Projects in ChemBE Unit Operations with Experiments

Membrane Filtration & Fluorescence

10/31/18

Group K

Diego Alba Burbano

Oliver Hickson

James Lubowsky

Joanna Wang

Abstract

PolyBioMolecular Inc. plans to launch their new line of dextran-based therapeutic agents. DexFID44 is a novel and effective blood plasma volume expander that needs FDA approval for human use. We have been contacted by PolyBioMoleuclar Inc. to separate the toxic Rhodamine B from their product solution.

In order to fulfil PolyBioMolecular Inc's request, we set out to define a diafiltration system capable of processing 10 kg/day of DexFID44. The first steps in this process were to find the optimal operating conditions and estimate the rejection coefficient for the involved species. Because of fluorescence interference between the particles, it was necessary to run separate set of experiments were the fluorescent molecules were not interacting. Due to molecular similarities, we determined that the results of independent experiments could be analyzed together as if the solution contained both molecules. We then sketched out a scale up solution that would meet PolyBioMolecular Inc's needs.

We found the final Rhodamine B product concentration and the amount of volume it took to dilute it were strongly dependent on the initial feed volume and concentration. With starting conditions of 0.0119 mg/mL of Rhodamine B and 0.381 mg/mL of Dextran-FITC in a 15.6 mL 1xPBS solution, we found that 7 dilution volumes were sufficient to reach a Rhodamine B concentration of 0.25 μg/mL (1% of the lethal dose). At the same time, we were able to recover 86% of the original product, and this number could be higher if PolyBioMolecular Inc decides to act on the recommendations we suggest, such as investing in membrane fouling research.

Table of Contents

Definition of Symbols

A	absorbance
C	concentration (mg/mL)
Cf	feed concentration (mg/mL)
Cp	permeate concentration (mg/mL)
Cr	retentate concentration (mg/mL)
I	intensity
I_0	incident intensity
J	permeate flux (LMH)
L	path length (cm)
N	number of dilution volumes
P_f	feed pressure (psig)
P_p	permeate pressure (psig)
P_r	retentate pressure (psig)
Q_f	feed flow rate (mL/min)
Q_p	permeate flow rate (mL/min)
Q_r	retentate flow rate (mL/min)
R	rejection coefficient
S	surface area (cm^2)
V_0	initial volutme (mL)
V_t	final volume (mL)
ε	molar absorptivity (L/(cm#mol))
Ƥ	permeability

Introduction

PolyBioMolecular Inc. (PBM) has envisioned a novel dextran derivative, DexFID44, for blood plasma volume expander. The synthesis of DexFID44 involves two backbone modifications: the addition of fluorescein isothiocyanate (FITC) and Rhodamine B (RhoB). New studies have shown that Rhodamine B could be carcinogenic, and even lethal under some doses.[1] Now the FDA has imposed strong regulations on drugs containing, and so RhoB must be cleaved and separated from DexFID44. PBM's synthetic chemists have been able to detach the RhoB from the dextrose backbone, but they are in need of chemical engineers that can filter it out. We have been commended with the task of producing 10 kg/day of clean product by using a semi-disposable membrane cartridge.

Background

In order to effectively separate the cleaved Rhodamine B from the DexFID44 product solution, it is imperative we understand the molecular differences between the two, the concentrations we will be handling, and what properties we ought to measure to know those concentrations.

Molecular Properties

Dextran is a polysaccharide, a glucose polymer, that is commonly used therapeutically as plasma volume expander and anticoagulant due to its biocompatible and biodegradable properties.[2] In particular, we will be working with a specific dextran derivative, DexFID44, which has been modified to incorporate a fluorescent dye, Fluorescein Isothiocyanate, (FITC), in its backbone. The molecular weight of this compound is 200 kD, and the solubility is 25 mg/mL, which sets an upper bound for the concentrations we can experiment with.

FITC is a fluorescent dye derived from fluorescein by adding an isothiocyanate group, which enables it to form bonds with amine and sulfhydryl groups on proteins.[3] This reactive property makes FITC extremely useful for labeling proteins and cells, which can then be quantitively analyzed with flow cytometry. In our case, FITC binds to the polysaccharide through a hydroxyl group, as seen in Figure 1. The molecular weight of FITC is much smaller than

dextran's: 389.3 g/mol for FITC compared to $2x10^6$ g/mol for dextran.[4] We also know that the ratio of FITC to dextran is 130:1, and Rhodamine B to dextran is 1:1. [4]

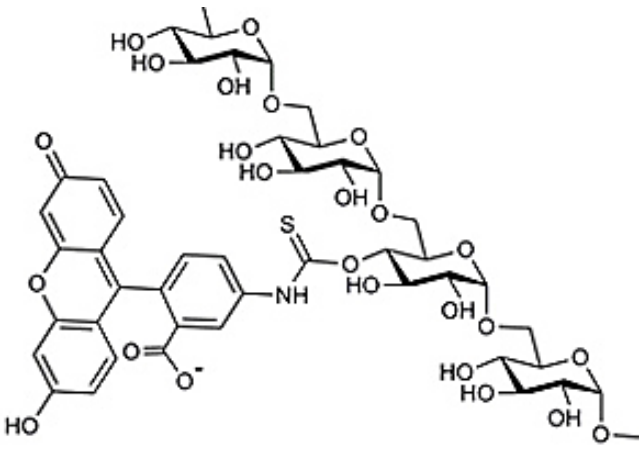


Figure 1: Molecular structure of FITC bound to the backbone of Dextran by an isothiocyanate group. There could be more FITC molecules attached to the backbone.[5]

Rhodamine B (RhoB, Figure 2) is a chemical compound commonly used as a fluorescent probe and a histological dye for the study of the anatomy of cells and tissue.[6] We can also modify it with an isothiocyanate group to bind to biomolecules.[7] In the same way as FITC, but in a much higher ratio, it binds to dextran in the synthesis process of DexFID44. It is necessary to cleave RhoB due to FDA regulations, since it is carcinogenic.[8] There are also studies showing that 25 μg/mL concentrations can be lethal.[1] Hence, we set the maximum acceptable RhoB concentration in the production solution to be 0.25 μg/mL. We can indirectly measure the concentrations of Dextran-FITC and RhoB by measuring their fluorescence.

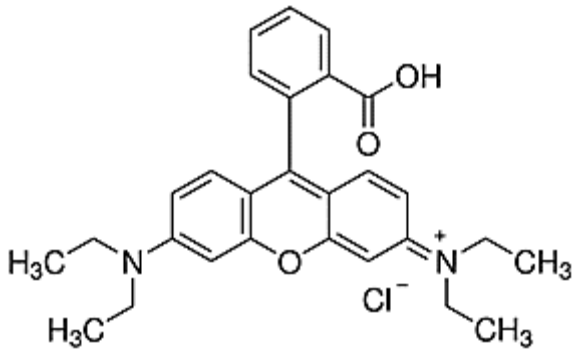


Figure 2: Molecular structure of Rhodamine B in ionic form. Note the chlorine is also in display.[9]

Fluorescence and Absorbance

Fluorescence is a type of photoluminescence where a substance emits light after having absorbed electromagnetic radiation (e.g. light). At the atomic level, this means that an excited electron relaxes to its ground state and emits a photon. This phenomenon is more conveniently described with the aid of a Jablonski diagram, such as the one depicted in Figure 3.[10]

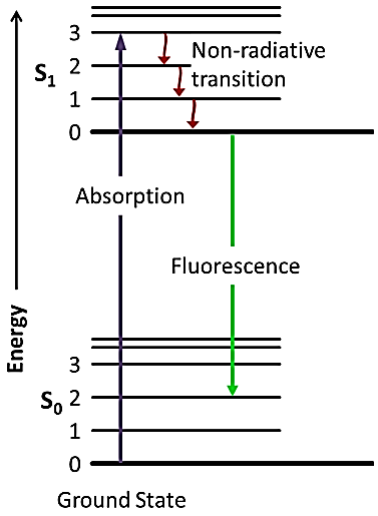


Figure 3: Example of a Jablonski diagram. Electromagnetic radiation would be absorbed by an electron, exciting it from the ground state to a higher state. The electron loses energy, until it relaxes back to the ground state, fluorescing. [10]

It is also important to consider the absorbance of the molecule, especially when dealing with more of one fluorescence substance. It could be the case that one of the species absorbs light at the same frequency that the other emits at, causing interferences and skewing the results. Spectroscopy data for both RhoB and FITC (Figure 4) suggests there are interferences between the two molecules. It is clear from the diagram that the FITC emits light at the same wavelength that RhoB absorbs it. It is important to note that these measurements were taken when the molecules where dissolved in ethanol, whereas they will be in 1x PBS (phosphate-buffered saline) during our experiments. Changing the environment of the molecules can influence the wavelengths at which molecules emit and absorb light: for instance, RhoB absorbs light of 542 nm and emits at 565nm when in ethanol, but absorbs at 562nm and emits at 583nm in water.[11] Without the information on how the mixture of RhoB and FITC in PBS behaves for different concentrations, it is hard, if not impossible, to predict if there would be interferences. Nevertheless, we should be

cautious when measuring the fluorescence of both species in solution and investigate walkarounds if they do interfere with one another.

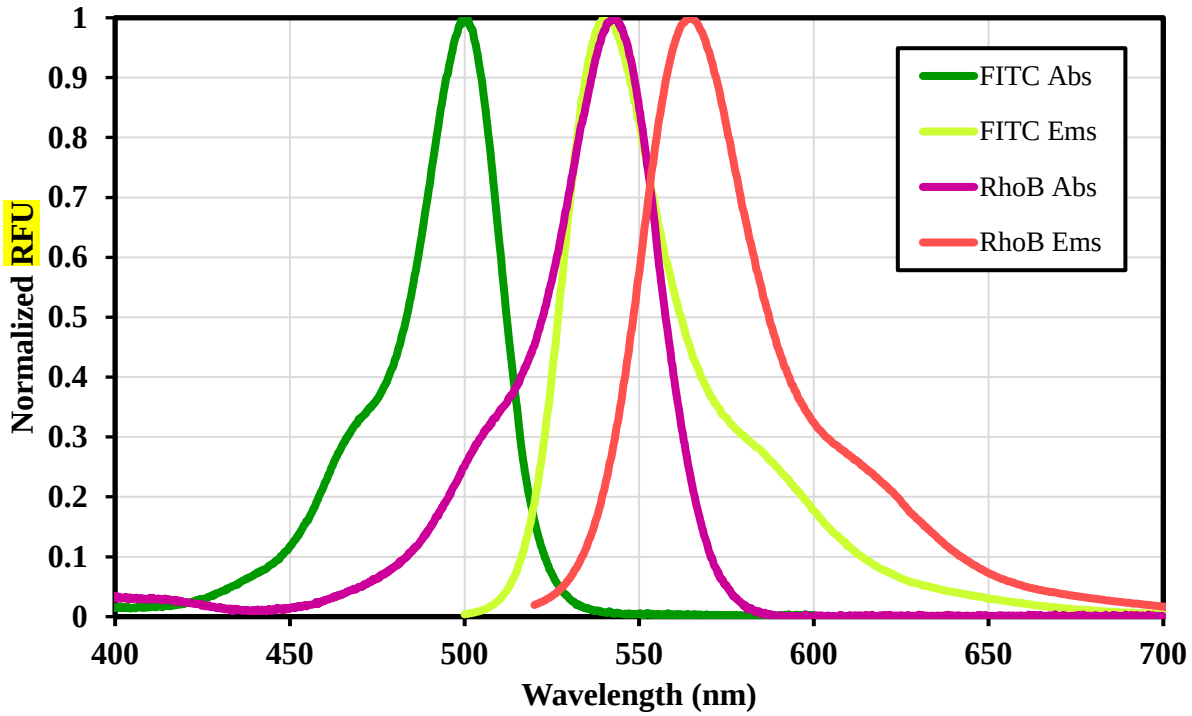


Figure 4: Spectroscopy data for both RhoB and FITC. Both species were in EtOH. There is a clear overlap between the emission wavelength of FITC (light green) and the RhoB absorption (purple). This overlap could cause interferences when trying to measure the concentrations of the species. The Y-axis is the normalized relative fluorescence units to ease the comparison.[11,12,13]

We can relate the amount of light absorbed to the concentration of a fluorescent specie though the Beer-Lambert Law:

$$A = log_{10}\left(\frac{I_0}{I}\right) = \varepsilon \cdot L \cdot C \qquad \textbf{[Equation 1]}$$

where the absorbance A is the ratio of the incident intensity I_0 to the transmitted intensity I, L is the length of the light path, C is the concentration of the chemical compound, and ε is the molar absorptivity coefficient. This equation indicates there is a linear correlation between absorbance and concentration: as concentration increases, absorbance should increase proportionally. However, there are limitations to the Beer-Lambert Law. Mainly, when the concentration becomes too high there are greater electrostatic interaction between the molecules that could affect the absorbance of light. As the concentration increases, the refractive index of the solution could change, also changing the absorbance.[14] For these reasons, Beer-Lambert Law applies when the concentrations are below 10mM, which is high enough for our purposes.

Fallowing the Beer-Lambert logic, we can postulate the relationship between fluorescence and concentration is also linear. In order to prove this, we can measure the fluorescence of solutions of known concentration and fit a line through the data.

Size exclusion Separation

A heterogenous mixture, such as the suspension of RhoB and Dextran-FITC in PBS, can be mechanically separated by exploiting the physical differences between its distinct components. There are several technologies available for the separation of a solid-liquid mixture, and we ought to choose the optimal one by considering the concentrations, flow rates, and particle dimensions we need to process (Figure 5). For instance, membrane filtration would be suitable since there is a large difference between the molecular sizes of the RhoB and Dextran-FITC. More specifically, because of the small dimensions of the particles, we will be operating in the ultrafiltration range.

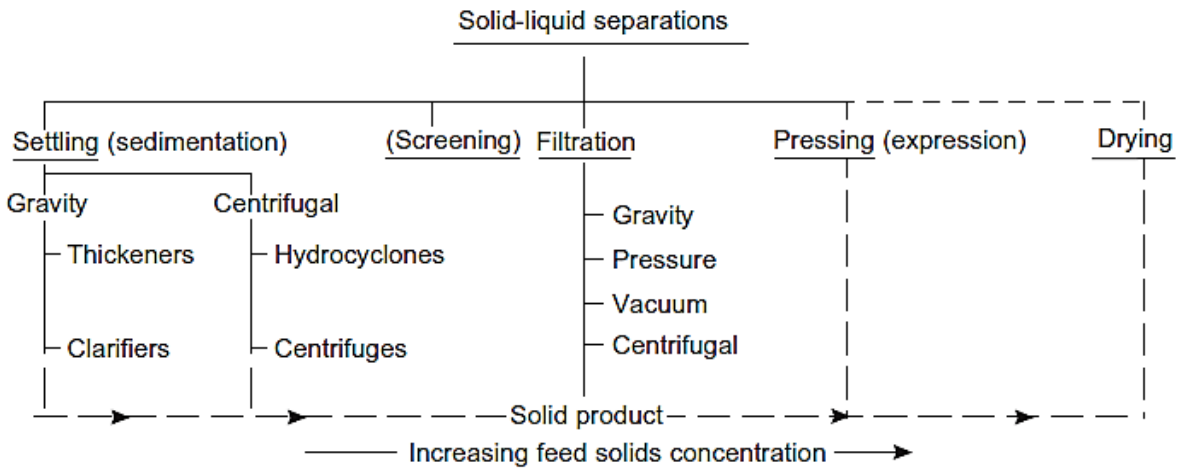


Figure 5: Several separation technologies for the separation of solid and liquid phases. They are ordered in increasing solid concentration from left to right.[15]

There are two methods employed in membrane separation: dead-end filtration, where all solution is forced perpendicularly through the membrane surface, and tangential flow filtration (TFF), where the solution flows parallel to the membrane. In dead-end filtration, the solids retained by the membrane accumulate at the surface and eventually plug the device. On the other hand, TFF allows for continuous removal of deposited material, and it is a more efficient method when the membrane could get easily plugged by big molecules, such as Dextran-FITC.

In a filtration process, the separation driving force is pressure. A portion of the feed is forced through the semipermeable membrane due to a lower pressure on the other side of the

membrane. What passes through the membrane is called filtrate or permeate, whereas what is retained is denominated retentate. The size of the membrane pores determines which molecules can pass to the permeate side, and therefore is a critical parameter when choosing a specific membrane. Only a small percentage of the feed becomes permeate, therefore the retentate is usually recycled back for a better separation. A typical diagram for membrane TFF is displayed in Figure 6.

