

Lesson 3. GLYCEROL TRANSPORT IN CELLS

Freezing cells for storage is an important commercial and medical process. One method for doing this is to infuse the cells with glycerol, which acts as an 'antifreeze'. A problem arises when it comes time to use the cells and the glycerol must be extracted. Since glycerol is a powerful osmolyte, there is a risk of lysing the cells, rendering them useless.

OBJECTIVES

1. How to construct a multi-compartment model with coupled flows of solute and solvent.
2. How to treat quantities that are conserved, such as total mass and volume.
3. How to convert units and maintain dimensional homogeneity in a model.
4. How to use Madonna™ to graph “static” functions that are not time dependent.
5. How to simplify a model when one flow is much faster than another.

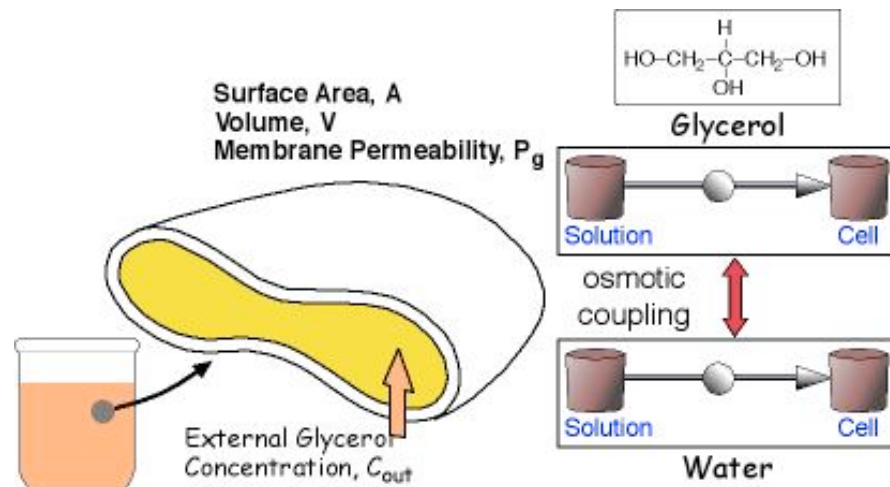


Figure 1

PROBLEM STATEMENT

165 *mmol* of glycerol is suddenly introduced into 1 liter of blood containing 45% (by volume) of red blood cells.

Model the swelling of the cells as they take up glycerol.

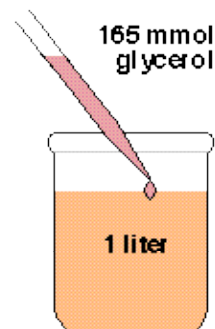


Figure 2

BACKGROUND

Glycerol transport

Assume that glycerol transport takes place by simple diffusion across the membrane (in human erythrocytes there is actually a carrier system for glycerol, but for our present purposes simple diffusion is a good approximation). Thus the net transport of glycerol, J_g , from outside the cell to inside is proportional to the difference in concentration across the membrane:

$$J_g = P_g A (c_{go} - c_{gi}) \quad (1)$$

where:

P_g = permeability of cell membrane [cm/s]

A = the area of the cells [cm²]

c_{go} = glycerol concentration outside [mmol/cm³]

c_{gi} = glycerol concentration inside [mmol/cm³]

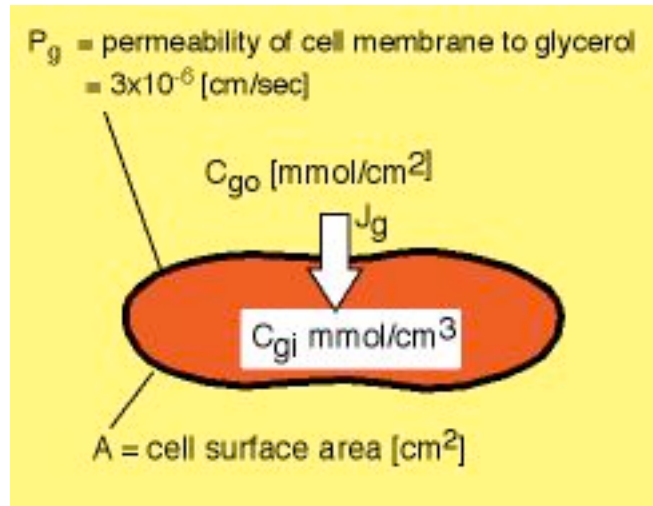


Figure 3

Water transport

Assume that the net flow of water, J_{vol} in [cm³/sec], into the cell is proportional to the difference in osmotic pressure on the two sides of the membrane. The osmotic pressure of any solution is proportional to the sum of the concentrations of all solutes in that solution.

