Tangential Flow Filtration for the Purification of Lucentis®

A thesis submitted in partial fulfillment of the
requirements for the degree of
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BACHELOR OF SCIENCE IN CHEMICAL ENGINEERING

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Abstract

The goal of the Genentech Capstone project is to design a process to manufacture and purify a “Lucentis®-like” (Lu-L) protein. This group has been working on the tangential flow filtration (TFF) portion of the project, which will be utilized multiple times throughout the separation and purification process. The primary purpose of tangential flow filtration is to exchange buffer solution. Exchanging buffer solution regulates the pH of the mixture in preparation for chromatography, and changes the mobile phase utilized in each different chromatography step. The secondary purpose is to remove impurities from the buffer, while keeping the desired product on the upstream side of the membrane. Finally, tangential flow filtration can be used to concentrate proteins, which is an important step in the creation of the Lu-L product, as it allows for a product with the necessary specifications of volume, concentration, and purity. The team conducted trials to concentrate the modeling analog, hemoglobin, to observe the anticipated behavior of the Lu-L protein throughout TFF. In addition to performing those trials, the as well as modeling the industrial scale up of the TFF process will prepare students to start working with the Genentech protein and create the optimized scale-up system for the Lu-L protein.
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Purpose

The overall Genentech task was to design a process capable of providing a 99% purity, 10-30 g/L Lu-L protein solution. This product must be generated through a series of growth and then separation process, initializing with the development of approximately 1000 L of *E. coli* cells, which inherently contain roughly 5 g/L Lu-L protein and 20-25% undesired solids. The separation must be conducted through no more than eight unit operations, each of which must run for no more than 24 hours each. Included unit operations are the batch bioreactor, homogenizer, continuous centrifuge, each unique manner of chromatography columns, and tangential flow filtration systems.

Since the process was divided into three independent group projects as Bioreactor, Chromatography, and Tangential Flow Filtration (TFF), the goal of the TFF group was to characterize the TFF unit operations for the separation and purification of the Lu-L protein. TFF performs three primary functions: perform buffer exchange, purify the protein, and concentrate the protein. Buffer exchange is required to enable different chromatography methods to be implemented, swapping the mobile phase that the protein moves through the column on. Purification is required to remove all salts, small proteins and other soluble impurities from the protein solution. Concentration is required to reduce the processing volume, as well as to achieve final product specifications. Thereafter, the goal was to develop a processing plant-scale block flow diagram of both the base and alternative cases and select the most optimal case. This included economic and efficiency considerations for the entirety of the processes.

Background

As the leading cause of vision loss in Americans 60 years of age and older, macular degeneration affects roughly 11 million Americans, today\textsuperscript{[1]}. Lucentis\textsuperscript{®} is a blood vessel growth inhibitor, and works by binding and neutralizing the vascular endothelial growth factor (VEGF). Due to this specialize binding, Genentech produces Lucentis\textsuperscript{®}, formally called ranibizumab\textsuperscript{[2]}, to halt the undesired growth of vascular tissues in the macula on the
retina, leading to loss of vision in the macula, and has industrialized the process to be able to provide the treatment to all patients who may need it.

Tangential flow filtration is an important step in research, product development, and production within the medical and biopharmaceutical industries. This step is used in upstream and downstream processing, as well as product formulation. The primary benefit of tangential flow filtration, is that unlike other types of filtration which operate in a batch manner, this system runs continuously. Other benefits include a higher overall liquid removal rate, and the production of a movable slurry. This prevents filter cake formation, and allows the product to undergo further processing.

Tangential flow filtration is possible at lab scale with the Pall Centramate® LV holder and 30 kDa filter, and the setup provided in the unit operations laboratory, shown later as Fig. 3. This filtration technique consists of a solution entering the feed side of the cassette, the product load (left side) in Fig. 1, which then passes tangentially along the surface of the membrane. The retentate, material which remains on the original side of the membrane, exits the opposite end of the cassette, on the same side of the membrane, the retentate in Fig. 1. The permeate, material which has passed through the membrane, leaves on the opposite side of the membrane, the permeate in Fig. 1. The separation is driven by transmembrane pressure (TMP), the difference in pressure across the membrane itself, which forces the components which are smaller than the membrane pores through the membrane, into the permeate stream. The retentate that flows past the membrane contains the desired product.
Due to the TMP, the product also attempts to pass through the membrane. Since the molecule is too large to pass through the pores, it can build up on the surface of the membrane. This accumulation is called gel polarization and always presents a challenge, since the layer provides an additional source of resistance for the undesired particles to pass through the membrane. Given a thick enough gel polarization layer, called a filter cake, the flow of permeate can drop to zero, completely stopping the filtration of desired molecules. To fix, or preferably to prevent, such an undesirable occurrence, the TMP can be reduced, and the cross-flow increased. Thus, the too-large molecules are not as thoroughly stuck to the membrane surface, and they are swept off from the surface when they do become affixed by the high cross-flow.
**Lucentis®- Like (Lu-L) Protein**

The *E. coli* have been genetically modified to produce a Lu-L molecule called ranibizumab, which is the protein of interest. This molecule\(^4\) is a monoclonal immunoglobulin G, or FAB. It has been designed to bind to and neutralize vascular endothelial growth factor (VEGF) which is associated with macular degeneration. Fig. 2 shows Lucentis® in action, interacting with and neutralizing the VEGF. The Lu-L protein is being considered essentially identical to Lucentis®, which has a molecular weight\(^6\) of 48 kDa, and a nonspherical diameter of 20 Å. It remains in the proper conformation in a pH of 7, matching biological pH in the human body.

![Model of the use of Lucentis® to bind to VEGF and treat “wet” macular degeneration](image)

The Lu-L molecule is believed to be unharmed during the TFF process. The size of the molecule indicates that a very effective separation will occur between buffer solutions and the protein, given the properly sized membrane is used, so both removal of contaminants and buffer exchange will be accomplished effectively with TFF.
**Full Theoretical Genentech System**

The block flow diagrams in Fig. 3 and 4 demonstrate the theorized processes for the entire synthesis and separation process of the Lu-L protein under the designated restrictions from Genentech. Fig. 3 and 4 illustrate the base and alternative case, respectively. TFF unit operations appear in both cases the same number of times, and are used for essentially the same purpose in each case. In both processes, TFF is mainly used to concentrate the protein and perform buffer exchange prior to the next unit operation.

The base case uses TFF to perform buffer exchange in between the anion ion exchange chromatography (AIEX) and the hydrophobic interaction chromatography (HIC) because the AIEX requires a specific salt-concentration of buffer that is not suitable for the HIC. On top of buffer exchange the TFF also concentrates the protein, and is used near the end of the process to concentrate the protein for the final product.

