a solution and thus protect the sterile filter from fouling. Experiments were performed with different pore size polyvinylidene fluoride (PVDF) prefilters before a 0.22 μm PVDF sterile filter. The larger pore size prefilters will allow the vaccine solution to flow through more easily, while the smaller pore size prefilters should be more effective at removing components that would otherwise foul the sterile filter.

Although there have been no previous studies of prefiltration of glycoconjugate vaccines, there have been a limited number of studies of the effects of prefilters on protein filtration. For example, Cutler et. al. showed that the use of a 1.0 μm pore size prefilter reduced the fouling of both 0.45 and 0.22 μm membranes when filtering a lysozyme protein solution[8]. Without a prefilter, there was a rapid decline in filtrate flux during the constant pressure filtration. When a prefilter was added to the process, the permeate flux through the sterile filter remained nearly constant, demonstrating a significant reduction in fouling. However, these experiments were run with polytetrafluoroethylene (PTFE) filters with a small protein (lysozyme), making it difficult to translate the results to PVDF filters and glycoconjugate vaccines. Therefore, the objectives of this study were to: (1) examine whether prefilters can be used to increase the capacity of the sterile filters when filtering glycoconjugate vaccines, and (2) develop a more fundamental understanding of the mechanisms involved in fouling during sterile filtration of these important vaccine products.

Chapter 2

Materials and Methods

2.1 Glycoconjugates and Filters

Glycoconjugate vaccine solutions from different serogroups were provided by Pfizer Inc. These are referred to as Serotype 2 and Serotype 3 based on the nomenclature developed as part of the collaboration between Penn State and Pfizer. As described earlier, these glycoconjugates are made up of the capsular polysaccharide of a pathogenic bacteria covalently bonded to a tetanus toxoid as the carrier protein. The glycoconjugates were stored in bulk 1 L storage containers, with Serotype 2 showing a yellowish tint while Serotype 3 is clear; both solutions were slightly more viscous than water.

Five different membranes were used throughout these experiments including the 0.22 µm (sterile filter); 0.45 µm, 0.65 µm, and 5 µm PVDF prefilters; and a 5 µm Isopore polycarbonate prefilter. The PVDF filters are symmetrical (often referred to as homogeneous), with the pore size remaining relatively uniform throughout the depth of the filter as seen in Figure 2. The membrane is made by a phase inversion process which provides a random distribution of irregular pores. The 0.22 µm pore rating indicates that the filter is of sterilizing grade, i.e., the filter provides a completely sterile filtrate when challenged with a suspension of *B. dimunita*, one of the smallest known bacteria.[10]

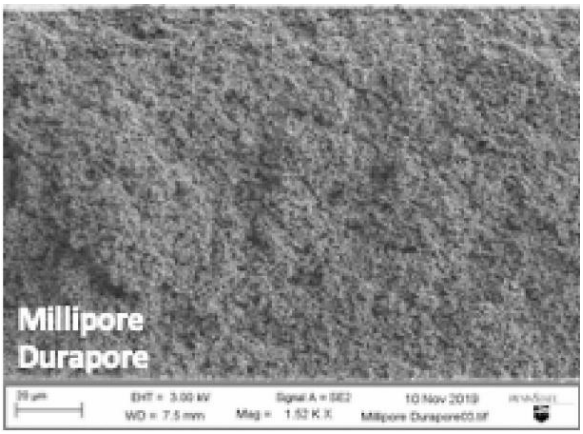

Figure 2: SEM cross section of 0.22 µm PVDF filter highlighting the homogeneous pore size.[9]

Isopore filters are made using a track-etch process in which a flat polycarbonate sheet is irradiated and then etched to form highly uniform cylindrical pores that pass completely through the depth of the filter material. These membranes are not used for large-scale commercial processing due to their higher cost, but they provide a very well-defined pore structure and retention properties for both laboratory work and more fundamental studies of membrane transport / separations.

2.2 Sample Preparation and Experimental Setup

Samples were prepared the same way for each experiment. First the 1 L bulk storage container was removed from the refrigerator used for storage of the glycoconjugate at 4 ^{o}C. The 1 L container was then carefully rotated both end over end and around the vertical axis multiple times to ensure that the solution was fully mixed without causing excessive agitation. This allows the solution to re-homogenize including resuspension of any large components that might have settled on the bottom. This mixing was important to ensure consistency from experiment to experiment with regards to concentration and size distribution. Fifty mL of solution was then poured into a 50 mL centrifuge tube and set on the counter for 30 minutes to allow the solution to reach room temperature.

