Ultrafiltration

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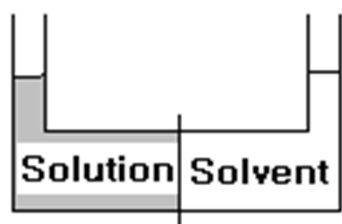
1 Basic Ideas

- Ultrafiltration is a effective separation method for proteins

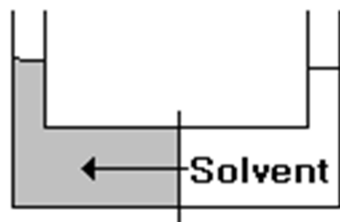
- Protein have two characteristics which are important for these separation

  - Large molecules
  - Conformation change with pH

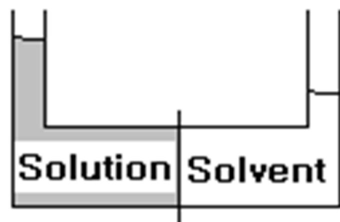
❖ Osmotic Pressure



- Macromolecule is uncharged
- Macromolecule can not pass through the membrane



- Solvent flows from right to left, diluting the macromolecular sol'n



- As the dilution takes place, the sol'n vol. increases and the level in the capillary rises

Figure 1. Osmosis pressure across a membrane. Solvent can diffuse across the membrane shown, but solute cannot.

Ideal state

$$\mu_2(pure) = \mu_2(solution)$$

$$\mu_2^0 + p^0\overline{V_2} = \mu_2^0 + p\overline{V_2} + RT\ln x_2$$

μ_2^0 : *chemical potential in STP*

$\overline{V_2}$: *partial molar volume of solvent*

x_2 : *mole fraction*

$$\Delta\Pi = p - p_0 = -\frac{RT}{\overline{\overline{V_2}}}\ln x_2$$

$\Delta\Pi$: *osmotic pressure*

If the macromolecular sol'n is dilute, then we can expand the logarithm in term of x_1

Dilute sol'n

$$\Delta\Pi = p - p_0 = -\frac{RT}{\overline{\overline{V_2}}}\ln x_2 = -\frac{RT}{\tilde{V}_2}\ln(1 - x_1)$$

$$\cong -\frac{RT}{\tilde{V}_2}(-x_1 - ....) \cong RTc_1$$: ***Van't Hoff's Law***

❖ Side chain of Proteins

- **Carboxylic acid (- COOH) : glutamic acid**
  in basic sol'n to form carboxylate (- COO^-) groups
- **Amine (- NH_2) : lysine**
  in acid sol'n to form ammonium (- $NH3^+$) groups
- **A function of the pH of the sol'n : the relative amount of these positive and negative charges**

- Low pH : more -NH_3^+ and -COOH, High pH : more -NH_2 and -COO^-

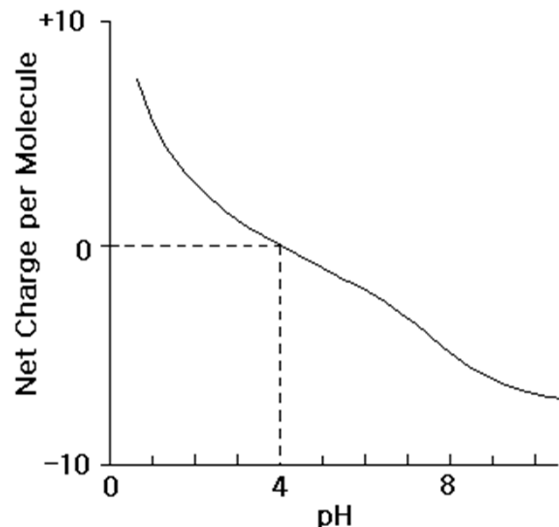


Figure 2. Charges on a protein.
At low pH, amine side chains are protonated to give a positive charge ; at high pH, carboxylic side chain ionizeto give a negative charge. The intermediate pH of no net charge is called the isoelectric point

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Table 1. Molecular weights and isoelectric pH values of Proteins