The alternate case in Fig. 4 exhibits how TFF is utilized in between the monolithic chromatography and cation ion exchange chromatography (CIEX) and also at the end of the process. The TFF is used in the alternative case for the same reasons that it was used in the base case. The team has considered another alternate case where, looking at the base case, TFF would be used between unit operation three and four as well as four and five to ensure that the proper buffer is swapped into the protein solution between every unit operation. The costs would remain unchanged, so this alternative offers no disadvantages beyond a possibly longer processing time, which has been approved by Genentech.

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![Figure 3: Flow Diagram from 2016 Genentech Project, the Base Case.](image-url)
Experimental Procedures and Methods

Lucentis® is grown in and harvested from a bioreactor. The Lucentis® undergoes the series of unit operations presented in Fig. 3 and 4 in the Full Theoretical Genentech System section. The homogenizer breaks up and distributes the bioreactor product evenly throughout the mixture. Next, the centrifuge separates the solids from the liquid solution. Following the centrifugation, the product undergoes monolithic chromatography, where a column filled with absorbent material forces the different components of the mixture to flow out of the column at a different flow rates based upon internal interactions. The next unit operation is TFF which is the ultrafiltration process that will be discussed in more detail presently. Cation IEX separates the mixture afterwards based upon their affinity to the ion exchanger. Multimodal chromatography is similar to monolithic, except, that to achieve separation, it utilizes multiple forms of interaction between the analytical and stationary phase. The result then undergoes TFF an additional time, with the Lucentis® and waste as the end products.

TFF can negate the effects of filter cake buildup, increasing the length of operation of the unit. To determine the effects that the TFF unit has upon the mixture, it is necessary to utilize the UV-Visible Spectrophotometer. A sample of the permeate and retentate should be taken every few minutes, and analyzed for absorbance of light within a range of wavelengths. The Beer-Lambert law relates optical depth to absorbance, to determine the concentration over time. The complete Standard Operating Procedure (SOP) is provided in Appendix A.
Safety Considerations

The hazards of this tangential flow system are minimal and manageable. The hazards can be separated into chemical and non-chemical hazards. For chemical hazards, the group will take special care with the cleaning chemicals. These will present the largest, yet still small, hazard to the group during regular operation. Cleaning chemicals include: 1% H₃PO₄, 1% NaOH, and 200 ppm NaHClO (dilute bleach). All three of the chemicals are corrosive, and irritating to the skin, lungs, and eyes. Special care should be taken with the 200 ppm NaHClO to limit its interaction with other chemicals. If the NaHClO is mixed with acid, such as the H₃PO₄, highly hazardous chlorine gas will be produced and released. If NaHClO is mixed with hydrogen peroxide, a fire hazard will be created. Non-chemical hazards are mainly associated with equipment and most of the process conditions are not highly dangerous. The equipment will not be operating beyond three times atmospheric pressure (42 psi). All the equipment will be operating near ambient temperature, so no special thermal insulation is needed. Primary non-chemical hazards include slips, trips, and falls. In order to prevent large spills from causing a hazard, water spills will be mopped up, and other aqueous spills will be covered with an inert absorber, and disposed of in the solids waste container.

Safety glasses or goggles should be worn when conducting the experiments and during cleaning cycles. If any of the chemicals were to come in contact with a person’s eye, the eye wash station should be used to flush both eyes for 15 minutes, and then seek additional medical aid as needed. Closed-toe shoes should be worn, and all areas of the body covered. In the event of skin contact with the chemicals, either the hand sink or the shower should be utilized to remove the harmful effects. Liquid waste was discarded in a five-gallon bucket labeled for EH&S disposal. NaOH and dilute bleach was contained in the same waste container. Phosphoric acid had its own waste bottle.

Tangential Flow Filtration (TFF)

With a general understanding of main goals of TFF provided in the Full Theoretical Genentech System section, specific characteristics of the TFF unit operation can be described in greater detail.
The experimental setup of TFF is shown in Fig. 5. TFF, also called cross flow filtration, is a type of membrane filtration. Flow is directed across a membrane rather than through a membrane as in typical filtration processes as previously described. As in dead end filtration (DEF), larger molecules cannot flow through the membrane and remain as retentate. TFF behaves differently than DEF on a molecular level at the membrane interface. Increasing the pressure on a DEF does not force more material to filter. Instead of increasing filtration, the pressure increase compresses the material\cite{5}. This compressed material is called a filter cake. TFF has the advantage of a longer lifespan because this filter cake is avoided since the retentate is recycled away from the membrane. The process diagram of the process is shown in Fig. 6.

Figure 5: Representation of the TFF Setup.
There are three parameters to control and design TFF: transmembrane pressure (TMP), flow rate, and operation time. Membrane surface area, or flux capacity, is useful to characterize the capacity of the process. It can be derived from Eq. 1\[^8\].

\[
A = \frac{V}{J \times T} \quad \text{Eq. 1}
\]

\(A = \text{membrane area (m}^2\) \)
\(V = \text{volume of permeate (liters)}\)
\(J = \text{permeate flux (liters/m}^2/\text{hour)}\)
\(T = \text{process time (hours)}\)

The transmembrane pressure is also related to the filtration process. TMP is calculated according to Eq. 2\[^8\].

\[
\text{TMP} = \left(\frac{P_{\text{feed}} + P_{\text{retenate}}}{2}\right) - P_{\text{permeate}} \quad \text{Eq. 2}
\]

\(\text{TMP} = \text{Transmembrane pressure (psi)}\)
\(P_{\text{feed}} = \text{Pressure of the feed (psi)}\)
\(P_{\text{permeate}} = \text{Pressure of the permeate (psi)}\)
$P_{\text{retentate}} = \text{Pressure of the filtrate (psi)}$

**Advantages of TFF**

There are several advantages of TFF compared to other separation processes such as size exclusion chromatography (SEC). The first advantage of TFF compared to SEC is that it can perform buffer exchange while concentrating with minimal loss of protein. The second advantage of TFF is that it has the highest fold ion removal compared to alternatives; specifically, SEC can only remove about $5 \times 10^5$ ions whereas TFF can remove up to $5 \times 10^{12}$ ions as shown in Fig. 7.