The experiments were performed with three different configurations: (1) sterile filtration alone (control), (2) sterile filtration after a batch prefiltration, and (3) sterile filtration after an inline prefiltration. The control setup is shown in Figure 3. The glycoconjugate solution was fed to the stirred cell from an acrylic pressurized reservoir. The stirred cell holds the sterile filter at the bottom as indicated by the orange disc in Figure 3.

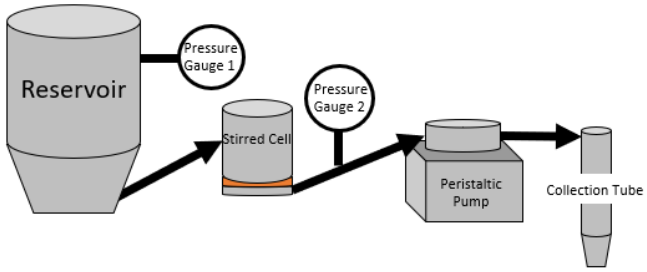

Figure 3: Regular single membrane sterile filtration experiment setup.

Two pressure gauges were used to monitor the transmembrane pressure (TMP) across the sterile filter: one connected to the reservoir and the other connected to the tubing between the stirred cell and the peristaltic pump that was used to control the filtrate flux. The transmembrane pressure was calculated by subtracting the second pressure from the first pressure.

$$TMP_1 = P_1 - P_2 \qquad (1)$$

The second experimental setup that was used is a batch filtration which uses the same configuration as the control except the filtration is done twice, first through the prefilter with the collected permeate then used as the feed for the sterile filtration as shown in Figure 4. The two filtration experiments were run sequentially using the same equipment, with the apparatus cleaned, the prefilter removed and discarded, and then the sterile filtration membrane placed at the bottom of the stirred cell before starting the second filtration.

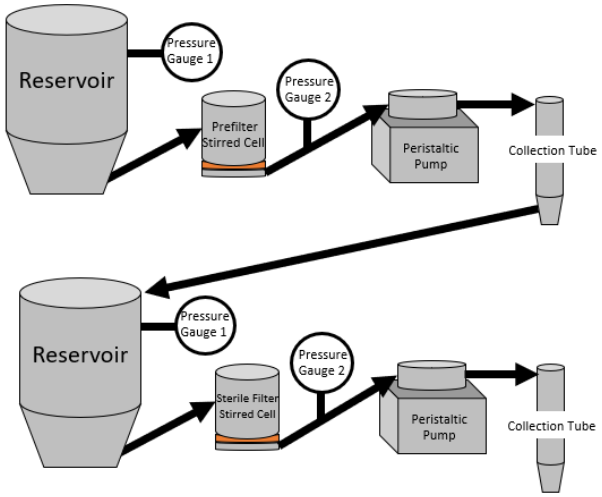

Figure 4: Batch filtration experimental setup

The third configuration used is referred to as inline filtration in which the prefiltration and sterile filtration are performed together in a continuous process. The filtrate obtained from the prefilter in the first stirred cell directly flows into a second stirred cell that holds the sterile filter as shown in Figure 5. This setup uses three pressure gauges, with Equation (1) used to evaluate the transmembrane pressure across the prefilter while the TMP across the sterile filter is evaluated as:

$$TMP_2 = P_2 - P_3 \qquad (2)$$

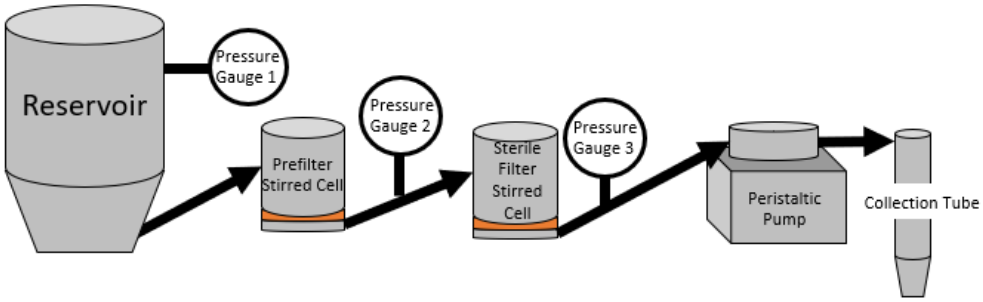

Figure 5: Experimental setup for inline filtration

Prior to each run, the system was flushed with 100 mM NaCl + 10 mM Tris-HCl pH 7.00 buffer. Buffer was flushed for about ten minutes at low pressure (under 2 psi) to ensure proper wetting of the membrane before the Serotype was added to the system. After the buffer flush, the excess buffer was removed by emptying the stirred cell(s) and then pumping air through the tubing. The serotype solution was then added into the reservoir, the lid was screwed on, and a small quantity of solution was allowed to flow through the system to remove any entrapped air. All pressure gauges were then zeroed to ensure any difference in pressure is recorded properly. The reservoir was then pressurized to 140 kPa = 20 psi. The system was allowed to equilibrate for several minutes, after which the peristaltic pump was turned on at the desired filtrate flow rate. The experiment was continued until the TMP exceeded 69 kPa (10 psi) or until the solution was fully emptied from the reservoir.

All experiments were performed at a filtrate flux of 5 μm/s, corresponding to a filtrate flow rate of 0.12 mL/min, which was maintained by the peristaltic pump at the exit of the stirred cell. The mass flow of the permeate was measured using an analytical balance placed underneath the collection tube that was zeroed before each experiment. This mass flowrate was converted to a filtrate flux using the density of water and a membrane area of 0.00041 m^2 and monitored throughout each experiment. The TMP profiles were also evaluated throughout the experiment and used to determine the extent of fouling of the membranes.

In addition, small samples of the initial feed and permeate solution were examined by dynamic light scattering (DLS) to evaluate the effective size distribution of the glycoconjugate samples. Sixty μL of sample were added to a quartz cuvette and analyzed using a Malvern Zetasizer Nano ZS90 DLS instrument. The system was allowed to equilibrate for 2 min at room temperature, with the light scattering intensity measured for several repeat runs to obtain accurate data for the diffusivity which was then converted into an effective size using the Stokes-Einstein equation.

Chapter 3

Results and Discussion

3.1 Reproducibility

Figure 6 shows results from two repeat experiments with the exact same setup for the filtration of Serotype 2 through the 0.22 µm PVDF sterile filter at a constant flux of 5 µm/s.

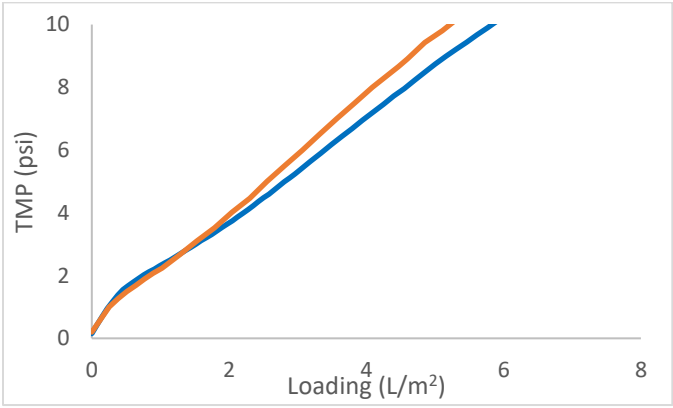

Figure 6: TMP profiles for repeat experiments showing Serotype 2 filtration through a 0.22 µm sterile filter

As seen in Figure 6, the two trials show very similar fouling behavior with a nearly linear increase in TMP over the course of the filtration. The capacities of the two filters, defined as the volumetric loading when the transmembrane pressure reached 10 psi, were 5.2 and 5.8 L/m^2, a difference of less than 12%, showing the good reproducibility of the fouling behavior.

3.2 DLS Measurements

The size distribution and average size of each Serotype were determined using dynamic light scattering (DLS), with typical results shown in Figures 7 and 8. Serotype 3 shows an average size of 140 nm while Serotype 2 shows an average size of 107 nm, both of which are smaller than the nominal 0.22 µm pore size of the sterilizing grade filters.