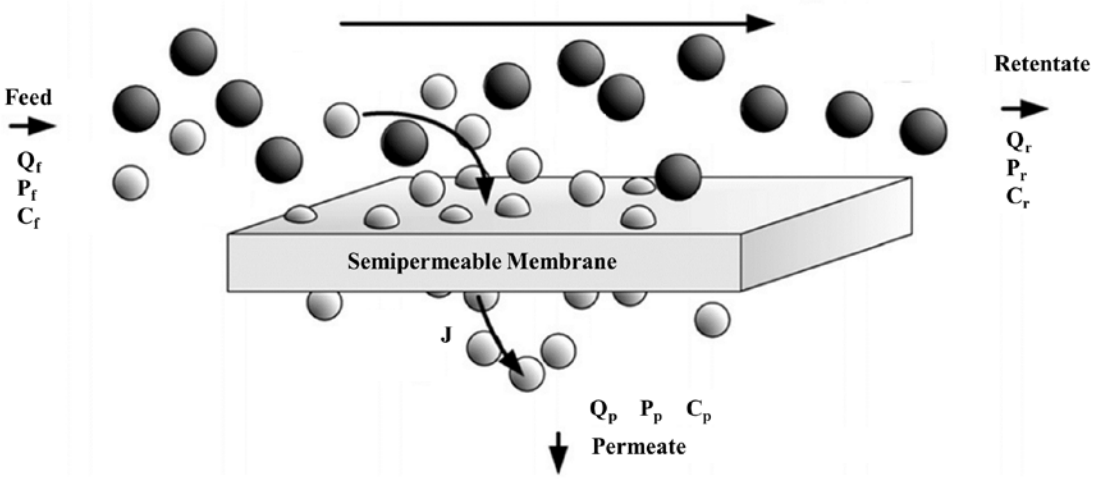


Figure 6: Simple diagram for a tangential flow filtration system. Particles suspended in the feed flow tangential to the membrane. Because of the difference in pressure on both sides of the membrane, particles smaller than the pores will flow into the permeate. The rest will continue as the retentate. [16]

Key parameters in process design and operation are the transmembrane pressure, rejection coefficient, feed flow rate, permeate flux, membrane area and pore size, and shear stress on the membrane. The three pressures (P_f, P_r, and P_p) can be controlled indirectly though other parameters, mainly the pump rate and the intrinsic resistance of the tubing and membrane.

The feed flow rate Q_f rate is determined by the pump, which induces a pressure drop across the system. We define the pressure drop as follows:

$$DP = P_f - P_r \qquad \textbf{[Equation 2]}$$

where *DP* is the pressure drop, and P_f, and P_r, are the feed and retentate pressures, respectively. As the pump rate increases, the pressure drop increases and a higher volumetric flow is achieved. The feed flow rate determines the shear stress on the membrane: the parallel force applied on the membrane surface by the fluid. Higher shear stresses are better for preventing plugging, since deposited material can be more easily scrapped, but could also damage the membrane if too high.

The rejection coefficient relates the concentration between the permeate and the retentate and serves a measure of how permeable the membrane is to a particular species. It is an intrinsic property of the membrane and the compound to separate, and it is defined as:

$$R = 1 - \frac{C_p}{C_r} \quad \textbf{[Equation 3]}$$

where R is the rejection coefficient, and C_p and C_r are the permeate and retentate concentration, respectively. When the rejection coefficient is equal to 1, the molecules cannot be filtered through the membrane and come out in the retentate. In contrast, the permeability $\mathcal{P}$ of a species defines how much passes to the permeate:

$$\mathcal{P} = \frac{C_p}{C_r} = 1 - R \quad \textbf{[Equation 4]}$$

The permeate flux defines how much feed is being filtered through the membrane per unit are per unit time. It can be calculated from the permeate volumetric flow rate:

$$J = \frac{Q_p}{S} \quad \textbf{[Equation 5]}$$

where J is the permeate flux, Q_p is the volumetric flow rate, and S is the membrane surface area. The flux is typically. spread in liter per square meter per hour, or LMH.

The transmembrane pressure is the separation driving force, as it is the difference in pressure across the membrane:

$$TMP = \frac{(P_r + P_f)}{2} - P_p \quad \textbf{[Equation 6]}$$

where TMP is the transmembrane pressure, and P_p is the permeate pressure. The TMP will dictate the permeate flux J, and therefore the permeate flow rate Q_p as well. As TMP increases, we expect the permeate flux to increase. However, the flux is not pressure dependent in the entire pressure domain: if the pressure is too high and the shear stress too low, particles will plug the pores and

will not be removed by the fluid, lowering the permeate flux. This phenomenon can be perceived in **Error! Reference source not found.**.

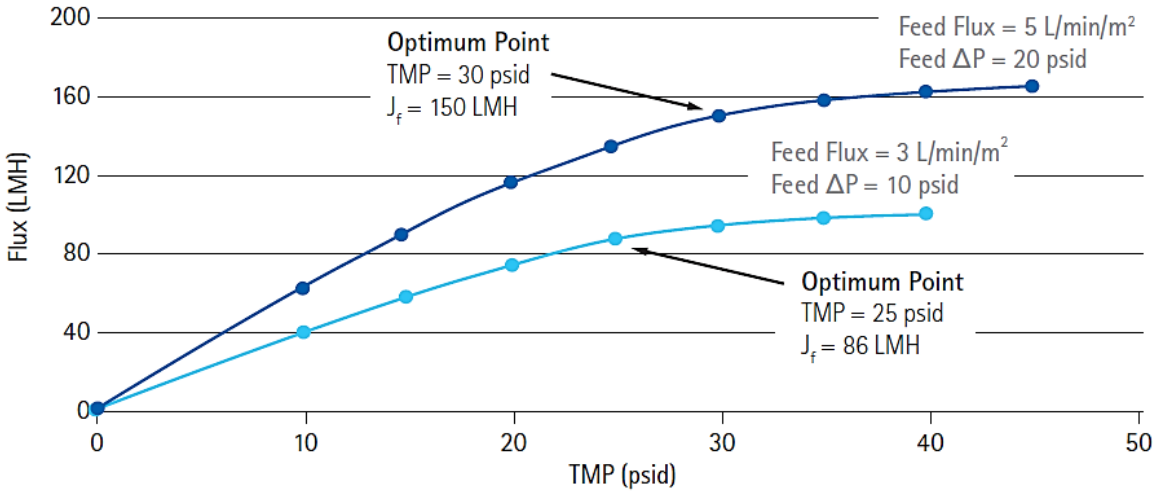


Figure 7: Permeate flux (in Liters per square Meter per Hour) as a function of TMP for different flow rate. The optimum point is defined as the point at which the slope decreases by more than 50%. When operating above the optimum point, fouling can be problematic.[17]

The point at which the flux starts to no longer be pressure dependent is the optimum point at which to run the process. We expect a higher optimum point for a higher feed flow rate. Operating above the optimum point could cause fouling, lowering the efficiency of the membrane and possibly damaging it. There are several types of fouling, but we are mainly concerned with caking: the formation of a solid layer that hinders permeate flux due to accumulation of particles on the membrane surface or in the membrane pores. High shear rates is a common way to combat caking, since the faster fluid will be able to scrape some of that layer off the membrane surface.

A way to check for fouling is to continuously monitor the flux, TMP, and rejection coefficient, since these are parameters that would greatly be affected by solids accumulating in the membrane. Moreover, we can apply the equation of continuity, assuming the density is constant, and component material balances for both fluorescent species to check for accumulation and leaks. The following equations should hold at steady state.

$$Q_f = Q_r + Q_p \qquad \textbf{[Equation 7]}$$

$$Q_f \cdot C_f = Q_r \cdot C_r + Q_p \cdot C_p \qquad \textbf{[Equation 8]}$$

Diafiltration

Diafiltration is a dilution process where clean buffer is added to the system while permeate is removed. It can be implemented in several ways, such as volume reduction or sequential dilution, but constant volume is often used due to its simplicity, scalability, and the fact that the concentration of the product in the retentate remains constant.[18] This can be beneficial for biomolecular processes where the product stability must be precisely controlled. It is also important to keep the concentration constant to avoid molecules precipitating out of solution.[19] However, a downside of constant volume diafiltration is that larger quantities of replacement buffer are usually required, and the set up must include a pump to continuously add buffer into the system.[18] In addition to the pump, most systems include a backpressure valve, or pressure adjustment valve (Figure 8), in the retentate side, which facilitates control over TMP.

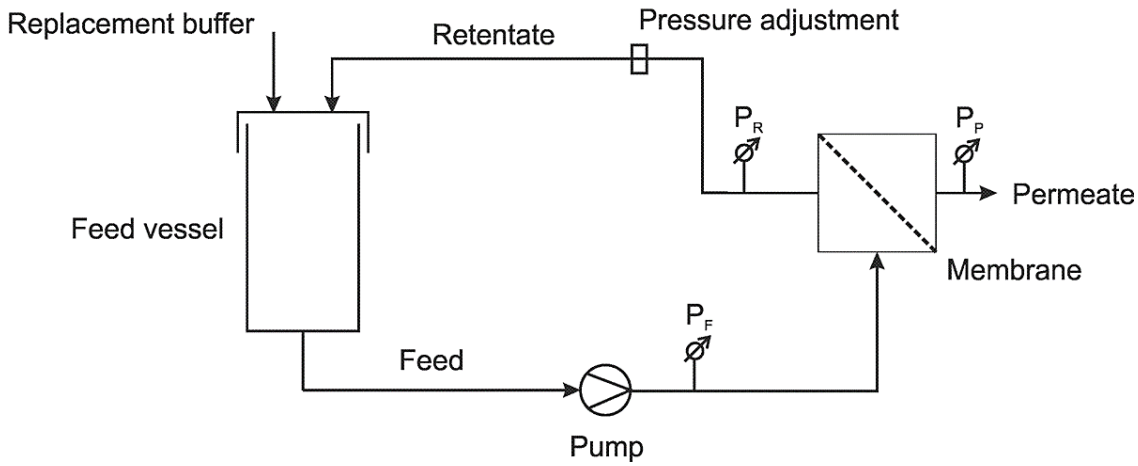


Figure 8: A flow diagram of a simple diafiltration process. New buffer is continuously being added into the system, while permeate leaves and retentate is recycled for better separation. The pressure adjustment valves usually controls the transmembrane pressure. [20]

We measure how much replacement buffer is added to the system in terms of DVs (diavolume, diafiltration volume, or dilution volume):

$$DV = \frac{V_t}{V_0} \qquad \textbf{[Equation 9]}$$

where V_0 is the original volume of the mixture, and V_t is the total buffer volume added to the system. Hence, 1 DV means the same volume of buffer as volume initially fed has been added to

the system and come out as permeate. Because the added buffer comes out as permeate and the retentate is recycled, the volume in the system does not change. Moreover, if the product (Dextran-FITC) cannot pass through the membrane, its concentration in the retentate will stay constant. On the other hand, the impurities (RhoB) will be washed away, and their concentrations will decrease in the retentate with each DV added to the system. The number of required DVs to reach a specific retentate concentration can be determined with the following equation:[17]

$$C_r = C_{f0} \cdot \exp((R-1) \cdot N) \qquad \textbf{[Equation 10]}$$

where C_r is the concentration in the retentate after *N* dilution volumes have been added, and C_{f0} is the initial concentration in the feed, before any buffer was added. From this equation, we observe that as the rejection coefficient increases we would need to add more buffer to the system to reach the same final retentate concentration. If the rejection coefficient equals 1, the concentration will remain constant no matter how much buffer is added. It is often convenient to express Equation 10 after rearranging the terms and taking the natural logarithm on both sides. Equation 11 can be now plotted linearly on *N*, where the slope is *R*-1 and the y-intercept is 0:

$$\ln\left(\frac{C_r}{C_{f0}}\right) = (R-1) \cdot N \qquad \textbf{[Equation 11]}$$

Dealing with Interferences

With the present information, we can elaborate a procedure in case there are interferences in the fluorescent signals of the two species, RhoB and Dextran-FITC. Since the molecular differences between Dextran-FITC and Dextran are minimal and there are no chemical interactions between Rhodamine B and Dextran or Dextran-FITC, we expect Dextran to behave identically to Dextran-FITC within our system. This means the rejection coefficient for both species should be the same, and much higher than that for RhoB. Therefore, we can run sets of experiments with solutions of RhoB and Dextran in PBS, and experiments with Dextran-FITC in PBS, and draw conclusions as if the mixture was RhoB and Dextran-FITC in PBS. This hypothesis could be verified if we had other concentration measurement tools at our disposal, since the concentration of Dextran cannot be measured through fluorescent spectroscopy, and if the stock solution of Dextran-FITC did not contain Dextran as well. However, we are confident in the logic of this assumption, and therefore in the validity of our results.

KR2i TFF System: Parameters and Features

To separate the cleaved Rhodamine B form the Dextran-FITC product, we employed the KR2i Tangential Flow Filtration system with automated process controls from Spectrum Labs. All the experiments were carried out with this system, and the data was collected through their own Excel-based software. We had direct control over the following parameters: feed composition and volume, feed flow rate, backpressure valve, and mode of operation. In contrast, several parameters were inherent to the system: all the tubing and fittings, the volume capacity of the reservoirs (feed, permeate, and buffer), and the membrane itself. The membrane used during all experiments was a modified polyethersulfone (mPES) hallow fiber with pore rating of 30kD and a surface area of

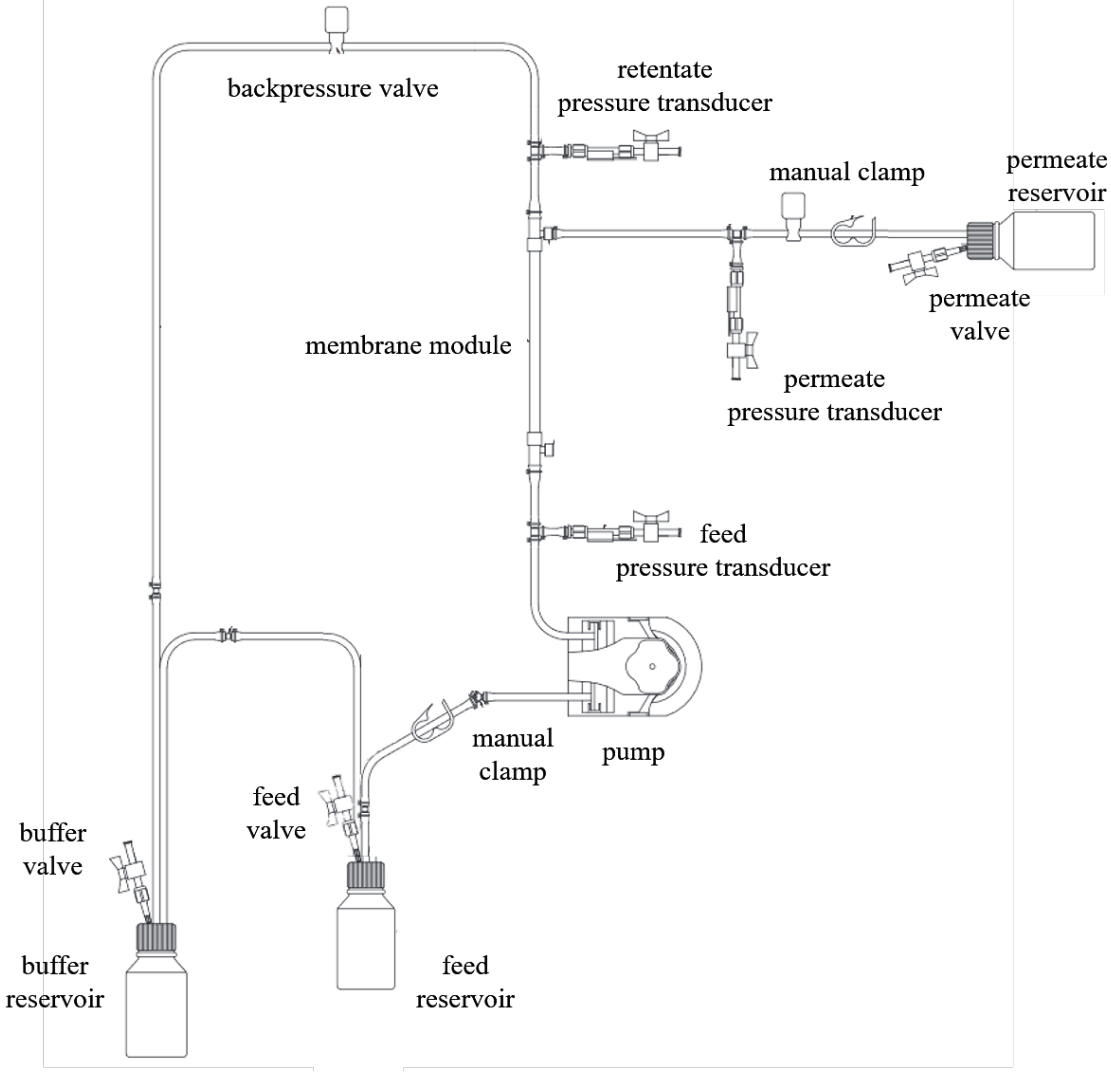


Figure 9: Full diagram for the KR2i TFF system. Most important components have been labeled accordingly. An inaccuracy in the diagram is that the retentate does not flow directly into the buffer reservoir, but into the feed. There is a T-junction before the retentate reaches the feed where the buffer is drawn from. [19]

115cm^2 (Spectrum Labs, part #: D02-E030-05-N). These fibers are packed into filter modules that can be easily exchanged and disposed. The full KR2i TFF set-up is represented in With the present information, we can elaborate a procedure in case there are interferences in the fluorescent signals of the two species, RhoB and Dextran-FITC. Since the molecular differences between Dextran-FITC and Dextran are minimal and there are no chemical interactions between Rhodamine B and Dextran or Dextran-FITC, we expect Dextran to behave identically to Dextran-FITC within our system. This means the rejection coefficient for both species should be the same, and much higher than that for RhoB. Therefore, we can run sets of experiments with solutions of RhoB and Dextran in PBS, and experiments with Dextran-FITC in PBS, and draw conclusions as if the mixture was RhoB and Dextran-FITC in PBS. This hypothesis could be verified if we had other concentration measurement tools at our disposal, since the concentration of Dextran cannot be measured through fluorescent spectroscopy, and if the stock solution of Dextran-FITC did not contain Dextran as well. However, we are confident in the logic of this assumption, and therefore in the validity of our results. .