![Figure 7: Fold Ion Removal vs. Diavolumes for TFF at Total Retention (R=1) and SEC at 95% and 99% yield][1]

One of the biggest advantages of TFF is the fact that it is the most cost efficient process compared to SEC and CCD. Since industrial scale TFF only requires three 1500 L vessels while SEC and CCD typically need five 4000 L vessels, TFF requires less space, utilities, and labor, leading to lower costs and higher efficiencies. The comparison costs between TFF and SEC are represented in Fig. 8.
Empirical Determination of Mass Transfer Coefficient

Equations relating gel layer thickness and permeate flux:

Starting with Eq. 3\(^{[10]}\) for calculating the flux filtrate,

\[
J_{\text{filtrate}} = k \times TMP = \left[ \frac{1}{\frac{1}{R_g} + \frac{1}{R_m}} \right] \times TMP \tag{Eq. 3}
\]

\(k\) = mass transfer coefficient
\(TMP\) = transmembrane pressure
\(R_g\) = resistance to mass transfer due to the gel layer
\(R_m\) = resistance to mass transfer due to the membrane itself

Setting the flux in Eq. 3 equal to that in Eq. 1, and solving for \(k\) yields,

\[
k = \frac{V}{TMP \times T \times A} \tag{Eq. 4}
\]

Thus, the mass transfer coefficient can be determined via known or easily measured process variables. By tracking \(k\) as a function of time, the resistance of the gel layer can be determined. Since \(k\) is inversely proportional to the combined resistance to mass transfer
of the membrane and gel layer, and the membrane resistance to mass transfer is essentially constant, as $k$ decreases, the gel layer resistance to mass transfer, and therefore the thickness of the gel layer, increases.

*Alternate calculations on mass transfer coefficient:*

The mass transfer coefficient is a dimensionless number according to Geankoplis.\[^{10}\] It is related by the dimensionless Sherwood, $N_{Sh}$, Schmidt, $N_{Sc}$, and Reynolds, $N_{Re}$, numbers in Eq. 5.

\[
J_D = \frac{N_{Sh}}{(N_{Re}N_{Sc})^{1/3}}
\]

Eq. 5

Where the Schmidt, Reynolds, and Sherwood Numbers\[^{10}\] are specified in Eq. 6, 7, and 8.

\[
N_{Re} = \frac{L*V*\rho}{\mu}
\]

Eq. 6

\[
N_{Sc} = \frac{\mu}{\rho*V*N_{AB}}
\]

Eq. 7

\[
N_{Sh} = Kc * \frac{L}{D_{AB}}
\]

Eq. 8

$L$ is the length of the retentate path through the filter, and $V$ is the velocity along that path. $N_{AB}$ is the molar flux, $D_{ab}$ the diffusivity constant. The concentration of protein is very low. As this is true, the physical properties of $\rho$ and $\mu$ (density and viscosity) are assumed to be equal to water’s. Here the value of $Kc$ in the Schmidt number has a constant value as that of the mass transfer coefficient $Kc$, not to be confused with $k$ from Eq. 3. This must be determined empirically.
By using mass transfer principles, the group determined the mass transfer coefficient. By using Eq. 9, the group found $K_c$. The simplest form of flux can be expressed as a driving force multiplied by a constant.

$$ Na = K_c (C_{a_2} - C_{a_1}) $$  
Eq. 9

$Na =$ flux $g \ m^2 \ s^{-1}$  
$Kc =$ mass transfer coefficient $m \ s^{-1}$  
$C_{a_2} =$ concentration at time 1 mole/L  
$C_{a_1} =$ concentration at point 1

The driving force for diffusion is the concentration gradient across the membrane. As the membrane is supposed to be preventing the diffusion of material across the membrane, $Kc$ is not very large. $Kc$ was found to be $40 \ m \ s^{-1}$.

**Modeling**

Modeling is a useful tool to help predict the concentration of the retentate after a change in operating conditions. This model is based on a component balance around the filter. Some assumptions had to be made about the operating conditions for the model to be useful. It was assumed that the filter rejects 99% of protein, and 70% of water in each pass stays on the retentate side. Eq. 10-12 are the two material balances for the system model$^{[10]}$.

$$ R_{hbn} = F_{hbn} * R_{jt_{hbn}} $$  
Eq. 10

$$ P_{hbn} = R_{hbn} * (1 - R_{jt_{hbn}}) $$  
Eq. 11

$$ V_{w,p} = V_{w,r} * (1 - R_{jt_{w}}) $$  
Eq. 12

Here $P$ means permeate, $F$ means feed, $R$ is retentate, $R_{jt}$ is rejection, $w$ is water, $V$ is volume, and $hbn$ is hemoglobin. Hemoglobin was the group’s initial modeling protein. Using the values modeled, the group was able to model and predict the concentration of hemoglobin in the permeate as a function of the concentration in retentate in Fig. 9.
Figure 9: Concentration of Hb in the permeate is plotted versus the concentration in the retentate. An increase in concentration in the permeate is not desired, but the concentration increase is minimal.

Fig. 9 shows that the model is fairly accurate. The blue model line has the same slope as the orange experimental line. If the group wished to proceed with higher concentrations in the retentate, the group could predict fairly accurately what the concentration in the permeate would be or vice versa. The slope of the line is .0175 which is the dimensionless concentration factor of the permeate. As this value is very small, this indicates not much protein traversed the membrane. This is ideal as the protein should stay in the retentate.

A secondary model the group was able to develop was the concentration in the feed, retentate and permeate versus time. A model of concentration versus time is not very useful for industry, as a product can be brought to any concentration if the process time goes to infinity or the volume of feed changes. For the group's purposes, however, it was effective in determining the accuracy of the model. Fig. 10 shows the concentration in the feed and retentate versus time. Fig. 11 shows the concentration in the permeate versus time.
Figure 10: Change in Feed and Retentate Concentration versus Time. The shapes of the model are incorrect, but the final concentration is fairly accurate.

Figure 11: Change of Permeate Concentration versus Time. Like in the previous figure, the shape is incorrect, but the final concentration is fairly accurate.

