ULTRAFILTRATION OF PROTEIN SOLUTIONS

A Laboratory Experiment

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Biology is playing an increasingly important role in the chemical engineering curriculum. During the summer of 2010, the Department of Chemical Engineering at Princeton University changed its name to the Department of Chemical and Biological Engineering due to the increasing presence of biological research within the department. Courses such as Quantitative Principles of Cell and Molecular Biology, Separations in Chemical Engineering and Biotechnology, and Metabolic Engineering have become permanent fixtures in the curriculum. Introducing biotechnology concepts into both the classes and the laboratory experience is crucial to prepare our students for careers in the pharmaceutical and biotech industries.

The Chemical Engineering Laboratory course is required for undergraduates in the department. The course reinforces concepts the students have learned in the classroom with hands-on laboratory experience. In addition, it has a major focus on communication and technical writing. We have recently implemented a new experiment in the course that requires students to design, model, and carry out separation experiments involving ultrafiltration of protein solutions. Here, we show how the ultrafiltration portion of the experiment is carried out and its learning objectives.

Ultrafiltration has traditionally played a role in biotechnology as a means to concentrate and purify proteins. Separation processes such as centrifugation and dialysis are batch processes that are difficult to scale up. With ultrafiltration, large volumes of solutions or suspensions can be concentrated and purified in a few hours with minimal product degradation. There are two modes of operation for membrane separations: concentration and diafiltration, as shown in Figure 1 (next page). In the concentration mode the solute of interest is retained by the membrane (unit 1); solvent passes through the membrane into vessel 6 where the mass is measured to determine transmembrane fluxes. The concentration of the retained solute (in reservoir 3) increases as the volume of the retentate decreases. In the diafiltration mode, solvent and a low molecular weight impurity pass through the membrane, the solute of interest is retained, and additional pure solvent is added to the solute volume (from reservoir 4) to keep the solute concentration constant. Diafiltration is a washing or solvent exchange process.

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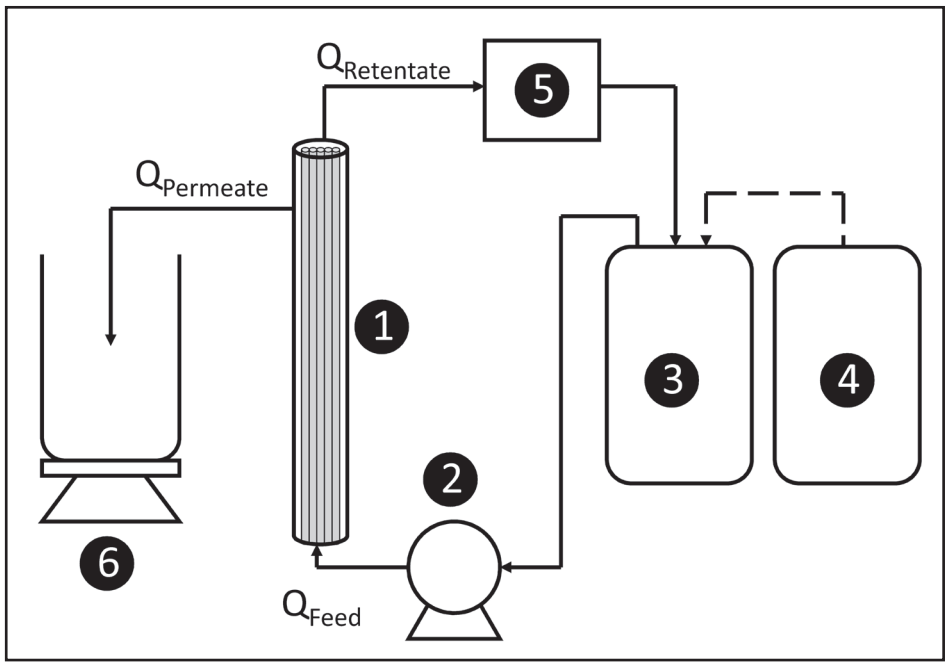


Figure 1. *Schematic and flow-path of the KrosFlo TFF Research IIi ultrafiltration system. (1) Hollow fiber membrane module; (2) peristaltic pump which defines the feed flow rate Q_{Feed} or Q_F ; (3) process reservoir containing the solution/suspension to be concentrated or diafiltered; (4) buffer reservoir where pure water or buffer is stored to be delivered to (3); (5) backpressure controller to set the transmembrane pressure; and (6) beaker and weigh scale to measure the mass of permeate collected, from which the permeate $Q_{Permeate}$ (or Q_P) and retentate $Q_{Retentate}$ (Q_R) are determined. The dashed line between (3) and (4) indicates the tube connected to the solvent reservoir (4) for constant volume diafiltration.*

