Optimizing Membrane Separations of Two-Component Protein Solutions

Problem Presented by

Skand Saksena
Genentech Corporation

Workshop contributors

Georges Belfort, Ron Buckmire, Don Cohen,
David A. Edwards, and Dale Larson.

Section 1: Introduction

Single-protein solutions are often needed during the production of pharmaceuticals. However, with current technology, directly producing such a solution is virtually impossible. Therefore, a standard industrial goal is to separate and prepare single-protein solutions from mixtures of multi-component solutions. Optimally, whatever process is used should achieve high *purification* and high *yield*. Total purification (the highest value of some measure of the purity) means that the solution prepared for distribution and use contains only the single protein of interest. Similarly, yield is a measure of the amount of the single protein extracted from the multi-component solution. It is easy to show that maximum purification and maximum yield cannot be obtained. Thus, an optimal strategy will necessarily involve trade-offs. Specifying an objective function to be maximized in any generic situation is impractical; the qualities of each protein dictate the utility values associated with purity and yield.

At Genentech Corporation, a filtration process is used, a schematic of which is shown in exhibit A. The solution to be filtered is recycled through the system, and additional solution is added to keep the volume on the retentate side constant. The Genentech representative presented data and observations for the membrane separation of two-component protein solutions. Two different basic situations are encountered, depending upon the length of time the process is run. As described in section 6, the filters become fouled. Initial fouling (within a few seconds) accounts for most of the filter degradation. After this initial stage, fouling proceeds at a much slower rate. Thus, over short periods, where the filter degradation is small, the filter performance can be assumed constant. It is for this case that we attempted to model the data and experimental results from Genentech; the fruits of these efforts are presented in sections 2-5. Genentech is also interested in longer runs where filter performance will change; we address this situation in section 6.

It is straightforward to model the situation in exhibit A in either case described above. Even with filter fouling, the modeling is not difficult provided that the fouling mechanism is well-understood (either phenomenologically or physically). As a result of the study of the production process at Genentech, various measures of purity and yield have evolved. These are used here although there appear to be more natural dimensionless parameters in the system. Some information is derived relating the measures of purity and yield to the other parameters in the problem. Since both maximum purity and maximum yield cannot be simultaneously achieved, knowledge of dependence of these quantities on all other parameters allows the selection of various optimal separation strategies.

Section 2: Mass Balance

We wish to obtain expressions for the sieving coefficients by using a mass balance across the membrane. A typical experimental apparatus is shown in figure 2.1.

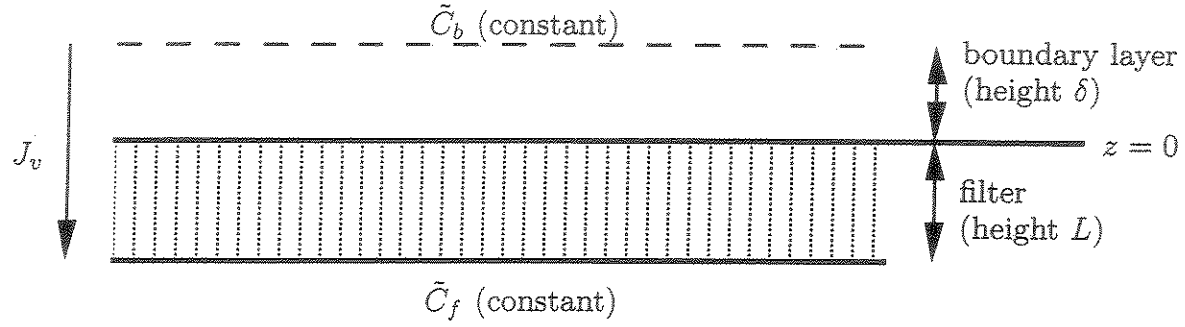

Figure 2.1. Experimental apparatus.

We hypothesize that the concentration changes only in a thin boundary layer near the filter on the retentate side. We denote this region by $0 < z < \delta$, where $z = 0$ denotes the position of the filter. In this region, there are two components to the flux $\mathcal{N}$ in the z-direction (that is, normal to the surface of the filter): a diffusive part and a convective part:

$$\mathcal{N}_{r,j} = -J_v \tilde{C}_{r,j} - D_j \frac{d\tilde{C}_{r,j}}{dz}, \tag{2.1}$$

where J_v is the flux of solvent through the membrane given in units of velocity, $\tilde{C}_{r,j}$ is the concentration of protein j in the thin boundary layer near the membrane, and D_j is the diffusivity of the protein in the fluid. Here we use a negative sign in front of J_v since we take J_v to be positive even though the velocity is acting downward. In practice, J_v is adjusted by changing the pressure drop across the membrane. However, the permeate rate can also be affected by the shear rate of the flow parallel to the membrane [1].

In the filtrate, we assume that the concentration is maintained at a constant value, and hence there is only a convective flux:

$$\mathcal{N}_{f,j} = -J_v \tilde{C}_{f,j}. \tag{2.2}$$

These two fluxes must be equal everywhere in z, so we have

$$J_v C_{f,j} = J_v C_{r,j} + D_j \frac{dC_{r,j}}{dz}, \tag{2.3}$$

where we have introduced the following scaling:

$$C_j = \frac{\tilde{C}_j}{\tilde{C}_{i,j}},$$

where $C_{i,j}$ is the initial concentration of protein j in the retentate.

At $z = \delta$, we obtain the boundary condition

$$C_{r,j}(\delta) = C_{b,j}, \tag{2.4}$$

where $C_{b,j}$ is the bulk concentration in the retentate of protein j. In the context of a true boundary layer analysis, (2.4) would become the matching condition for the layer solution. Solving (2.3) subject to (2.4), we have the following:

$$C_{r,j} = C_{f,j} + (C_{b,j} - C_{f,j}) \exp\left[\frac{J_v(\delta - z)}{D_j}\right]. \tag{2.5}$$

Let $C_{w,j}$ be the concentration of protein j in the boundary layer at $z = 0^+$, sometimes called the *wall concentration.* Then we have

$$C_{w,j} = C_{f,j} + (C_{b,j} - C_{f,j}) \exp\left(\frac{J_v \delta}{D_j}\right). \tag{2.6}$$

At this stage we introduce the *actual* and *observed sieving coefficients* $S_{a,j}$ and $S_{o,j}$, defined by

$$S_{a,j} = \frac{C_{f,j}}{C_{w,j}}, \qquad S_{o,j} = \frac{C_{f,j}}{C_{b,j}}. \tag{2.7}$$

$S_{a,j}$ is called the actual sieving coefficient since it measures the actual proportion of protein which makes it from one side of the membrane to the other. However, one cannot measure $C_{w,j}$, since the boundary layer near the membrane is so thin. Therefore, the best one can measure is $C_{b,j}$, the bulk retentate concentration, and hence one can calculate only the observed sieving coefficient $S_{o,j}$. Since $C_{b,j} < C_{w,j}$, we have that $S_{o,j} > S_{a,j}$.