Experimental Methods

In order to characterize the properties of our system (e.g. rejection coefficient, optimal transmembrane pressure and feed flow rate) and find out how much replacement buffer should be added to reach a safe concentration of 0.25 μg/mL RhoB in the product, we designed and executed two sets of experiments. We also made a calibration curve to relate the fluorescent measurements to concentrations.

KR2i TFF System Operation and Sampling Procedure

To operate the system at constant volume, we add enough buffer to the reservoir and open the buffer valve, so the reservoir is always at atmospheric pressure. We add the solution to the feed reservoir and close the feed valve. This ensures that when the pump draws feed, buffer is also drawn out of the buffer reservoir and into the feed, keeping the volume of the feed reservoir constant and avoiding air in the reservoir from getting into the pump. After the solution passes through the membrane, the permeate is collected in the permeate reservoir, which is also open to the atmosphere, and the retentate is recirculated into the buffer. Both the buffer reservoir and the permeate reservoir sit on weighing scales connected to the computer (Windows Tablet) that

controls the system and collects the data. The computer logs the data (pressures, weights, and feed flow rate) every minute, and we failed to find an option to change this. Through the Excel control panel, we set the desired feed flow rate and the backpressure. It is also possible to control the pump ramp rate, and to set a threshold for when to stop pumping either in terms of the buffer reservoir weigh or the permeate reservoir weigh. This become useful when we need to sample after 1 DV has passed through the system.

Before every experiment, we run a solution of 1x PBS to clean the system and ensure there are no contaminants from previous experiments. We use higher flow rates (up to 300 mL/min) and transmembrane pressures (up to 12 psig) to achieve a higher shear stress on the membrane surface, removing any deposited material, and a higher permeate flux, unclogging membrane pores.

When we need to sample the retentate and permeate to measure the concentration of RhoB and Dextran-FITC, we stop the pump, and proceed to close all valves and manual clamps. This ensures no liquid is lost when we open the permeate and feed reservoirs. From each reservoir, we pipette 100 μL into 5mL centrifuge tubes to be sampled with the spectrometer. We close the reservoirs, tar the permeate balance so the computer stops again after 1 DV, open the clamps and valves (except the feed valve), and start the pump again.

If it is necessary to change the backpressure or flow rate, we found optimal to stop the pump and open the backpressure valve first. Then, we can set the new parameters through the control panel, close the backpressure valve, and start the pump again. This ensures the backpressure is set to the desired pressure for the entire duration of the experiment.

Sample Preparation

We diluted the 10x PBS stock solution to 1x PBS by adding 900 mL DI water to 100 mL 10xPBS. From the other three available stock solutions (0.05 mg/mL RhoB in 1xPBS, 20 mg/mL Dextran in 1xPBS, and 19 mg/mL Dextran plus 1 mg/mL Dextran-FITC in 1x PBS), we made feed solutions in 1:1:0.621 volumetric ratio (PBS:Dextran/Dextron-FITC:RhoB). This yields a solution of 0.0119 mg/mL of RhoB and 0.381 mg/mL of Dextran-FITC. We also made feed solutions with just RhoB or Dextran-FITC but maintaining the same final concentrations. In the case of the clean RhoB solution, we substituted the Dextran/Dextran-FITC with just Dextran, and in the case of the clean Dextran-FITC solution, we substituted RhoB with PBS (1.621:1:0). Similarly, we also made

PBS solution with Dextran in a 1.621:1 volumetric ratio. We also made solutions to make the calibration curve for fluorescence by diluting the stock solutions with PBS several times.

Measuring Concentration and Constructing a Calibration Curve

To make measure the concentration of the fluorescent species, we used the ThermoFisher Scientific Nanodrop 3300. This spectrometer was able to measure the fluorescence in relative fluorescence units. In order to use the unit, we first selected which light source to use and which wavelength to measure at, based on the consistency of the measurements at for different setups. We used the white light source since it yielded the best results. After measuring the fluorescence of the known concentration solutions, we plotted the data in Excel and drew a trendline, setting the y-intercept to 0.

Constructing TMP v Flux

To determine the ideal transmembrane pressure for a given flow rate, we set out to measure the permeate flux at different TMPs. We used the Dextran in PBS solution for this experiment. We placed 40 mL of the solution in the feed reservoir and filled the buffer reservoir with 1x PBS. We allowed the system to reach the desired TMP and waited until at least 3 stable measurements were recorded in the Excel spreadsheet. Then, we stopped the system and changed the TMP as described in the system operation section. We increased the transmembrane pressure until it was noticeable that the permeate flux had plateaued. Often it we had to take a new measurement in between previous TMP values to achieve a better resolution. We repeated this process for three feed flow rates: 50 mL/min, 150 mL/min, and 250 mL/min.

Constructing Rejection Coefficient

To determine the rejection coefficient of both species, we collected retentate and permeate samples after 1 DV, for several DVs. We used three different solutions: RhoB and Dextran/Dextran-FITC in 1x PBS, RhoB and Dextran in 1xPBS, and Dextran/Dextran-FITC in 1x PBS. The solutions were fed at 150 mL/min and the transmembrane pressure was set to 6 psig through the Excel control panel. The pump automatically stopped after 1 DV, and we sampled the reservoirs as explained in the sampling procedure section. The process run for 4 to 7 DVs, depending on time constrains. We repeated this procedure for each of the three solutions. The 5 mL centrifuge tubes were then taken to the spectroscopy section and analyzed with the nanodrop**.**

Excel Calculations

Conveniently, the KR2i system records all parameters except concentration. At the end of each experiment, we had an Excel table with information on the time, pressures (feed, retentate, permeate, TMP, and DP), flow rates (feed, retentate, and permeate), mass in the permeate and buffer reservoirs, permeate flux, and shear. We also had data from the spectrometer when necessary. All the calculations were therefore done in Excel.

The Excel file automatically calculates TMP, DP, permeate flow rate, retentate flow rate, and permeate flux. DP, permeate flux, and TMP are calculated as explained in Equation 2, Equation 5, and Equation 6, respectively. Its noteworthy that to calculate the permeate flow rate, the Excel spreadsheet calculates the change in mass in the permeate reservoir over the change in time, assumes the density of the solutions equals 1 g/mL. For the retentate flow rate, the Excel does a material balance as seen in Equation 7.

To find the optimal TMP for each flow rate, we found the first order derivative by calculating the forward, finite difference between our flux measurements and divided by the corresponding change in TMP. From the first derivative graph, we were able to visually determine the optimal TMP more clearly that with the plotted raw data.

To calculate the rejection coefficient of each species, we transformed the concentration data as indicated in Equation 11. We then plotted the number of dilution volumes against the natural logarithm of the ratio of concentrations and fitted a line through the data. The rejection coefficient was then the slope of the trendline minus 1. To find the required replacement buffer needed to achieve 0.25 μg/mL concentration of RhoB in the retentate, we solved for the number of dilution volumes in Equation 10.

Safety and Waste Disposal

In accordance with the material safety data sheet found on Fisher Scientific's webpage, Dextran can cause eye and skin irritation (Fisher). Dextran should be used in an area with proper ventilation and any spills should be cleaned up immediately. Eyes should be flushed out with water for at least 15 min if they come into contact with this chemical. Additionally, if the skin meets with Dextran, it should be flushed with water for at least 15 min and any contaminated clothing should be removed. (Fisher) When handling Dextran, protective equipment should be worn. This

includes goggles, lab coats, and closed toed shoes (Fisher). The safety information regarding Dextran FITC and Rhodamine B is the same. Moreover, RhoB is believed to be carcinogenic, so extreme caution will be taken so as to not cause any splashes or spills. When disposing of these chemicals, they will be stored in a closed, labeled, container designated specifically for the waste of that kind.

The second main safety issue when operating the KR2i TFF system is the pressure, and the possible pressure build up. The membrane cartridge has been designed to hold up between 20-30 psig. Operating above those pressures will not only damage or rupture the membrane, but it could also make the tubbing and fittings pop. This can become a hazard for anyone within a 15 feet radius from the machine.

We must also be careful with the electric circuitry of the system, since it could get short-circuited if it came in contact with liquid. The main power adapter is encased inside a plastic box, but liquid should never be near it. We tried keeping all the solutions on the opposite side of the table to ensure this measure.

Results

Here we discuss the findings related to the optimal operating parameters and the rejection coefficient of the species. We also present the calibration curves necessary to measure concentration. We made the raw data available in *Appendix C: Raw Data.*

Fluorescence calibration curves

Based on our operating concentrations of 0.381 mg/mL of Dextran-FITC and 0.0119 mg/mL of RhoB, we measured the RFUs in the surrounding concentration neighborhood. The resulting calibration curves relating concentration to fluorescence for both Dextran-FITC and Rhodamine B are displayed in Figure 10. As long as the concentrations are low, the relationship should be linear. We fitted a trendline for both sets of data, setting the y-intercept to 0, as there should be no fluorescence signal when there are no fluorescent molecules. Setting the y-intercept did not affect the trendline for Dextran-FITC. For RhoB, however, the trendline changed greatly. We decided on setting the y-intercept to 0 because of the physical significance behind the equation.

This model is more accurate for Dextran-FITC (R^2 of 0.9887) than for RhoB (R^2 of 0.8366). For comparison, the average relative standard error for the Dextran-FITC measurements was 5.23%, whereas for RhoB it was almost double: 9.76%. With the slope of the trendlines we can readily transform relative fluorescent units (RFUs) measured with the nanodrop into concentrations. The slopes were 10414 RFU/(mg/mL) for Dextran-FITC and 214939 RFU/(mg/mL) for RhoB.

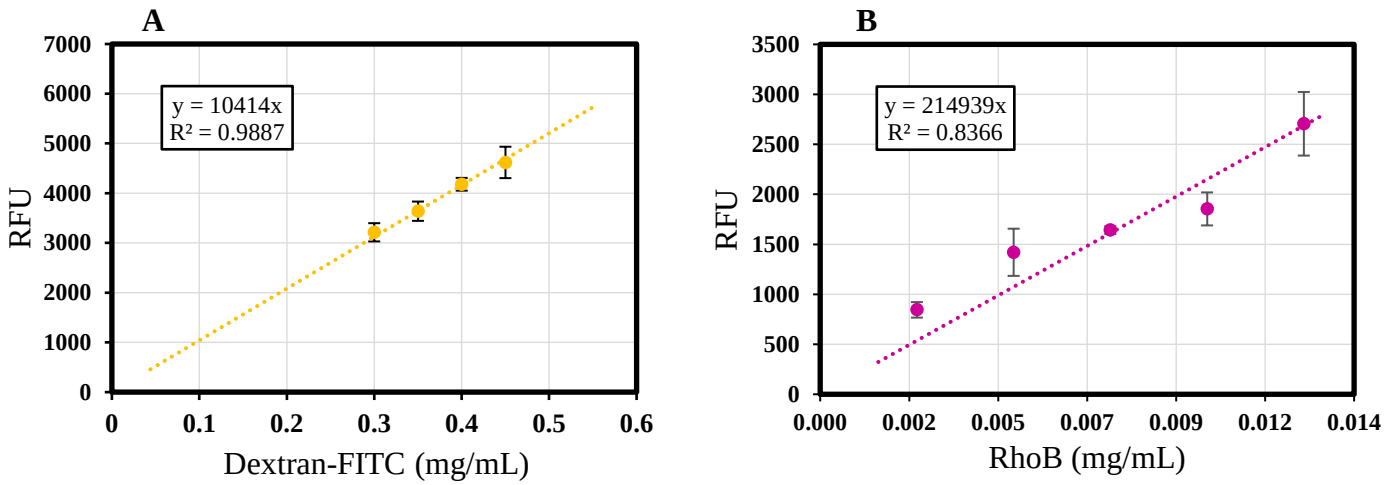


Figure 10: Calibration curves for A: Dextran-FITC; and B: Rhodamine B. The measurements were more consistent at higher concentrations (A), which is reflected in the R^2 values. It is worth nothing that the slopes of the regression lines are different by around 20-fold. The y-intercept was set to 0 for both cases, since there should be no fluorescence signal when there are no fluorescent species present in solution.

Finding the Optimal Transmembrane Pressure

The transmembrane pressure is the driving force for separation. Operating at the optimal TMP is crucial to achieve high efficiencies. In order to determine the optimal TMP, it is useful to check the permeate flux. The measured permeate fluxes at different transmembrane pressures are presented in **Error! Reference source not found.**. The three different curves represent the three different flow rates tested, color coded for readability. We also show the derivative used to find

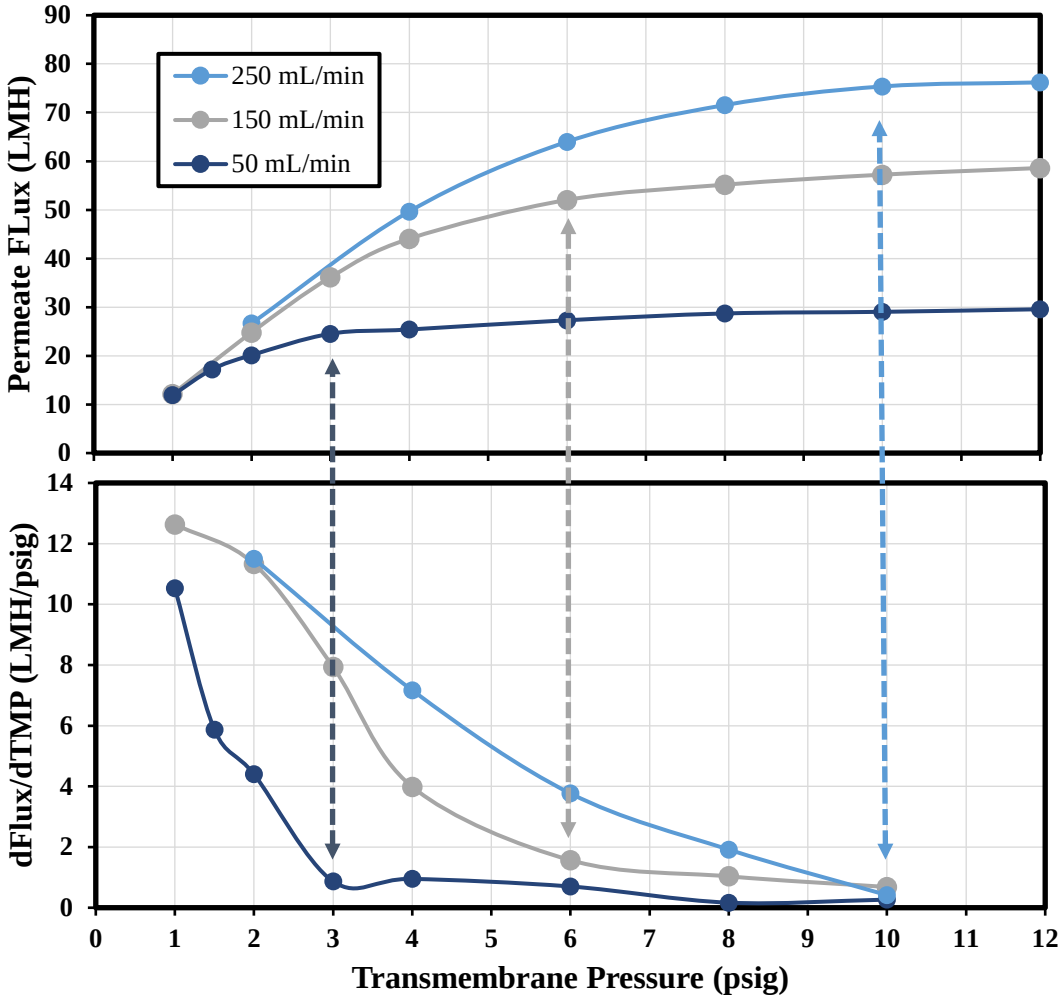


Figure 11: Permeate flux, and its derivative, as a function of transmembrane pressure. By taking the derivative, we can much clearly identify which TMP values are optimal. This task would be better if more measurements at different TMPs had been taken.

the optimal TMP. The three arrows point to the chosen TMP for each feed flow rate: 3 psig for 50 mL/min, 6 for 150 mL/min, and 10 psig for 250 mL/min.

Finding the Rejection Coefficient

We present the measurements taken to find the rejection coefficient of both fluorescent species in Figure 12. The data represents three different feed solutions: “mixed” indicates that both RhoB and Dextran-FITC were present, whereas “clean” indicates that only one fluorescent component was in solution. The data should be the same if there are no interferences between species. The first data point, the initial concentration before any buffer is added (corresponding to the origin), was deliberately ignored when analyzing the data.

Since the rejection coefficient is an intrinsic property of the membrane-particle pair, we fitted the data with a linear relationship, following Equation 11. The rejection coefficient is then 1 minus the slope of the line. We provide all equations for the trendlines, R^2 values, and corresponding rejection coefficients in Table 1. Since the “mixed” data is clearly different, we proceeded to use the rejection coefficients obtained for the “clean” solutions. For RhoB, the rejection coefficient is 0.4316, while for Dextran-FITC it is 0.9709. Because Dextran and Dextran-FITC are molecularly almost identical, we expect the rejection coefficient of Dextran to be 0.9709 as well.

