

Red Blood Cell: A Steady State Osmotic Balance/ElectroNeutrality (OBEN) Model

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Overview

This osmotic model, built in Mathematica[®], is based on the equations outlined in Joshi and Palsson's "*Metabolic Dynamics in the Human Red Cell. Part II – Interactions with the Environment*" (Journal of Theoretical Biology (1989). **141**, 529-545). The purpose of the present document is to detail the equations used in the OBEN model, describe how they were solved, and illustrate some interesting analyses that can be done with the OBEN model.

Figure 1 illustrates the main principles behind the osmotic balance and electroneutrality equations. Some ions and proteins are impermeable to the membrane; hence their concentrations only change relative to changes in internal and external volume (denoted by V_i and V_e , respectively). Na^+ and K^+ are exchanged via the Na/K pump, which pumps 2 K^+ in and 3 Na^+ out of the cell per ATP utilized. This pump is a major player in maintaining the concentration gradients across the membrane and balances the Na^+ leakage into the cell and the K^+ leakage out of the cell. The anions, conversely, are free to move across the membrane and are distributed based on the Donnan ratio.

The red cell has to satisfy both osmotic balance and electroneutrality, resulting from the confinement of high concentrations of hemoglobin, by adjusting the pump activity and the corresponding Donnan ratio (r), the distribution of anions across the membrane.

Red Blood Cell OBEN Model

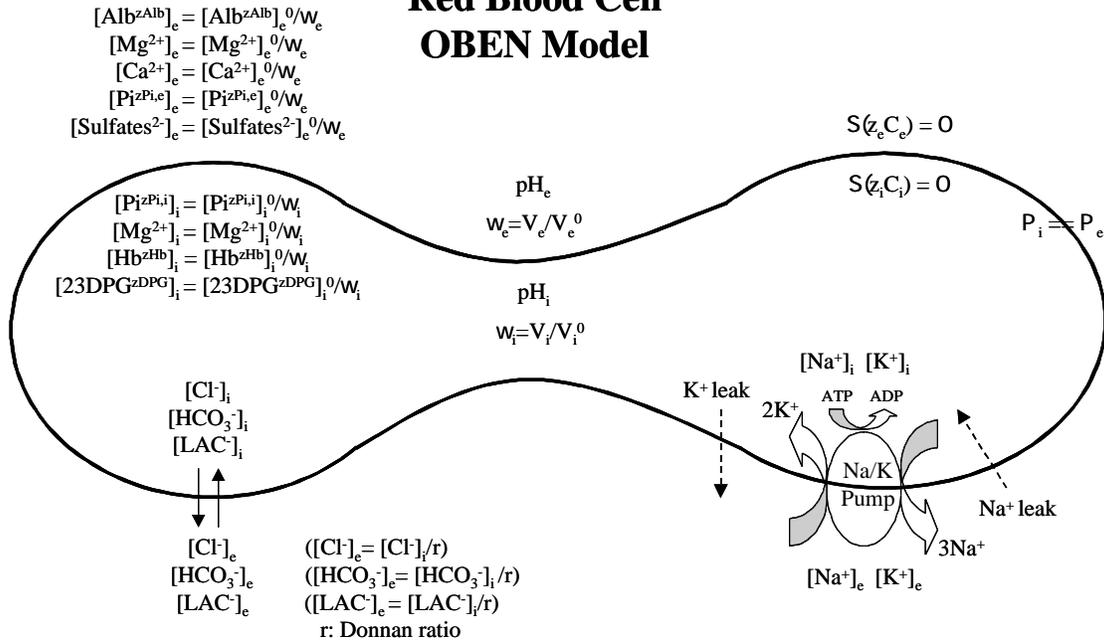


Figure 1: A schematic of the main constituents in the osmotic balance/electroneutrality model

The following sections outline, in detail, the equations used to build the model based on the original, 1989 Joshi and Palsson paper. At the end of this document, several interesting results and conclusions from the OBEN model are shown and discussed.

Model Equations

We now summarize the equations that describe the various biochemical and physico-chemical processes that are accounted for in this model. All the equations and parameter values used are those of the original Joshi and Palsson paper. A full list of the model parameters and their values are given at the end of this section.