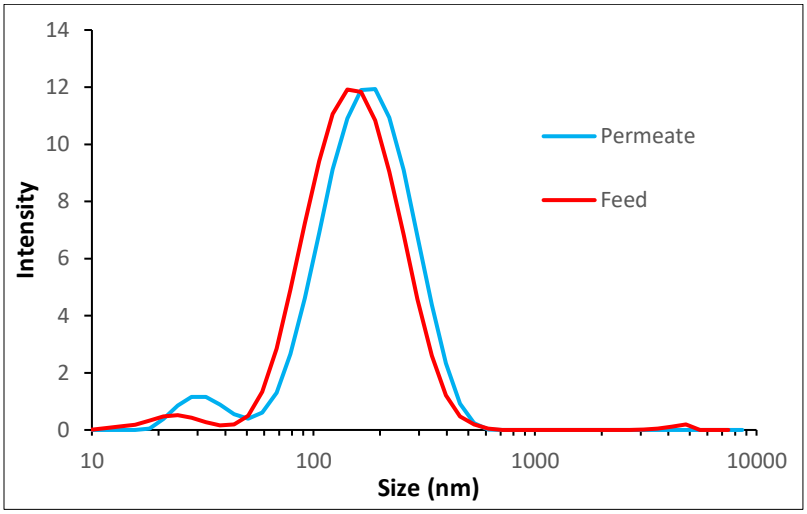

Figure 7: Serotype 3 size distribution before and after prefiltration through the 5 µm PVDF membrane

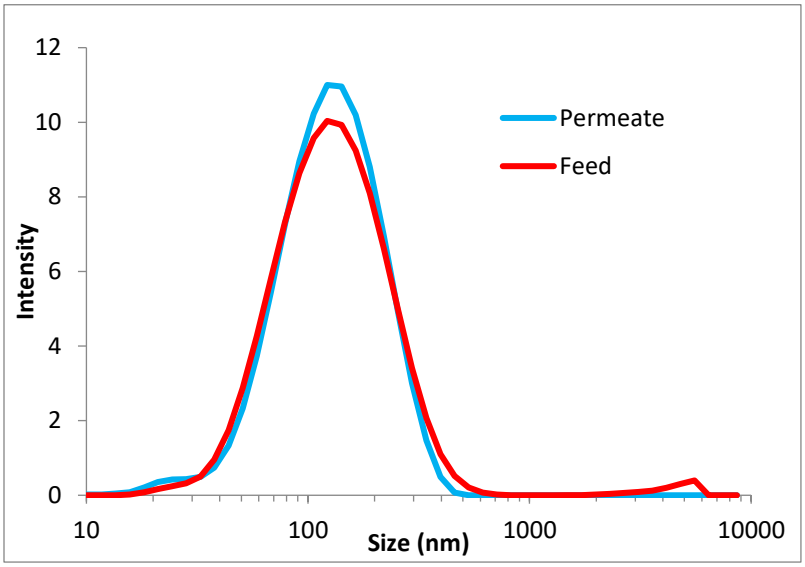

Figure 8: Serotype 2 size distribution before and after prefiltration through the 5 µm PVDF membrane

However, both serotypes show a very broad size distribution, with some components as small as 20 nm and a few being larger than 600 nm. This includes a small peak around 5 µm in size that is easily visible in the feed for both Serotype 2 and 3. It is these large particles that will likely foul the membranes during the sterile filtration step. This can be seen in the difference in the DLS results for the feed and permeate samples obtained through the 5 µm PVDF prefilters. Both serotypes show a significant reduction in the size of the peak around 5 µm, as well as a small shift in the location of the main peak for Serotype 3. The net result is that the Z-average size of both serotypes is very slightly reduced by the prefiltration process, with Serotype 3 going from 144 nm to 138 nm and Serotype 2 going from 107 to 106 nm. This clearly indicates that some of the larger particles are being captured in the prefilter.

3.3 Fouling Behavior of Serotype 3 – Inline Prefiltration

The first set of filtration experiments focused on Serotype 3. Figure 9 shows the transmembrane pressure versus the volumetric loading of the 0.45 and 5 µm prefilters compared to that of the 0.22 µm

sterile filter alone. The volumetric loading is defined as the cumulative filtrate volume divided by the membrane area.