Hence we write:

$$J_{vol}[\text{cm}^3/\text{s}] = P_{\text{water}} A [\sum(\text{solute concentrations})_{\text{in}} - \sum(\text{solute concentrations})_{\text{out}}] \quad (2)$$

where

$$P_{\text{water}} = \text{osmotic water permeability of cell membrane} = 0.00036 [\text{cm}^4/\text{mmole}\cdot\text{s}] \quad (3)$$

(see the Appendix for a discussion of the units of P_{water}). The difference in total solute concentration on the two sides of the membrane drives the water flow (osmosis).

$$\begin{aligned} \square (\text{solute concentrations})_{\text{in}} &= \frac{m_{gi}(t) + m_{si}}{V_i(t)} \\ \square (\text{solute concentrations})_{\text{out}} &= \frac{m_{go}(t) + m_{so}}{V_o(t)} \end{aligned} \quad (4)$$

where

$V_i(t)$ and $V_o(t)$ = inside and outside water volumes, respectively [cm³].

m_{gi} and m_{go} = mmol of glycerol inside and outside the cell [mmol/cm³]*

m_{si} and m_{so} = mmol of all impermeable solutes [mmol/cm³]

All other solutes permeate slowly compared to glycerol and water so that they can be considered "impermeable" for the duration of this simulation. Both m_{si} and m_{so} remain constant throughout the simulation. Since they are constant their values can be estimated at any time we choose. It is most convenient to estimate them at the very beginning when the cells are at equilibrium with normal plasma and before the glycerol has been added. Let $V_i(0)$ and $V_o(0)$ represent the inside and outside water volumes at $t = 0$, just an instant before the glycerol is added. Then the corresponding impermeable solute concentrations will be $C_{si}(0) = m_{si}/V_i(0)$ and $C_{so}(0) = m_{so}/V_o(0)$, or solving for m_{si} and m_{so} we have:

$$\text{Solutes inside: } m_{si} = C_{si}(0) \cdot V_i(0); \quad \text{Solutes outside: } m_{so} = C_{so}(0) \cdot V_o(0)$$

At $t = 0$ the cells are assumed to be in equilibrium with an isotonic solution. (An isotonic solution has the same osmotic pressure as plasma, which is approximately equivalent to the osmotic pressure of a 300mM solution of sucrose.)

$$C_{si}(0) = C_{so}(0) = 300 \text{ mmol/Liter} = 0.3 \text{ mmol/cm}^3$$

Thus the expression for the osmotic force is:

$$\Sigma(\text{solute conc})_{in} = [m_{gi}(t) + 0.3 V_i(0)] / V_i(t)$$

$$\Sigma(\text{solute conc})_o = [m_{go}(t) + 0.3 V_o(0)] / V_o(t)$$

where the $m_g(t)$ = mmol of glycerol at time t , and $V(0)$ = initial volume. The numerical values are given in the table below.

Summary of Experimental Data

QUANTITY	SYMBOL	UNITS	VALUE
Initial plasma glycerol concentration	C_{go}	mmol/cm ³	0.3
Initial cell water	$V_i(0)$	cm ³	315
Initial plasma volume		cm ³	550
Total cell surface area	A	cm ²	7×10^6
initial impermeable solute concentration	$C_{si}(0) = C_{so}(0)$	mmol/cm ³	0.3
membrane permeability to glycerol	P_g	cm/sec	3×10^{-6}
membrane osmotic permeability	P_{water}	cm ⁴ /mmol·sec	0.00036

Simulations

1. Set up the model to simulate and plot the movement of cell water volume and cell glycerol. First, run the model so that glycerol is impermeable by setting $P_g = 0$. This will wipe out the glycerol transport and allow you to estimate how fast water equilibrates. To do this, extend the

* Note that osmotic pressure depends only on the *number* of molecules, not their size or weight. So millimoles/cm³ are the right units for concentration.

simulation time until the water equilibrates. Then try different step sizes (dt) to explore the compromise between speed and accuracy.