Product losses can occur *if irreversible gel layers* are formed at the membrane surface (membrane fouling) as proteins irreversibly aggregate and denature. However, careful optimization of process parameters can mitigate this risk and product recoveries greater than 90% are achieved in practice.[1, 2] *Reversible protein concentration near the membrane surface*, called concentration polarization, also affects membrane performance. This is the major concept for the students to understand from this laboratory experiment: how convective flux through the membrane creates a concentrated protein layer near the membrane surface, how this layer limits transmembrane flux, and how tangential flow minimizes concentration polarization. The phenomenon is shown schematically in Figure 2, where at lower solute concentrations and higher tangential flow rates there is no polarization. At higher concentrations, higher transmembrane fluxes, and lower tangential flow rates, polarization occurs. The concentrated, polarized layer causes a decrease in transmembrane flux due to increased osmotic pressure at the membrane surface, and/or the reduced hydraulic permeability of the gel layer on the membrane surface. For further information on membrane technologies, we recommend three excellent references: *Ultrafiltration and Microfiltration Handbook*,[3] *Basic Principles of Membrane Technology*,[4] and *Membrane Technology and Applications*.[5]

Experiments carried out by the students have the goal of optimizing a process for the concentration and purification of a bovine serum albumin (BSA) solution. The learning objectives for the experiment are:

1) *To determine the membrane resistance for solvent flux, and its dependence (or lack thereof) on tangential flow.*
2) *To determine concentration polarization for protein ultrafiltration by measuring the transmembrane flux as a function of protein concentration, transmembrane pressure, and tangential flow rate. To have a physical picture of how these variables interact.*
3) *To understand the differences between concentration and diafiltration operations.*
4) *To design an optimized processing scheme to purify and concentrate a protein solution based on an understanding of the phenomena of concentration polarization and the results from the previous experiments.*

Separations processes have long been an integral part of the chemical engineering discipline, especially in the areas of membrane separations and chromatography. However, because such technologies are difficult to implement in undergraduate lab courses, there have been relatively few examples of their use.[6] The growing importance of biotechnology and bioseparations has prompted several pedagogical articles on integrating such processes into the undergraduate lab experience.[7-10] Excellent articles on how to instruct students in membrane separations have focused on reverse osmosis,[11, 12] ultrafiltration,[6, 13, 14] and multi-stage ultrafiltration and modeling.[15] Similarly, articles focusing on affinity[9, 16-18] and ion exchange[19] chromatography are available as well. The ultrafiltration articles tend to focus on hardware design and setup, testing different filter geometries, flow rates as a function of solute concentration and pressure, and modeling to determine mass transfer coefficients. In this experiment we emphasize the fundamental challenge of process engineering, which is optimization of a process. The student must balance the conflicting phenomena of concentration polarization, which is significant

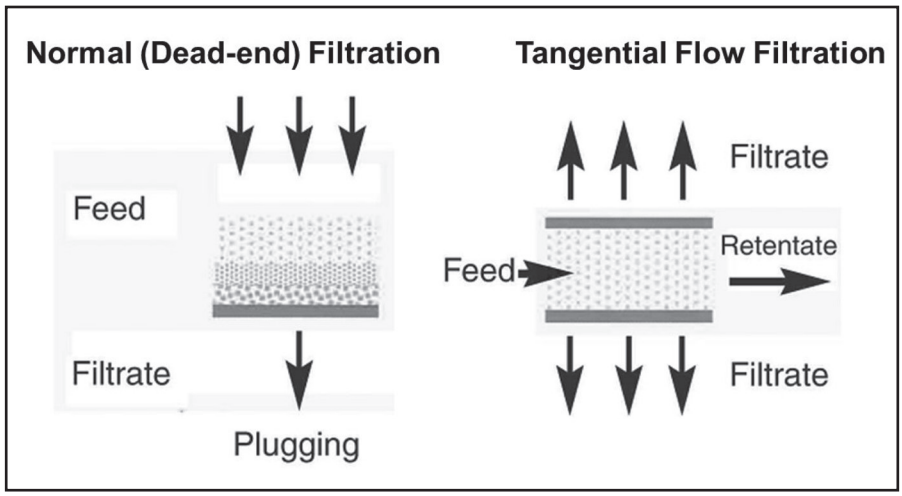


Figure 2. *In dead-end filtration, concentration polarization occurs, and the permeate flux drops at low transmembrane fluxes since the feed flow and permeate flow are the same direction. This problem is mitigated by tangential flow where the feed flow and permeate flow are perpendicular to each other. The tangential shear at the membrane surface sweeps away the polarized layer and minimizes protein concentration at the membrane surface. Much higher transmembrane fluxes can be obtained at equivalent transmembrane pressures. Adapted from Spectrum Labs.*[30]

at high concentrations and high transmembrane fluxes, with the need to minimize the volume of fluid processed in order to minimize process cycle time. This experiment does an excellent job of highlighting the tradeoffs inherent in optimization. Basic fluid mechanics and mass transfer models are applied to determine operating parameters, and key process parameters are also optimized to emphasize the importance of minimal processing time and materials usage.

EQUIPMENT, MATERIALS, AND METHODS

Materials and equipment

While it would be possible to construct the laboratory apparatus from components, we chose to purchase a KrosFlo Research IIi system (SYR2-U20-01N) with 10 kD hollow fiber membranes (D02-E010-05-N) from Spectrum Labs, Inc. The unit comes with integrated pressure transducers, an electronic balance to measure permeate flow rates, and computer software to capture, analyze, graph, and print data. A schematic of the apparatus is shown in Figure 1. The cost of the KrosFlo Research IIi system was approximately $12,000, with each membrane module costing about $170.