The models in both Fig. 10 and 11 predict the final concentrations accurately. While the shape is incorrect, the final concentration was the important condition to model. By being fairly accurate with the ending concentration, this model was able to corroborate the results in Fig. 9.
**Diafiltration**

Diafiltration is a process where buffer exchange (BE) occurs. The objective is to minimize the consumption of the diafiltration buffer and to keep the processing time short. For the overall process, BE usually consists of initial concentration to reduce the amount of buffer required to achieve thorough diafiltration. The diafiltration factor offers a quantitative assessment of the extent to which original buffer has been replaced by a new buffer. Eq. 13 demonstrates how the diafiltration factor is determined.

\[
\text{Diafiltration factor} = \frac{\text{Rinsing Buffer Volume}}{\text{Starting Volume}} \quad \text{Eq. 13}
\]

For example:

\[
\text{Diafiltration Factor} = \frac{5L}{1L} = 5
\]

For the diafiltration process, there are several different approaches for how a new buffer is added to the feed to replace the old buffer. Two common approaches include the continuous and discontinuous diafiltration. Continuous diafiltration is where the volume of liquid in the feed reservoir is kept constant by adding the new buffer to the feed reservoir at the same rate as liquid is removed in the permeate\[^8\]. Alternatively, discontinuous diafiltration consists of periodically, but not continuously, adding new buffer to replenish the feed reservoir. Finally, single bulk dilution consists of concentrating the feed fully, and then utilizing a single addition of new buffer to dilute the saturated protein to the original volume. As shown in Fig. 12, the bottom plot of continuous diafiltration\[^8\] is the most efficient form of diafiltration, compared to the middle discontinuous and the top single bulk dilution plots. Discontinuous is less efficient because a larger volume of finishing buffer is required to obtain the same diafiltration factor. Each plot represents the final concentration of contaminant divided by initial concentration of contaminant on the y-axis, compared to diafiltration factor on the x-axis.
Determining the diafiltration optimization parameter enables the optimum concentrations for diafiltration in both the starting and final buffers. There is a tradeoff between the flux and diafiltration buffer volume that will create an optimum bulk concentration to perform diafiltration. This can be calculated by using the diafiltration optimization parameter at each data point\(^8\). Calculating the diafiltration optimization parameter is demonstrated\(^{11}\) in Eq. 14.

\[
DF = \frac{Optimization\ Parameter\ Conc.\ [g/L] \times Flux\ [LMH]}{[g/L] \times Flux\ [LMH]}\ 
\]

Eq. 14

For example:

\[
55.0\ g/L \times 14.54\ LMH = 800
\]

The original contaminant in the retentate at each diavolume can be calculated from retention values by using\(^{11}\) Eq. 15:

\[
\text{Remaining Contaminant} \% = 100 \times e^{-\frac{Retention}{N}}\ 
\]

Eq. 15

\(N = \text{number of diavolumes, the number of original feed reservoir volumes}\)
For example, after five diavolumes:

\[
100 \times e^{(0.75-1) \times 5} = 28.65 \%
\]

In terms of product recovery, the actual yield, Eq. 16, is used to determine how much protein of interest is present in the retentate after diafiltration has occurred:

\[
Yield = 100 \times \frac{(V_{retentate} \times C_{retentate})}{V_{initial} \times C_{initial}} \quad \text{Eq. 16}
\]

For example:

\[
100 \times (0.94 \times 0.09) / (0.95 \times 0.1) = 90 \%
\]

**Scale-up**

The general parameters for an industrial scale TFF are shown in Table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ranges/Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure</td>
<td>16 - 45 psi</td>
</tr>
<tr>
<td>Volume</td>
<td>250 - 1500 L</td>
</tr>
<tr>
<td>Surface area</td>
<td>0 - 6 m²</td>
</tr>
<tr>
<td>Corresponding flux</td>
<td>0 - 25 LMH</td>
</tr>
<tr>
<td>Temperature</td>
<td>15 - 30 °C</td>
</tr>
<tr>
<td>Operating time</td>
<td>1 - 7 hrs</td>
</tr>
<tr>
<td>Membrane Materials</td>
<td>Polymer/Polymer</td>
</tr>
<tr>
<td></td>
<td>Polymer/Inorganic Composite</td>
</tr>
<tr>
<td></td>
<td>Amphiphilic Copolymer</td>
</tr>
</tbody>
</table>

There are two main types of configurations regarding the processes for scaled-up TFF, batch and fed-batch\textsuperscript{12}. The batch system has a lower average concentration. As a result, less membrane surface area required\textsuperscript{13}. The volume concentration factor has a maximum
achievable value of 20x. As for the fed-batch system, the volume concentration factor is estimated to remain no higher than 10 - 15x. In addition, minimization of the fed-batch ratio must occur in order to minimize the membrane area.

Alternatively to diafiltration is gel filtration, a technique that separates molecules on the basis of molecular size. Common usages for gel filtration are desalting and buffer exchange. Although gel filtration is simple to run, the size difference between the substances being separated should be large. Furthermore, there are significant limitations with gel filtration. Continuous diafiltration has a severe advantage over gel filtration\textsuperscript{[14]}. One of the advantages of diafiltration is the fact that the volume remains constant throughout the process. In addition, after diafiltration occurs, the product can be further concentrated in the same system.

At the end of the large-scale diafiltration, a buffer flush is used to rinse the system of residual product. The entire amount of buffer added to the system for the flush is collected along with the product\textsuperscript{[15]}, which inevitably dilutes the final product. The dilution needs to be compensated for during any concentration steps of the process to ensure that the final diluted product concentration meets any specification. A buffer flush system\textsuperscript{[15]} is outlined in Fig. 13.

![Buffer Flush Flowpath](image)

**Figure 13: Buffer Flush System Diagram\textsuperscript{[15]}**
A system drain is often the simplest form of product recovery. It is especially most effective if the system has a low drain port\textsuperscript{[15]}. In terms of removing all product from the system, the system drain works better for some devices than others. An outlined schematic which combines a drain and blowdown system is depicted below in Fig. 14.

![Drain and Blowdown Flowpath](image)

**Figure 14: Drain and Blowdown Flow Path\textsuperscript{[15]}**

**Testing Results**

Initially, the pump standards which correlate pump power level with flow rate were determined via flushing with DI water, as seen in Appendix B Fig. B.1. These calibrations enabled the team to set desired flow rates through the system with greater precision.

Thereafter, the TFF system was operated at different pump settings to determine the flux of water through the membrane as a function of TMP. The 30 kDa membrane was tested for each setting Appendix B Fig. B.2 shows the fluxes for each TMP, at each power setting. This testing is necessary as a later reference for analyzing the integrity of the membrane after experiments have been conducted.
After initial system characterization, an experiment was conducted to aid in modeling. A 1.5 micromolar mixture of hemoglobin was run through the TFF system. Hemoglobin was selected since its molecular weight is similar to that of the Lu-L protein, and therefore hemoglobin would be kept in the retentate with roughly the same efficiency. Furthermore, the coloration of the hemoglobin provided a visual indication about the success of the filtration, since the hemoglobin is a rich, reddish color. Since the permeate reservoir contains only clear liquid, and feed reservoir’s contents became far darker in color, it is clear that very little hemoglobin passed into the permeate, and the hemoglobin in the retentate became more concentrated over time. Despite the first trial showing signs of the clogging in the filter, definitive separation was observed as shown in Fig. 15. Note the lack of color in the right container, which indicates that little to no hemoglobin is present, and the darkening of color in the left container, which indicates that the hemoglobin is becoming more concentrated in the feed/retentate reservoir.