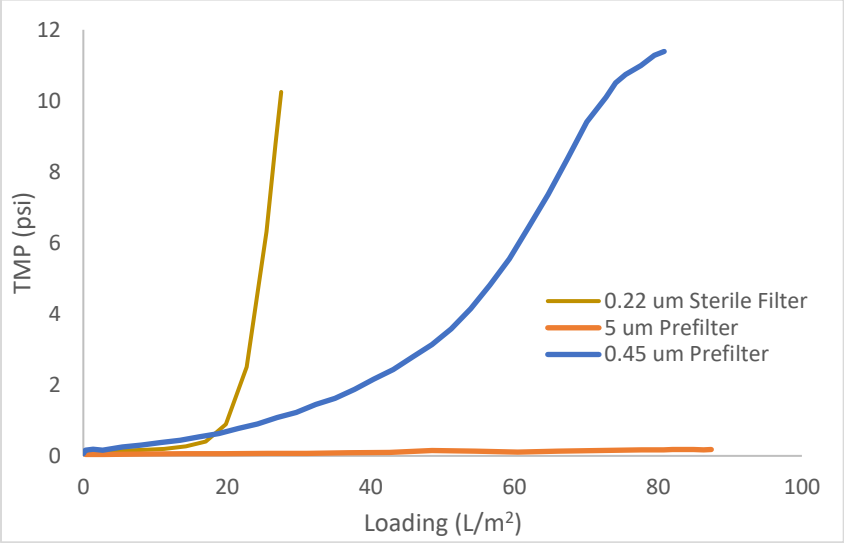

Figure 9: TMP as a function of Loading for Serotype 3 through the 0.45 and 5 µm prefilters and a separate 0.22 µm sterile filter at a flux of 5 µm/s.

Since these experiments were performed at a filtrate flux of 5 µm/s, the volumetric loading of 80 L/m^2 corresponded to a filtration experiment of approximately 5 hr. There was no measurable retention of Serotype 2 by any of the filters, with measured recoveries of >95%. The larger pore size 5 µm PVDF filter did not show any significant fouling throughout the entirety of the experiment, with the TMP increasing from 0.04 psi to 0.18 psi over 88 L/m^2. In contrast, the 0.22 µm sterile filter started to foul quite rapidly at about 20 L/m^2, with the TMP exceeding 10 psi after only 28 L/m^2. The 0.45 µm prefilter showed an unusual S-shaped TMP curve that lies between the results for the 0.22 and 5 µm filters as expected, with the TMP = 10 psi after 81 L/m^2.

Figure 10 shows experimental data for filtration of Serotype 3 through the 0.22 µm sterile filter either alone or in combination with an inline prefiltration through either the 0.45 or 5 µm PVDF prefilter.

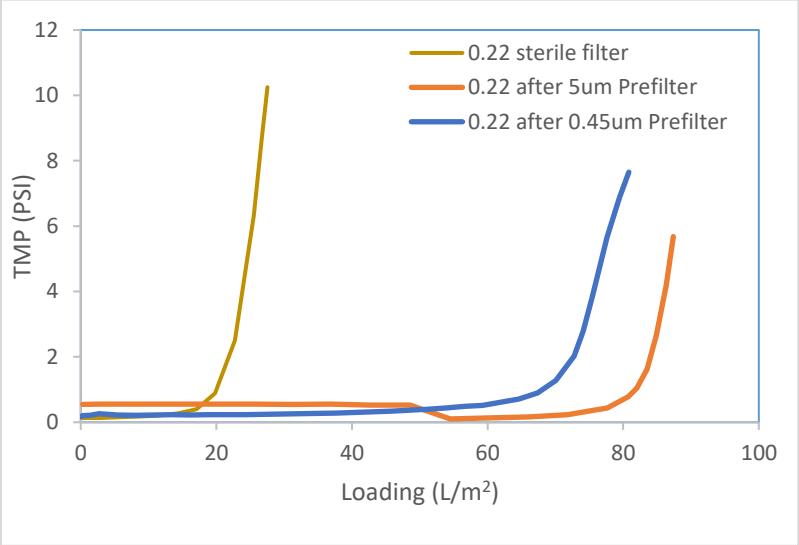

Figure 10: TMP profiles for Serotype 3 through the 0.22 µm sterile filter with and without inline prefiltration through different pore size prefilters. Flux is set at 5 µm/s.

The sterile filter fouled the least when the 5 µm PVDF prefilter was used prior to the sterile filter with a capacity of more than 80 L/m^2. In contrast, when the 0.22 µm membrane was used on its own the capacity was less than 30 L/m^2. Interestingly, the sterile filter after the 0.45 µm prefilter fouled more rapidly than the sterile filter after the 5 µm prefilter even though one would expect the 0.45 µm prefilter to provide greater overall removal of large foulants. This could simply be run-to-run variability in the data, although it is also possible that there is some adsorptive removal of foulants within the depth of the 5 µm prefilter.