Bovine serum albumin (BSA, A2153), sodium phosphate (71640), phosphoric acid (438081), NaOH (72082), and sodium salicylate (S3007) were purchased from Sigma Aldrich and used as received. Denatured ethanol (A407P-4) was purchased from Fisher Scientific. Water was obtained from a Barnstead NanoPure system (Barnstead Corp.) that includes 0.22 μm filtration. UV-vis absorbance measurements were taken on the Evolution 300 spectrophotometer purchased from Thermo Fisher Scientific.

We chose the 10 kD molecular weight cutoff (MWCO) hollow fiber membranes to retain all of the 66.5 kD BSA protein while allowing smaller molecular "contaminants" on the order of 100-500 D to pass through the membrane easily. Other acceptable membranes cutoffs available from Spectrum Labs include 3, 5, and 30 kD MWCO. The fibers are composed of hydrophilic polyethersulfone 0.5 mm in diameter. Hydrophobic polysulfone membranes require higher pressures to filter aqueous solutions and are more prone to protein fouling.

METHODS

Ethanol wetting and water permeability test

A new hollow fiber membrane unit purchased from Spectrum Labs was first wetted with 40% EtOH solution by circulating 50 mL throughout the membrane for 20-25 minutes at a feed rate of 150 mL/min. Then the system was flushed with water (2 mL water per cm^2 of membrane area). Since membranes are re-used, this procedure is required only for the first use of the membrane. To establish the intrinsic membrane permeability, a water permeability test was performed. The tangential flow rate through the hollow fiber module (*i.e.*, the "feed rate") was set to 100, 200, and 300 mL/min and the transmembrane pressure (TMP) was varied from 10-25 psi using the supplied automatic backpressure controller. Data were recorded directly from the KrosFlo instrument using the supplied software.

Starting BSA feed composition

The starting BSA solution consisted of 1 wt% BSA (10 mg/mL) in 5 mM phosphate buffer at pH 7.0. A process volume of 200 mL of BSA solution was used for TMP optimization and diafiltration optimization experiments. For the diafiltration portion of the experiment, the BSA solution consisted of 1 wt% BSA with 1 mM sodium salicylate in 5 mM phosphate buffer at pH=7.0.

BSA TMP optimization

TMP optimization was carried out by running the unit in diafiltration mode (with a fresh buffer reservoir connected to the process reservoir). The feed flow rates were set to 300, then 200, then 100 mL/min with the TMP varied from 10-25 psi at each flow rate. Following this, the fresh buffer line was

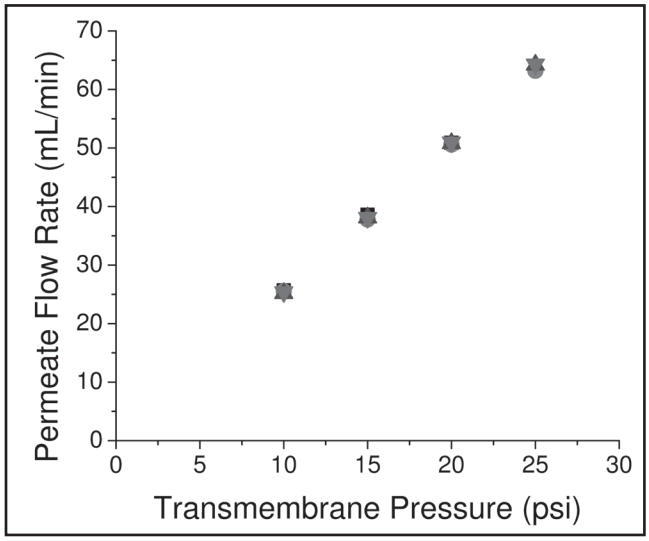


Figure 3. *The results of the water permeability test demonstrate that the permeate flow rate is linearly dependent on P_{TMP} and independent of the feed flow rate. (Q_{feed} = ■ 50 mL/min | ● 150 mL/min| ▲ 250 mL/min | ▼ 300 mL/min).*

disconnected to concentrate the BSA solution from 9.6 to 65.9 mg/mL (6.9 fold concentration). Once the approximate concentration was achieved, the buffer line was reconnected and the flow rates and TMPs repeated once more at this higher concentration. Final BSA concentrations were verified by UV absorbance measurements at 280 nm.

BSA concentration optimization

The feed flow rate was set at 300, 200, and 100 mL/min and the membrane cleaned between each run (see section on membrane cleaning procedures) to within ~15% of the starting water permeability values. The starting BSA solution was concentrated from 10 mg/mL to approximately 150 mg/mL for each run as measured by UV absorbance measurements at 280 nm.