![Figure 15: Separation of Hemoglobin and Water, at Initial and Final TFF Processing.](image)

The team observed a rise in TMP over time, throughout the filtration of the hemoglobin mixture as shown in Fig. 16. Fig. 17 shows the rise in permeate flow rate over time for
the filtration of hemoglobin. The concentration of Hemoglobin as a function of time can be seen in Fig. 18.

![Hemoglobin TMP vs. Time](image1.png)

**Figure 16:** Hemoglobin TFF trial TMP versus of Time

![Hemoglobin Flowrate vs. Time](image2.png)

**Figure 17:** Permeate Flow Rate of Hemoglobin versus Time
Experimental Data Analysis

The flux through the membrane as a function of TMP was determined, and is shown in Appendix B Fig B.2. This gives the team the ability to determine whether the membrane is broken, clogged, or if gel polarization has occurred via comparison to the calibrations.

A 1.5 micromolar mixture of hemoglobin and water was next utilized for TFF. Although the membrane successfully filtered the mixture to zero detectable hemoglobin in the permeate, experimental issues still arose. Based on the combination of behaviors quantified through Fig. 16 and 17, it was determined that the hemoglobin was clogging the membrane. The TMP rose throughout the experiment, Fig. 16, yet the pressure valve was not adjusted. Furthermore, the permeate increased in coordination with the increase in TMP, indicating that more fluid was being directed through the membrane. Together, this indicates that the retentate pathway had been clogged by the hemoglobin. Upon visual inspection, there were traces of red clumps of hemoglobin lodged in the filter, so the solution was pre-filtered. The filtering was clearly necessary, as shown in Fig. 19, where the red material is the same as was observed in the membrane pathway. Even with these challenges, the flux vs. concentration factor was found, and the nearly 100% separation and concentration of hemoglobin was observed.
After the prefiltration, the hemoglobin solution was usable, but still contained stringy particles floating within it. So a second round of prefiltration was conducted. The repeated prefiltration was only 90% effective, and thus the team was hesitant to continue with experimentation for fear of damaging the cassette. In the future, an in-line pre-filter[16] should be used to remove large particulate and prevent the cassette from being subjected to the high pressures experienced as a result of clogging.

As observed in Fig. 18, the concentration of hemoglobin was doubled in half an hour. A visual representation of the concentrated solution is shown in Fig. 15. This picture displays the high concentration of hemoglobin in the feed reservoir, and the clear DI water buffer which was removed from the system. The ultrafiltration process was so effective, that there was no detectable hemoglobin in the permeate. This experiment served as the basis of the majority of the Lu-L protein modeling buffer exchange.
Scale-Up
Linear scaling is utilized to keep a number of characteristics the same for a full-scale process. For an ultrafiltration process, linear scaling is the most common method of scale-up\[15\]. This means that the concentration profile and operating profile will remain constant during the scale-up. For the TFF process, these include the following: flux, mass transfer coefficient, $k$, membrane material, and process time.

![Diagram of UF Process](image)

However, the module width, $w$, changes from small scale to large scale\[15\]. An example of linear scaling in a block diagram is provided as Fig. 20. As the UF process scales up in volume from 10 to 1000 liters, the only value that is changing is the volume of the filtrate and retentate ($V_F$ and $V_R$).
Table 2: Heuristics of Scale-Up

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lab-Scale</th>
<th>Industrial-Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Vol. → Final Vol.</td>
<td>2 L → 300 mL</td>
<td>1000 L → 100L</td>
</tr>
<tr>
<td>Time Required</td>
<td>30 minutes</td>
<td>3 hours</td>
</tr>
<tr>
<td>Membrane Surface Area</td>
<td>0.02 m²</td>
<td>3 m²</td>
</tr>
<tr>
<td>Filter Pore Size</td>
<td>30 kDa</td>
<td>30 kDa (maybe use 16 kDa)</td>
</tr>
<tr>
<td>Operating Pressure</td>
<td>20 psi TMP, 40 max</td>
<td>20 psi TMP, 40 max</td>
</tr>
<tr>
<td>Buffer Feed (continuous)</td>
<td>33.33 mL/min</td>
<td>5 L/min</td>
</tr>
<tr>
<td>Feed Flow</td>
<td>117 mL/min</td>
<td>585 L/hour</td>
</tr>
<tr>
<td>Flow Rate of Retentate</td>
<td>83.67 mL/min</td>
<td>418 L/hour</td>
</tr>
</tbody>
</table>

The heuristics of scale-up are depicted in Table 2. The importance of the heuristics of scale-up is that they allow for the transition from lab-scale to industrial-scale processes. Several parameters increase due to scale-up. However, the operating pressure and filter pore size remain the same. This is because these process characteristics do not inherently change during scale-up. If the process was to increase the time required for operation to 24 hours, the membrane surface area would decrease, and the feed flow rate, and therefore flow rate of retentate and buffer feed, would all decrease.

For the scale-up process, determining the number and arrangement of the TFF are crucial aspects to consider. One way of arrangement is to connect several single-pass tangential flow filters (SPTFF) in series[17]. These are configured either internally or externally through the use of staging plates. Two important parameters for SPTFF in series are the specific membrane area of the channel and dimensionless length.

Specific membrane area of the channel is shown in Eq. 18.

\[ \sigma_c = \frac{\text{Membrane Area of Flow Channel [cm}^2\text{]}}{\text{Void Volume of Flow Channel [cm}^3\text{]}} \]

Eq. 18
Dimensionless length is shown in Eq. 19 and 20.

$$\lambda_{\text{Stage}} = \text{Specific Membrane Area } (\sigma_c) \times \text{Channel Length } (L_{\text{Stage}}) \quad \text{Eq. 19}$$

$$\lambda_{\text{System}} = \text{Specific Membrane Area } (\sigma_c) \times \text{Channel Length } (L_{\text{Total}}) \quad \text{Eq. 20}$$

Fig. 21 considers the effect of multiple SPTFF in a process. A linear trend is witnessed from the graph. If productivity is defined as the mass throughout of the SPTFF, then increasing the number of SPTFF in series will increase the productivity. In the same manner, on the same Fig. 21, the productivity of the system can be defined as a function of the dimensionless length of the system. From Fig. 21, the optimal SPTFF performance occurs at three or more cassettes connected in series\[17\]. This also implies that systems operating with one or two cassettes in-series are inferior in terms of performance to a system with 3 or more connected in series\[17\].