Internal pH

The internal pH is a function of the external pH (which is fixed for this model) and the Donnan ratio:

$$pHi = pHe + \log(r) - 0.034$$

where,

pH_i = internal pH
 pH_e = external pH
 r = Donnan ratio

(Equation 31 in J&P)

Relative Volume

Both the internal (cellular) and external (plasma) volumes are expressed as relative volumes based on their initial states. The initial states are defined based on the blood hematocrit (Hc) as follows:

$$V_i^0 = Hc$$

$$V_e^0 = 1 - Hc$$

where,

V_i^0 = Initial internal volume

V_e^0 = Initial external volume

We define the parameter η , such that,

$$\eta = \frac{V_i^0}{V_e^0}$$

Now the relative volumes can be defined as:

$$\omega_i = \frac{V_i}{V_i^0} \quad \text{and} \quad \omega_e = \frac{V_e}{V_e^0} = \eta (1 - \omega_i) + 1$$

where,

ω_i = Relative internal volume

ω_e = Relative external volume

(Equations 14 and 15 in J&P)

The permeable ions

Chloride, bicarbonate, and lactate are permeable to the cell membrane and hence are free to move from one side to the other. They are distributed based on the Donnan ratio such that,

$$r = \frac{[Anion]_i}{[Anion]_e}$$

Each ion must also satisfy the conservation of mass such that the total initial amount is conserved (although their concentrations will change). The following equations are the basis for calculating the concentrations of these ions on each side of the membrane:

$$Cl_i = \frac{Cl_i^0 + \frac{Cl_e^0}{\eta}}{\omega_i + \frac{1-\eta(\omega_i-1)}{r\eta}}$$

$$HCO_{3,i} = \frac{HCO_{3,i}^0 + \frac{HCO_{3,e}^0}{\eta}}{\omega_i + \frac{1-\eta(\omega_i-1)}{r\eta}}$$

$$Cl_e = \frac{Cl_i}{r}$$

$$HCO_{3,e} = \frac{HCO_{3,i}}{r}$$

$$Lac_i = \frac{Lac_i^0 + \frac{Lac_e^0}{\eta}}{\omega_i + \frac{1-\eta(\omega_i-1)}{r\eta}}$$

$$Lac_e = \frac{Lac_i}{r}$$

(Based on Equations 16-20 in J&P)

The impermeable constituents

Many of the model constituents are impermeable to the membrane, they are confined either to the intracellular or extracellular environment. Hence their concentrations fluctuate based on changes in the relative cell and plasma volumes.

The external components include calcium, magnesium, phosphates, sulfates, and albumin. Their concentrations change according to the following equations:

$$Ca_e = \frac{Ca_e^0}{\omega_e}, \quad Mg_e = \frac{Mg_e^0}{\omega_e}, \quad Phos_e = \frac{Phos_e^0}{\omega_e}, \quad S_e = \frac{S_e^0}{\omega_e}, \quad Alb_e = \frac{Alb_e^0}{\omega_e}$$

The internal constituents include hemoglobin, ATP, phosphates, magnesium, and 23DPG. Their concentrations change in an analogous manner:

$$Hb_i = \frac{Hb_i^0}{\omega_i}, \quad ATP_i = \frac{ATP_i^0}{\omega_i}, \quad Mg_i = \frac{Mg_i^0}{\omega_i}, \quad Phos_i = \frac{Phos_i^0}{\omega_i}, \quad 23DPG_i = \frac{23DPG_i^0}{\omega_i}$$

Variable charges

Hemoglobin, albumin, 23DPG, and the internal and external phosphates all have charge values that are functions of various environmental parameters. Each ion's charge can be calculated as follows:

$$z_{Hb} = m_{Hb} [pH_i - (pI_{T=0} - 0.0167 T)]$$

$$z_{Alb} = m_{Alb} (pH_e - pK_{Alb})$$

$$z_{23DPG} = \frac{10^{pK_1 - pH_i} + 2 \cdot 10^{2(pK_2 - pH_i)}}{1 + 10^{pK_1 - pH_i} + 2 \cdot 10^{2(pK_2 - pH_i)}} - 5.0$$

$$z_{phos,i} = -\frac{2 \cdot 10^{pH_i - pK_p} + 1}{10^{pH_i - pK_p} + 1}$$

$$z_{phos,e} = -\frac{2 \cdot 10^{pH_e - pK_p} + 1}{10^{pH_e - pK_p} + 1}$$

(Equations 27-30 in J&P)