Rearranging (2.6) using (2.7), we have the following:

$$S_{o,j} = \frac{S_{a,j}}{S_{a,j} + (1 - S_{a,j}) \exp(-J_v/k_j)}, \qquad k_j = \frac{D_j}{\delta}. \tag{2.8}$$

Here the k_j are *mass transfer coefficients*, which have units of velocity. These measure the effective transfer of mass through the membrane, which can be changed by adjusting δ. This is possible since the boundary layer width varies with the cross-stream velocity in the bulk flow in the retentate. It is our eventual goal to track relevant quantities in the problem as functions of the k_j.

Next we solve the equation inside the filtering membrane, which we denote as the region $-L < z < 0$. Here each of the terms in the flux is modified by a constant of proportionality, so we have

$$N_{m,j} = -K_{c,j} J_v C_{m,j} - K_{d,j} D_j \frac{dC_{m,j}}{dz}, \tag{2.9}$$

where the subscript m refers to the membrane and where $K_{c,j}$ and $K_{d,j}$ are constants. In practice, these constants are unknown and unmeasurable. We suppose that there is

some partition coefficient ϕ corresponding to the filter. This coefficient basically acts like a porosity, since the concentration inside the membrane is smaller than the concentration outside due to the presence of pores, which reduce the volume in which the proteins can reside. Therefore, we see that at $z = -L$, we have the following boundary condition:

$$C_{m,j}(-L) = \phi C_{f,j}. \tag{2.10}$$

Equation (2.9) must also match the flux at the filter given by (2.2), so we have

$$-\frac{dC_{m,j}}{dz} - \frac{K_{c,j}J_v}{K_{d,j}D_j}C_{m,j} = -\frac{J_v}{K_{d,j}D_j}C_{f,j}$$

$$C_{m,j} = \frac{C_{f,j}}{K_{c,j}} + \left(\phi C_{f,j} - \frac{C_{f,j}}{K_{c,j}}\right)\exp\left[-\frac{K_{c,j}J_v(z+L)}{K_{d,j}D_j}\right]. \tag{2.11}$$

At $z = 0^-$, we see that the concentration in the membrane must be the product of the partition coefficient and the wall concentration, so we have the following boundary condition:

$$C_{m,j}(0^-) = \phi C_{w,j}. \tag{2.12}$$

Using (2.7) and (2.11) in (2.12) yields

$$S_{a,j} = \frac{S_{\infty,j}\exp(A_jJ_v)}{\exp(A_jJ_v) + S_{\infty,j} - 1}, \qquad A_j = \frac{K_{c,j}L}{K_{d,j}D_j}, \qquad S_{\infty,j} = \phi K_{c,j}. \tag{2.13}$$

Since one cannot measure the constants K with any degree of accuracy, the values of A can only be estimated.

We may combine equations (2.8) and (2.13) to yield

$$S_{o,j} = \frac{1}{1 + \gamma_j[1 - \exp(-A_jJ_v)]\exp(-J_v/k_j)}, \qquad \gamma_j = \frac{1 - S_{\infty,j}}{S_{\infty,j}}. \tag{2.14}$$

Section 3: Yield and Purity

Now that we have obtained expressions for our sieving coefficients, we wish to relate them to various experimental quantities, such as the yield and purity of our filtrate. In the retentate, the concentration varies only in a small region near the filter, as described in section 2. Tracking the mass balance across the membrane, we have

$$-V_b \frac{d\tilde{C}_{b,j}}{d\tilde{t}} = J_v \mathcal{A}_m \tilde{C}_{f,j}, \tag{3.1}$$

where $\mathcal{A}_m$ is the area of the membrane, and V_b is the volume of the bluk flow in the retentate, which is kept constant by addition of pure solvent through a feed stream, as shown in exhibit A.

We introduce the following nondimensionalization:

$$N = \frac{J_v \mathcal{A}_m \tilde{t}}{V_b}. \tag{3.2}$$

N is called the *diavolume*, and it basically measures the proportion of solvent pushed through the filter as compared to the bulk volume. Using (3.2) in (3.1), we obtain

$$-\frac{dC_{b,j}}{dN} = C_{f,j}. \tag{3.3}$$

We note from equations (2.13) and (2.14) that $S_{a,j}$ and $S_{o,j}$ are independent of time. Using (2.7) and the fact that the sieving coefficients are constant, we have the following:

$$\begin{aligned} \frac{dC_{b,j}}{dN} &= -S_{o,j} C_{b,j} \\ C_{b,j} &= \exp(-N S_{o,j}). \end{aligned} \tag{3.4}$$

If one wished, one could redefine N to include the $S_{o,j}$, thus making the exponential in (3.4) simpler. However, for the purposes of the optimization we wish to do, (3.4) is more attractive.

The overall mass balance for each protein for the entire system is given by

$$V_b C_{i,j} = V_b \tilde{C}_{b,j} + \int_0^{\tilde{t}} \tilde{C}_{f,j}(\tilde{t})\, d\tilde{V}_f(\tilde{t}). \tag{3.5}$$

The left-hand side of (3.5) is the initial mass; the right-hand side is the sum of the mass in the retentate at time $\tilde{t}$ and the mass in the filtrate at time $\tilde{t}$. Here $\tilde{V}_f$ is the volume of filtrate at time $\tilde{t}$, which we normalize by V_b to yield

$$1 = C_{b,j} + \int_0^N C_{f,j}(N)\, dV_f(N). \tag{3.6}$$

Without loss of generality, we select protein 1 as the one which we would like to be filtered out of the retentate. Therefore, we define the integral in (3.6) for $j = 1$ to be the *yield of protein 1 in filtrate*, denoted by Y_1. Using (3.4) in (3.6), we obtain

$$Y_1 = 1 - \exp(-NS_{o,1}). \tag{3.7}$$

Similarly, protein 2 is the one we would like to stay in the retentate. Therefore, we define the first term in the right-hand side of (3.6) for $j = 2$ to be the *yield of protein 2 in retentate*, denoted by Y_2. Using (3.4), we have

$$Y_2 = \exp(-NS_{o,2}). \tag{3.8}$$

The purification factor P_1 for protein 1 in the filtrate is then defined by the ratio of Y_1 to the yield of protein 2 *in the filtrate*, which is simply $1 - Y_2$:

$$P_1 = \frac{Y_1}{1 - Y_2} = \frac{1 - \exp(-NS_{o,1})}{1 - \exp(-NS_{o,2})}. \tag{3.9}$$