Figure 21: Number-in-Series versus Productivity
The robustness of the SPTFF is due to several important parameters. These parameters are the feed flow rate, residence time, and pressure drop\textsuperscript{[17]}. Design curves of constant concentration depicted in Fig. 22 can be constructed to determine the operating space required for specific objectives\textsuperscript{[17]}. Longer flow path configurations (7, 8, or 9-in-series) are due to better utilization of SPTFF design principles. Furthermore, from Fig. 22, the relationship between the average flux and concentration factor is exponential for higher configurations. This pattern does not apply for configurations with 4-in-series or less, which exhibit a linear relationship.

**Economics**

One of the important questions to consider when calculating the economic costs of the tangential flow process is determining whether single-use or reusable filters should be used. Single-use regimes offer several advantages over re-use filters\textsuperscript{[13]}, including decreases in system preparation and overall cost. An annual CoG (Cost of Goods) is provided below in Fig. 23 for a 250 L system\textsuperscript{[13]}. 

![Figure 22: Average Flux versus the Concentration Factor](image)
There are several important conclusions from the annual CoGs. One of the prominent conclusions is that single use is 27% less costly than re-use in terms of the annual CoG. As for the several aspects listed in Fig. 23, single-use continues to demonstrate less of a financial cost compared to the re-use. These areas include the capital cost, cost of materials, and total labor. However, there is one cost where the re-use is overwhelmingly more cost-effective than the single-use. This is the consumables portion of the CoG. Even with the large difference in that particular category, single-use filters are still more financially appealing\(^{[13]}\). For a 1000 L scale system, the CoG would still exhibit the same trends as witnessed for the 250 L system. The main difference would be an increase in the total cost, while the percentage differences would approximately remain the same.

Another way of examining the comparison of single-use filters versus reusable filters is done through a sensitivity analysis. Conducting a sensitivity analysis for the number of batches per year and batch volume allows the percent savings of the single use filter to be quantified\(^{[13]}\). Fig. 24 demonstrates the relationship for a batch system with 250 L.
The optimal number of batches for a 250 L batch single-use TFF system is 20. Another piece of information that the graph provides is that a single-use TFF system is more cost effective than a re-use filter when the number of batches is less than 50 per year. If this analysis were to apply to a system of 1000 L, there would be slight differences to determine the optimal batch. For example, since the batch volume has increased, the advantage that single-use filters have decreases slightly\textsuperscript{13}. A particular instance that could occur is that the batches per year could increase to approximately 25 batches per year. Therefore, a larger volume has a noticeable effect on the optimal number of batches per year.
Fig. 25, demonstrates the effect of various batch sizes when just 20 batches are required per year for a single-use TFF system\cite{13}.

For the system, the optimal single-use option is 250 L. However, there is a size limit for when single use becomes the less economically viable option compared to multi-use TFF systems: between 1500 L and 2000L multi-use TFF systems become more economically viable\cite{13}. Compared to the targeted scale up of 1000 L, the single-use presents an advantage of 10% in terms of cost, but that is only if 20 batches are performed per year. Unless the targeted batch volume increases to 2000L, the single-use filter will be the better alternative for these number of campaigns per year. For the particular scale up for 1000 L, the single-use filter appears to be the more viable choice compared to the re-use filter.

For an overall analysis, the purchase cost of the unit operations for each of the processes to scale up production of Lucentis® to 1000 L can be seen in Table 3\cite{18}.
Table 3: Economic Capital Cost of Base and Alternative Case

<table>
<thead>
<tr>
<th>Unit Operations (Industrial)</th>
<th>Cost Base Case</th>
<th>Cost Alternate Case</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioreactor</td>
<td>$1,190,000</td>
<td>$1,190,000</td>
</tr>
<tr>
<td>Homogenizer</td>
<td>$140,000</td>
<td>$140,000</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>$615,000</td>
<td>$615,000</td>
</tr>
<tr>
<td>Cation IEX</td>
<td>$20,052</td>
<td>$20,052</td>
</tr>
<tr>
<td>Anion IEX</td>
<td>$35,000</td>
<td>0</td>
</tr>
<tr>
<td>TFF</td>
<td>$233,750-$467,500</td>
<td>$233,750-$467,500</td>
</tr>
<tr>
<td>HIC</td>
<td>$327,250</td>
<td>0</td>
</tr>
<tr>
<td>Monolithic Chroma.</td>
<td>0</td>
<td>$33,161</td>
</tr>
<tr>
<td>Multimodal Chroma.</td>
<td>0</td>
<td>$24,874</td>
</tr>
<tr>
<td>Total Cost</td>
<td>$2,561,000-$2,795,000</td>
<td>$2,226,000-$2,491,000</td>
</tr>
</tbody>
</table>

**Return on Investment**

Return on investment is a deciding measure for investment into a new project. Return on Investment, also known as ROI, is determined by subtracting the cost of investment from the gain on investment and dividing the quantity by the cost of investment\[^{20}\], Eq. 20.

\[
ROI = \frac{Gain - COI}{COI}
\]  
Eq. 20
The average price of Lucentis® per dose is $2000[19]. One ophthalmologist billed 37,000 doses in 2012[19]. This results in a gain of $74 million. The cost of investment, COI will be the total costs from Table 3 above. The ROI for the base case system on a single doctor is 2,650% to 2,889%. The alternate case has an ROI between 2,970% and 3,280%. Both cases result in a highly profitable venture, especially as this is gain based on dosages utilized by a single doctor for a single year. The higher ROI for the alternate case suggests that the alternate cases should be pursued rather than the base case.

**Project Management:**

In the first week of the tangential flow filtration (TFF) project, it was essential that all the students on the team become familiar with information about TFF and how it works. Each student was responsible for performing literature review on specific topics, such as design issues, environmental, health, and safety hazards (EH&S), and heuristics. A detailed schedule and Gantt chart shown in Fig. 26 was created to ensure that the project proceeded in a timely manner. Students also reviewed a previous years’ thesis to gauge what parameters prior teams used and how the current team could optimize those parameters and improve the system. The most important component of the first week was for the students to become tempered to their colleagues and the TFF system.
In the second week, the team continued their in-depth research for informational articles about the TFF process. The students also maintained a high level of organization by creating a collaborative Google Drive folder in order to compile all their articles and other scholarly resources. This compilation enabled all the current and possible future students of the TFF project to have the resources and information of the current team members. The students reflected on the knowledge they learned in the prior week and strove to gain more useful knowledge such as information on current membrane properties and limitations, the Lu-L protein, and gel polarization, and specific parameters or solutions that would solve possible design issues they might face. The team gained a greater understanding and created a more specific standard operating procedure (SOP). The last task of the week, according to the schedule, was to set-up the TFF system—which the team had to redo because the system was assembled correctly the first time. Overall, the team generally stayed on schedule for week two and was ready to continue to week three.