Protein	Source	Mol Wt.	Isoelectric Point
Salmin (protamin)	Salman specrm	5,600	12.0 ~ 12.4
Cytochrome C	Beef heart	15,600	9.7
Myoglobin	Muscle	17,200	7.0
Lactalbumin	Cow milk	17,400	5.1
Trypsin	Pancreatic juice	34,000	5.0 ~ 8.0
Pepsin	Pig stomach	35,500	2.75 ~ 3.0
Insulin	Pig pancreas	40,900	5.30 ~5.35
Lactoglobulin	Cow milk	41,800	4.5 ~ 5.5
Ovalbumin	Hen egg	43,800	4.84 ~4.90
Hemoglobin	Horse blood	66,700	6.79 ~6.83
Serum albumin	Horse blood	70,100	4.88
Serum globulin	Horse blood	167,000	5.4 ~ 5.5
Gelatins	Pig skin	10,000- 1000,000	4.8 ~ 4.85
Myogen	Muscle	150,000	6.2 ~ 6.4
Casein	Cow milk	375,000	4.6
Urease	Jack bean	480,000	5.0 ~ 5.1
Myosin	Miscle	1,000,000	6.2 ~ 6.6
Bushy stunt virus	Tomato	7,600,000	4.11

❖ Transport Equations

- **Ultrafiltration : the species transported - solvent**
  Chief force - pressure

⟶ **Ultrafiltration from membrane separations, and not from convectional filtration**

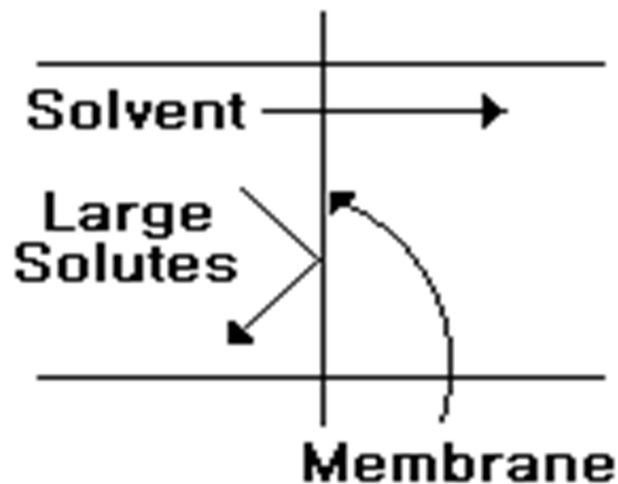

Figure 3. Ultrafiltration from a pressure difference.

Solvent velocity ∝ force on solvent

$$j_v = L_P \Delta P$$

j_v : *the volume of solvent per area per time*

L_P : *permeability*

$$v = \left(\frac{k}{l\mu}\right)\Delta P \quad \text{: Darcy's Law}$$

k : *the Darcy's law permeability*

l : *the bed of thickness*

Basic Eq'n for Ultrafiltration

$$j_v = L_P(\Delta P - \sigma \Delta \Pi)$$

σ : *reflection coefficient*

If the membrane rejects all solutes, then σ= 1 .

If the membrane passes both solvent and solute, then σ= 0

Chemical potential includes all forms of energy acting on the solute

$$\mu_1 = \mu_1^0 + k_B T \ln c_1 + \left(\frac{\pi}{6} d^3\right)(\rho_S - \rho) g z + \frac{z_1 \Im \phi}{\tilde{N}}$$

Reference energy for a single protein sphere

Free energy of mixing
If the sol'n is dilute, C is const. and the resulting term $k_B T \ln C$ is lumped into the μ_1^0.

The effect of gravitational force

The solute charge and the electrical potential

μ_1 : *the solute's chemical potential* =

$\longrightarrow$ *the partial molar Gibbs free energy*

μ : *viscosity of the solvent*

d : *the diameter of the solute sphere*

z_1 : *the solute charge*

ϕ : *the electrical potential*

1. Charged solute in the absence of any gravitatinal field

$$-v_1 = \left(\frac{k_B T}{3\pi\mu d}\right)\left(\frac{1 dc_1}{c_1 dz} + \frac{\pi}{6} d^3 \frac{(\rho_s - \rho)g}{k_B T} + \left(\frac{z_1 \Im}{RT}\right)\frac{d\phi}{dz}\right)$$