- In setting up the Madonna™ model, remember that reservoirs should represent *conserved* quantities. Since no chemical reactions are taking place we can use numbers of molecules (measured in millimoles) and volume as the conserved flow quantities.
- Set P_g equal to its normal value, but now set $P_{water} = 0$; this wipes out water transport. Comparing these two simulations allows you to compare the rates of the two processes.
- Now run the simulation with the given permeabilities. It may be difficult to find an appropriate step size (dt) that will accommodate both fast and slow processes. If so, try reducing P_{water} by a factor of 10 (this will correspond to the water permeability of a lipid bilayer) so that the relaxation times of the water and glycerol are more equal.
- A *linear* system has the following characteristic: when you change the input by some factor (i.e. the initial glycerol concentration) the response (cell volume or cell glycerol concentration)

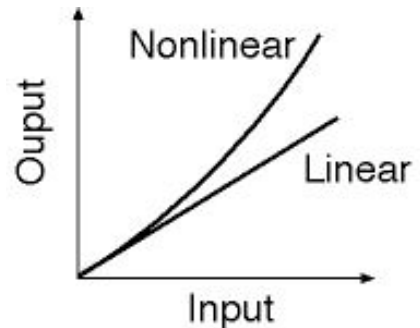
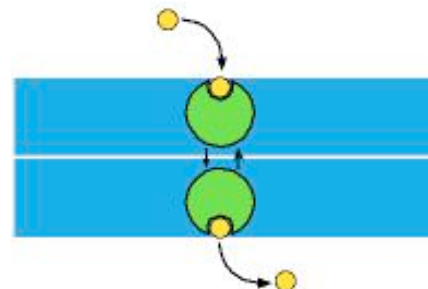


Figure 4

- changes by a constant factor. Is this model a linear system?
- FACILITATED DIFFUSION.** The glycerol molecule contains 3 polar OH⁻ groups making it insoluble in lipids; thus simple diffusion of glycerol through the bilayer is very slow. A realistic model of glycerol transport takes into account specialized proteins in the membrane which make transport much faster. Since there are a only a limited number of these transporters, as the glycerol concentration is raised the glycerol molecules begin to compete for access to these *carrier* molecules, and the transport *saturates*. If there is no metabolic energy coupled to the transport it is called *facilitated diffusion*.



Simulate this type of transport by replacing J_g with the saturating function:

$$J_p = P_g A K \left[\frac{C_{go}}{K + C_{go}} - \frac{C_{gi}}{K + C_{gi}} \right], \tag{5}$$

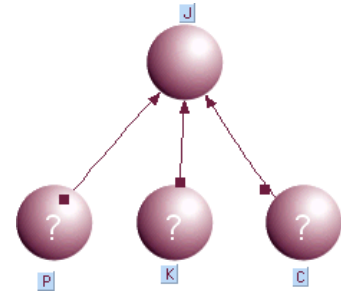
where K is related to the dissociation constant of the binding of glycerol to the transport site. Note that $\lim_{K \rightarrow \infty} J_p = P_g A (C_{go} - C_{gi})$ so that we can consider our original model (simple diffusion) a limiting case of carrier transport. A typical value of K for glycerol transport is 0.7 mmol/cm^3 .

Try out values for $K = (0.01, 0.1, 1.0, 10.0)$ to see how these affect the rate of equilibration. How big does K have to be for the difference between facilitated and simple diffusion to be negligible?

PLOTTING AN ALGEBRAIC EQUATION IN MADONNA™

Sometimes you will want to see what an algebraic function looks like. Madonna™ can plot functions as follows. To illustrate the influence of K directly on the expression for J_g , let $C_{gi} = 0$ and plot J_g as a function of C_{go} . This is done with formulas. We call the primary formula J_g and feed it with formulas that contain values for P_g and K and C_{go} . Into the primary formula we enter

$J_g = P_g K \frac{C_{go}}{K + C_{go}}$ [i.e. type: $P * K * (C / (K + C))$] and choose J_g as the variable to be plotted. To generate different values for C_{go} , you must set $C_{go} = \text{TIME}$, so that when TIME goes from 0 to say 3, C_{go} will also go from 0 to 3.



Problem: how to freeze cells without killing them

Prior to freezing cells they are equilibrated with 3M glycerol. This suppresses ice formation and the consequent cell dehydration that results from removal of water into the ice crystals, and the perforation of the cell membrane by growing ice crystals. However, before cells can be used for, say, transfusion this high glycerol concentration must be removed, or they will burst (hemolyse): if the cells are placed in the glycerol-free plasma of the body then water will rapidly enter due to the high concentration of intracellular glycerol. If cell water increases to about 1.8 times the normal value the cells swell, become spherical, and the membrane begins to leak solutes or rupture. It is also deleterious for cells to shrink below 0.38 times their normal volume.

Devise a strategy to reduce the intracellular glycerol to safe levels (say <100 mmol). This requires that the cell volume remains between 0.38 and 1.8 x normal, and will probably take more than one "wash". A wash consists of equilibrating the cells with a medium of your choice and then centrifuging them to separate the cells from the wash medium. They are then transferred to the next solution for the next wash. To devise a successful procedure you will want to change the salt concentration, $C_{so}(0)$, in your initial wash medium.