In the third week, the team focused on more quantitative analysis of the TFF system. During this week, the team gained another team member and immediately got him up to speed. The team worked hard to ensure that the new team member had all the resources from the Google Drive and answered any questions he had. After assembling the system correctly, the team analyzed modeled results using optimization product recovery and flux equations. They also investigated the advantages of TFF over other separation processes such as Size Exclusion Chromatography (SEC) and TFF’s ability to prevent gel polarization. Week three was the first week that the students started to conduct experiments. The team calibrated the pump’s flow rate according the pump power level and created a standard linear graph. This graph will provide the students with an accurate linear relationship between the power setting and the flowrate of the pump. They also constructed a flushing gasket in order to start cleaning the system, as will be required for future use. The team did not strictly follow the timeline for week three, but the completion of the tasks of week three were imperative before continuing to week four.

In the fourth week, the team began creating standard curves for the hemoglobin using a spectrophotometer. While the standards were being made, the team also continued to run
trials to measure permeate flow rates and transmembrane pressures. The students achieved running a trial of hemoglobin and tracked the concentration change as a function of time; unfortunately, the membrane became clogged, and the run had to be stopped. Due to the clog, that data was questioned on the basis of its validity, and therefore the students resolved to conduct more trials in the next week.

During the sixth week, the team conducted trials of hemoglobin separation. The team performed the separation with the hemoglobin for the purpose of observing the behavior because hemoglobin has a similar molecular weight to the Lu-L protein. These trials enabled the team to better visualize and model the Lu-L protein separation system.

The seventh week was disrupted by the fact that the hemoglobin suspension was frozen and accumulated red string-like follicles. The team was unable to conduct any TFF experiments that week, instead, the team carried out gravity filtration on the suspension in attempt to remove the follicles. After gravity filtration, the follicles were mostly removed. After the freezing and filtration, the team questioned whether the suspension will behave differently, but Mr. Mike Kivistik confirmed that it should behave the same as long as the suspension was not heated, which would denature the proteins.

During the eighth week, the students focused highly on modeling. Instead of conducting more trials with hemoglobin, the team sat down and discussed the best orientations, sizes, and cost of scale-up models and attempted to identify which was the best to utilize. Students also signed up for Innovation Day and discussed ideas of what the team would do and tasks that needed to be completed in order to prepare for Innovation Day.

The ninth week the students were on spring break. The students returned to the lab in the tenth week and worked together to flush the system, perform theoretical calculations, centrifuge the Lu-L protein solution, and prepare for future experimentation. The team gathered information relating to the economics and scale-up process, and focused on modeling the concentration factor as a function of time.
In weeks eleven through fourteen, the team compiled data in preparation for the upcoming events. The team presented to the entire chemical engineering department in week eleven. Throughout weeks twelve and thirteen, the team then submitted and revised the final presentation which was presented to Genentech on April 28th. In week thirteen, the team prepared for the presentation at Innovation Day on May 5th. Throughout weeks eleven through fourteen, the team condensed all of the information into the final report.

The team was able to stay on track with help from the Gantt chart, and weekly assignments. The team received feedback from Professor Fuchs regarding the weekly assignments on alternating weeks to help stay on track and improve. The feedback helped ensure that the team was utilizing time and resources efficiently, in order to fully understand, model, and work with the tangential flow filtration system. However, the project was not completed without challenges.

The setup and calibration of the system was confusing and time consuming to begin with. However, the team was able to figure it out, and began testing of hemoglobin. The team experienced clogging of hemoglobin within the cassette in the fourth week of experimentation. For this reason, experimentation was delayed until the following week, where the team made tremendous headway in the separation of hemoglobin. In the eighth week, the team experienced an issue with the storage of the hemoglobin. The mixture was stored in a freezer by accident between weeks seven and eight. Unfortunately, the hemoglobin could not be heated up rapidly without risk of denaturation. For this reason, no results were taken in week seven.

Following week seven, the team learned that the initial concentration for the Lu-L could not be determined with the equipment in the Unit Operations Lab. The team also discovered that attempts to separate Lu-L using the TFF system did not produce any noticeable color change. Without any way to calibrate and work with Lu-L in the TFF system, the team began to focus more on modeling and economics. From here, the team ran into issues pricing the equipment, as manufacturers generally provide quotes, but lacked the incentive to do so for students.
With persistence and time, the team eventually gathered all of the information necessary to complete the quantitative results. Towards the end of the semester, the team did not stay on track according to a strict interpretation of the Gantt chart, as the results would have been inconclusive and pointless. Instead the team focused efforts on presentations, modeling, economics, scale up, and final compilation of the results.

**Conclusions:**

Tangential flow filtration (TFF) serves numerous roles in the synthesis and separation of proteins in the pharmaceutical industry. For the Genentech Lucentis® purification project, TFF has been selected to perform buffer exchange as a method of preparing the protein solution for chromatography, and for removing impurities. Additionally, TFF has been selected to achieve desired levels of concentration throughout and at the end of the proposed production-scale processes.

Through the course of this project, the tangential flow filtration system was characterized. The system was used to remove a portion of the buffer, water, out of a hemoglobin solution, resulting in the concentration of the hemoglobin solution. By utilizing the data from the hemoglobin trial in combination with modeling methods gathered from the literature, the separation of Lucentis® and the “Lucentis®-like” proteins were modeled. Therein, the theoretical capability of the system to perform buffer exchange and concentrate the proteins of interest was determined.

Through the modeling of TFF, it was determined that a diafiltration factor of 5 L of new buffer would be required for every 1 L of starting solution. Furthermore, it was determined that the water, and therefore most aqueous buffers, operated according to a mass transfer coefficient of 40 m/s. The protein in solution, however, experienced a far lower mass transfer coefficient, which accounted for the approximate 90% yield of modeled protein. In order to meet the specifications laid out by Genentech, it would be advantageous if yields greater than 90% were achieved. Therefore, it is recommended that for the future, a membrane with pore size of 15 kDa, or even 5 kDa, since Genentech indicates that is the size they use, in order to decrease the mass transfer coefficient of the proteins to near-zero.
It must be acknowledged that utilizing hemoglobin as a model protein is problematic. Although the proteins have relatively close molecular weights, the shapes are radically different. Hemoglobin is quite spherical, while Lucentis® is more linear. Due to this, it would be better to utilize a membrane with smaller pore size than larger. Also, the different shape may interact with the membrane retentate channels differently, so clogging may become more of an issue. Also, hemoglobin is easy to detect via UV-vis spectroscopy, due to the distinctive absorbance of the heme groups. However, Lucentis® does not have such a distinctive group, so detection is far more challenging.