$\longrightarrow 0$ $\longrightarrow 0$

Gravitational force = 0 **The solute charge = 0**

$$-c_1 v_1 = \left(\frac{k_B T}{3\pi\mu d}\right)\left(\frac{dc_1}{dz}\right) = -j_1 = D\frac{dc_1}{dz}$$ **:** ***Fick's Law***

j_1 : *the diffusion flux*

D : *the diffusion coefficient, with* dim*ensions of area per time*

2. The concentration of an uncharged solute is uniform

$$-v_1 = \left(\frac{k_B T}{3\pi\mu d}\right)\left(\frac{1}{c_1}\frac{dc_1}{dz} + \frac{\pi}{6}d^3\frac{(\rho_s-\rho)g}{k_B T} + \left(\frac{z_1\Im}{RT}\right)\frac{d\phi}{dz}\right)$$

$\frac{1}{c_1}\frac{dc_1}{dz} \rightarrow 0$, $\left(\frac{z_1\Im}{RT}\right)\frac{d\phi}{dz} \rightarrow 0$

$$-v_1 = \left(\frac{d^2(\rho_s-\rho)}{18\mu}\right)g = \left(\frac{d^2(\rho_s-\rho)}{18\mu}\right)\omega^2 r = s\omega^2 r$$

g : *the acceleration*

$\omega^2 r$: *the ultracentrifuge*

$$s = \left(\frac{D}{k_B T}\right)\left(\frac{\pi}{6}d^3\right)(\rho_S-\rho)$$ ***: Sedimentation coefficient, with dimensions of time***

3. The concentration differences and gravitational forces are minor

$$-v_1 = \left(\frac{k_B T}{3\pi\mu d}\right)\left(\frac{1 dc_1}{c_1 dz} + \frac{\pi}{6} d^3 \frac{(\rho_s - \rho) g}{k_B T} + \left(\frac{z_1 \Im}{RT}\right)\frac{d\phi}{dz}\right)$$

$$\frac{1 dc_1}{c_1 dz} \rightarrow 0 \qquad \frac{\pi}{6} d^3 \frac{(\rho_s - \rho) g}{k_B T} \rightarrow 0$$

$$-v_1 = \left(\frac{z_1 \Im}{3\pi\mu d}\right)\left(\frac{d\phi}{dz}\right) = D\left(\frac{z_1 \Im}{RT}\right)\left(\frac{d\phi}{dz}\right)$$

D : *the diffusion coefficient*

$$-v_1 = m\left(\frac{d\phi}{dz}\right)$$ **: *Engineering form***

m : *the mobility*

Table 2. Mobility Spectrum for Normal Human plasma Components

Component	Concentration (g / 100 ㎖)	Percent Total Solutes	Mobility m (10^5cm^2 /V sec)
Albumin	4.04	60	-5.9
α 1-globulin	0.31	5	-5.1
α 2-globulin	0.48	12	-4.1
β -globulin	0.81	12	-2. 8
Fibrinogen	0.34	5	-2.1
γ -globulin	0.74	11	-1.0

Example 1 Yeast Ultrafiltration

Ultrafiltration of a well stirred suspension containing 0.1 vol% yeast suspension gives a flux of 36 gal/ft² day under a pressure difference of 130 psi.

(a) What is the value of L_p ?

The yeast cells will have a very high molecular weight, so that their molar
Concentration and the resulting osmotic pressure will be small.
At the same time, this large size leads to high rejection, so σ= 1.
As a result from

$$j_v = L_P(\Delta P - \sigma\Delta\Pi), \quad \sigma\Delta\Pi \to 0$$

$$\frac{36gal}{ft^2 day} = L_P(130psi) \qquad \underline{L_p = 0.28gal \ / ft^2 day \ \ psi}$$

(b) What is the water velosity through the membrane?

To find the velocity through the membrane, we need only convert the units of the flux

$$v = j_v =$$

$$\frac{36gal}{ft^2 day}\frac{3758cm^3}{gal}\left(\frac{ft}{30.5cm}\right)^2\frac{day}{24\times3600\sec} = \underline{0.0017cm/\sec}$$

Example 2 The Transport of Ovalbumin

Imagine a solution of 25℃ containing 0.004g/cm^3 of ovalbumin, a protein of molecular weight 45,000. The solution is buffered at a pH of 3.5 and has the viscosity close to water, 8.9×10^3 g/cm sec. Under these conditions, the protein has a charge of +10, a diffusion coefficient of 7.8×10^{-7} cm^2/sec, and a sedimentation coefficient of 3.5×10^{-13} sec.