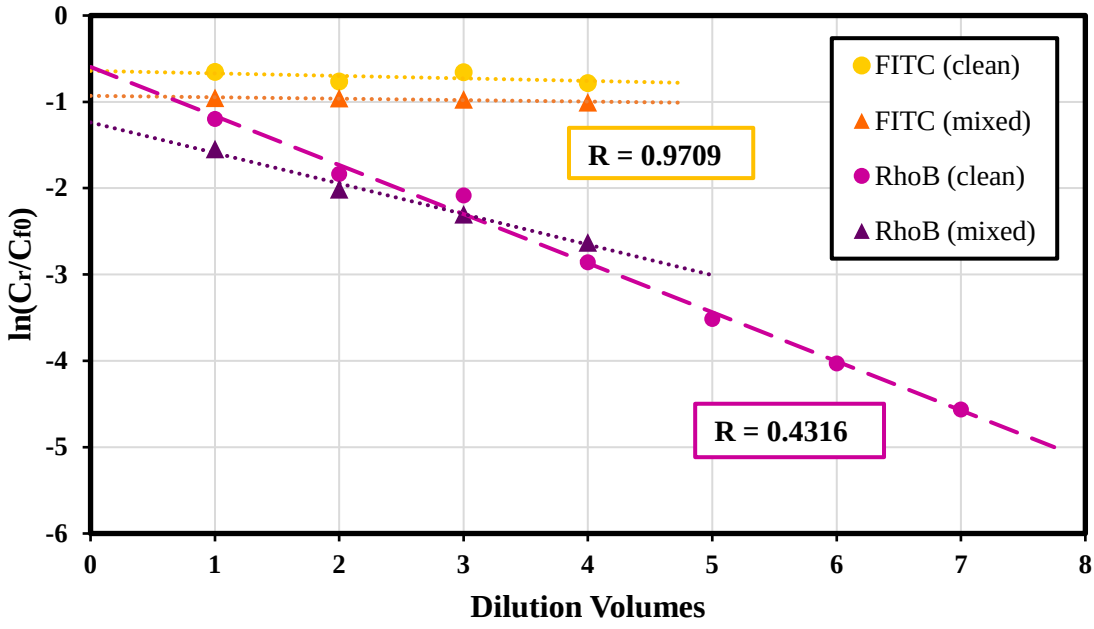


Figure 12: Rejection coefficients for both RhoB and Dxtran-FITC. We also compare the case when they are both in solution (“mixed”). The regression lines do not pass through the origin, indicating that C_{f0} is smaller than what we measure. The equation this graph is based on is Equation 11. It is important to note that the normalization is done within a dataset, not across datasets.

Table 1: Regression lines, R^2 values, and rejection coefficients for the 4 datasets. We can observe that there are barely no differences between both Dextran-FITC cases.

	Dextran-FITC (clean)	Dextran-FITC (mixed)	RhoB (clean)	RhoB (mixed)
Line	y = -0.0291x - 0.6411	y = -0.0166x - 0.9302	y = -0.5684x - 0.5953	y = -0.3539x - 1.2378
R^2	0.2983	0.8538	0.9928	0.9884
R	0.9709	0.9834	0.4316	0.6461

System Operation and Mass Balances

It is important to analyze both how much of the Dextran-FITC product is lost through the membrane and how much RhoB is in the retentate, and under which conditions these results were obtained. The results can then be extrapolated to the dimensions we wish to operate. Note that this analysis was only carried out for the "clean" solutions.

The overall material balance, just comparing the replacement buffer coming in and the permeate coming out, checks out. By looking at the reported mass of each weighing scale, we found that the total mass of the system did not change over time. This data is presented in *Appendix A: Supplementary Material*, Figure S1. The material balance closed with a relative standard error of 0.073% for the "clean" RhoB experiment, and 0.0.180% for the "clean" Dextran-FITC case.

When evaluating the components separately, we had to make use of the calibration curve to find the concentrations in the permeate, retentate, and feed. This induced some error on the analysis. The initial concentrations of RhoB and Dextran-FITC were 0.0107 ± 0.0006 mg/mL and 0.299 ± 0.014 mg/mL, respectively. It is important to note these concentrations were measured before the feed entered the system. From Figure 12, we can infer that the concentration drops once its in the system (y-intercept does not equal 0). We hypothesize this is mainly due to leftover volume inside the system (in the tubes and membrane) that gets mixed with the feed through the first DV.

From the y-intercept, we back-calculated what the actual initial concentration in the system was. Since the feed volume is known, we can calculate the leftover volume in the system. We found this volume to be 12.29 mL from the "clean" RhoB y-intercept and 14.01 mL from the "clean" Dextran-FITC y-intercept. These values are in agreement with the estimated leftover

volume calculated from tubing and membrane cartridge dimensions (13 mL). This extra volume becomes important when we define the product volume, since it will be the sum of both the initial feed volume (15.6 mL) and the system volume. If not, product would be left behind in the system.

We tabulated the masses of both RhoB and Dextran-FITC after 7 and 4 dilution volumes in Table 2. We included the starting mass for comparison, and the closure error, i.e. the percentage of mass unaccounted for. The fractional recovery of Dextran-FITC in the retentate was 0.867 while the fractional recovery of RhoB in the permeate was 0.832. This is equivalent to a final RhoB concentration of 0.112 μg/mL of in the product, well below our target concentration of 0.25 μg/mL.

Table 2: Component material balances for both "clean" experiments. Closure error indicates the percentage away from 0 in the mass balance (unaccounted mass). Note all values are taking into account the system volume.

	Initial (mg)	DV	Permeate (mg)	Retentate (mg)	Closure Error
RhoB	0.167 ± 0.009	7	0.139*	0.003*	14.75%
DexFITC	4.664 ± 0.218	4	0.839*	4.046*	4.72%

***** Main error associated with fluorescence

These results were obtained under the following conditions: feed flow rate of 150 mL/min, operating TMP of 5.86 ± 0.46 psig, permeate flux of 33.10 ± 0.63 LMH, and feed volume of 15.6 mL. The total buffer volume greatly exceeded the required volume (about 260mL, only used 60-100mL), and the average DV took 2.41 ± 0.09 min to run. This data can be visualized in *Appendix A: Supplementary Material*, Figure S2**.**

Necessary Dilution Volumes and Scale Up

From the collected data, we can calculate precisely how many dilution volumes we would need to reach the desired target concentration. Then, we can find how much product we recover, and how long it takes. We will also scale our current process to meet the customer requirements.

Reevaluating Equation 10 with our measured rejection coefficients and initial concentrations, we solved for the number of dilution volumes it would take to achieve a 0.25 μg/mL RhoB product concentration. We found the ideal number of DVs to be 6.6. In order to account for start-up and performance variations, we set round to 7 DVs. This means that the

Dextran-FITC product concentration will be 0.244 mg/mL (initial concentration being 0.299 mg/mL and considering system volume). In terms of mass, that is 7.22 mg out of the 8.85 mg originally fed (81.6% recovery). The process would use 207 mL of fresh buffer, and it would run for 17 min and 27 sec.

Assuming the process can be parallelized, and that it can operated for 18 hrs a day (allowing for 6 hrs of downtime), it would require 22,379 units to produce 10 kg of Dextran-FITC a day. In terms of membrane area, that would be 257 m^2, or 60% of a basketball court.

Discussion

In this section we analyze the robustness of our results and the logic behind the assumptions made along the process. We also present the weaknesses of our methodology, and future experiments to improve the filtration system.

Fluorescence Spectroscopy

We determined the nanodrop to be the main source of error in all experiments involving concentrations of fluorescent species. The measurement error was considerably large, between 5.23% and 9.76%. The error clearly became greater at low concentrations, which makes this spectrometer unsuitable for projects that require accurate and precise measurement of small quantities of dangerous compounds, such as Rhodamine B.

The error on the fluorescence signal can explain the big discrepancies between the closure errors of RhoB and Dextran-FITC (14.75% and 4.72%). The fact that the slope of the RhoB regression line is more than 20 times the slope of Dextran-FITC indicates that RhoB is more sensitive: smaller differences in concentration make a greater impact on fluorescence. This becomes important when we take the natural logarithm of the concentration, since the function enlarges small errors.

We can attribute the poor performance of the nanodrop to its age, constant use, and perhaps improper operation and maintenance by the student body. Since the Nanodrop 3300 model came out, ThermoFisher Scientific has upgraded their line of fluorescent spectrometers and now offers a wider range of products. Replacing the nanodrop with a newer model should yield better data that would reinforce our results.

Optimal Feed Flow Rate and TMP

In order to choose the ideal feed flow rate, more experiments regarding fouling should be carried out. When operating the filtration unit for long periods of time, it is possible for materials to get deposited in the membrane surface. This does not only hinder separation, but it can also become a safety hazard because of pressure buildup. To avoid this scenario, we operate at high enough flow rates to produce high shears on the membrane, peeling deposited material away. Under the time scale we operated at, we did not observe any kind of accumulation on the membrane. We envision future experiments were the system runs for longer and the accumulation effects might become noticeable. It would also be ideal to operate at different concentrations to find a relationship between feed concentration and fouling, making it easier to determine the optimal feed flow rate.

When analyzing the permeate flux data, we realized there is a mismatch in the predicted flux (around 50 LMH) to the measured flux (about 33 LMH). The small variation in TMP are not enough to explain the discrepancy. When looking into the raw data, we found that the concentration of the solution used in the optimal TMP experiment was 0.1x of what was intended. This error would explain the difference in permeate flux values, since a more dilute solution contains more PBS that can pass through the membrane. However, it is important to note that the reported TMP values are still valid in the case were diluting the initial feed to 0.1x is an option.

The consequences of committing this mistake are that we were not operating under optimal conditions for our later experiments and we introduced inconsistencies in our methodology. More experiments would be needed to determine the effect on the calculated rejection coefficients and dilution volumes. Theoretically, the reaction coefficient is an intrinsic property of the membrane and filtrate, and should not be affected by the flow rate or transmembrane pressure. However, we hypothesized that the *apparent* rejection coefficient might be dependent on these variables and fouling, since accumulated material would prevent filtrate to pass through the membrane, increasing the rejection coefficient.

When analyzing our assumption that Dextran and Dextran-FITC would behave the same within the system, we compared the permeate flux rates for both solution and found them to be the same. The fact that the stock solutions is mostly Dextran also supports are claim. Hence, because throughout our experiments we did not observe accumulation in the system and the calculated

rejection coefficients are well correlated to our data, we believe to have successfully model the behavior of RhoB and Dextran-FITC.

Rejection Coefficient and System Volume

The steps we took when analyzing the rejection coefficient data were non-trivial. The main reason we decided to do regression on the averaged values, instead of doing regression on all the data, was that we must normalize the data by the initial concentration, and there would be no way of determining that concentration for each data point. We also chose to ignore the first data point, which corresponds to the origin, and did not set the y-intercept to 0, although, theoretically, the trendline should pass through that point. This led us to the conclusion that the feed gets diluted within the system, and we were able to find precisely by how much. Setting the y-intercept would yield incorrect results: because of the unaccounted volume, the concentrations would be smaller, and the rejection coefficient would seem smaller than what it actually is.

From the data, we were also able to show the interaction between RhoB and Dextran-FITC. As Dextran-FITC fluoresces, RhoB is able to absorb that energy and fluoresces itself. This means that the concentration of Dextran-FITC would be lower, and that the RhoB concentration would be higher. Graphically, the “mixed” Dextran-FITC shows a lower concentration compared to the “clean” Dextran-FITC, and the “mixed” RhoB has a greater slope (or greater rejection coefficient), indicating that the measured RhoB in the retentate is an overestimate. We believe that with future studies it would be possible to model the interplay between the two fluorescence signals, which would enable us to run experiments with both species in solution.

Scale up

The final dilution volumes needed to achieve a safe RhoB concentration and the mass of the product, will depend on the initial starting concentration. Without proper data on the properties of the feed, such as volume and concentration, it is hard to offer more than mere guesses for the fresh buffer needed, the operating time, and the necessary membrane area. Once the system has more constrains, determining the parameters should be simple scale up laws. However, to verify that one can scale the system as described, it is necessary to check the operating plant resources: it may not have the available space for so many units or the capacity to hold the required buffer volume.

Conclusion and Recommendations

In order to produce 10kg/day of DexFID44 with a safe Rhodamine B concentration of 0.25 μg/mL, we recommend PolyBioMolecular Inc to parallelized 22,379 KR2i TFF units with 30kD mPES membranes. For accurate results, they should replicate the procedures and parameters presented above, including the initial concentrations.

Before scaling for full production, we encourage PBM to allow for more research to be done in two of the main following areas: membrane fouling and best prevention methods, and optimal feed flows. For a more accurate optimal transmembrane pressure, we recommend a higher sampling frequency when repeating the TMP experiment. This would increase the resolution of the first derivative, allowing for a finer tuning around the optimal point.

Given the realization about the system volume, we recommend PBM investigating using a poor DextFID44 solvent to extract the leftover volume in the system without getting air inside. After the desired RhoB concentration is reached in the retentate, the buffer could be replaced with this solvent to push the rest of the product out. Using a liquid in which DexFID44 does not dissolve would avoid having to extract the product from the new phase. We also believe this effect to only be considerable when operating small feed volumes, in the same scale as the system volume. In our experiment, the volumes were almost equal, meaning that 50% of the product would be left in the system. As the ration between the feed volume to the system volume increases, this effect should become negligible.

Lastly, we recommend the PolyBioMolecular Inc directives to investigating the possible options for scale up. Where thousands of small membranes units seem impractical, larger ones able to handle greater volumes become optimal. However, due to the smaller surface area to volume ratio, bigger modules could be less efficient, requiring more dilution volumes and more time to achieve the same results. With more data on membrane sizing and production volumes, it should be possible to visualize the solution space and even constrain it with price functions to find an optimal solution.

Literature Cited

1. Kaji, Toshiyuki, et al. “Inhibitory Effect of Rhodamine B on the Proliferation of Human Lip Fibroblasts in Culture.” *Toxicology*, vol. 68, no. 1, 1991, pp. 11–20., doi:10.1016/0300-483x(91)90058-9.

2. “Dextran.” *National Center for Biotechnology Information. PubChem Compound Database*, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/dextran#section=Top.

3. Fluorescein isothiocyanate. (2018, September 27). Retrieved from https://en.wikipedia.org/wiki/Fluorescein_isothiocyanate

4. “FITC-Labelled Polysaccharides.” *Sigma-Aldrich*, www.sigmaaldrich.com/technical-documents/articles/chemistry/fluorescently-labeled-dextrane.html.

5. (PDF*) Labeled Dextran Conjugates*, FluoProbes, http://www.interchim.fr/ft/1/18938B.pdf?fbclid=IwAR2ZSoSdCDlY0iLibdz_RV9EE-6X_oRjcvChd5jAzO9Q3L9MgD7GGGcyrIE pubchem.ncbi.nlm.nih.gov/compound/18730#section=Top.

6. Brelje, T. Clark, and Robert L. Sorenson. “Histology Guide.” *Histology Guide*, www.histologyguide.com/.

7. “Rhodamine B.” *National Center for Biotechnology Information. PubChem Compound Database*, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/rhodamine_b#section=Top.

8. “*(PDF) Material Safety Data Sheet Rhodamine B,* https://pim-resources.coleparmer.com/sds/95510.pdf

9. “Rhodamine B R6626.” *Sigma-Aldrich*, www.sigmaaldrich.com/catalog/product/sigma/r6626?lang=en®ion=US.

10. “Jablonski Diagram.” *Wikipedia*, Wikimedia Foundation, 9 Jan. 2018, en.wikipedia.org/wiki/Jablonski_diagram.

11. "Lifetime Data of Selected Fluorophores." *ISS Data Tables | Lifetime Data of Selected Fluorophores*, www.iss.com/resources/reference/data_tables/LifetimeDataFluorophores.html.

12. "PhotochemCAD 3: Diverse Modules for Photophysical Calculations with Access to Multiple Spectral Databases,"Taniguchi, M.; Du, H.; Lindsey, J. S. Photochem. Photobiol. **2018**, 94, 277–289.

13. "Database of Absorption and Fluorescence Spectra of >300 Common Compounds for Use in PhotochemCAD," Taniguchi, M.; Lindsey, J. S. Photochem. Photobiol. **2018**, 94, 290–327.

14. Newton, et al. "Ultraviolet-Visible (UV-Vis) Spectroscopy – Limitations and Deviations of Beer-Lambert's Law | Analytical Chemistry." *PharmaXChange.info*, 27 June 2016, pharmaxchange.info/2012/05/ultraviolet-visible-uv-vis-spectroscopy-–-limitations-and-deviations-of-beer-lambert-law/.

15. *Chemical Engineering Design - Elsevier*. booksite.elsevier.com/samplechapters/9780080966595/Front_Matter.pdf.

16. *(PDF) Overview on Porous Inorganic Membranes for Gas ...* www.researchgate.net/publication/315797652_Overview_on_porous_inorganic_membranes_for_gas_separation.

17. *(PDF) A Hand On Guide to Ultrafiltraion/Diafiltration Optimization using Pellicon Cassettes*, Millipore

18. (*PDF) Diafiltration: A Fast, Efficient Method for Desalting or Buffer Exchange of Biological Samples*, https://laboratory.pall.com/content/dam/pall/laboratory/literature-library/non-gated/02.0629_Buffer_Exchange_STR.pdf

19. (PDF) KrosFLow Research II TFF System, Product Information and Operating Instructions, access from Blackboard.