The lab-scale specific parameters were utilized to develop a scaled-up set of parameters. It would be possible to utilize a 3 m² membrane, with pores of approximately 15 kDa at a full-scale level. The operating TMP would remain between 20 and 40 psi, with a feed flow rate of 585 L/hour to maintain an operating period of 135 minutes.

Based upon this analysis, the economic advantage for the production-scale plant goes to the alternative case. The primary cost differential lies with the types of chromatography being utilized, since both processes will use the same homogenizer, centrifuge, bioreactor, and TFF systems. However, additional analysis must be referred to the chromatography based upon the costs of resins utilized in the chromatography column, as well as the relative costs of buffers for each chromatography method.

**Future Steps:**

Additional tests ought to be run to solidly reinforce the models developed. Similarly, experiments needs to be carefully developed at each flow rate wherein permeate flow rate is plotted as a function of TMP. These experiments can be utilized to determine the optimal TMP for each flow rate: the TMP just prior to the relationship becoming linear.

Many more water tests need to be performed on the membranes, both when they are new and after each use. Through the use of Eq. 4, the k term for water-only, and by the use of Eq. 3, \( R_m \), the resistance to mass transfer from the membrane, can be determined. By
tracking $R_m$, the health of the membrane can be tracked. If $R_m$ increases, that means that the membrane resistance to mass transfer is increasing, which could be because the membrane is becoming permanently gelled, and needs to be cleaned thoroughly. If it does not recover after cleaning, it is an indication of the wear on the membrane. Or, if $R_m$ goes down, then that indicates that perhaps the membrane is clogging, and, again, needs to be cleaned thoroughly. Alternatively, it could mean that the membrane has a puncture, and will not operate properly ever again. If cleaning cannot return the resistance to normal, then the membrane may no longer be suitable for use.

As a corollary, Eq. 4 can be used, along with the value of $R_m$ and Eq. 3, to determine $R_g$, or the resistance to mass transfer due to the gel layer. Thus, as $R_g$ increases, the gel layer can be assumed to be thickening, so TMP needs to be decreased, and cross-flow increased. Overall, TFF is a very versatile, instrumental component of protein separation and purification from a biological source. It is a unit operation which is well-suited to use in the pharmaceutical industry. Through the implementation of such an efficient purification process, greater supplies of medicines could be produced at a decreased cost and distributed to citizens in need.
APPENDIX A

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Appendix A.1- Small Scale Sample Preparation

*Small-Scale TFF:*
Initially, an attempt was made to run water through an old white cassette-holder membrane, a Pellicon® XL. The cassette would be used as a sacrificial, learning membrane, since the cassette was dried out, it expected to be obsolete. It would still serve to verify that the system was setup properly. The membrane was completely destroyed: a water integrity test was conducted, and the qualitative results indicated that the membrane contained an internal leak, and the retentate flow path was wholly plugged. An attempt to dislodge a plug by switching the feed and retentate lines was unsuccessful. A high pressure drop occurred between the feed and retentate stream, for no other reason than the plugged flow path, and the permeate flowed at far too-high a rate for an intact membrane. However, the test still served to demonstrate to the team which lines needed to be re-routed to achieve proper measurement of parameters.

Prior to testing the new system’s membranes, the Pall Centramate® LV, a flushing gasket was constructed using a spare gasket according to Fig. A.1[7]. The flushing gasket enables the cassette holder to be washed clean of storing oils and machining metal residues. Furthermore, the tubing is also washed clean in this manner.

![Figure A.1: Adaptation of Spare Gasket to a Flushing Gasket.](image-url)
Once the gasket was constructed, calibration of the pump began. The pump started at the lowest power level (1), and was increased until the last power level (9), was reached. To calculate the flow rate, the time elapsed to allow 100 mL of water to flow was determined, and therein a flow rate could be related to the power setting of the pump. Cleaning of the system occurred after calibration. The system was flushed with DI water, and then with 1% Alconox® detergent to clean out any residue left in tubes. Once the 1% detergent had run through the system, a large quantity of DI was used to rinse the system once again.

After initial testing, the following SOP was developed and implemented for every run.

*TFF sample preparation:*

It is beneficial for the health of the membrane to pre-filter the feed solution[21]. This removes the majority of the large contaminants which would otherwise clog the membrane retentate pathway. Utilizing chemically neutral, clean-in-place pre-filters extends the lifetime of the more expensive TFF filters considerably, even up to a tenfold increase. The implementation[21] of inexpensive pre-filters results in considerable financial savings over the course of the life of the plant. Such filters are capable of removing 99% of the solid particulate to within 0.4 microns of desired liquid passage, and options are available to install self-cleaning filters in preparation for systems that use excessively fouled feeds.
Appendix A.2 - Standard Operating Procedure\textsuperscript{[4]}

1) Install 30 kDa membrane into clamps

2) Fill feed vessel with solution to be purified
   a) The solution should be in a buffer 50 mM sodium phosphate monobasic-0.1 M NaCl
   b) Vessel should be large enough to accommodate 1000 L
   c) Run time will be around 4 hours

3) Attach feed, retentate and permeate lines
   a) Lead retentate line back to feed solution for recycle
   b) Lead permeate line to a different collection vessel

4) Set pump pressure to 23 psig

5) Allow system to reach steady state with no bubbles in the lines

6) Ensure the membrane pressure drop is approximately 12 psig

7) Collect separate samples of permeate and retentate every five minutes for fifty minutes
   a) 2 mL of each sample will be characterized from 200-800 nm by UV-VIS spectroscopy

8) After 4 hours, allow lines to clear of liquid into their respective containers, or a waste container

9) Turn off pump

10) Swap supply vessel for buffer

11) Turn pump back on, allow buffer to recirculate

12) Turn pump off, disconnect lead lines to membrane, seal membrane

13) Dispose of waste buffer solution, excess retentate, etc...
APPENDIX B

Appendix B.1- Pump Calibrations................................................................. 45
Appendix B.1- Pump Calibrations

Figure B.1: Pump Output Standard Correlation with each Power Level.

Figure B.2: Flux versus TMP as Various Power Levels.
References

4. Dinh, Justing; Heck, Greg; Lynam, Philip; Mar, Diane; Marshall, Kevin; Mischel, Nolan; Olson, Jared; Ramirez, Adrian Z. Monoclonal Antibody Separation: Lucentis® from Genentech. https://scholarworks.unr.edu/handle/11714/358 (accessed May 1, 2017).