(a) Estimate the diameter of the protein.

The easiest way to estimate the protein's size is from the diffusion coefficient, as suggested by

$$D = \left(\frac{k_B T}{3\pi\mu d}\right) \quad , \quad 7.8\times10^{-7}\,\frac{cm^2}{\sec} = \frac{1.38\times10^{-16}(g\ cm^2/\sec^2 K)298K}{3\pi(0.0089g\ /cm\ \sec)d}$$

$$\underline{d = 62\,\mathring{A}}$$

The protein's diameter is about 20 times larger than that of a water molecule and about 200 times smaller than that of a bacterium.

(b) What are the flux and velocity when this protein diffuses from the solution across a 1 cm film into pure water?

The flux is found by integrating $-c_1 v_1 = \left(\frac{k_B T}{3\pi\mu d}\right)\left(\frac{dc_1}{dz}\right) = -j_1 = D\frac{dc_1}{dz}$ **across the film to find**

$$j_1 = \frac{D}{l}c_{10}$$

D : *the diffusion coefficient*

c_{10} : *the solution concentration*

$$j_1 = \left(\frac{7.8\times10^{-7}\ cm^2/\sec}{1\ cm}\right)\frac{0.004\ gm/cm^3}{45{,}000\,g/mol} = \underline{7\times10^{-14}\ mol/cm^2\sec}$$

The average velocity can be estimated either by integrating $-j_1 = D\frac{dc_1}{dz}$ **or by dividing the flux by the average conc. The latter is easier :**

$$v_1 = \frac{j_1}{c_{10}/2} = \underline{8\times10^{-7}\ cm/\sec}$$

(c) What is the protein's velocity under the influence of gravity?

The velocity in a gravitational field is found from $v_1 = s\omega^2 r$ **where the acceleration is the that due to gravity :**

$$v_1 = s\omega^2 r = 3.5\times10^{-13}\,\text{sec}\,\frac{980cm^2}{\text{sec}} = 0.003\times10^{-7}\,\frac{cm}{\text{sec}}$$

This velocity is much less than that due to diffusion. In fact, as we expect, the mixing due to diffusion (I.e., to Brownian motion) swamps any separation due to gravity. It is only in the ultracentrifuge, where the acceleration far exceeds that of gravity, where these effects become significant.
In the words of our students, "moles are worth more than gravity."

(d) What are the flux and velocity due to a force of 1 volt/cm?

The velocity under an electric field can be calculated from

$$-v_1 = \left(\frac{z_1\Im}{3\pi\mu d}\right)\left(\frac{d\phi}{dz}\right) = D\left(\frac{z_1\Im}{RT}\right)\left(\frac{d\phi}{dz}\right)$$

$$v_1 = D\left(\frac{z_1\Im}{RT}\right)\left(\frac{d\phi}{dz}\right) = \left(\frac{Dz_1\Im}{RT}\right)\frac{\Delta\phi}{l}$$

$$= \frac{7.8\times10^{-7}\ cm^2/\sec(+10)96{,}500\mathrm{C}/mol(1\mathrm{V})}{8.31\mathrm{J/mol\ K}\ (298\ \mathrm{K})\ \mathrm{C\ V/J}\ (1\ cm)}$$

$$= 3{,}000\times10^{-7}\ \frac{cm}{\sec}$$

In each second, the protein moves 100 diameters.
The flux can be found by multiplying the velocity times the conc.

$$j_1 = c_1 v_1 = 2.7\times10^{-11}\ \mathrm{mol}/cm^2\ \sec$$

The flux under an electric field is several hundred times that due to diffusion.
Again according to our students, "volts are worth more than moles"

In passing, we can use the data given to calculate the mobility, as suggested by

$$-v_1 = m\left(\frac{d\phi}{dz}\right) \text{ where } m = \frac{Dz_1\Im}{RT}$$

$$m = \frac{Dz_1\Im}{RT} = \frac{7.8\times10^{-7}\,cm/\sec(+10)96{,}500\text{C}/mol}{(8.31\text{C V}/mol\text{ K})\ \ 298\text{ K}}$$

$$= 3.0\times10^{-4}\,\frac{cm^2}{\sec\ \ \text{V}}$$

This value is highter than normal because of the large on the protein at this pH.