The effects of the different inline prefilters on the sterile filter capacity are summarized in Table 1. The experiments with the two prefilters did not reach the desired 10 psi due to the limited pool of filtrate collected before the pressure drop through the prefilter became too large. Instead, the capacities for the sterile filters in those experiments were evaluated from the final volumetric throughput at the end of the filtration, corresponding to a transmembrane pressure of 7.7 psi for the run with the 0.45 µm prefilter and

5.7 psi for the run with the 5 µm prefilter. Both prefilters increase the capacity of the sterile filter by about 200%.

Table 1: Membrane capacity during sterile filtration of Serotype 3 with different prefilters

Experiment	Loading (L/m^2)	Percent Change
No prefilter	28	
0.45 µm Prefilter	>81	>190%
5 µm Prefilter	>87	>220%

3.4 Fouling Behavior of Serotype 2 – Batch Filtration

The transmembrane pressure profiles for the four different prefilters used for the Serotype 2 filtration experiments are shown in Figure 11.

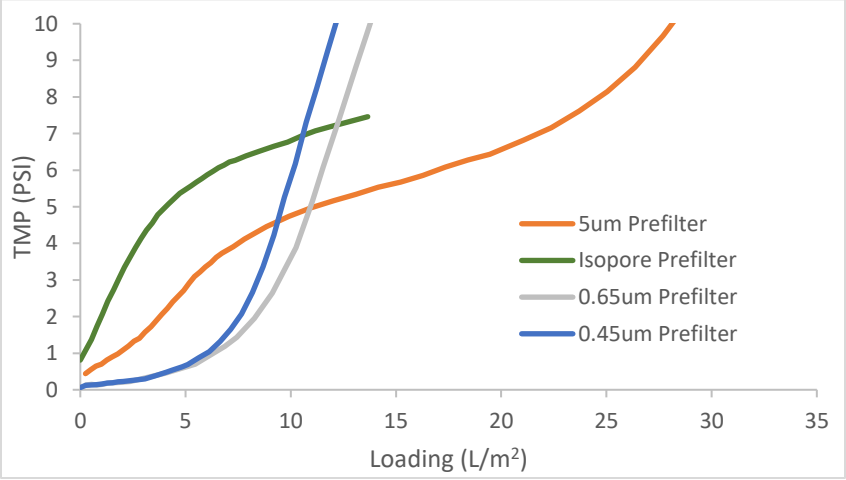

Figure 11: Fouling behavior for the different batch prefilters for Serotype 2. The flux was set at 5 µm/s.

The capacities obtained with Serotype 2 are much smaller than the corresponding values for Serotype 3 (shown previously in Figure 9). The 0.45 and 0.65 µm prefilters showed similar fouling

behavior, with the 0.65 µm filter having a slightly larger capacity as expected. The concave up pressure profile seen with these two prefilters is similar to that seen with the 0.22 µm sterile filter. However, the fouling behavior of the two 5 µm prefilters were very different. These larger pore size prefilters showed a very rapid initial increase in TMP followed by a "plateau region" leading to an S-shaped pressure profile. The 5 µm pore size PVDF prefilter had the largest capacity, with the pressure reaching 10 psi after filtration of 28 L/m^2.

The fouling of the sterile filters after the different batch prefilters is shown in Figure 12.