BSA diafiltration

The BSA solution was first concentrated to an intermediate concentration as determined by the optimization parameter described in the Results and Discussion section at the previously determined optimal TMP (20 psi). Once the intermediate concentration was reached, the buffer line was connected and diafiltration commenced. Approximately 10-15 diavolumes were processed before the buffer line [tube connecting reservoirs (3) and (4) in Figure1] was disconnected and concentration resumed until the final desired concentration was achieved. Throughout this process, small (1-2 mL) samples were taken from the permeate line to track the concentration of sodium salicylate "impurity." Sample concentrations were measured by UV absorbance at 295 nm. Absorbance values were converted to concentration by using Beer's Law.

Membrane cleaning procedures

The membrane should be cleaned between experiments to achieve maximal flow rates and reproducibility. Cleaning procedures vary depending on the nature of the suspension filtered prior. First, the membrane should be flushed with pure water (following the 2 mL per cm^2 membrane area rule), then blown down with air. For protein solutions, we have found dilute bleach (standard Clorox bleach mixed 1:1 with water) provides a sufficiently oxidative environment to degrade and remove proteins from the membrane wall. The dilute bleach (50 mL) should be recirculated at a medium shear rate (5000-8000 s^{-1}) for about 20 minutes. The effectiveness of the cleaning is assessed by flushing the system with water (2 mL/cm^2) then running a water permeability test at one of the points previously established (reference crossflow rate and TMP). Achieving permeate water flow rates within 15% of the original values is considered acceptable. Although the membranes are technically disposable single-use devices, we have routinely used membranes for 4-6 sessions with this cleaning procedure. The replacement frequency is driven by the fact that we force the system into a "failure mode," *i.e.*, high amounts of fouling to show the limits of operation. If one wants even longer membrane lifetime, then not forcing the system to strong fouling will prolong the membrane life. However, the students learn by finding the limits of operation.

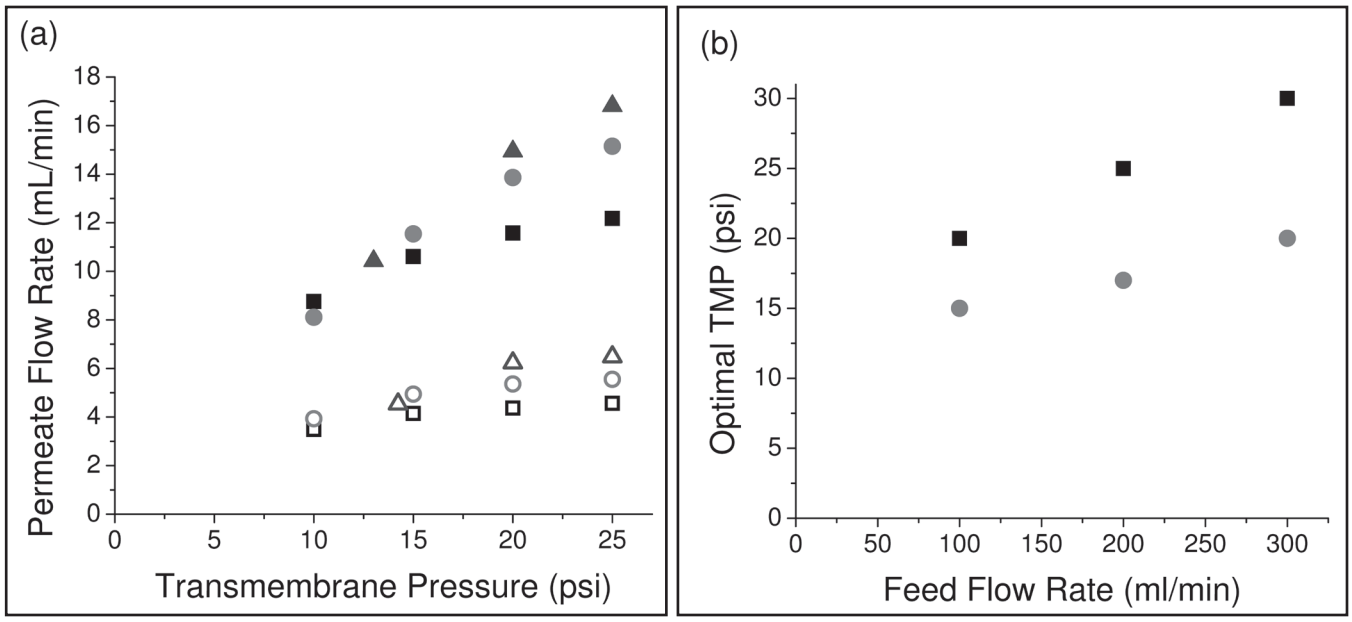
RESULTS AND DISCUSSION

Membrane wetting protocol

Initially the pores of the membrane are filled with air. The students should be taught about the Laplace pressure across a curved interface, and the contact angle at an air/liquid solid surface. If the membrane is used without the prewetting, at the operating pressures of the hollow filter module approximately 20% of the membrane pores will remain air filled. The pressures are not great enough to displace air from the pores since the water does not adequately wet the membrane surfaces. The apparent water permeability will be reduced. Ethanol or isopropanol does wet the polymer membrane and all air will be displaced. If the students have adequate time they can perform an initial permeability measurement on a new membrane with buffer solution, then wet the membrane and re-measure the water permeability. The students only need to perform alcohol membrane wetting on a brand new membrane. Since membranes are re-used during the course of the lab, not all groups need to perform this.