Similarly, the purification factor P_2 for protein 2 in the retenate is defined by

$$P_2 = \frac{Y_2}{1 - Y_1} = \frac{\exp(-NS_{o,2})}{\exp(-NS_{o,1})}. \tag{3.10}$$

Since we have chosen protein 1 to be the one that is most easily filtered, we see that if we define

$$\Delta S \equiv S_{o,1} - S_{o,2}, \tag{3.11}$$

we may expect that $\Delta S > 0$. However, in section 5 we will obtain the rather surprising result that this is not always the case. For now, we note that a ΔS near 1 would mean the filter excludes nearly all of protein 2 while filtering nearly all of protein 1. Using (3.11), it can easily be seen that (3.10) may be written as

$$P_2 = \exp(N\Delta S). \tag{3.12}$$

In addition, we see that (3.9) becomes

$$P_1 = \frac{Y_1}{1 + (Y_1 - 1)\exp(NS_{o,1} - NS_{o,2})} = \frac{Y_1}{1 + (Y_1 - 1)\exp(N\Delta S)}. \tag{3.13}$$

The *selectivity* ψ is given by the ratio of the sieving coefficients:

$$\psi = \frac{S_{o,1}}{S_{o,2}}. \tag{3.14}$$

Therefore, a high value of ψ corresponds to a membrane which excludes much of protein 2 while filtering much of protein 1. Using (3.14), it can be easily seen that (3.10) may be written as

$$P_2 = \frac{Y_2}{Y_2^{S_{o,1}/S_{o,2}}} = Y_2^{1-\psi}. \tag{3.15}$$

Also, we see that (3.9) becomes

$$P_1 = \frac{Y_1}{1-(1-Y_1)^{S_{o,2}/S_{o,1}}} = \frac{Y_1}{1-(1-Y_1)^{1/\psi}}. \tag{3.16}$$

Therefore, depending on what we wish to use for our set of parameters, we may use (3.9) and (3.10), (3.12) and (3.13), or (3.15) and (3.16).

For completeness, we use (2.14) in (3.14) to construct a more useful version of ψ:

$$\psi = \frac{1+\gamma_2[1-\exp(-A_2 J_v)]\exp(-J_v/k_2)}{1+\gamma_1[1-\exp(-A_1 J_v)]\exp(-J_v/k_1)}. \tag{3.17}$$

Section 4: Selectivity

Now we endeavor to find extrema for our functions. The method is as follows: we note from (3.17) that ψ is a function of J_v only. We know from our P-Y curves (see exhibit B) that a higher value of ψ induces a higher yield, so we maximize ψ with respect to J_v. The other parameter is now considered to be $N\Delta S$. However, we note that ΔS depends only on J_v. Therefore, we see from (3.2) that we may adjust $\tilde{t}$, the length of the run, to obtain a value of $N\Delta S$ which achieves a desirable balance between the yield and the purification factor.

As an aside, we find the maximum of $S_{o,j}$ with respect to J_v. We begin with $S_{o,2}$:

$$S_{o,2} = \frac{1}{1 + \gamma_2[1 - \exp(-A_2 J_v)]\exp(-J_v/k_2)}. \tag{4.1}$$

For simplicity, we define the following variables:

$$x = \frac{J_v}{k_2}, \qquad \beta_j = A_j k_2, \qquad \lambda = \frac{k_2}{k_1} = \frac{D_2}{D_1}. \tag{4.2}$$

We note that though k_1 and k_2 may be varied since δ may change as a result of changes in the experimental setup, λ must remain constant for any bulk mixture.

It is believed that $x = O(1)$ in this problem. Using (4.2) in (4.1), we have

$$S_{o,2} = \frac{1}{1 + \gamma_2(1 - e^{-\beta_2 x})e^{-x}}, \tag{4.3}$$

$$\frac{dS_{o,2}}{dx} = \frac{\gamma_2[1 - (1 + \beta_2)e^{-\beta_2 x}]e^{-x}}{[1 + \gamma_2(1 - e^{-\beta_2 x})e^{-x}]^2}. \tag{4.4}$$

Setting (4.4) equal to zero, we obtain

$$x = \frac{\log(1 + \beta_2)}{\beta_2}, \tag{4.5}$$

$$J_v = \frac{\log(k_2 A_2 + 1)}{A_2}. \tag{4.6}$$

This is a slight correction to the calculation by Saksena on the handouts. However, we see that if β_2 is small (which is true; see Appendix), then we have

$$x \sim 1, \tag{4.7a}$$

$$J_v \sim k_2, \tag{4.7b}$$

as claimed on the handouts.

Since the problem is symmetric, we see that when we maximize $S_{o,1}$ with respect to J_v, we have the following:

$$J_v = \frac{\log(k_1 A_1 + 1)}{A_1}, \tag{4.8a}$$

$$x = \frac{\log(1 + \beta_1/\lambda)}{\beta_1}. \tag{4.8b}$$

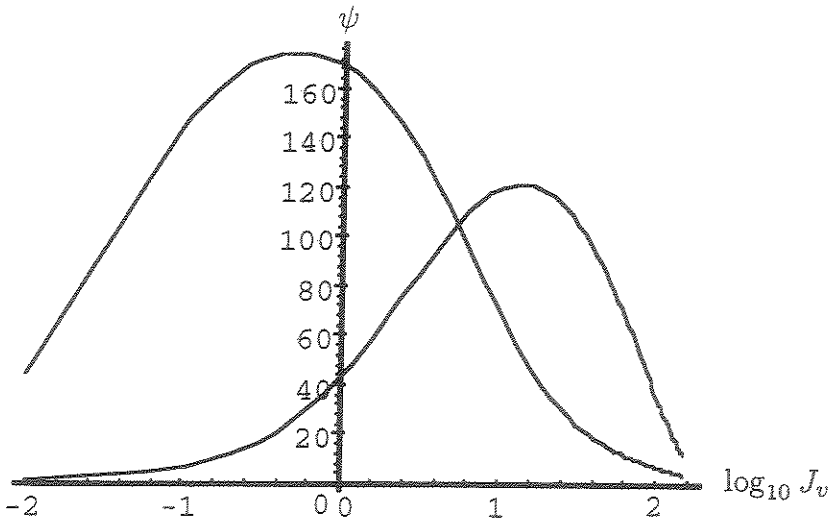

Figure 4.1. ψ *vs.* $\log_{10} J_v$ with $\gamma_1 = 999$, $\gamma_2 = 2 \times 10^4$, $\lambda = 5/7$. First curve: $\beta_1 = 6.94 \times 10^{-1}$, $\beta_2 = 6.94$. Second curve: $\beta_1 = 6.94 \times 10^{-3}$, $\beta_2 = 6.94 \times 10^{-2}$.