20. "Diafiltration." *Wikipedia*, Wikimedia Foundation, 7 Feb. 2017, en.wikipedia.org/wiki/Diafiltration.

Appendix A: Supplementary Material

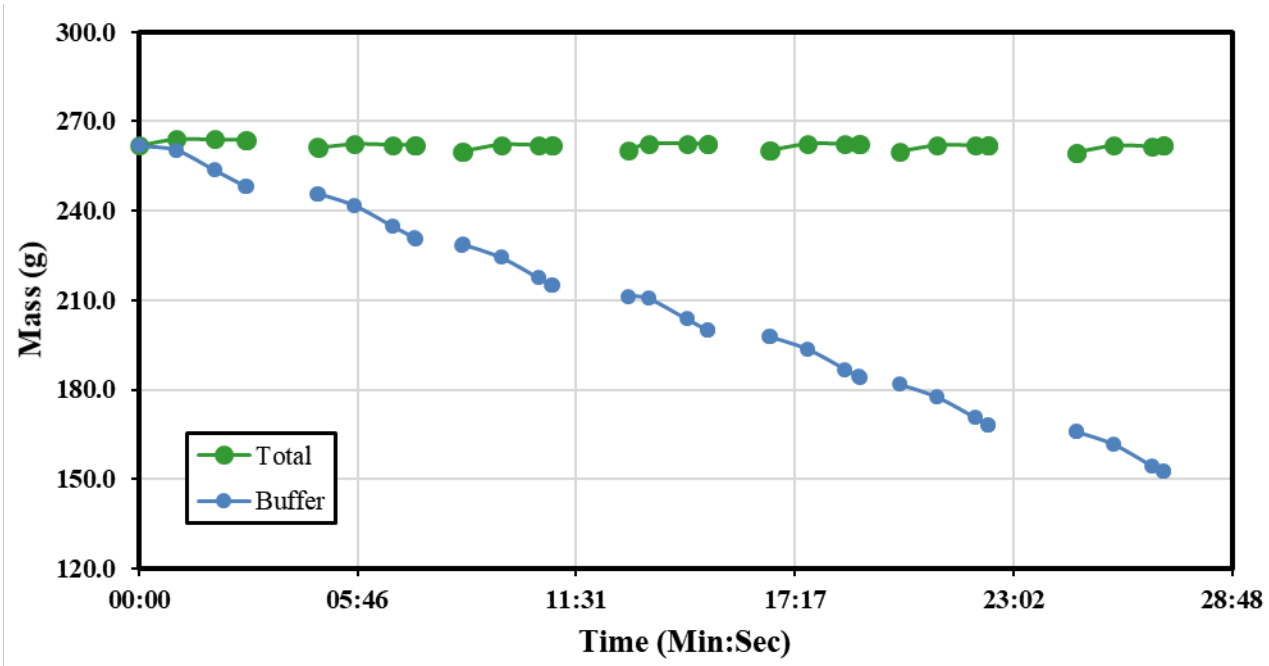


Figure S 1: Overall material balance. There virtually no change in mass over time (Total). The buffer mass decreases continuously. Each segment represents 1 DV. Empty spaced is down time for sampling.

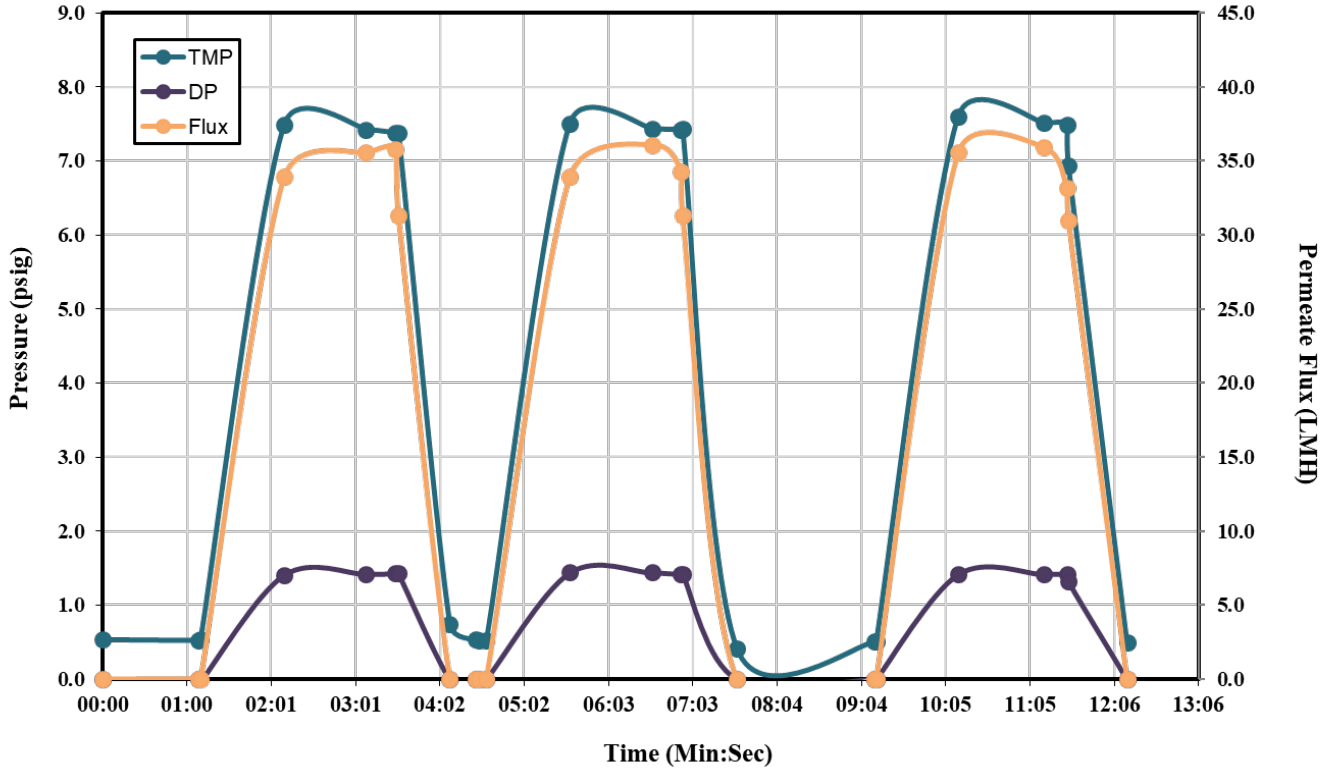


Figure S 2: TMP, DP (on left axis), and Flux (on right axis), for three different DVs. The values reach a constant, steady state, within one minute of startup, and stay there until the system is stopped for sampling.

Appendix C: Raw Data

Calibration curves

Rhod-B

mg/mL	RFU	Avg RFU	Std Dev	Error
0.0025	744.4	844.425	155.0254	77.51268
	879.1			
	705.7			
	1048.5			
0.005	770.9	1420.225	472.0692	236.0346
	1729.4			
	1806.5			
	1374.1			
0.0075	1648	1641.4	78.07603	39.03802
	1547.2			
	1737.8			
	1632.6			
0.01	2263.8	1854.075	330.1463	165.0731
	1878.9			
	1815.8			
	1457.8			
0.0125	3155.7	2705.75	636.3254	318.1627
	2255.8			

Dex - FITC

mg/mL	RFU	Avg RFU	Std Dev	Error
0.45	3861.9	4618.975	631.127	315.5635
	4927.8			
	4381.6			
	5304.6			
0.4	3889.9	4179.85	256.4317	128.2159
	4408.7			
	4038.7			
	4382.1			
0.35	4142.3	3637.85	388.2597	194.1298
	3694.6			
	3493.4			
	3221.1			
0.3	3369.9	3213.8	366.6355	183.3177
	3303.5			
	2678.2			
	3503.6			

Optimal TMPs

TMP (psig)	Flow Rate 1		Flow Rate 2	Flow Rate 3	Average Flow Rate	Err	deriv 50
1	11.5	12.3	12	11.93333333	0.23333333		
1.5	16.7	17.4	17.5	17.2	0.25166115	10.5333333	
2	20.3	19.8	20.3	20.13333333	0.16666667	5.86666667	
3	26.1	23.5	24	24.53333333	0.79652021	4.4	
4	25.7	25.5	25	25.4	0.2081666	0.86666667	
6	27.7	27.1	27.1	27.3	0.2	0.95	
8	29.2	28.2	28.7	28.7	0.28867513	0.7	
10	29.2	28.7	29.2	29.03333333	0.16666667	0.16666667	
12	31.8	29.2	27.7	29.56666667	1.19768295	0.26666667	

TMP (psig)	Flow Rate 1	Flow Rate 2	Flow Rate 3	Average Flow Rate	Err	deriv 150
1	12.7	12.1	11.7	12.16666667	0.29059326	
2	24.9	25	24.5	24.8	0.15275252	12.6333333
3	36	36	36.4	36.13333333	0.13333333	11.3333333
4	44.3	43.8	44.1	44.06666667	0.14529663	7.93333333
6	51.8	52.5	51.8	52.03333333	0.23333333	3.98333333
8	55.3	55.3	54.9	55.16666667	0.13333333	1.56666667
10	58.4	57.4	55.9	57.23333333	0.72648316	1.03333333
12	59	57.8	59	58.6	0.4	0.68333333

TMP (psig)	Flow Rate 1		Flow Rate 2	Flow Rate 3	Average Flow Rate	Err	deriv 250
2	25.6	26.7	27.7	26.66666667	0.60644685		
4	50.1	49.6	49.3	49.66666667	0.23333333	11.5	
6	63.7	64.2	64.1	64	0.15275252	7.16666667	
8	71.1	70.8	72.7	71.53333333	0.58972687	3.76666667	
10	75.1	76.4	74.6	75.36666667	0.53644923	1.91666667	
	12	75.7	76.5	76.4	76.2	0.25166115	0.41666667

Rejection Coefficients

Clean RhoB

Retentate

	1	2	3	4	AVERAGE	Err	C	ln Ct/Ci
0	2411.7	2433	2047.7		2297.467	125.0346	0.010689	0
1	858.9	599.3	622.5		693.5667	82.93751	0.003227	-1.19771
2	263.7	460.1	375.1		366.3	56.86628	0.001704	-1.83611
3	293.4	317.6	275.4	257.9	286.075	10.58789	0.001331	-2.08331
4	124.1	106.2	165.6		131.9667	17.59264	0.000614	-2.85701
5	101.9	54.3	46.3	71.2	68.425	15.0293	0.000318	-3.51382
6	30	40.1	52.4		40.83333	5.608996	0.00019	-4.03006
7	17.1	20.5	34.3		23.96667	4.554485	0.000112	-4.5629

Permeate

				AVERAGE	Err	C
1	0	6.7	10.8	5.833333	2.725955	2.71E-05
2	41.5	99.9	68.2	69.86667	14.61783	0.000325
3	350.5	375.2	296	340.5667	20.26181	0.001584
4	466.2	368.1	365.8	400.0333	28.65678	0.001861
5	385	454.2	384.2	407.8	20.09278	0.001897
6	375.3	215.9	159.7	250.3	55.92039	0.001165
7	471.3	222.4	172.9	288.8667	79.95937	0.001344

Clean Dextran-FITC

Retentate

	1	2	3		AVERAGE	Err	C	Cf/Ci
0	2998.1	2942.1	3402.1		3114.1	144.9046	0.299	0
1	2003.9	1524.6	1519.3	1446	1623.45	128.0785	0.155891	-0.65139
2	1553.7	1275.5	1529.1		1452.767	88.91736	0.139501	-0.76247
3	1689.4	1535.9			1612.65	76.75	0.154854	-0.65806
4	1343	1503.1			1423.05	80.05	0.136648	-0.78314

Permeate

1	154.5	154.6		154.55	0.05	0.014841	0.231513	
2	163.7	167.1	173.9	168.2333	2.998518	0.016155	0.504022	
3	137.1	150.9		144	6.9	0.013828	0.647129	
4	150.1	129.9		140	10.1	0.013443	0.838871	

Mixed Experiment

0	2708.4	2369.1	2107.8	2395.1	122.9411	0.011143	0	0
0	2665.6	2357.9	2262.4	2428.633	86.01837	0.233209	0	0
	443.5	616.3	472	510.6	37.8207	0.002376	-1.54559	1
	854.5	989.4	962.3	935.4	29.13248	0.089821	-0.95411	1
	297.1	317.2	345.1	319.8	9.840986	0.001488	-2.01348	2
	846.2	955.8	997.1	933.0333	31.83668	0.089594	-0.95664	2
	239.5	248.8	230.7	239.6667	3.695117	0.001115	-2.30193	3
	925.6	971.2	861.3	919.3667	22.54123	0.088282	-0.9714	3
				172.8		0.000804	-2.62905	4
				889.4		0.085404	-1.00454	4