Water permeability test

The water permeability test measures permeate flow rate as a function of TMP [Eq. (1)] and feed flow rate. This allows for the intrinsic resistance of the membrane to be calculated according to Eq. (2). Since there is no gel layer formation with pure water, the term R_g is equal to 0 and the permeate flow rate should be directly proportional to the TMP as shown in Figure 3.



Figures 4. *a) TMP Optimization for BSA concentrations of 9.59 mg/mL (solid symbols) and 65.9 mg/mL (open symbols) at various feed flow rates (Q_{feed} = ■ 100 mL/min | ● 200 mL/min | ▲ 300 mL/min).*
b) Summary of TMP Optimization results for various feed flow rates and BSA concentrations ([BSA] = ■ 9.59 mg/mL | ● 65.9 mg/mL).

Since the TMP is independently controlled via the automatic backpressure controller, the feed flow rate does not affect the permeate flux. The students will see this in the data they generate, which will be similar to that shown in Figure 3. However, the feed flow rate (or crossflow rate) does affect the filtration of macromolecular solutions since higher crossflow rates result in higher shear rates (up to approximately 12,000 s^{-1} at 300 mL/min). The transmembrane pressure driving force is given by

$$P_{TMP} = \frac{P_{feed} + P_{retentate}}{2} - P_{permeate} \quad (1)$$

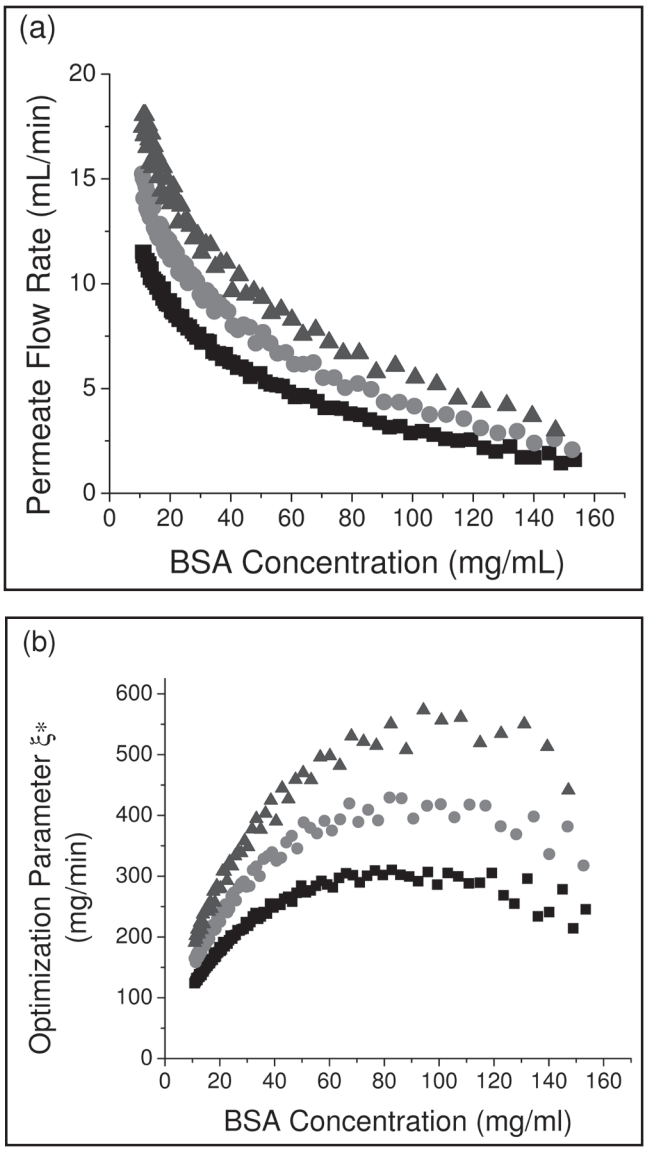
and the permeate flux through the membrane, J, defines the intrinsic membrane resistance, R_m, and the gel layer resistance, R_g,

$$J = \frac{P_{TMP}}{\mu\left(R_m + R_g\right)} \quad (2)$$

where J is permeate flux ($mL \bullet min^{-1} \bullet cm^{-2}$), P_{TMP} is transmembrane pressure [defined in Eq. (1)], μ is fluid viscosity (Pa•s), R_m is the intrinsic membrane resistance, and R_g is gel layer resistance.[20] The slope of the linear fit in Figure 3 gives the value for $R_m = 2.06 \times 10^9\ m^{-1}$. The students should be asked to justify Eq. (1) as the single "transmembrane pressure" when there are three pressures involved in the calculation. More inquisitive students can be directed to the derivation of the equations for flow in a porous tube,[21] and can then be asked to justify the linear approximation given in Eq. (1).