Next we try to solve the problem at hand: namely, maximizing ψ with respect to J_v. Using (4.2) in (3.17), we see that we have

$$\psi = \frac{1 + \gamma_2(1 - e^{-\beta_2 x})e^{-x}}{1 + \gamma_1(1 - e^{-\beta_1 x})e^{-\lambda x}}. \tag{4.9}$$

Using the numbers in the Appendix, we plotted an exact computation of ψ as a function of various parameters. Figure 4.1 shows the results as ψ *vs.* $\log_{10} J_v$, as in the Genentech graph. (Here J_v is measured in LMH, which are $\mathrm{L}/(\mathrm{m}^2 \cdot \mathrm{h})$.) Figure 4.2 shows the results as ψ *vs.* x. Note that the position of the maximum is highly sensitive to changes in the β_j, as can be expected since they are exponents in the expression for ψ.

We begin by calculating the derivative of (4.9) with respect to x:

$$\frac{d\psi}{dx} = \frac{\gamma_1[1 - (\beta + 1)e^{-\beta_2 x}]e^{-x}}{1 + \gamma_1(1 - e^{-\beta_1 x})e^{-\lambda x}} - \frac{\gamma_1[\lambda - (\beta_1 + \lambda)e^{-\beta_1 x}]e^{-\lambda x}[1 + \gamma_2(1 - e^{-\beta_2 x})e^{-x}]}{[1 + \gamma_1(1 - e^{-\beta_1 x})e^{-\lambda x}]^2}.$$

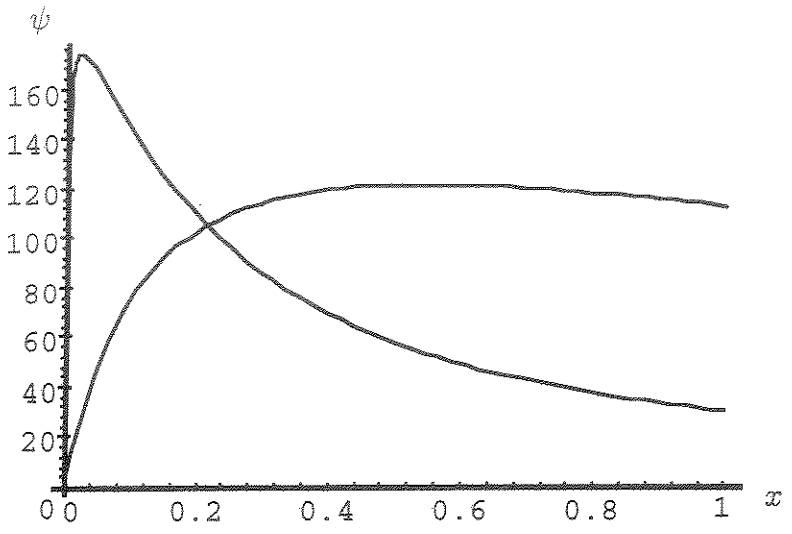

Figure 4.2. ψ *vs.* x with $\gamma_1 = 999$, $\gamma_2 = 2\times10^4$, $\lambda = 5/7$. First curve: $\beta_1 = 6.94\times10^{-1}$, $\beta_2 = 6.94$. Second curve: $\beta_1 = 6.94 \times 10^{-3}$, $\beta_2 = 6.94 \times 10^{-2}$.

Setting this expression equal to zero, we have

$$\gamma_1[\lambda-(\beta_1+\lambda)e^{-\beta_1 x}][1+\gamma_2(1-e^{-\beta_2 x})e^{-x}] = \gamma_2 e^{-x}[1-(\beta_2+1)e^{-\beta_2 x}][1+\gamma_1(1-e^{-\beta_1 x})e^{-\lambda x}]. \tag{4.10}$$

Equation (4.10) is a complicated transcendental equation for which we would like to find roots as various parameters become large and small. From the Appendix we see that the β_j are small, while λ is moderately sized. Therefore, we may asymptotically expand (4.10) with respect to the β_j to yield

$$\gamma_1[-\beta_1(1-\lambda x)](1+\gamma_2\beta_2 x e^{-x}) = \gamma_2 e^{-x}[-\beta_2(1-x)](1+\gamma_1\beta_1 x)e^{-\lambda x}.$$

However, we note from the Appendix that $\beta_2\gamma_2 \gg 1$, so we can neglect the 1 in the last set of parentheses on the left-hand side, and hence we have

$$\gamma_1\beta_1 x e^{-x}(\lambda x - 1) = e^{-x}(x-1)(1+\gamma_1\beta_1 x e^{-\lambda x}). \tag{4.11}$$

However, this is still not solvable in closed form. Therefore, if one has to use a computer anyway, it is probably best to solve (4.10) directly.

Figure 4.3 shows a computation of the value of J_v at which ψ attains a maximum *vs.* k_2 for the listed parameters. Actually, the x-value of the maximum decreases with increasing k_2, but since $J_v = xk_2$, the net effect is an increase in J_v. This increase is slightly slower than linear growth. Note that λ has been held fixed, as required.

Figure 4.4 shows a computation of the maximum attained value of ψ *vs.* k_2 for the listed parameters.

Another approach tried was to do a logarithmic expansion for our maximum x. Upon rearranging, equation (4.9) can be written as

$$\psi = \frac{e^x + \gamma_2(1-e^{-\beta_2 x})}{e^x + \gamma_1(1-e^{-\beta_1 x})e^{\epsilon x}}, \qquad \epsilon = 1-\lambda. \tag{4.12}$$

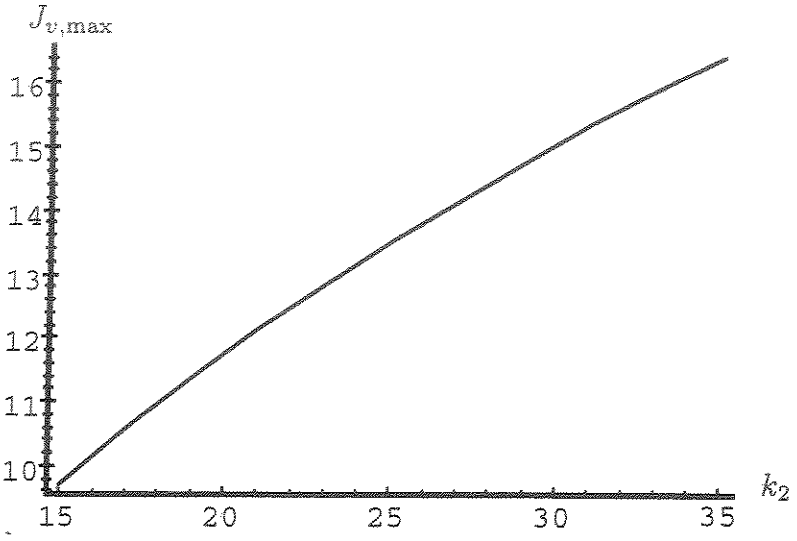

Figure 4.3. $J_{v,\max}$ *vs.* k_2 with $\gamma_1 = 999$, $\gamma_2 = 2 \times 10^4$, $\lambda = 5/7$, $A_1 = 10^3$, $A_2 = 10^4$.