Runs

TMP - 50

Time	P_{inlet} psig	$P_{retentate}$ psig	$P_{permeate}$ psig	TMP psig	DP psig	Q_{inlet} ml/min	$Q_{permeate}$ mL/min	$Q_{retentate}$ ml/min	$M_{permeate}$ g	M_{feed} g	LMH (Filt. Flux)	VT mL/cm²	Pump rpm	Temp °C	Conc Factor	Diafilt Vols	Shear
														Measure			
0:00:00	0.1	0.1	0.0	0.1	0.1	50.0			-0.9	432.7		0.0000	63.		1.00	0.00	
0:00:58	2.2	1.8	0.0	2.0	0.3	50.0	2	48	0.9	432.3	9.7	0.0157	63.		1.00	0.00	1816
0:01:59	2.0	1.6	0.0	1.8	0.4	50.0	4	46	5.1	428.6	21.6	0.0522	63.		1.00	0.00	1730
0:02:59	2.2	1.8	0.0	2.0	0.4	50.0	4	46	9.0	424.7	20.3	0.0861	63.		1.00	0.00	1739
0:03:59	2.2	1.8	0.0	2.0	0.4	50.0	4	46	12.8	420.8	19.8	0.1191	63.		1.00	0.00	1743
0:04:59	2.1	1.7	0.0	2.0	0.4	50.0	4	46	16.7	416.7	20.3	0.1530	63.		1.00	0.00	1739
0:05:59	0.5	0.1	0.0	0.3	0.4	50.0	0	50	17.1	416.3	2.1	0.1565	63.		1.00	0.00	1871
0:06:59	4.9	4.6	0.0	4.8	0.3	50.0	4	46	21.2	412.1	21.4	0.1922	63.		1.00	0.00	1732
0:07:59	4.3	3.9	0.0	4.2	0.4	50.0	5	45	26.6	406.4	28.2	0.2391	63.		1.00	0.00	1683
0:09:00	4.2	3.8	0.0	4.0	0.4	50.0	5	45	31.6	401.3	25.7	0.2826	63.		1.00	0.00	1701
0:09:59	4.2	3.8	0.0	4.0	0.4	50.0	5	45	36.4	396.4	25.5	0.3243	63.		1.00	0.00	1702
0:10:59	4.2	3.8	0.0	4.0	0.4	50.0	5	45	41.2	391.4	25.0	0.3661	63.		1.00	0.00	1705
0:11:59	0.6	0.2	0.0	0.4	0.4	50.0	1	49	42.0	390.9	4.2	0.3730	63.		1.00	0.00	1856
0:13:00	6.3	6.0	0.0	6.2	0.3	50.0	6	44	48.1	384.2	31.3	0.4261	63.		1.00	0.00	1660
0:13:59	6.3	5.9	0.0	6.1	0.4	50.0	6	44	54.2	378.0	32.4	0.4791	63.		1.00	0.00	1652
0:14:59	6.2	5.8	0.0	6.0	0.4	50.0	5	45	59.5	372.7	27.7	0.5252	63.		1.00	0.00	1686
0:15:59	6.2	5.8	0.0	6.0	0.4	50.0	5	45	64.7	367.4	27.1	0.5704	63.		1.00	0.00	1690
0:16:59	6.2	5.8	0.0	6.0	0.4	50.0	5	45	69.9	362.2	27.1	0.6157	63.		1.00	0.00	1690
0:17:59	0.6	0.3	0.0	0.5	0.4	50.0	1	49	70.7	361.7	4.2	0.6226	63.		1.00	0.00	1856
0:18:59	7.5	7.2	0.0	7.4	0.3	50.0	7	43	78.1	353.7	38.6	0.6870	63.		1.00	0.00	1607
0:19:59	8.3	8.0	0.0	8.1	0.4	50.0	7	43	84.6	347.0	33.9	0.7435	63.		1.00	0.00	1641
0:20:59	8.1	7.8	0.0	8.0	0.4	50.0	6	44	90.2	341.4	29.2	0.7922	63.		1.00	0.00	1675
0:21:59	8.2	7.8	0.0	8.0	0.4	50.0	5	45	95.6	335.8	28.2	0.8391	63.		1.00	0.00	1683
0:22:59	8.2	7.8	0.0	8.0	0.4	50.0	6	44	101.1	330.2	28.7	0.8870	63.		1.00	0.00	1679
0:23:59	0.6	0.2	0.0	0.4	0.4	50.0	1	49	102.0	330.0	4.7	0.8948	63.		1.00	0.00	1852
0:24:59	10.8	10.4	0.0	10.6	0.3	50.0	8	42	109.6	321.5	39.7	0.9609	63.		1.00	0.00	1600
0:25:59	10.1	9.7	0.0	9.9	0.3	50.0	7	43	116.6	314.3	36.5	1.0217	63.		1.00	0.00	1622
0:26:59	10.1	9.8	0.0	9.9	0.4	50.0	6	44	122.5	308.3	30.8	1.0730	63.		1.00	0.00	1664
0:27:59	10.2	9.8	0.0	10.0	0.4	50.0	6	44	128.1	302.6	29.2	1.1217	63.		1.00	0.00	1675
0:29:00	10.2	9.8	0.0	10.0	0.4	50.0	6	44	133.7	296.9	28.7	1.1704	63.		1.00	0.00	1678
0:30:00	10.2	9.8	0.0	10.0	0.4	50.0	6	44	139.3	291.3	29.2	1.2191	63.		1.00	0.00	1675
0:30:59	0.5	0.1	0.0	0.3	0.4	50.0	2	48	141.0	290.5	9.0	1.2339	63.		1.00	0.00	1821
0:31:59	10.9	10.6	0.0	10.7	0.3	50.0	7	43	147.8	282.6	35.5	1.2930	63.		1.00	0.00	1630
0:32:59	12.0	11.7	0.0	11.9	0.3	50.0	8	42	155.8	274.4	41.7	1.3626	63.		1.00	0.00	1584
0:33:59	12.1	11.8	0.0	12.0	0.3	50.0	6	44	161.9	268.3	31.8	1.4157	63.		1.00	0.00	1656
0:34:59	12.2	11.9	0.0	12.0	0.3	50.0	6	44	167.5	262.6	29.2	1.4643	63.		1.00	0.00	1675
0:35:59	12.1	11.8	0.0	12.0	0.3	50.0	5	45	172.8	257.2	27.7	1.5104	63.		1.00	0.00	1686
0:36:59	0.6	0.3	0.0	0.4	0.4	50.0	1	49	173.7	257.1	4.7	1.5183	63.		1.00	0.00	1852
0:37:59	14.1	13.9	0.0	13.9	0.2	50.0	9	41	183.0	246.3	48.5	1.5991	63.		1.00	0.00	1535
0:38:59	14.2	14.1	0.0	14.1	0.2	50.0	8	42	190.9	238.5	41.2	1.6678	63.		1.00	0.00	1588
0:40:00	14.2	14.0	0.0	14.1	0.2	50.0	6	44	196.7	232.7	29.8	1.7183	63.		1.00	0.00	1671
0:40:59	0.6	0.2	0.0	0.4	0.4	50.0	1	49	197.3	233.5	3.2	1.7235	63.		1.00	0.00	1863
0:41:59	1.9	1.6	0.0	1.7	0.4	50.0	2	48	199.5	231.3	11.5	1.7426	63.		1.00	0.00	1803
0:42:59	1.8	1.4	0.0	1.6	0.4	50.0	3	47	202.7	228.0	16.7	1.7704	63.		1.00	0.00	1766
0:43:59	1.7	1.4	0.0	1.5	0.4	50.0	3	47	205.9	224.6	16.7	1.7983	63.		1.00	0.00	1766
0:45:00	1.7	1.4	0.0	1.5	0.4	50.0	3	47	209.3	221.2	17.4	1.8278	63.		1.00	0.00	1760
0:45:59	1.7	1.4	0.0	1.5	0.4	50.0	3	47	212.6	217.9	17.5	1.8565	63.		1.00	0.00	1760
0:46:59	0.4	0.1	0.0	0.2	0.4	50.0	2	48	214.9	215.6	12.0	1.8765	63.		1.00	0.00	1800
0:48:00	0.5	0.2	0.0	0.3	0.3	50.0	0	50	215.0	215.5	0.5	1.8774	63.		1.00	0.00	1883
0:48:59	1.3	1.0	0.0	1.1	0.4	50.0	2	48	216.5	213.9	8.0	1.8904	63.		1.00	0.00	1829
0:49:59	1.2	0.9	0.0	1.0	0.4	50.0	2	48	218.7	211.7	11.5	1.9096	63.		1.00	0.00	1803
0:51:00	1.2	0.9	0.0	1.0	0.4	50.0	2	48	221.1	209.3	12.3	1.9304	63.		1.00	0.00	1797
0:52:00	1.2	0.9	0.0	1.0	0.4	50.0	2	48	223.4	206.9	12.0	1.9504	63.		1.00	0.00	1800
0:52:59	0.6	0.2	0.0	0.4	0.4	50.0	1	49	224.0	206.3	3.2	1.9557	63.		1.00	0.00	1863
0:53:59	2.9	2.5	0.0	2.6	0.4	50.0	4	46	228.3	201.8	22.4	1.9930	63.		1.00	0.00	1724
0:54:59	3.2	2.9	0.0	3.0	0.4	50.0	5	45	233.3	196.8	26.1	2.0365	63.		1.00	0.00	1698
0:55:59	3.2	2.9	0.1	3.0	0.4	50.0	4	46	237.8	192.2	23.5	2.0757	63.		1.00	0.00	1717
0:56:59	3.2	2.9	0.1	3.0	0.4	50.0	5	45	242.4	187.5	24.0	2.1157	63.		1.00	0.00	1713
0:57:59	3.2	2.9	0.1	3.0	0.4	50.0	4	46	246.9	182.9	23.5	2.1548	63.		1.00	0.00	1717
0:59:14	0.0	0.0	0.1	0.0	0.0	0.0	5	-5	252.7	181.4	24.2	2.2052	0.		1.00	0.00	-175

TMP – 150

Time	P_{inlet} psig	$P_{retentate}$ psig	$P_{permeate}$ psig	TMP psig	DP psig	Q_{inlet} ml/min	$Q_{permeate}$ mL/min	$Q_{retentate}$ ml/min	$M_{permeate}$ g	M_{feed} g	LMH (Filt. Flux)	VT mL/cm²	Pump rpm	Temp °C	Conc Factor	Diafilt Vols	Shear
														Measure			
0:00:00	0.3	0.1	0.0	0.2	0.1	150.0			0.0	428.4		0.0000	188.		1.00	0.00	
0:00:59	1.5	0.4	0.0	1.0	1.1	150.0	2	148	2.4	427.3	12.7	0.0209	188.		1.00	0.00	5567
0:02:01	1.5	0.4	0.0	1.0	1.1	150.0	2	148	4.8	424.9	12.1	0.0417	188.		1.00	0.00	5571
0:03:00	1.5	0.4	0.0	1.0	1.1	150.0	2	148	7.0	422.5	11.7	0.0609	188.		1.00	0.00	5574
0:04:00	1.5	0.4	0.0	1.0	1.1	150.0	2	148	8.7	420.9	8.9	0.0757	188.		1.00	0.00	5595
0:05:01	2.6	1.5	0.0	2.1	1.1	150.0	3	147	12.1	417.4	17.4	0.1052	188.		1.00	0.00	5533
0:06:00	2.5	1.4	0.0	2.0	1.1	150.0	5	145	16.8	412.7	24.9	0.1461	188.		1.00	0.00	5479
0:07:00	2.5	1.4	0.0	2.0	1.1	150.0	5	145	21.6	407.8	25.0	0.1878	188.		1.00	0.00	5478
0:08:00	2.5	1.4	0.0	2.0	1.1	150.0	5	145	26.3	403.0	24.5	0.2287	188.		1.00	0.00	5482
0:09:01	1.9	0.9	0.0	1.4	1.1	150.0	2	148	28.5	400.8	11.3	0.2478	188.		1.00	0.00	5577
0:10:00	3.2	2.2	0.0	2.7	1.1	150.0	5	145	33.4	395.8	26.0	0.2904	188.		1.00	0.00	5471
0:11:00	3.5	2.4	0.0	3.0	1.1	150.0	7	143	40.3	388.8	36.0	0.3504	188.		1.00	0.00	5399
0:12:00	3.5	2.5	0.0	3.0	1.1	150.0	7	143	47.2	381.8	36.0	0.4104	188.		1.00	0.00	5399
0:13:01	3.5	2.5	0.0	3.0	1.1	150.0	7	143	54.3	374.7	36.4	0.4722	188.		1.00	0.00	5395
0:14:00	2.7	1.7	0.0	2.2	1.1	150.0	3	147	57.0	372.0	14.3	0.4957	188.		1.00	0.00	5555
0:15:00	3.6	2.6	0.0	3.1	1.1	150.0	6	144	63.0	365.7	31.3	0.5478	188.		1.00	0.00	5432
0:16:01	4.6	3.5	0.0	4.1	1.1	150.0	8	142	71.6	357.0	44.1	0.6226	188.		1.00	0.00	5340
0:17:00	4.6	3.5	0.0	4.1	1.1	150.0	9	141	80.0	348.6	44.6	0.6957	188.		1.00	0.00	5337
0:18:00	4.5	3.5	0.0	4.0	1.1	150.0	8	142	88.5	339.9	44.3	0.7696	188.		1.00	0.00	5338
0:19:00	4.5	3.5	0.0	4.0	1.1	150.0	8	142	96.9	331.6	43.8	0.8426	188.		1.00	0.00	5342
0:20:01	4.5	3.5	0.0	4.0	1.1	150.0	8	142	105.5	322.9	44.1	0.9174	188.		1.00	0.00	5340
0:21:00	2.3	1.3	0.0	1.8	1.1	150.0	3	147	108.2	320.4	14.3	0.9409	188.		1.00	0.00	5555
0:22:00	5.5	4.5	0.0	5.0	1.1	150.0	9	141	116.8	311.5	44.9	1.0157	188.		1.00	0.00	5334
0:23:00	6.7	5.6	0.0	6.1	1.1	150.0	10	140	126.7	301.5	51.7	1.1017	188.		1.00	0.00	5285
0:24:01	6.5	5.4	0.0	6.0	1.1	150.0	10	140	136.8	291.4	51.8	1.1896	188.		1.00	0.00	5284
0:25:00	6.6	5.5	0.0	6.0	1.1	150.0	10	140	146.7	281.4	52.5	1.2757	188.		1.00	0.00	5279
0:26:01	6.6	5.5	0.0	6.0	1.1	150.0	10	140	156.8	271.3	51.8	1.3635	188.		1.00	0.00	5284
0:27:00	1.5	0.5	0.0	1.0	1.1	150.0	1	149	157.9	270.5	5.8	1.3730	188.		1.00	0.00	5617
0:28:00	9.2	8.1	0.0	8.6	1.0	150.0	8	142	166.3	261.4	43.8	1.4461	188.		1.00	0.00	5342
0:29:00	8.6	7.5	0.0	8.0	1.1	150.0	11	139	176.9	250.9	55.3	1.5383	188.		1.00	0.00	5259
0:30:00	8.6	7.5	0.0	8.0	1.1	150.0	11	139	187.5	240.1	55.3	1.6304	188.		1.00	0.00	5259
0:31:01	8.6	7.5	0.0	8.0	1.1	150.0	11	139	198.2	229.3	54.9	1.7235	188.		1.00	0.00	5262
0:32:01	1.5	0.5	0.0	1.0	1.1	150.0	2	148	199.9	228.2	8.9	1.7383	188.		1.00	0.00	5595
0:33:00	11.0	10.0	0.1	10.4	1.0	150.0	9	141	208.8	218.4	47.2	1.8157	188.		1.00	0.00	5317
0:34:01	10.2	9.2	0.1	9.6	1.1	150.0	11	139	219.7	207.5	55.9	1.9104	188.		1.00	0.00	5254
0:35:00	10.6	9.5	0.1	10.0	1.1	150.0	11	139	230.7	196.3	58.4	2.0061	188.		1.00	0.00	5237
0:36:00	10.6	9.5	0.1	10.0	1.1	150.0	11	139	241.7	185.3	57.4	2.1017	188.		1.00	0.00	5244
0:37:01	10.6	9.5	0.1	10.0	1.1	150.0	11	139	252.6	174.3	55.9	2.1965	188.		1.00	0.00	5254
0:38:01	0.0	0.0	0.1	0.0	0.0	0.0	1	-1	253.4	173.3	4.2	2.2035	0.		1.00	0.00	-30

TMP -250

Time	P_{inlet} psig	$P_{retentate}$ psig	$P_{permeate}$ psig	TMP psig	DP psig	Q_{inlet} ml/min	$Q_{permeate}$ mL/min	$Q_{retentate}$ ml/min	$M_{permeate}$ g	M_{feed} g	LMH (Filt. Flux)	VT mL/cm²	Pump rpm	Temp °C	Conc Factor	Diafilt Vols	Shear
														Measure			
0:00:00	0.1	0.1	0.2	-0.1	0.0	250.0			15.3	416.7		0.0000	44.		1.00	0.00	
0:00:59	2.8	1.1	0.3	1.7	1.7	250.0	3	247	18.5	416.4	17.0	0.0278	313.		1.00	0.00	9309
0:01:59	3.4	1.7	0.5	2.0	1.7	250.0	5	245	23.4	411.4	25.6	0.0704	313.		1.00	0.00	9247
0:03:00	3.7	2.0	0.8	2.0	1.7	250.0	5	245	28.6	406.3	26.7	0.1157	313.		1.00	0.00	9238
0:04:00	3.9	2.2	1.1	1.9	1.7	250.0	5	245	33.6	401.0	26.1	0.1591	313.		1.00	0.00	9243
0:05:00	4.1	2.5	1.4	1.9	1.7	250.0	5	245	38.7	395.4	26.6	0.2035	313.		1.00	0.00	9239
0:06:00	4.5	2.8	1.7	2.0	1.7	250.0	5	245	44.0	390.5	27.7	0.2496	313.		1.00	0.00	9231
0:07:00	3.6	1.9	1.8	0.9	1.7	250.0	0	250	44.1	390.2	0.5	0.2504	313.		1.00	0.00	9428
0:08:00	6.0	4.3	2.1	3.1	1.7	250.0	6	244	50.4	383.5	32.9	0.3052	313.		1.00	0.00	9194
0:09:00	7.6	5.9	2.7	4.1	1.7	250.0	10	240	60.4	373.4	52.2	0.3922	313.		1.00	0.00	9054
0:10:01	7.8	6.1	3.4	3.6	1.7	250.0	10	240	70.1	363.7	49.8	0.4765	313.		1.00	0.00	9071
0:11:00	7.6	5.9	1.8	4.9	1.7	250.0	11	239	81.2	352.6	58.9	0.5730	313.		1.00	0.00	9006
0:12:00	6.2	4.5	0.0	5.3	1.7	250.0	12	238	93.0	340.9	61.6	0.6757	313.		1.00	0.00	8986
0:13:00	4.9	3.2	0.0	4.1	1.7	250.0	10	240	102.9	331.0	51.7	0.7617	313.		1.00	0.00	9058
0:14:00	4.8	3.1	0.0	4.0	1.7	250.0	10	240	112.5	321.5	50.1	0.8452	313.		1.00	0.00	9069
0:15:00	4.9	3.2	0.0	4.0	1.7	250.0	10	240	122.0	311.9	49.6	0.9278	313.		1.00	0.00	9073
0:16:01	4.9	3.2	0.0	4.0	1.7	250.0	9	241	131.6	302.0	49.3	1.0113	313.		1.00	0.00	9075
0:17:00	3.2	1.5	0.0	2.4	1.7	250.0	3	247	135.0	298.8	18.0	1.0409	313.		1.00	0.00	9301
0:18:00	6.3	4.6	0.0	5.4	1.7	250.0	9	241	143.9	290.1	46.4	1.1183	313.		1.00	0.00	9096
0:19:00	7.0	5.3	0.0	6.1	1.7	250.0	12	238	156.4	277.7	65.2	1.2270	313.		1.00	0.00	8960
0:20:00	6.9	5.2	0.0	6.0	1.7	250.0	12	238	168.6	265.3	63.7	1.3330	313.		1.00	0.00	8971
0:21:00	6.9	5.2	0.0	6.0	1.7	250.0	12	238	180.9	252.8	64.2	1.4400	313.		1.00	0.00	8967
0:22:01	6.9	5.2	0.0	6.0	1.7	250.0	12	238	193.4	240.0	64.1	1.5487	313.		1.00	0.00	8968
0:23:00	3.3	1.6	0.0	2.4	1.7	250.0	4	246	197.5	236.1	21.8	1.5843	313.		1.00	0.00	9274
0:24:01	7.5	5.8	0.1	6.6	1.7	250.0	12	238	209.2	223.7	60.0	1.6861	313.		1.00	0.00	8997
0:25:00	8.9	7.2	0.1	8.0	1.7	250.0	14	236	222.6	210.6	71.1	1.8026	313.		1.00	0.00	8917
0:26:01	8.9	7.2	0.1	8.0	1.7	250.0	14	236	236.4	196.4	70.8	1.9226	313.		1.00	0.00	8919
0:27:00	9.0	7.2	0.1	8.0	1.7	250.0	14	236	250.1	182.8	72.7	2.0417	313.		1.00	0.00	8906