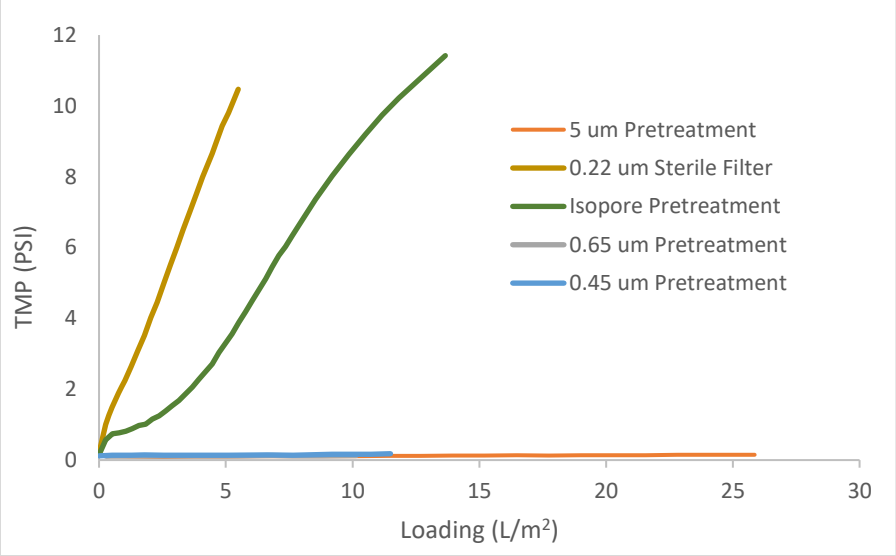

Figure 12: Sterile filtration data with TMP as a function of Loading for Serotype 2. The flux set at 5 µm/s.

The 0.22 µm sterile filters after the 0.65 µm, 0.45 µm, and 5 µm pretreatments showed minimal fouling as the TMP stayed below 0.2 psi throughout the entirety of the experiments. For example, the final pressure for the experiment using the 5 µm Durapore prefilter was 0.15 psi compared to the initial pressure of 0.12 psi. This behavior is quite different than the rapid fouling that was seen for the 0.45 and 0.65 µm prefilters in Figure 11. After the Isopore prefilter, the sterile filter showed fairly rapid fouling, achieving a loading of <15 L/m^2 before the TMP exceeded 10 psi.

Chapter 4

Conclusions

This thesis examined the effects of different prefilters on the sterile filtration of two different glycoconjugate vaccine serotypes. As shown by the DLS data, the average effective size of both serotypes was above 100 nm, which is more than half the diameter of the 0.22 µm pores of the sterilizing grade filter. More importantly, both serotypes displayed a small peak that was approximately 5 µm in size. This peak was consistently observed in all of the DLS data for the fresh samples and likely reflects a key foulant that can block the membrane pores during sterile filtration. This was confirmed by the significant reduction in the height of this peak (and in some cases the complete elimination of the peak) during passage through the different prefilters. This indicates that larger components present in the glycoconjugate solutions were being caught in the prefilter, thereby protecting the sterile filter from fouling when the feed was first passed through a prefilter.

The results obtained with Serotype 3 and Serotype 2 were generally very similar with a few exceptions. In both cases, the different pore sized prefilters showed the same trends, with the capacity of the prefilter increasing with increasing pore size as shown in Figures 9 and 11. For Serotype 3, the 0.45 µm prefilter was able to process almost four times the material of the sterile filter while the 5 µm prefilter showed minimal signs of fouling even after processing the same amount of the glycoconjugate solution. In contrast, for Serotype 2 the 0.45 µm and 0.65 µm prefilters were able to process about two times as much material as the sterile filter, while the 5 µm prefilter was able to process about five times as much material as the sterile filter. This is important as the goal is to not only reduce the fouling of the sterile filter but also to increase the capacity of the entire filtration process as a whole. If the prefilters themselves foul too rapidly, any benefit to the sterile filter will be difficult to realize in an actual process.

For Serotype 3, prefiltration through either the 0.45 µm or 5 µm prefilters was able to increase the membrane capacity by approximately 200% compared to the sterile filter alone. An even greater increase in capacity was seen for Serotype 2 where the 0.45 µm, 0.65 µm, and 5 µm prefilters pretreatments almost

completed eliminated fouling of the sterile filter. For example, the feed that was prefiltered through the 5 µm pore size prefilter showed less than a 0.1 psi increase in pressure over more than 25 L/m^2 filtration through the sterile filter. No matter the size of the prefilter, the large fouling particles were able to be removed effectively as shown by the DLS data and then the substantial increase in sterile filter capacity.

Although the sterile filtration trials showed a dramatic increase in capacity when a prefilter was used, the capacities were limited by the amount of material that was able to be processed through the sterile filter. None of the prefilter treatment groups were able to process enough material to fully foul the sterile filters to the maximum TMP of 10 psi. To better understand the true impact the prefilters have on the capacity of the sterile filter, full fouling of the membrane needs to be observed. However, these results clearly support the hypothesis that the prefilter has the potential to provide a very large increase in the capacity of the sterile filters.