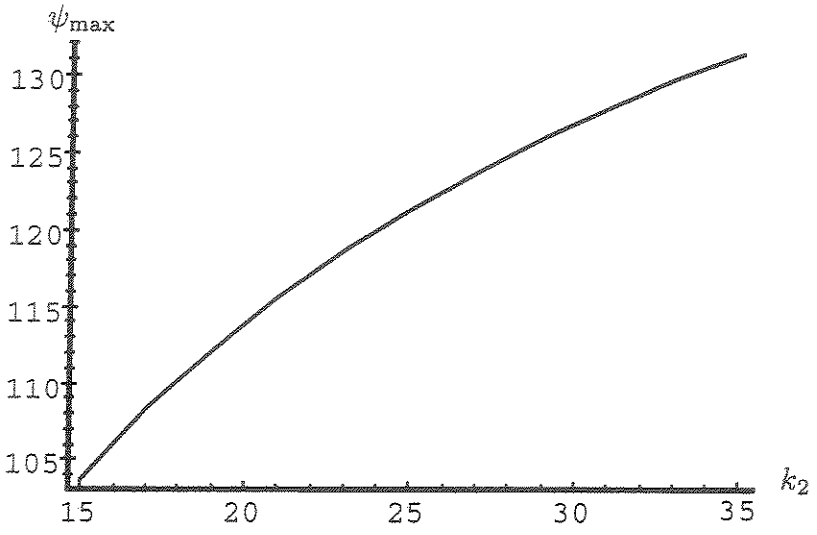

Figure 4.4. $\psi_{\max}$ *vs.* k_2 with $\gamma_1 = 999$, $\gamma_2 = 2 \times 10^4$, $\lambda = 5/7$, $A_1 = 10^3$, $A_2 = 10^4$.

Differentiating (4.12) and setting the result equal to zero yields

$$(e^x+\gamma_2\beta_2 e^{-\beta_2 x})[e^x+\gamma_1(1-e^{-\beta_1 x})e^{\epsilon x}] = \{e^x+\gamma_1[\epsilon+(\beta_1-\epsilon)e^{-\beta_1 x}]e^{\epsilon x}\}[e^x+\gamma_2(1-e^{-\beta_2 x})]. \tag{4.13}$$

We surmise from our calculations for the week that the value of $x_{\max}$ is highly sensitive to the parameters in the problem. Since λ is a fixed quantity, we see that ϵ is as well. Though $\epsilon = 2/7$, that is still small enough to perhaps get a reasonable feel for our solution. We try a solution of the form

$$x = a\log\epsilon + \log b + f(\epsilon), \qquad a < 0, \qquad f(\epsilon) \ll 1. \tag{4.14}$$

Using (4.14) in (4.13), we have, to leading orders

$$[b\epsilon^a(1+f)+o(1)][b\epsilon^a(1+f)+\gamma_1+o(1)] = [b\epsilon^a(1+f)+o(1)][b\epsilon^a(1+f)+\gamma_2+o(1)], \quad (4.15)$$

which has a solution only if $\gamma_1 = \gamma_2$. This constraint arises naturally from the high degree of symmetry in (4.12).

However, we note from the Appendix that $\beta_2 \ll \epsilon$. It is our hope that a solution of the form of (4.14) will work if one replaces ϵ by β_2 and then treats ϵ as an $O(1)$ quantity. Given the form of (4.10), we may have to try something more complicated as a gauge function: for instance, the W function, where

$$W(\epsilon^{-1})e^{W(\epsilon^{-1})} = \epsilon^{-1}, \qquad \epsilon \to 0. \quad (4.16)$$

Note that $W(\epsilon^{-1})$ diverges more slowly than the logarithm.

Another problem we ran into was the expansion of the exponentials in the fractions. For instance, consider the function $y = e^{-x} - e^{-2x}$, which has a maximum at $\log 2$, which for the purposes of this example can be considered to be small. But we may also write

$$y = \frac{1-e^{-x}}{e^x} \sim \frac{x}{1+x},$$

which has no extrema whatsoever. However, carrying terms to the next order, we have

$$y \sim \frac{x - x^2/2}{1 + x - x^2/2},$$

which does have an extremum. Therefore, a more careful expansion of the exponentials may be necessary.

Section 5: The Difference of the Sieving Coefficients

Another question posed to the group was how to maximize $J_v \Delta S$. This question naturally arises from the fact that $N\Delta S$, which is directly related to $J_v \Delta S$, is another important parameter in the problem. We see from (4.2) and (4.3) that we have

$$J_v \Delta S = \frac{k_2 x}{1 + \gamma_2 (1 - e^{-\beta_2 x}) e^{-x}} - \frac{k_2 x}{1 + \gamma_1 (1 - e^{-\beta_1 x}) e^{-\lambda x}}. \tag{5.1}$$

A graph of the behavior of this function for the *old* parameters from section 4 are plotted in Figure 5.1. (We used the old parameters since we wanted to replicate a Genentech graph. Once again, J_v is measured in units of LMH.)