Time	P_{inlet} psig	$P_{retentate}$ psig	$P_{permeate}$ psig	TMP psig	DP psig	Q_{inlet} ml/min	$Q_{permeate}$ mL/min	$Q_{retentate}$ ml/min	$M_{permeate}$ g	M_{feed} g	LMH (Filt. Flux)	VT mL/cm²	Pump rpm	Temp °C	Conc Factor	Diafilt Vols	Shear
														Measure			
0:00:00	0.1	0.1	0.0	0.1	0.0	250.0			0.0	430.1		0.0000	30.		1.00	0.00	
0:01:00	6.3	4.6	0.0	5.5	1.7	250.0	4	246	3.6	428.3	18.8	0.0313	313.		1.00	0.00	9296
0:02:00	9.4	7.6	0.0	8.5	1.7	250.0	12	238	15.7	415.6	63.1	0.1365	313.		1.00	0.00	8975
0:03:01	10.7	9.0	0.0	9.9	1.7	250.0	14	236	30.1	401.1	73.9	0.2617	313.		1.00	0.00	8897
0:04:01	10.8	9.1	0.0	10.0	1.7	250.0	14	236	44.5	386.8	75.1	0.3870	313.		1.00	0.00	8888
0:05:00	10.9	9.1	0.0	10.0	1.7	250.0	15	235	58.9	372.0	76.4	0.5122	313.		1.00	0.00	8879
0:06:00	10.9	9.1	0.0	10.0	1.7	250.0	14	236	73.2	357.7	74.6	0.6365	313.		1.00	0.00	8892
0:07:00	4.7	3.0	0.0	3.8	1.7	250.0	5	245	77.9	353.3	24.5	0.6774	313.		1.00	0.00	9254
0:08:00	11.7	9.9	0.0	10.8	1.7	250.0	13	237	91.0	339.8	68.3	0.7913	313.		1.00	0.00	8937
0:09:00	12.9	11.2	0.0	12.0	1.7	250.0	15	235	105.5	324.6	75.7	0.9174	313.		1.00	0.00	8884
0:10:01	12.9	11.2	0.0	12.0	1.7	250.0	15	235	120.4	309.4	76.5	1.0470	313.		1.00	0.00	8879
0:11:00	12.8	11.2	0.0	12.0	1.7	250.0	15	235	134.8	294.9	76.4	1.1722	313.		1.00	0.00	8879
0:12:00	0.1	0.1	0.0	0.1	0.0	0.0	4	-4	138.9	289.8	21.4	1.2078	0.		1.00	0.00	-155
0:13:00	4.1	3.3	0.0	3.7	0.9	150.0	5	145	143.7	285.9	25.0	1.2496	188.		1.00	0.00	5478
0:14:01	13.0	12.0	0.0	12.5	1.0	150.0	12	138	155.6	273.1	61.1	1.3530	188.		1.00	0.00	5217
0:15:00	12.4	11.4	0.0	11.9	1.1	150.0	11	139	166.3	262.3	56.8	1.4461	188.		1.00	0.00	5248
0:16:00	12.5	11.5	0.0	11.9	1.0	150.0	11	139	177.4	251.3	57.9	1.5426	188.		1.00	0.00	5240
0:17:00	12.6	11.5	0.0	12.0	1.0	150.0	11	139	188.7	240.0	59.0	1.6409	188.		1.00	0.00	5233
0:18:01	12.6	11.6	0.1	12.1	1.0	150.0	11	139	199.7	228.9	56.5	1.7365	188.		1.00	0.00	5251
0:19:00	12.6	11.6	0.1	12.0	1.0	150.0	11	139	210.6	218.1	57.8	1.8313	188.		1.00	0.00	5241
0:20:00	12.5	11.5	0.1	12.0	1.0	150.0	11	139	221.9	206.6	59.0	1.9296	188.		1.00	0.00	5233
0:21:01	0.0	0.0	0.0	0.0	0.0	0.0	1	-1	222.8	205.7	4.6	1.9374	0.		1.00	0.00	-33

Rejection Coef -Mixed

Time	P_{inlet} psig	$P_{retentate}$ psig	$P_{permeate}$ psig	TMP psig	DP psig	Q_{inlet} ml/min	$Q_{permeate}$ mL/min	$Q_{retentate}$ ml/min	$M_{permeate}$ g	M_{feed} g	LMH (Filt. Flux)	VT mL/cm²	Pump rpm	Temp °C	Conc Factor	Diafilt Vols	Shear
														Measure			
0:00:00	0.9	0.9	-0.2	1.1	0.0	150.0			0.0	226.2		0.0000	72.		1.00	0.00	
0:00:59	6.5	5.2	-0.4	6.3	1.3	150.0	10	140	10.1	218.4	53.6	0.0878	188.		1.00	0.00	5271
0:01:53	6.4	5.1	-0.4	6.1	1.3	150.0	6	144	15.5	212.9	31.3	0.1348	188.		1.00	0.00	5432
0:01:55	6.4	5.1	-0.4	6.1	1.3	0.0	3	-3	15.6	212.8	15.7	0.1357	0.		1.00	0.00	-113
0:02:00	2.0	1.6	-0.4	2.2	0.4	0.0	1	-1	15.7	210.6	6.3	0.1365	0.		1.00	0.00	-45
#N/A																	
0:08:29	0.1	0.1	-0.4	0.5	0.0	150.0			0.0	209.7		0.0000	27.		1.00	0.00	
0:08:31	0.2	0.2	-0.4	0.6	0.0	150.0	0	150	0.0	209.7	0.0	0.0000	63.		1.00	0.00	5659
0:09:29	7.9	6.6	-0.4	7.6	1.3	150.0	7	143	6.8	204.8	36.7	0.0591	188.		1.00	0.00	5393
0:10:29	7.8	6.5	-0.4	7.5	1.3	150.0	6	144	13.3	198.1	33.9	0.1157	188.		1.00	0.00	5414
0:10:51	7.8	6.5	-0.4	7.5	1.3	150.0	6	144	15.6	195.8	32.7	0.1357	188.		1.00	0.00	5422
0:10:52	6.8	5.7	-0.4	6.6	1.1	0.0	6	-6	15.7	195.7	31.3	0.1365	0.		1.00	0.00	-226
0:11:30	0.2	0.2	-0.4	0.5	0.0	0.0	0	0	15.6	193.4	-0.8	0.1357	0.		1.00	0.00	6
#N/A																	
0:15:34	0.2	0.2	-0.4	0.5	0.0	150.0			0.0	193.4		0.0000	46.		1.00	0.00	
0:15:36	0.2	0.2	-0.4	0.5	0.0	150.0	0	150	0.0	193.4	0.0	0.0000	83.		1.00	0.00	5659
0:16:37	8.0	6.7	-0.4	7.7	1.3	150.0	4	146	4.2	190.8	21.6	0.0365	188.		1.00	0.00	5503
0:17:36	8.0	6.7	-0.4	7.7	1.3	150.0	7	143	10.6	181.5	34.0	0.0922	188.		1.00	0.00	5413
0:18:22	7.9	6.7	-0.4	7.7	1.3	150.0	6	144	15.5	179.1	33.3	0.1348	188.		1.00	0.00	5418
0:18:23	7.9	6.7	-0.4	7.7	1.3	0.0	12	-12	15.7	178.9	62.6	0.1365	0.		1.00	0.00	-453
0:18:36	0.1	0.1	-0.4	0.5	0.0	0.0	2	-2	16.1	176.1	9.6	0.1400	0.		1.00	0.00	-70
#N/A																	
0:21:22	0.2	0.2	-0.4	0.5	0.0	150.0			0.0	177.0		0.0000	23.		1.00	0.00	
0:21:24	0.8	0.6	-0.4	1.1	0.1	150.0	-6	156	-0.2	177.0	-31.3	-0.0017	132.		1.00	0.00	5885
0:22:23	8.1	6.9	-0.4	7.9	1.3	150.0	7	143	6.6	172.0	36.1	0.0574	188.		1.00	0.00	5398
0:23:24	8.1	6.8	-0.4	7.8	1.3	150.0	6	144	13.1	165.2	33.4	0.1139	188.		1.00	0.00	5418
0:23:48	8.0	6.8	-0.4	7.8	1.3	150.0	6	144	15.6	162.6	32.6	0.1357	188.		1.00	0.00	5423
0:23:49	8.0	6.8	-0.4	7.8	1.3	0.0	12	-12	15.8	162.5	62.6	0.1374	0.		1.00	0.00	-453
0:24:23	0.1	0.1	-0.5	0.6	0.0	0.0	1202	-1202	697.0	160.2	6271.9	6.0609	0.		1.00	0.00	-45351
#N/A																	
0:25:33	0.1	0.1	-0.4	0.5	0.0	150.0			0.0	160.1		0.0000	30.		1.00	0.00	
0:25:35	0.5	0.4	-0.4	0.8	0.1	150.0	0	150	0.0	160.1	0.0	0.0000	102.		1.00	0.00	5659
0:26:34	8.2	6.9	-0.4	7.9	1.3	150.0	7	143	7.0	155.3	37.1	0.0609	188.		1.00	0.00	5390
0:27:35	8.1	6.8	-0.4	7.9	1.3	150.0	7	143	13.7	148.9	34.4	0.1191	188.		1.00	0.00	5410
0:27:52	8.1	6.8	-0.4	7.8	1.3	150.0	6	144	15.5	146.9	33.1	0.1348	188.		1.00	0.00	5419
0:27:53	8.1	6.8	-0.4	7.8	1.3	0.0	12	-12	15.7	146.8	62.6	0.1365	0.		1.00	0.00	-453
0:28:35	0.1	0.1	-0.4	0.6	0.0	0.0	1574	-1574	1117.4	144.6	8211.4	9.7165	0.		1.00	0.00	-59375
#N/A																	
0:30:48	0.1	0.1	-0.4	0.5	0.0	150.0			0.0	144.9		0.0000	30.		1.00	0.00	
0:30:49	0.3	0.1	-0.4	0.6	0.1	150.0	0	150	0.0	144.9	0.0	0.0000	67.		1.00	0.00	5659
0:31:03	8.3	7.0	-0.4	8.0	1.2	150.0	7	143	1.6	145.2	35.8	0.0139	188.		1.00	0.00	5400
0:31:04	8.3	6.9	-0.4	7.9	1.4	0.0	12	-12	1.8	-4327.5	62.6	0.0157	0.		1.00	0.00	-453
0:31:48	0.1	0.1	-0.4	0.5	0.0	0.0	0	0	1.7	142.7	-0.7	0.0148	0.		1.00	0.00	5
#N/A																	
0:32:53	0.1	0.1	-0.4	0.5	0.0	150.0			1.4	142.7		0.0122	4.		1.00	0.00	
0:32:55	0.3	0.2	-0.4	0.6	0.0	150.0	0	150	1.4	142.7	0.0	0.0122	76.		1.00	0.00	5659
0:33:54	8.2	6.9	-0.4	7.9	1.3	150.0	7	143	8.4	137.8	37.1	0.0730	188.		1.00	0.00	5390
0:34:54	8.1	6.9	-0.4	7.9	1.3	150.0	6	144	14.8	131.3	33.4	0.1287	188.		1.00	0.00	5417
0:35:00	8.1	6.9	-0.4	7.9	1.3	150.0	7	143	15.5	130.5	36.5	0.1348	188.		1.00	0.00	5395
0:35:01	8.1	6.9	-0.4	7.9	1.3	0.0	6	-6	15.6	130.4	31.3	0.1357	0.		1.00	0.00	-226
0:35:56	0.1	0.1	-0.4	0.6	0.0	0.0	1	-1	16.8	128.1	6.8	0.1461	0.		1.00	0.00	-49