BSA transmembrane pressure (TMP) optimization

During the course of filtration, a decline in permeate flux is observed as the concentration of BSA increases. In general, this flux decline can be attributed to a combination of concentration polarization and fouling. Concentration polarization results in a high osmotic pressure at the membrane surface. This osmotic pressure "subtracts" from the measured hydrostatic pressure difference across the membrane, thereby reducing the solvent flux. Membrane fouling (*i.e.*, membrane surface adsorption, membrane pore plugging, and gel layer formation on the membrane surface) also reduces flux.[22] In general, permeate flux increases linearly with TMP up to some value of the TMP where effects from concentration polarization become significant.[23] At a BSA concentration of 9.59 mg/mL and at feed flow rates above 200 mL/min, the relationship between permeate flow rate and TMP is close to linear, which suggests that there is negligible concentration polarization/fouling [see Figure 4(a)]. The data show that a critical TMP (where "critical" is defined as the point of deviation from the linear regime) occurs at a TMP of 17 psi at a feed flow rate of 200 mL/min for the 65.9 mg/mL BSA solution. At a higher feed flow rate of 300 mL/min the critical TMP increases to 20 psi. Higher tangential flow decreases polarization. Note that the permeate flow rates at 9.59 mg/mL are at least twice as high as the permeate flow rates at 65.9 mg/mL, which demonstrates that concentration polarization and membrane fouling are strongly dependent on BSA concentration. The approximate critical TMPs for



Figures 5. *Permeate flow rate (a) and optimization parameter ξ* (b) vs. BSA concentration at* Q_{feed} = ■ *100 mL/min* | ● *200 mL/min* | ▲ *300 mL/min at P = 20 psi. High crossflow rates allow for higher permeate fluxes at the same BSA concentration and thus, higher ξ*.*

the six possible combinations of BSA concentration and feed flow rates are plotted in Figure 4(b).

BSA diafiltration optimization

Ultrafiltration is used to both concentrate and purify macromolecular solutions or suspensions, thus involving two modes of operation: concentration and diafiltration. In concentration the solute of interest is retained by the membrane and solvent passes through the membrane. In diafiltration, a low molecular weight impurity passes through the membrane, the solute of interest is retained by the membrane, and fresh solution phase is continuously added to the circulating fluid to keep the volume of the ultrafiltered solution constant. The process of continuous diafiltration can be modeled as a CSTR with pure water or buffer as the input. This yields an exponentially decreasing impurity concentration as a function of the number of diavolumes [see Eq. 6)]. The process provides an interesting optimization problem to the students: minimize the total processing time while purifying and concentrating a protein solution to some desired level. In one limit, diafiltering before concentration might yield higher flow rates but requires larger fresh buffer volumes to be processed, which requires more time. In the other limit, diafiltering after concentrating reduces the volume of fresh buffer required but the permeate flow rate is lower because at higher concentration of protein, concentration polarization is more significant. The optimal method is a compromise: Concentration is performed first to some intermediate concentration, then diafiltration, then concentration again. The intermediate concentration is determined by the optimization parameter ξ(t)* [Eq. (3)][24, 25]:

$$\xi(t)^* = C(t)_{BSA} \times J(t)_{perm} \tag{3}$$

Conceptually, this optimization parameter reflects that you want to process at as high a protein concentration as possible and with as high a solvent flux as possible. However, going too high in concentration decreases the flux more than linearly in concentration, and decreasing concentration too much requires processing excessive volumes of solvent. The required diafiltration time (t_{DF}) and number of diavolumes (*i.e.*, volumes of permeate fluid relative to the fluid volume in the reservoir and flow tubing) [N(t)] are given by Eqs. (4) and (5):

$$t_{DF} = \frac{NV_{process}}{Q_P} \tag{4}$$

$$N(t) = \frac{V_{buffer}(t)}{V_{process}} \tag{5}$$

where C_{BSA} is the bulk BSA concentration at any time t, $J(t)_{perm}$ is the permeate flux (mL min^{-1} cm^{-2}) or flow rate Q_p (mL min^{-1}), t_{DF} is the diafiltration time required for a given number of diavolumes [Eq. (4)], N(t) is the number of diavolumes [Eq. (5)], $V_{process}$ is the process volume, and Q_P is the permeate flow rate (constant during diafiltration). The results of the Diafiltration Optimization experiments are shown in Figures 5. Q_P decays nonlinearly with increasing BSA concentration. Higher feed flow rates (and thus higher shear rates) allow for higher permeate fluxes due to reduced membrane fouling. Plotting the optimization parameter ξ*(t) against BSA concentration [Figure 5(b)] reveals the optimal concentration for beginning diafiltration. At a feed flow rate of 300 mL/min and a TMP of 20 psi, the BSA concentration is 94 mg/mL.

Since the software only measures the amount of permeate on the weighing scale, the BSA concentration over time must

be calculated from the collected permeate volume by Eq. (6):

$$[\mathrm{BSA}] = \frac{\mathrm{Mass}_{\mathrm{BSA,initial}}}{\mathrm{V}_{\mathrm{initial}} - \left(\mathrm{V}_{\mathrm{permeate}} + \mathrm{V}_{\mathrm{holdup}}\right)} \quad (6)$$

where $V_{permeate}$ is the volume collected on the scale and V_{holdup} is the volume held up in the membrane unit, tubing, and permeate exit line.