The 5 µm PVDF filter has a fairly broad distribution in pore size with a highly interconnected pore structure. This likely allowed the 5 µm filter to capture large foulants while still maintaining nearly complete transmission of both serotypes during prefiltration. The capture of the large foulants not only reduced the average size of the serotype it also increased the capacity of the sterile filter substantially. The smaller pore size prefilters were also able to remove large foulants, but these prefilters fouled more quickly than the 5 µm filter; thus, a larger prefilter area would be required in an actual process. Somewhat surprisingly, the data in Figure 10 showed a slightly greater capacity for the 0.22 µm sterile filter after prefiltration through the 5 µm pore size prefilter compared to the 0.45 µm pore size, although this could have simply been due to the inherent run-to-run variability in the results**.**

Chapter 5

Future Studies

Based on these filtration experiments, processing the Serotypes via a prefilter before the sterile filter significantly increases the capacity of the sterile filter. A trend was observed that the larger pore size prefilters were able to process more material, i.e., showed less fouling, while providing the greatest increase in the capacity of the sterile filter that was used after the prefilter. In fact, the sterile filters fouled so slowly that they never reached their final capacity. However, there are still many areas to explore further to gain a better understanding of this topic.

The first set of experiments would be exploring a wider range of pore sizes for the prefilters. For Serotype 3, since the sterile filters after the 5 µm and 0.45 µm pretreatments showed similar membrane capacities and the 5 µm prefilter itself showed no fouling behavior, there might be a prefilter with a pore size closer to 1 or 2 µm that could yield even better results. Ideally the 1 µm pore sized prefilter would yield a better balance between foulant capture and resisting fouling better than either the 0.45 µm or 5 µm prefilters.

The next set of experiments would be exploring the fouling behavior of Serotype 2 more thoroughly since the sterile filter didn't reach its maximum capacity in any of the experiments using the prefiltered glycoconjugate. This made it impossible to determine which prefilter truly allows the sterile filter to have the largest capacity. To fully explore this question further, more material would need to be processed through the prefilters to ensure the sterile filters have enough prefiltered feed solution to foul to a final pressure of ≥10 psi. This could be done by using a larger filter area for the prefiltration step or possibly by performing multiple prefiltration runs using the small disk filters to increase the volume of permeate available as feed to the sterile filter.

An additional factor that could also affect the results is the membrane chemistry. This work used one of the most common sterile filters for all of the experiments, a Durapore PVDF membrane. In addition, almost all of the experiments used PVDF prefilters. Thus, any foulants that would likely bind to the sterile filter due specific chemical interactions (e.g., charge, hydrophobicity, etc), would probably be removed via the prefilter. The situation could be very different if the prefilter were to have a different membrane chemistry than the sterile filter. Limited experiments with the Isopore polycarbonate prefilter did show very different behavior than the 5 µm pore size PVDF membrane even though both of these prefilters have very similar pore size. Although the origin of this result is not currently understood, it can be speculated that the differences are likely due to the Isopore membrane having a different chemistry and pore structure than the PVDF membrane. In particular, the Isopore membrane has straight through cylindrical pores while the PVDF membrane has a very irregular pore shape with a highly torturous flow path. The net result is that the 5 µm Isopore prefilter is not able to remove the foulants as effectively as the 5 µm Durapore membrane; thus, providing much less protection for the PVDF sterile filter. Studies performed with other 5 µm pore size prefilters could be used to separate out the effects of the prefilter chemistry and pore structure on the overall performance of the prefilter.

Some final factors that could affect the filtration of glycoconjugates through both a prefilter and sterile filter are the filtrate flux and actual Serotype. A flux of 5 µm/s was utilized for this set of experiments based on results from previous studies, but changing the filtrate flux could change the fouling behavior of the glycoconjugates. Additionally, changing the glycoconjugate itself could yield very different results due to the different size distributions and chemical interactions with the membranes.

More experiments that focus on the membrane type, pore size, flux, and glycoconjugate would allow a much more detailed investigation into the topic of using a prefilter prior to sterile filtration. These studies would build directly on the insights that were gained through the work conducted in this thesis demonstrating that a prefilter has the potential to drastically improve the capacity of the sterile filter for processing of glycoconjugate vaccine products.

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- Investigated the sterile filtration of glycoconjugate vaccine solutions under Dr. Andrew L. Zydney to examine different conditions for increased membrane capacity
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- Automated small-scale buffer formulation with Python on the Tecan Freedom EVOware Liquid Handler for high throughput affinity chromatography buffer screening
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