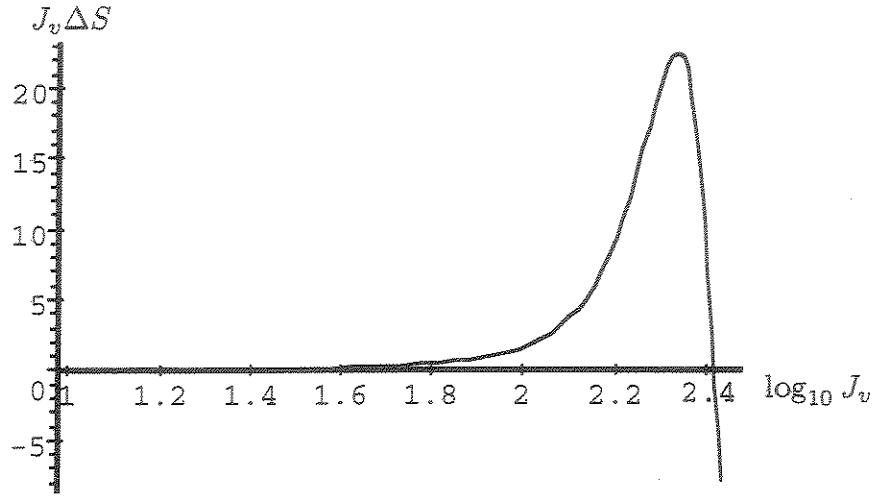

Figure 5.1. $J_v \Delta S$ *vs.* $\log_{10} J_v$ with $\gamma_1 = 999$, $\gamma_2 = 2 \times 10^4$, $\lambda = 5/7$, $\beta_1 = 6.94 \times 10^{-1}$, $\beta_2 = 6.94$.

Note that the maximum is sharper than before, and that the graph goes negative. This simply corresponds to $S_{o,2} > S_{o,1}$, which also corresponds to $\psi < 1$. Therefore, contrary to what we may expect, we may actually change the preferred protein for the filter by increasing the flux J_v.

Taking the derivative of (5.1) once again leads to an intractable mess. However, we may plot the solutions numerically, as shown in figures 5.2 and 5.3. Note that these graphs are the maxima using the new parameters from section 4, rather than the parameters which produced figure 5.1.

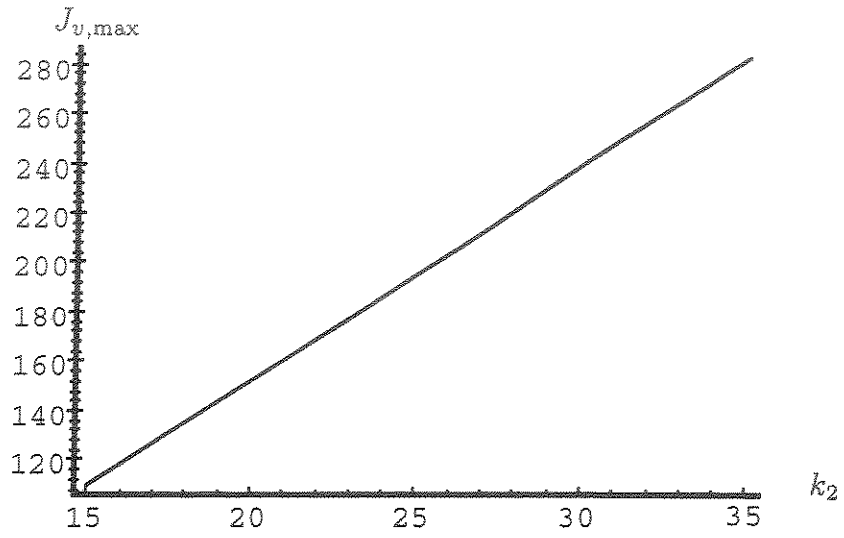

Figure 5.2. $J_{v,\max}$ *vs.* k_2 with $\gamma_1 = 999$, $\gamma_2 = 2 \times 10^4$, $\lambda = 5/7$, $A_1 = 10^3$, $A_2 = 10^4$.

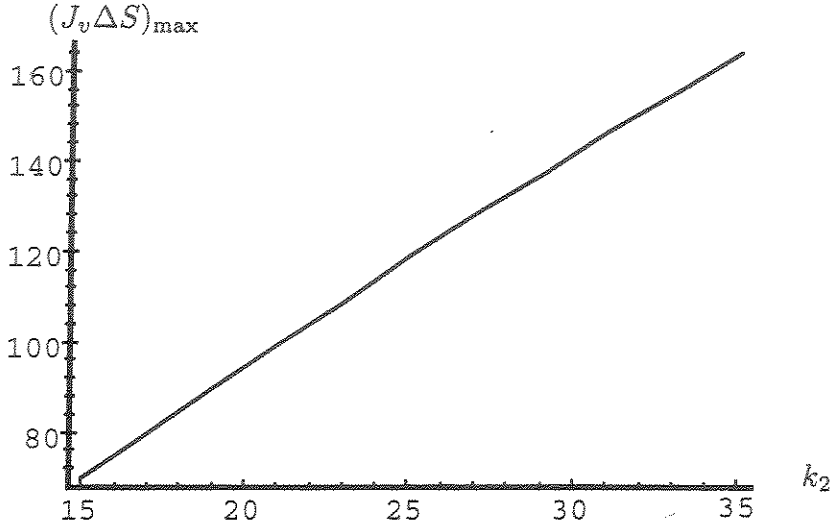

Figure 5.3. $(J_v \Delta S)_{\max}$ *vs.* k_2 with $\gamma_1 = 999$, $\gamma_2 = 2 \times 10^4$, $\lambda = 5/7$, $A_1 = 10^3$, $A_2 = 10^4$.

Section 6: Further Research

There are several possible areas in which further research could be pursued. First, using the full partial differential equations which hold in the retentate, membrane, and filtrate, one could rigorously establish the presence and width of the boundary layer directly above the filter surface. Once this has been completed, a careful derivation of the expressions for the concentration could be obtained. These would be preferable to the current mass balance equations since they can then be extended to include other effects. In addition, performing asymptotics on differential equations is more standard than performing them on transcendental ones.

An important additional effect in these systems is that of *fouling*, where protein molecules deposit on top of the filter, blocking pores and effectively reducing J_v. As the protein molecules accrete, J_v drops sharply, but then asymptotes to a steady state (see exhibit C). There are several ways of modeling this, each introducing a new level of complication.

At the most basic level, J_v can be modeled with an exponential decay curve. However, doing this eliminates the interpretation of many of our parameters, particularly the sieving coefficients, as constants. Therefore, the simple exponential form of our solutions in section 3 is eliminated. J_v can also be modeled as following a logistic decay curve, which would correspond to a chemical-reaction model for the site-occupation phenomenon. Lastly, a full reaction-diffusion equation could be used for the site occupation. This would incorporate the effect that often the depth of the deposition varies as one moves along the filter, with the deepest layer occurring near the recycling inlet.

In addition, Mark Hurwitz from Pall has some models for this type of process. These models indicate the effects of shear rate upon permeate rates [1], as well as models of fouling in cross-flow filtration [2].