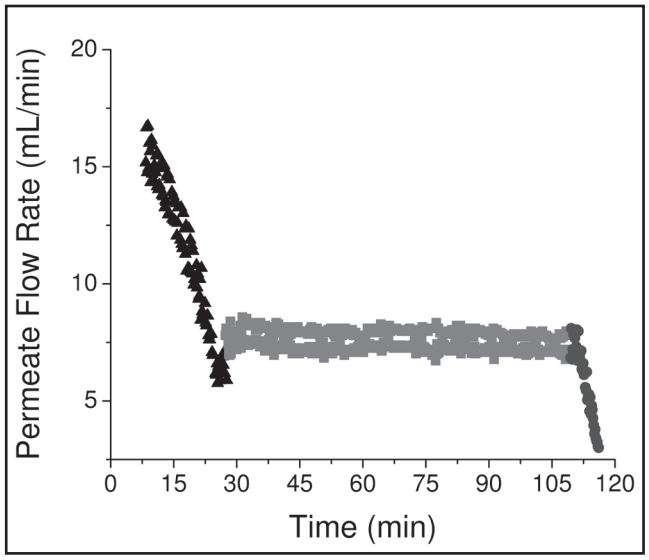


Figure 6. *The three stages of diafiltration: first concentration step (▲), diafiltration step (■), and the second concentration step (●). The diafiltration process is run at a feed flow rate of 300 mL/min and at a TMP of 20 psi.*

BSA continuous diafiltration

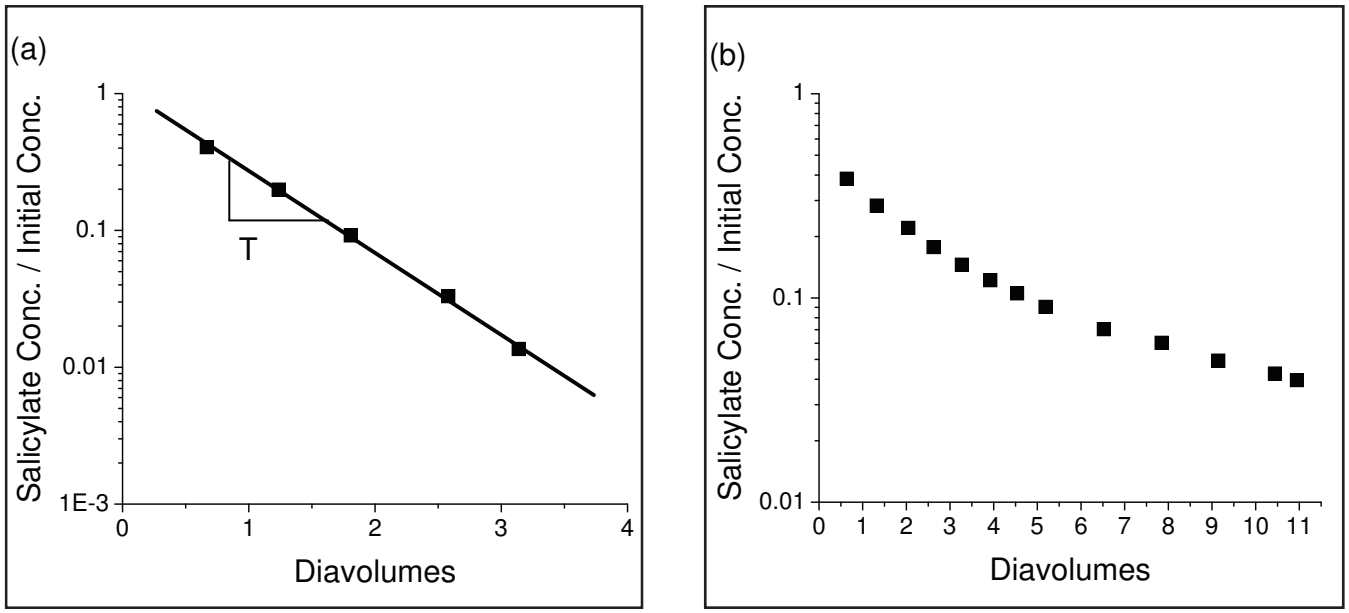
The goal of continuous (or constant volume) diafiltration is to remove undesirable permeable solutes ("impurities").[26] The concentration and diafiltration process has three phases, depicted in Figure 6: the first concentration phase, the diafiltration phase, and the second concentration phase. During the first concentration step, the BSA solution is concentrated at a TMP of 20 psi and a feed flow rate of 300 mL/min until it reaches the optimal concentration that was determined in the diafiltration optimization experiment. During the diafiltration step, approximately 10-15 diavolumes of fresh buffer are processed to remove the majority of the impurity. Since the buffer enters at the same rate as permeate leaves, the concentration of the feed remains constant. Finally, after sufficient buffer has been processed, the buffer line is disconnected and the solution in the feed reservoir is concentrated to the final desired concentration.