9. 2 Ultrafiltration

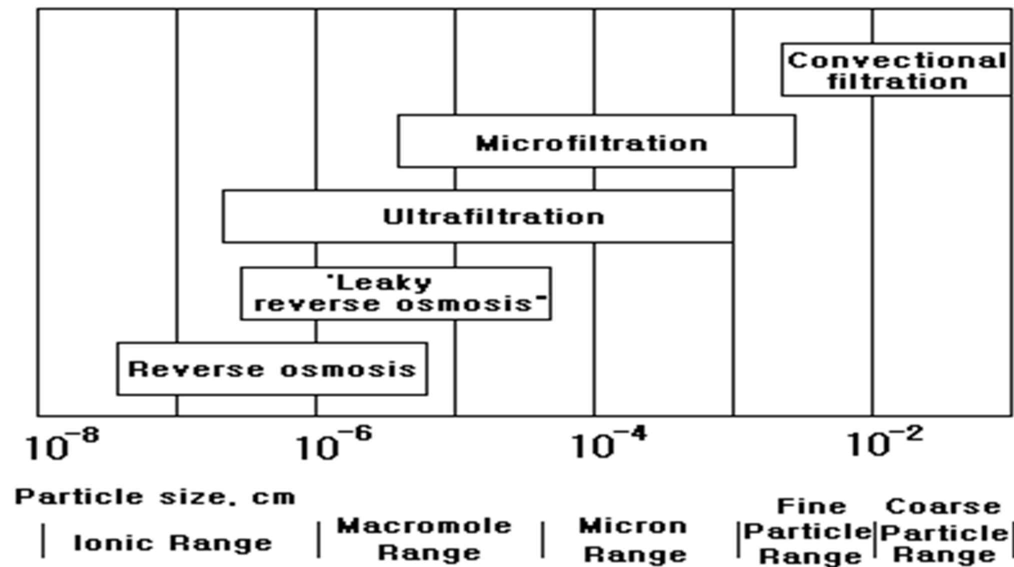

Figure 3.
Different types of ultrafiltration. These processes are most easily classified by the size of solutes being separated.
The difference between them are dominated by differences in membranes used
(After Lonsdale, *J. Memb. Sci.* **10** 81 (1982))

- ❖ **Ultrafiltration is a membrane process.**
- ❖ **Such a process depends on the ability of a permeable membrane to differentiate between solutes of different size.**
- ❖ **Three distinctive characteristics**
  - **Use a high cross flow**
  - **Dominated by the membrane**
  - **Depend on the membrane geometry in the actual equipment**

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❖ Crossflow

- Ultrafiltration almost always involves a large flow across the membrane surface, perpendicular to the flux through the membrane.
- When solid particles are being ultrafiltered, the cross flow minimizes the development of a filter cake which would retard the process
- When a marcromolecular sol'n is being ultrafiltered, the cross flow reduces marcromolecule accumulation near the membrane surface.

→ concentration polarization

❖ Membranes

- Made by spreading a thin layer of organic sol'n on water, glass, or an inert support
- Porosity : about 80%
- Average pore size : 0.1㎛ ~ 1.0 ㎛

Figure 4. Ultrafiltration membranes - 1

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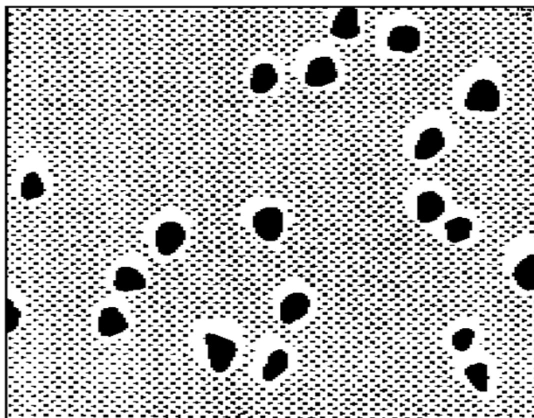
- Made by drawing warm, nonporous films of polymers like PP.
- Porosity : 35%
- Thickness : 0.003 cm