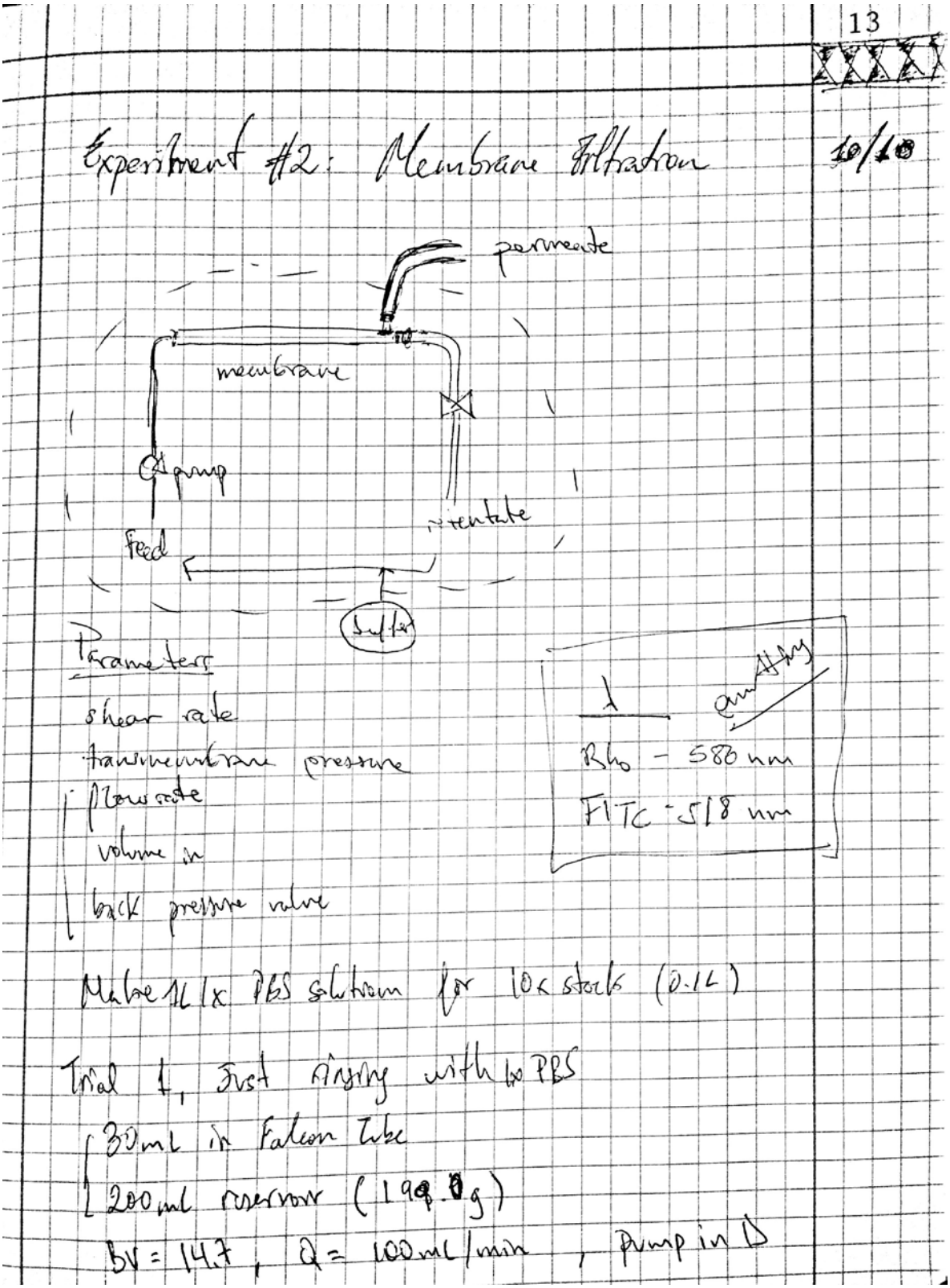
RhoB – Clean

Time	P_{inlet} psig	$P_{retentate}$ psig	$P_{permeate}$ psig	TMP psig	DP psig	Q_{inlet} ml/min	$Q_{permeate}$ mL/min	$Q_{retentate}$ ml/min	$M_{permeate}$ g	M_{feed} g	LMH (Filt. Flux)	VT mL/cm²	Pump rpm	Temp °C	Conc Factor	Diafilt Vols	Shear
														Measure			
0:00:00	0.2	0.2	-0.4	0.5	0.0	150.0			0.0	261.8		0.0000	81.		1.00	0.00	
0:00:59	4.3	3.0	-0.4	4.0	1.4	150.0	4	146	3.7	260.4	19.6	0.0322	188.		1.00	0.00	5517
0:01:59	6.0	4.6	-0.4	5.7	1.4	150.0	6	144	10.2	253.8	33.9	0.0887	188.		1.00	0.00	5414
0:02:48	6.0	4.6	-0.4	5.7	1.4	150.0	7	143	15.6	248.3	34.5	0.1357	188.		1.00	0.00	5409
0:02:50	6.0	4.6	-0.4	5.7	1.4	0.0	3	-3	15.7	248.2	15.7	0.1365	0.		1.00	0.00	-113
0:02:59	0.2	0.1	-0.4	0.5	0.0	0.0	1	-1	15.9	245.9	7.0	0.1383	0.		1.00	0.00	-50
#N/A																	
0:04:41	0.2	0.2	-0.4	0.5	0.0	150.0			0.0	245.8		0.0000	9.		1.00	0.00	
0:04:43	0.3	0.2	-0.4	0.6	0.1	150.0	-9	159	-0.3	245.8	-47.0	-0.0026	82.		1.00	0.00	5998
0:05:41	7.2	5.7	-0.4	6.8	1.4	150.0	5	145	5.0	241.8	28.6	0.0435	188.		1.00	0.00	5452
0:06:41	7.1	5.7	-0.4	6.8	1.4	150.0	7	143	11.7	234.9	35.0	0.1017	188.		1.00	0.00	5406
0:07:16	7.2	5.8	-0.4	6.9	1.4	150.0	7	143	15.5	231.0	34.0	0.1348	188.		1.00	0.00	5413
0:07:17	7.2	5.8	-0.4	6.9	1.4	0.0	6	-6	15.6	230.8	31.3	0.1357	0.		1.00	0.00	-226
0:07:42	0.2	0.2	-0.4	0.6	0.0	0.0	3609	-3609	1519.2	228.3	18827.7	13.2104	0.		1.00	0.00	-136138
#N/A																	
0:08:31	0.2	0.2	-0.4	0.6	0.0	150.0			0.0	228.6		0.0000	29.		1.00	0.00	
0:08:33	0.5	0.4	-0.4	0.8	0.1	150.0	0	150	0.0	228.7	0.0	0.0000	101.		1.00	0.00	5659
0:09:32	7.4	6.0	-0.4	7.1	1.4	150.0	7	143	6.6	224.4	35.0	0.0574	188.		1.00	0.00	5406
0:10:32	7.4	6.0	-0.4	7.1	1.4	150.0	7	143	13.4	217.5	35.5	0.1165	188.		1.00	0.00	5402
0:10:52	7.4	6.0	-0.4	7.1	1.4	150.0	7	143	15.6	215.3	34.4	0.1357	188.		1.00	0.00	5410
0:10:53	7.4	6.0	-0.4	7.1	1.4	0.0	6	-6	15.7	215.2	31.3	0.1365	0.		1.00	0.00	-226
0:11:32	0.2	0.2	-0.4	0.6	0.0	0.0	3297	-3297	2159.0	212.6	17203.7	18.7739	0.		1.00	0.00	-124396
#N/A																	
0:12:26	0.1	0.1	-0.4	0.5	0.0	150.0			0.0	212.9		0.0000	60.		1.00	0.00	
0:12:28	0.8	0.6	-0.1	0.8	0.2	150.0	0	150	0.0	212.9	0.0	0.0000	132.		1.00	0.00	5659
0:12:43	7.8	6.5	3.6	3.5	1.3	150.0	6	144	1.5	214.3	31.3	0.0130	188.		1.00	0.00	5432
0:12:45	7.5	6.2	1.7	5.1	1.3	0.0	462	-462	16.9	214.2	2410.4	0.1470	0.		1.00	0.00	-17429
0:12:52	1.4	1.2	-0.4	1.7	0.2	0.0	-128	128	2.0	211.4	-666.3	0.0174	0.		1.00	0.00	4818
0:12:54	0.5	0.4	-0.4	0.9	0.1	0.0	0	0	2.0	211.4	0.0	0.0174	0.		1.00	0.00	0
0:13:26	7.8	6.4	-0.4	7.5	1.4	150.0	6	144	5.2	210.6	31.3	0.0452	188.		1.00	0.00	5432
0:14:26	7.8	6.3	-0.4	7.4	1.4	150.0	7	143	12.1	203.7	36.0	0.1052	188.		1.00	0.00	5399
0:14:58	7.8	6.3	-0.4	7.4	1.4	150.0	7	143	15.6	200.1	34.2	0.1357	188.		1.00	0.00	5411
0:14:59	7.8	6.3	-0.4	7.4	1.4	0.0	6	-6	15.7	200.0	31.3	0.1365	0.		1.00	0.00	-226
0:15:27	0.2	0.2	-0.4	0.5	0.0	0.0	0	0	15.7	197.4	0.0	0.1365	0.		1.00	0.00	0
#N/A																	
0:16:36	0.1	0.1	-0.4	0.5	0.0	150.0			0.0	197.8		0.0000	22.		1.00	0.00	
0:16:37	0.1	0.2	-0.4	0.5	0.0	150.0	0	150	0.0	197.8	0.0	0.0000	58.		1.00	0.00	5659
0:17:37	7.8	6.4	-0.4	7.5	1.4	150.0	6	144	6.5	193.6	33.9	0.0565	188.		1.00	0.00	5414
0:18:36	7.8	6.3	-0.4	7.4	1.4	150.0	7	143	13.2	186.9	35.5	0.1148	188.		1.00	0.00	5402
0:18:57	7.7	6.3	-0.4	7.4	1.4	150.0	7	143	15.6	184.4	35.8	0.1357	188.		1.00	0.00	5400
0:18:59	7.7	6.3	-0.4	7.4	1.4	0.0	6	-6	15.8	184.2	31.3	0.1374	0.		1.00	0.00	-226
0:19:36	0.2	0.2	-0.6	0.7	0.0	0.0	-68	68	-26.1	181.7	-354.5	-0.2270	0.		1.00	0.00	2563
0:19:55	0.2	0.2	-0.4	0.5	0.0	0.0	82	-82	0.0	181.7	430.0	0.0000	0.		1.00	0.00	-3109
0:19:57	0.2	0.2	-0.4	0.5	0.0	0.0	0	0	0.0	181.7	0.0	0.0000	0.		1.00	0.00	0
#N/A																	
0:20:02	0.1	0.1	-0.4	0.5	0.0	150.0			0.0	181.8		0.0000	70.		1.00	0.00	
0:21:02	7.9	6.4	-0.4	7.5	1.4	150.0	6	144	6.5	177.5	33.9	0.0565	188.		1.00	0.00	5414
0:22:01	7.8	6.4	-0.4	7.4	1.4	150.0	7	143	13.3	170.7	36.1	0.1157	188.		1.00	0.00	5398
0:22:22	7.8	6.4	-0.4	7.4	1.4	150.0	7	143	15.6	168.3	34.3	0.1357	188.		1.00	0.00	5411
0:22:23	7.8	6.4	-0.4	7.4	1.4	0.0	6	-6	15.7	168.2	31.3	0.1365	0.		1.00	0.00	-226
0:23:02	0.2	0.2	-0.2	0.4	0.0	0.0	1544	-1544	1019.4	165.7	8056.5	8.8643	0.		1.00	0.00	-58254
#N/A																	
0:24:41	0.2	0.2	-0.4	0.5	0.0	150.0			0.0	165.9		0.0000	34.		1.00	0.00	
0:24:42	0.2	0.2	-0.4	0.5	0.0	150.0	0	150	0.0	165.9	0.0	0.0000	70.		1.00	0.00	5659
0:25:41	8.0	6.5	-0.4	7.6	1.4	150.0	7	143	6.7	161.6	35.5	0.0583	188.		1.00	0.00	5402
0:26:42	7.9	6.5	-0.3	7.5	1.4	150.0	7	143	13.7	154.3	35.9	0.1191	188.		1.00	0.00	5399
0:26:59	7.9	6.4	-0.3	7.5	1.4	150.0	6	144	15.5	152.7	33.1	0.1348	188.		1.00	0.00	5419
0:27:00	7.3	5.9	-0.3	6.9	1.3	0.0	12	-12	15.7	152.5	62.6	0.1365	0.		1.00	0.00	-453
0:27:42	0.1	0.1	-0.4	0.5	0.0	0.0	0	0	15.7	150.0	0.0	0.1365	0.		1.00	0.00	0

Dextran -FTIC- Clean

Time	P_{inlet} psig	$P_{retentate}$ psig	$P_{permeate}$ psig	TMP psig	DP psig	Q_{inlet} ml/min	$Q_{permeate}$ mL/min	$Q_{retentate}$ ml/min	$M_{permeate}$ g	M_{feed} g	LMH (Filt. Flux)	VT mL/cm²	Pump rpm	Temp °C	Conc Factor	Diafilt Vols	Shear
														Measure			
0:00:00	0.2	0.2	-0.3	0.4	0.0	150.0			0.0	230.5		0.0000	57.		1.00	0.00	
0:00:59	5.3	3.5	-0.4	4.8	1.8	150.0	3	147	3.3	229.4	17.5	0.0287	188.		1.00	0.00	5532
0:01:59	6.3	4.4	-0.4	5.7	1.9	150.0	5	145	8.8	224.0	28.7	0.0765	188.		1.00	0.00	5451
0:02:59	6.9	5.1	-0.4	6.4	1.9	150.0	5	145	14.0	218.9	27.1	0.1217	188.		1.00	0.00	5463
0:03:18	7.1	5.2	-0.4	6.5	1.9	150.0	5	145	15.7	217.2	28.0	0.1365	188.		1.00	0.00	5456
0:03:19	7.2	5.3	-0.4	6.6	1.9	0.0	6	-6	15.8	217.1	31.3	0.1374	0.		1.00	0.00	-226
0:04:00	0.2	0.2	-0.4	0.6	0.0	0.0	748	-748	526.8	212.2	3901.6	4.5809	0.		1.00	0.00	-28211
#N/A																	
0:04:36	0.1	0.1	-0.4	0.5	0.0	150.0			0.0	212.3		0.0000	5.		1.00	0.00	
0:04:37	0.1	0.1	-0.4	0.5	0.0	150.0	0	150	0.0	212.3	0.0	0.0000	40.		1.00	0.00	5659
0:05:36	6.7	4.9	-0.4	6.1	1.8	150.0	5	145	4.9	208.8	26.0	0.0426	188.		1.00	0.00	5471
0:06:36	7.5	5.6	-0.4	6.9	1.8	150.0	5	145	10.4	203.1	28.7	0.0904	188.		1.00	0.00	5451
0:07:32	7.6	5.7	-0.4	7.0	1.8	150.0	6	144	15.6	197.7	29.1	0.1357	188.		1.00	0.00	5449
0:07:33	7.6	5.7	-0.4	7.0	1.8	0.0	6	-6	15.7	197.7	31.3	0.1365	0.		1.00	0.00	-226
0:07:36	5.2	3.9	-0.4	4.9	1.3	0.0	2	-2	15.8	196.2	10.4	0.1374	0.		1.00	0.00	-75
0:08:33	0.2	0.2	-0.4	0.5	0.0	0.0	-17	17	-0.2	195.6	-87.9	-0.0017	0.		1.00	0.00	635
0:08:34	0.2	0.2	-0.4	0.5	0.0	0.0	0	0	-0.2	195.6	0.0	-0.0017	0.		1.00	0.00	0
#N/A																	
0:08:38	0.1	0.1	-0.4	0.5	0.0	150.0			0.0	195.7		0.0000	74.		1.00	0.00	
0:09:36	6.9	5.0	-0.4	6.3	1.8	150.0	5	145	5.0	192.3	27.0	0.0435	188.		1.00	0.00	5464
0:10:36	6.9	5.0	-0.4	6.3	1.9	150.0	5	145	10.5	186.8	28.7	0.0913	188.		1.00	0.00	5451
0:11:33	7.0	5.1	-0.4	6.4	1.9	150.0	5	145	15.6	181.5	28.0	0.1357	188.		1.00	0.00	5456
0:11:34	6.9	5.0	-0.4	6.3	1.9	0.0	12	-12	15.8	181.4	62.6	0.1374	0.		1.00	0.00	-453
0:11:37	4.7	3.4	-0.4	4.4	1.3	0.0	-200	200	5.8	179.9	-1043.5	0.0504	0.		1.00	0.00	7545
#N/A																	
0:12:51	0.1	0.1	-0.4	0.5	0.0	150.0			0.0	179.3		0.0000	53.		1.00	0.00	
0:12:52	0.4	0.3	-0.4	0.7	0.1	150.0	0	150	0.0	179.3	0.0	0.0000	88.		1.00	0.00	5659
0:13:52	7.2	5.3	-0.4	6.7	1.9	150.0	6	144	5.6	175.5	29.2	0.0487	188.		1.00	0.00	5448
0:14:51	7.2	5.3	-0.4	6.7	1.9	150.0	6	144	11.1	170.0	29.2	0.0965	188.		1.00	0.00	5448
0:15:39	7.1	5.2	-0.4	6.5	1.9	150.0	6	144	15.5	165.6	28.7	0.1348	188.		1.00	0.00	5451
0:15:40	7.1	5.2	-0.4	6.5	1.9	0.0	6	-6	15.6	165.5	31.3	0.1357	0.		1.00	0.00	-226
0:15:52	0.1	0.1	-0.5	0.6	0.0	0.0	1	-1	15.8	163.3	5.2	0.1374	0.		1.00	0.00	-38
#N/A																	
0:16:46	0.1	0.1	-0.4	0.5	0.0	150.0			0.0	163.4		0.0000	3.		1.00	0.00	
0:16:48	0.3	0.2	-0.4	0.6	0.1	150.0	0	150	0.0	163.4	0.0	0.0000	75.		1.00	0.00	5659
0:17:48	7.2	5.4	-0.4	6.7	1.8	150.0	6	144	6.0	159.7	31.3	0.0522	188.		1.00	0.00	5432
0:18:48	7.2	5.4	-0.4	6.7	1.8	150.0	6	144	11.7	154.1	29.7	0.1017	188.		1.00	0.00	5444
0:19:31	7.2	5.4	-0.4	6.7	1.9	150.0	5	145	15.5	150.1	27.7	0.1348	188.		1.00	0.00	5459
0:19:32	7.2	5.4	-0.4	6.7	1.9	0.0	12	-12	15.7	150.0	62.6	0.1365	0.		1.00	0.00	-453
0:19:48	0.1	0.1	-0.6	0.6	0.0	0.0	-3	3	15.0	147.9	-13.7	0.1304	0.		1.00	0.00	99

Appendix D: Lab Notebook



Meeting #1

- Calibration curve for RFU
- TMP vs Flux curve (dextran)
- R and DV

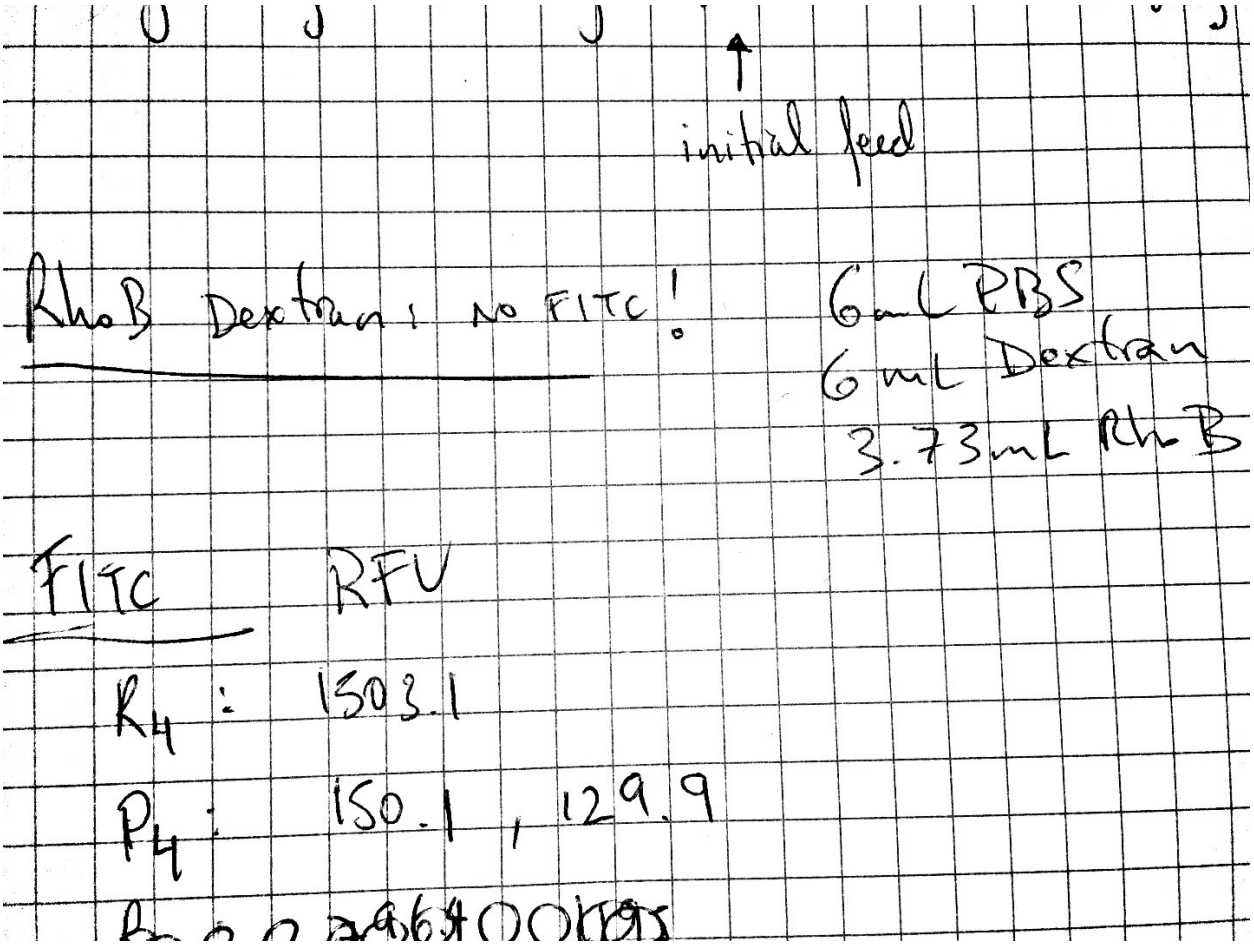
Lab Day #2

Aimed for → R: 0.0114 mg/mL
→ DFITC: 0.381 mg/mL
in PBS ($1:1_{PBS}$ 1_{DFITC})

(Based on 480 sites per dex

TMP curve: Dextran (no FITC) + PBS
[7.62 mg/mL]
40mL solution = x() + y PBS
7.62 mg/mL × 40mL = 30.48 mg Dextran
from 20mg/mL ⇒ 1.5 mL stock
+ 38.5 mL PBS

Flowrates: [50, 100, 150, 200] mL/min
TMP: [2 ; 2 : 20] psig


initial feed
RhoB Dextran: NO FITC!
6mL PBS
6 mL Dextran
3.73mL RhB
FITC RFU
R4: 1503.1
P4: 150.1, 129.9