APPENDIX

Numerical values

INITIAL GLYCEROL CONCENTRATION IN PLASMA = 0.3 MMOL/CM^3

In 1 L of blood there will be 450 mL of blood and 550 mL of plasma. 165 mmol dissolved into 550 mL of plasma yields a concentration (mmol/vol) = $165/0.55 = 300 \text{ mmol/L} = 0.300 \text{ mmol/mL} = 0.300 \text{ mmol/cm}^3$.

INITIAL CELL WATER = 315 CM^3

About 30% of the average cell is made of solid material (70% water) so that the cellular water = $0.7 \times 450 = 315 \text{ cm}^3$

INITIAL PLASMA VOLUME = 550 CM^3

55% of 1L = 550 cm³.

TOTAL CELL SURFACE AREA = $7 \times 10^6 \text{ CM}^2$.

One liter of blood at 45% hematocrit contains approximately 5×10^{12} cells. Each cell has a surface area of approximately $140 \mu\text{m}^2 = 1.4 \times 10^{-6} \text{ cm}^2$. Multiplying these two numbers gives a total surface area (available for glycerol transport) = $7 \times 10^6 \text{ cm}^2$.

Units

In general, we will use the cgs system of units:

length [cm], **area** [cm²], **volume** [cm³]

mass [M] = mmol (= number) \times molecular weight [gm/M] = [gm]. *Note:* Sometimes, the molecular weight is not mentioned explicitly, and mmol is used as a mass unit!

time = [sec]

The permeability coefficient for **solutes** is generally written as $P \text{ [cm/s]} = D/\Delta x$, where $D \text{ [cm}^2/\text{s]}$ is the diffusion coefficient of the solute and $\Delta x \text{ [cm]}$ is the thickness of the membrane. Multiplying by the cross-sectional area of the flow, $A \text{ [cm}^2]$, gives the total volumetric flow in $[\text{cm}^3/\text{s}]$.

The units for **water permeability**, P_{water} , can be confusing, however. It is important to distinguish whether the water flow is measured in terms of **numbers** [mmol/sec] or **volume** [cm³/sec (= ml/sec)]. When the solute concentrations are given in number concentration [mmol/cm³], the water permeability coefficient for red cells is approximately $2 \times 10^{-2} \text{ [cm/sec]}$.

Using this for P_{water} gives the number flux across 1 cm² surface. To convert to volume flow, i.e. cm³ of water, multiply by the volume occupied by 1 millimole of water = 18 cm³/1000 (i.e. one mol of water weighs 18 grams and water has a density = 1). So

$$P_{\text{water}} = (2 \times 10^{-2} \text{ cm/sec}) (18 \text{ cm}^3/\text{mol})/1000 = 0.00036 \text{ cm}^4/\text{mmol-sec}$$

At first sight the units seem strange, but, remember that we will multiply P_{water} by Area (cm^2) and by solute conc (mmol/cm^3), so that the mmol will cancel and multiplying P_{water} by cm^2 (area) and then dividing it by cm^3 (conc) will leave cm^3/sec for the units of J_{vol} —just what we want.

Quasi-equilibrium: when one variable changes much faster than the others

One way to speed things up is to assume that the fast process (water equilibration) reaches its equilibrium instantly. (You can use the more exact solution to verify the validity of this approximation) Then

$$\begin{aligned} \square \text{ (solute conc)}_{in} &= \square \text{ (solute conc)}_o \\ \frac{m_{gi}(t) + m_{si}}{V_i(t)} &= \frac{m_{go}(t) + m_{so}}{V_o(t)} \end{aligned} \tag{6}$$

In addition to the above, we know that the total amount of water is conserved.

$$\begin{aligned} V_i(t) + V_o(t) &= V_{total} = 315 + 550 = 865 \text{ cc}, \\ \text{or} \quad V_o(t) &= V_{total} - V_i(t) \end{aligned} \tag{7}$$

Use this to eliminate $V_o(t)$ in equation (2), cross multiply, and solve for $V_i(t)$:

$$V_i(t) = V_{total} \frac{m_{gi}(t) + m_{si}}{m_{go}(t) + m_{so} + m_{gi}(t) + m_{si}} \tag{8}$$

$V_{total} = 865 \text{ ml}$. and the m_s 's are calculated as indicated above. You can now omit the volume reservoirs and simply use converters to calculate the volumes algebraically from (3) and (4). This approximation allows you to make dt large enough to obtain rapid solutions.