Figure 5. Ultrafiltration membranes - 2



- Made by exposing nonporose pores films of mica or polycarbonate to α radiation.
- Then etched away with HF acid sol’n
- Lowest porosity : ~3%
- Similar permeability to the other types

Figure 6. Ultrafiltration membranes - 3

❖ Equipment

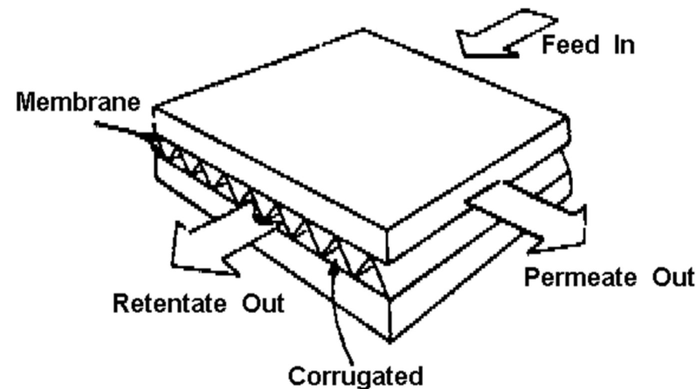


Figure 7. Membrane geometries for Ultrafiltration - Flat Sheets

- It consists of alternate layers of membrane, Support screen, and distribution chambers for feed and permeate
- It has the smallest area per Vol. of the common types, and so tends to give low ultrafiltration fluxes per Vol.

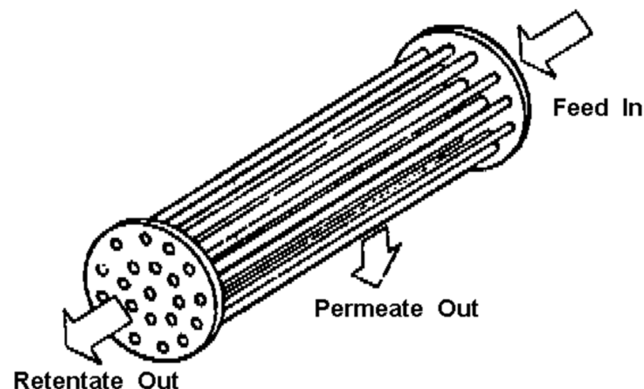


Figure 8. Membrane geometries for Ultrafiltration - Shell and Tube

- The feed stream enters the lumen of the tubes, the permeate passes through the walls, and the retentate passes out other end of the tubes
- It is harder to clean and service than the plate and flat sheet and it has lower area per Vol. and hence lwer fluxes than the spiral wound and hollow fiber geometries

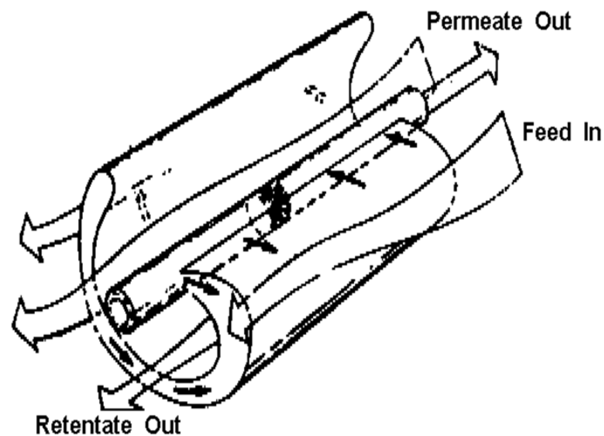


Figure 9. Membrane geometries for Ultrafiltration - Spiral Wound

- This device is like a huge envelop made of membrane and containing a feed spacers
- The device give higher filtration rates per Vol.
- They are much harder to clean, and often must be discarded if even part of the membrane fails
- They tend to be used when the feed is relatively pure, as in the production of ultrapure water by reverse osmosis