Appendix

The values of the constants S_∞ are as follows:

$$S_{\infty,1} = 10^{-3}, \qquad S_{\infty,2} = 5 \times 10^{-5}. \tag{A.1}$$

From the definition in (2.13) it is clear that S_∞ is a function of the composition of the filter. However, it is unclear whether these values can change very much with changing experimental setups. Using (A.1) to compute the γ parameters, we have

$$\gamma_1 = \frac{1 - S_{\infty,1}}{S_{\infty,1}} = \frac{1 - 10^{-3}}{10^{-3}} = 999, \tag{A.2a}$$

$$\gamma_2 = \frac{1 - S_{\infty,2}}{S_{\infty,2}} = \frac{1 - 5 \times 10^{-5}}{5 \times 10^{-5}} = 2 \times 10^4. \tag{A.2b}$$

For the mass transfer coefficients, we have

$$k_1 = 35 \text{ LMH} = \frac{35 \text{ LMH} \cdot \text{m/s}}{3.6 \times 10^6 \text{ LMH}} = 9.72 \times 10^{-6} \text{ m/s}, \tag{A.3a}$$

$$k_2 = 25 \text{ LMH} = \frac{25 \text{ LMH} \cdot \text{m/s}}{3.6 \times 10^6 \text{ LMH}} = 6.94 \times 10^{-6} \text{ m/s}. \tag{A.3b}$$

For any given protein pair and experimental setup, these may be varied, but not independently: their ratio must remain constant:

$$\lambda = \frac{k_2}{k_1} = \frac{D_2}{D_1} = \frac{5}{7}. \tag{A.4}$$

The only way to change D_j would be to introduce a new solvent in which the proteins are dissolved.

In addition, we have the following values which relate to N:

$$V_b = 10^3 \text{ L} = 1 \text{ m}^3, \tag{A.5}$$

$$\mathcal{A}_m = 200\text{–}600 \text{ ft}^2 \times \frac{(0.305 \text{ m})^2}{\text{ft}^2} = 18.6\text{–}55.8 \text{ m}^2, \tag{A.6}$$

$$\tilde{t} = 3 \text{ h} = 1.08 \times 10^4 \text{ s}, \tag{A.7}$$

$$N = 50\text{–}150. \tag{A.8}$$

The problem is very sensitive to the values of the A_j. Unfortunately, it is unclear as to even the proper order-of-magnitude estimates for them. The new, better values are given by

$$A_1 = 10^3 \text{ s/m} = \frac{10^3 \text{ s/m} \cdot \text{m/s}}{3.6 \times 10^6 \text{ LMH}} = 2.78 \times 10^{-4} (\text{LMH})^{-1}, \tag{A.9a}$$

$$A_2 = 10^4 \text{ s/m} = \frac{10^3 \text{ s/m} \cdot \text{m/s}}{3.6 \times 10^6 \text{ LMH}} = 2.78 \times 10^{-3} (\text{LMH})^{-1}. \tag{A.9b}$$

Using the new values and computing our β parameters, we have

$$\beta_1 = A_1 k_2 = 6.94 \times 10^{-3}, \tag{A.10a}$$
$$\beta_2 = A_2 k_2 = 6.94 \times 10^{-2}. \tag{A.10b}$$

In addition, in general the following three statements are equivalent:

$$S_{\infty,1} > S_{\infty,2}, \tag{A.11a}$$
$$k_1 > k_2, \tag{A.11b}$$
$$A_1 < A_2. \tag{A.11c}$$

Since k_j is proportional to D_j, it is easy to see how (A.11b) could imply (A.11c), all other things remaining constant. However, (A.11a) opposes this trend, and we see from (A.1) and (A.4) that the ratio of the sieving coefficients is much larger than λ^{-1}. Therefore, we see that the change in $K_{d,j}$ must be large enough to counteract the change in the $K_{c,j}$ (and hence the $S_{\infty,j}$). This is reasonable, since the variation in the $S_{\infty,j}$ is large, and we would expect both $K_{d,j}$ and $K_{c,j}$ to vary over the same orders of magnitude.

The old values of A, now in disrepute, are

$$A_1 = 10^5 \text{ s/m} = \frac{10^3 \text{ s/m} \cdot \text{m/s}}{3.6 \times 10^6 \text{ LMH}} = 2.78 \times 10^{-2} (\text{LMH})^{-1}, \tag{A.12a}$$

$$A_2 = 10^6 \text{ s/m} = \frac{10^3 \text{ s/m} \cdot \text{m/s}}{3.6 \times 10^6 \text{ LMH}} = 2.78 \times 10^{-1} (\text{LMH})^{-1}. \tag{A.12b}$$

Using these old values and computing our β parameters, we have

$$\beta_1 = A_1 k_2 = 6.94 \times 10^{-1}, \tag{A.13a}$$
$$\beta_2 = A_2 k_2 = 6.94. \tag{A.13b}$$

Nomenclature

Variables and Parameters

Units are listed in terms of length (L), mass (M), or time (T). The equation number where a particular quantity first appears is listed. If the same variable appears with and without tildes, the variable with tildes has units, while the variable without tildes is dimensionless.

A: dimensionless constant (2.13).
$\mathcal{A}$: area, units L^2 (3.1).
$\tilde{C}$: concentration of protein, units M/L^3 (2.1).
D: diffusivity of protein through the solvent in the retentate, units L^2/T (2.1).
J: flux of solvent through the filter, expressed in units of velocity, units L/T (2.1).
k: mass transfer coefficient, defined as D/δ, units L/T (2.8).
K: coefficient which changes the components of the flux in the membrane, dimensionless (2.9).
L: width of the membrane, units L/T (2.10).
N: diavolume, which plays the role of a dimensionless time variable (3.2).
$\mathcal{N}$: mass flux, units M/L^2T (2.1).
P: purification factor, dimensionless (3.9).
S: sieving coefficient, dimensionless (2.7).
$\tilde{t}$: time, units T (3.1).
$\tilde{V}$: volume of bulk stream, units L^3 (3.1).
W: that function which satisfies $W(z)\exp[W(z)] = z$ (4.16).
x: scaled flux variable, dimensionless (4.2).
y: arbitrary function.
Y: yield factor, dimensionless (3.7).
z: height as measured from the top edge of the filter, units L (2.1).
β: dimensionless ratio, defined as Ak_2 (4.2).
γ: dimensionless ratio, defined as $(1 - S_\infty)/S_\infty$ (2.14).
δ: width of the boundary layer in the retentate above the membrane, units L.
ϵ: dimensionless parameter, defined as $1 - \lambda$ (4.12).
λ: dimensionless ratio, defined as A_2/A_1 (4.2).
ϕ: partition coefficient of the membrane, dimensionless (2.10).
ψ: selectivity of the membrane, dimensionless, defined as $\psi = S_{o,1}/S_{o,2}$ (3.14).