Modeling the system as a CSTR yields the following:

$$\mathrm{C(N)} = \mathrm{C_0 e^{-TN(t)}} \quad (7)$$

$$\mathrm{T} = \frac{\mathrm{C_{impurity,permeate\ side}}}{\mathrm{C_{impurity,process\ side}}} \quad (8)$$

where C is the concentration of permeable solute ("impurity") on the process side, C_0 is the starting concentration of permeable solute in the process side, T is the transmission coefficient [Eq. (8)], and N(t) is the number of diavolumes processed. The transmission coefficient is a measure of how "hard" it is for an impurity to pass from the process side to the permeate side and can be calculated from the slope of the curve in Figure 7(a).



Figures 7. *(a) Concentration of sodium salicylate leaving the permeate line when no BSA is present (control experiment). (b) Concentration of sodium salicylate impurity as it is removed from the BSA solution via diafiltration. The non-linearity shows the binding interaction that occurs between sodium salicylate and BSA.*

Manipulating Eq. (7) gives the formula for impurity removal [27]:

$$\text{Removal } \%=1-\left[e^{TN}\right]^{-1} \qquad (9)$$

Thus, at 100% transmission, only 4.5 diavolumes are needed achieve 99% impurity removal. At 50% transmission, however, approximately 9.1 diavolumes are needed for the same purity. T values in Figure 7(a) are for small molecules not interacting with the solute in the reservoir or the gel layer and thus T ~ 1. Manipulating Eq. (7) further, the concentration of the impurity leaving the permeate line is given by:

$$\ln=\left(\frac{C(N)_{impurity,permeate}}{C_{impurity,feed,initial}}\right)=-TN(t)+\ln T \qquad (10)$$

where $C_{impurity,\,permeate}$ is the impurity concentration in the permeate, $C_{impurity,\,feed,\,initial}$ is the initial impurity concentration on the process side, T is the transmission coefficient, and N is the number of diavolumes.

Figure 7(a) shows the control experiment where salicylate is diafiltered out of solution with no BSA present and closely follows the standard exponential decay from Eq. (7). The transmission coefficient is determined to be T = 1.05, which is within experimental uncertainty to the expected value of 1 for a permeable solute being diafiltered in the absence of interactions. The data in Figure 7(b) show the concentration of salicylate during diafiltration from a BSA/salicylate mixture. The data do not fit an exponential curve and therefore a single transmission coefficient cannot be derived. The reason for this deviation is that there is a binding interaction between BSA and sodium salicylate that slows the rate of removal of sodium salicylate.[28] As a result, many additional diavolumes are needed to remove the desired fraction of the impurity.[27,29] It is possible to model the separation when the impurity is binding to the retained solute, but that is beyond the scope of an undergraduate laboratory experiment. Note that fluorescein can also be used as the impurity. It gives a visual impression of the amount of purification, but it will interact even more strongly with BSA than does the salicylate.

CONCLUDING REMARKS ON IMPLEMENTING THE EXPERIMENT AND STUDENT RESPONSE

The experiment is in its second semester of operation. It has been well received by the students, since 40% of our students are in our "bio" engineering track and they are motivated by experiments they feel are relevant to their interests. The aspect of optimization means that there is not one correct result for the experiment. The onset of membrane fouling by gel layer formation depends on the operating conditions chosen by the students and the care in preparing the initial BSA solution. Therefore, the results for each group are different. It has been interesting reading the laboratory reports because the groups have emphasized different aspects of the experiment depending on the observations they found most engaging. This experiment is more open-ended than many in the laboratory. Some students find this disconcerting, because they want a simple recipe to follow, but the good students stand out. One group looked in the literature for binding constants between BSA and solutes and developed a model of equilibrium binding effects on diafiltration. Another group found convection/diffusion models for polarization at membrane surfaces and data on the osmotic pressure of BSA solutions. They modeled the concentration at the membrane surface and calculated the reduction in TMP due to the osmotic pressure effect. The grades on this experiment have been consistently higher than the grades on other experiments in the lab. This reflects the engagement of the students in experiment and evaluation of the results. The experiment requires a good teaching assistant to help the students get started on the Spectrum Labs software, and to provide oversight to ensure that the students don't irreversibly damage the membranes. The base cleaning of the membranes between lab periods is somewhat inconvenient, but necessary to obtain reproducible results.

Safety in the laboratory

Safety is given the highest priority in the undergraduate core lab course. All students are required to take a 1-day course run by the Department of Environmental Health and Safety at Princeton University that covers the basics of laboratory safety such as chemical handling and disposal, use of personal protective equipment, and emergency procedures. In addition we begin each lab period with a "safety moment" (1-2 minutes) where we ask a safety question to a group: "What are safety concerns about your (experiment, emergency shutdown procedures, etc.)?", or "What do you do in (various emergency scenarios)?" Students are required to wear personal protective equipment at all times when in the laboratory. In this experiment, the chemicals used are relatively safe and disposed according to EH&S guidelines. Students wear a face shield, lab coat, and gloves when handling acids and bases used for membrane cleaning and pH adjustment. In addition, the fluid pressures are relatively low.

We can provide copies of the experimental handouts we give the students for this experiment. Please send an email to Prof. Robert Prud'homme (prudhomm@princeton.edu).

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