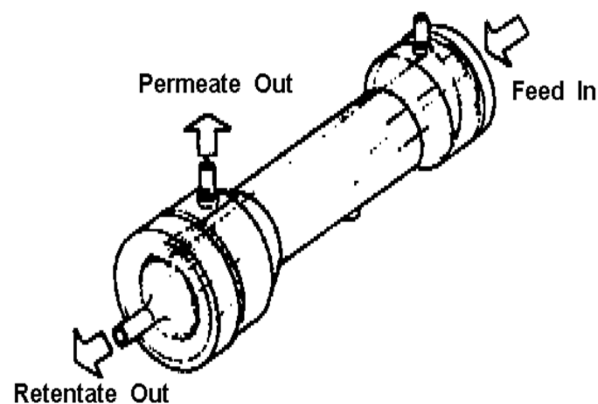


Figure 10. Membrane geometries for Ultrafiltration - Hollow Fiber

- The fibers are typically 0.01 cm on diameter, while the tubes are around 1 cm diameter
- Which configuration is best depends on the specific situation, but os rarely obvious

❖ Analysis

- To find this time, we first must find the solvent velocity through the membrane
  This velocity is given by

$$j_v = L_P(\Delta P - \sigma\Delta\Pi)$$

If the solute is completely rejected by the membrane, the reflection coefficient $\sigma = 1$; if the sol'n is dilute, the osmotic press. $\Delta\Pi = RTc_{10}$, where c_{10} is the solute conc. at the surface of the membrane

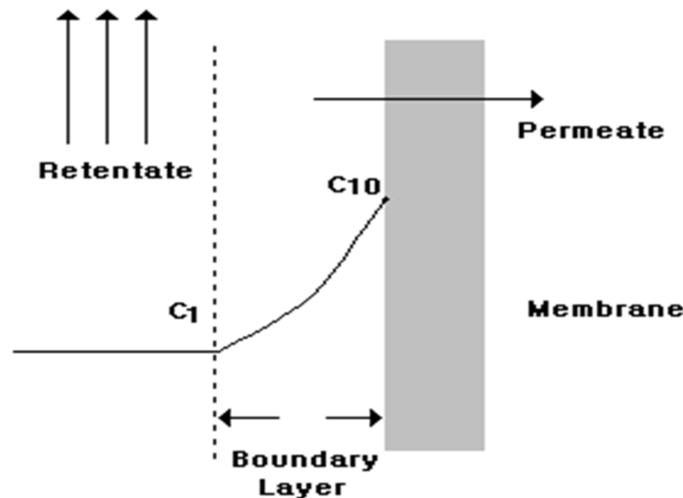


Figure 11. Conc. polarization.

- Higher solute concentration

$$-c_1 j_v = -D\frac{dc_1}{dz}$$

Boundary condition

$$Z = 0, \quad c_1 = c_{10}$$
$$Z = l, \quad c_1 = c_1$$

Integrating

$$j_v = \frac{D}{l}\ln\frac{c_{10}}{c_1}$$

$$j_v = \frac{D}{l}\ln\frac{c_{10}}{c_1}$$

A plot of flux versus the logarithm of reservoir conc. c_1 should be a straight line

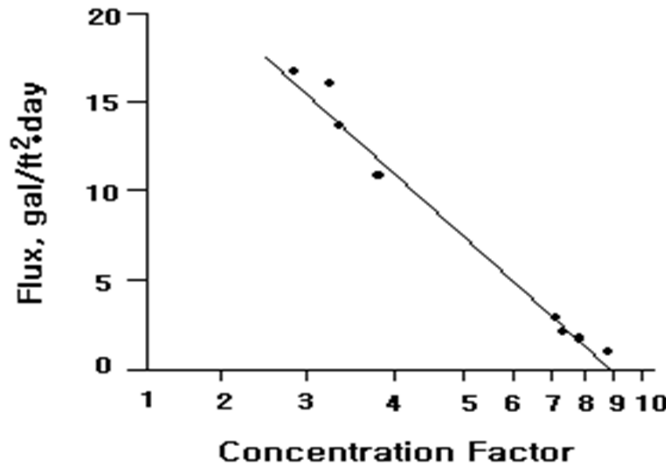


Figure 12. Flux versus reservoirr conc. These data, for the ultrafiltration of *B. Thuringlensis*, support the analysis which leads to

$$j_v = \frac{D}{l}\ln\frac{c_{10}}{c_1}$$

- **Estimating the time to filter a given volume**

$$\frac{D}{lj_v} \rangle\rangle\rangle 1$$

In this case , $c_{10} = c_1$

$$\frac{dV}{dt} = -Aj_v = -AL_P\Delta P\left(1 - \frac{RTc_1}{\Delta P}\right)$$

Because the membrane rejects all the solute, the ($n_1 = c_1 V$)is const.