Other Notation

a: as a subscript, used to indicate the actual sieving coefficient (2.7).
b: as a subscript, used to indicate the concentration in the bulk feed stream (2.4).
c: as a subscript, used to indicate a coefficient of the convective term (2.9).
d: as a subscript, used to indicate a coefficient of the diffusive term (2.9).
f: as a subscript, used to indicate the filtrate (2.2).
i: as a subscript, used to indicate the initial state.
$j = 1, 2$: as a subscript, used to indicate either protein 1 or protein 2 (2.1).
m: as a subscript, used to indicate the membrane (2.9).
max: as a subscript, used to indicate a maximum value.
o: as a subscript, used to indicate the observed sieving coefficient (2.7).
r: as a subscript, denotes concentrations in the thin boundary layer in the retentate (2.1).
v: as a subscript, indicates units of velocity (2.1).
w: as a subscript, denotes the "wall concentration" measured at the top of the membrane filter (2.6).
Δ: denotes the difference of a quantity (3.11).
∞: as a subscript, used to indicate a characteristic value of the sieving coefficient (2.13).

References

[1] Cole, J., and J. Brantley. "Dynamic Membrane Filtration in Cell and Protein Production." *BioPharm*, 66–71 (1996).

[2] Hurwitz, M., and J. Brantley. To appear.

EXHIBIT A

MEMBRANE DIAFILTRATION FOR PROTEIN FRACTIONATION

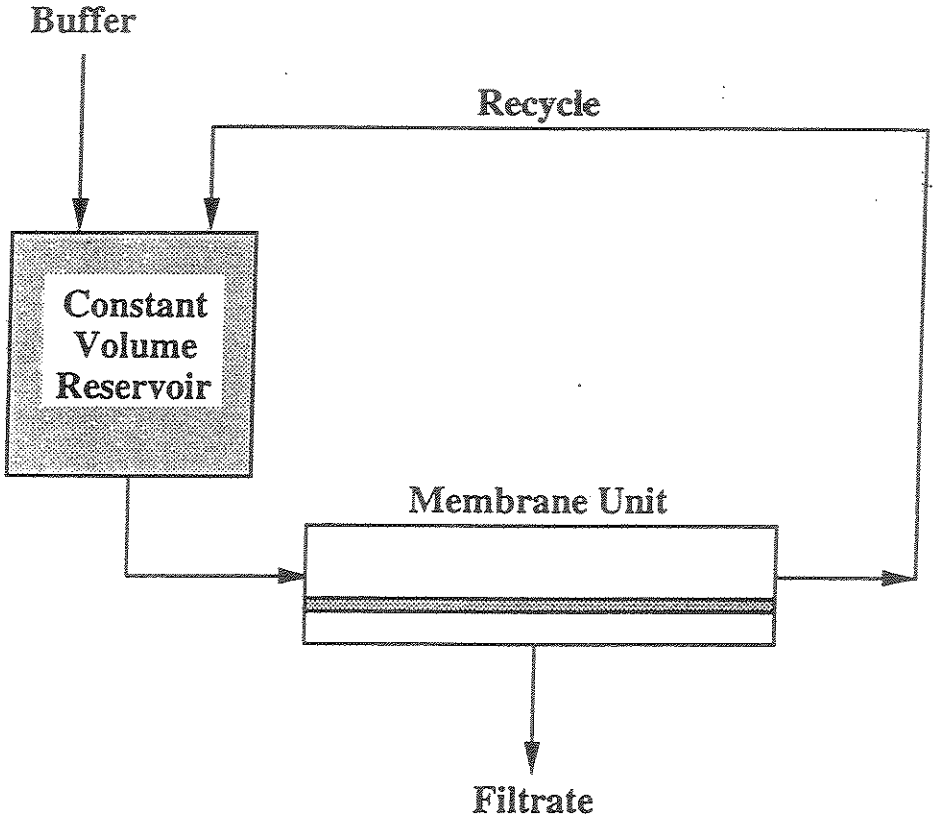

EXHIBIT B

PROCESS DIAGRAM FOR THE PRODUCT IN THE FILTRATE

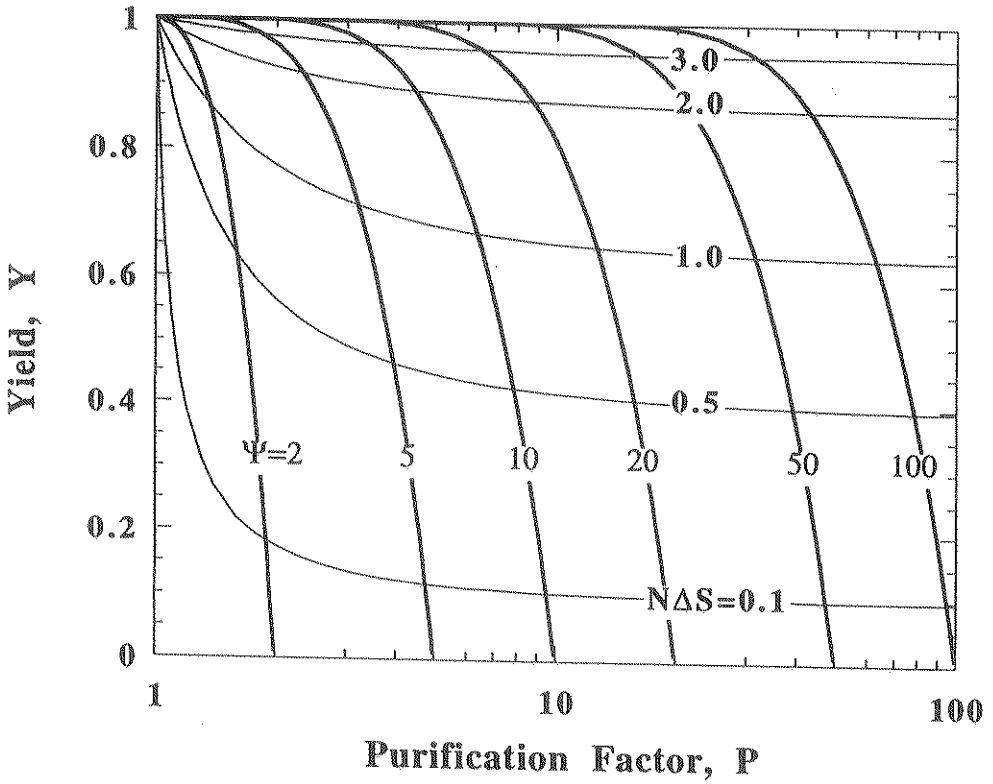

EXHIBIT C

DECAY OF ULTRAFILTRATE FLUX WITH TIME