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$$\frac{dV}{dt} = -(AL_P\Delta P)\left(1 - \frac{RTn_1/\Delta P}{V}\right)$$

$$\frac{dV}{dt} = -(AL_P\Delta P)\left(1 - \frac{RTn_1/\Delta P}{V}\right)$$

Initial condition **$t = 0$, $V = V_0$**

Integrating

$$t = \left(\frac{1}{AL_P\Delta P}\right)\left((V_0 - V) + \left(\frac{RTn_1}{\Delta P}\right)\ln\left(\frac{V_0 - RTn_1/\Delta P}{V - RTn_1\Delta P}\right)\right)$$

Example 2   The Surface Conc. For Chymotrypsin Ultrafiltration

We are carrying the ultrafiltration of chymotrysin in a spiral wound module at a rate of 1.3×10^{-3} cm / sec (28 gal / ft^2 day). The sol'n conc. is 0.44 wt%, the protein's diffusion coefficient is 9.5×10^{-7} cm^2 / sec, and the boundary layer is about 180×10^{-4} cm thick. How high is the surface conc. ?

$$j_v = \frac{D}{l}\ln\frac{c_{10}}{c_1}$$

$$1.3\times10^{-3}\frac{cm}{\sec} = \frac{9.5\times10^{-7}cm^2/\sec}{0.018cm}\ln\frac{c_{10}}{c_1}$$

$$\frac{c_{10}}{c_1} = 1.3$$

The conc. at the wall is 30% higher than that in the bulk

Example 3 Estimating the Time for Vaccine Ultrafiltration

We want to ultrafilter 840 liters of a solution containing 0.061 wt% of a protein used as a vaccine for herpes. The protein has a diffusion coefficient of 1.1×10^{-6} cm^2/sec and a molecular weight of 16,900. We would like to get the conc. up to about 2% by weight. The ultrafilter which we hope to use has eight hollow fiber cartridge, each of which has a surface area of 1.20 m2. It is cooled to 4℃. The membrane in these cartridges gives an pressure drop of 31 atm.

(a) Assuming negligible conc. polarization, estimate the time to complete this filtration

$$\frac{RTn_1}{\Delta P} = \frac{0.082 l \text{ atm / mol K } (277 \text{ K}) \left[(840\times10^3 g)0.00061\, g\right]/\, g}{31 \text{ atm } (16{,}900 \text{ g/mol})} = 0.02\, l$$

Since this is much less than the volume being filtered, even at the end of the filtration, we neglect this term

$$t = \left(\frac{1}{AL_p\Delta P}\right)\left((V_0 - V) + \left(\frac{RTn_1}{\Delta P}\right)\ln\left(\frac{V_0 - RTn_1/\Delta P}{V - RTn_1\Delta P}\right)\right)$$

→ 0

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$$t = \left(\frac{1}{AL_p \Delta P}\right)(V_0 - V)$$

$$= \left(\frac{1}{8(1.2\times10^4 cm^2)5.7\times10^{-5} cm/\sec}\right)\left(840\times10^3 cm^3 - 840\times10^3 cm^3\left(\frac{0.00061}{0.02}\right)\right)$$

$$= 1.5\times10^5 \sec$$

This ultrafiltration will take slightly less than two days-if conc. polarization remains unimportant.

(b) Test whether conc. polarization is significant

To see if conc. polarization is unimportant, we turn to $\frac{D}{lj_v} \rangle\rangle\rangle 1$ **to find**

$$\frac{D}{lj_v} = \frac{1.1\times10^{-6} cm^2/\sec}{l0.01 cm(5.7\times10^{-5} cm/\sec)} = 2 \rangle\rangle\rangle 1$$

This is greater than one, but not much greater than one. We would expect at least some effect of conc. polarization and would certainly make a laboratory test before risking all 840 liter of feed