Hemoglobin's osmotic coefficient

Hemoglobin's osmotic coefficient, ϕ_{Hb} , also varies and is a function of the hemoglobin concentration:

$$\phi_{Hb} = 1 + 0.0645Hb + 0.0258Hb^2$$

(Equation 24 in J&P)

The Na/K Pump

The following rate law describes the Na/K pump flux in units of mM/hr per ATP utilized:

$$v_{pump} = \frac{ATP}{ATP + K_{ATP}^{pump}} \frac{\left(\frac{v_m}{2}\right) \left[K_e^2 + B_2 K_e \frac{\xi}{2} \right]}{B_1 B_2 + 2 B_2 K_e + K_e^2 + \left(\frac{B_3}{Na_i} + 1\right)^3 \left[B_1 B_2 \frac{k_2}{k_1} + \frac{k_3}{k_1} (K_e^2 + \xi B_2 K_e) \right]}$$

(Equation 6 in J&P)

The Na⁺ and K⁺ leaks

Na⁺ and K⁺ are constantly leaked down their concentration gradients (i.e. Na⁺ leaks into the cell and K⁺ leaks out). The leaks are defined as follows:

$$v_{leak,Na} = k_{Na} \ln(r) \frac{Na_e - Na_i}{r - 1} + v_{m,Na} \frac{Na_e}{K_{m,Na} + Na_e} - \frac{r Na_i}{K_{m,Na} + r Na_i}$$

$$v_{leak,K} = k_K \ln(r) \frac{K_e - K_i}{r - 1} + v_{m,K} \frac{K_e}{K_{m,K} + K_e} - \frac{r K_i}{K_{m,K} + r K_i}$$

(Equation 4 in J&P)

Na⁺ and K⁺ Mass Balances

As with the permeable ions, conservation of mass equations can be written relating internal and external concentrations of Na⁺ and K⁺:

$$Na_i = \frac{1}{\omega_i} Na_i^0 + \frac{Na_e^0}{\eta} - \frac{Na_e \omega_e}{\eta}$$

$$K_i = \frac{1}{\omega_i} K_i^0 + \frac{K_e^0}{\eta} - \frac{K_e \omega_e}{\eta}$$

(Based on Equation 16-18 in J&P)

Steady State Na⁺ and K⁺ Balances

Under steady state conditions there is a balance between the leak and pump fluxes for both Na⁺ and K⁺ such that:

$$v_{leak,Na} - 3v_{pump} = 0 \quad (1)$$

$$v_{leak,K} + 2v_{pump} = 0 \quad (2)$$

These equations come from the fact that for each ATP utilized, there are 3 Na⁺ pumped out and 2 K⁺ pumped into the cell. These are 2 of the 4 equations that will be used to solve for the 4 unknowns in the model: the Donnan ration, the relative internal volume, and the external Na⁺ and K⁺ concentrations.

(Based on Equations 7,8 in J&P)

Satisfying electroneutrality

Electroneutrality states that there must be no net charge either inside or outside the cell, hence the positive and negative ions must balance.

Internally:

$$Cl_i^- + HCO_{3,i}^- + Lac_i^- = Na_i^+ + K_i^+ + 2Mg_i^{2+}$$

$$+ (z_{Hb} Hb) + (z_{23DPG} 23DPG) + (z_{Phos,i} Phos_i)$$

Externally:

$$Cl_e^- + HCO_{3,e}^- + Lac_e^- = Na_e^+ + K_e^+ + 2(Ca_e^{2+} + Mg_e^{2+}) + (z_{Alb} Alb) + (z_{Phos,e} Phos_e) - 2S_e$$

The Donnan ratio is defined as:

$$r = \frac{Cl_i^- + HCO_{3,i}^- + Lac_i^-}{Cl_e^- + HCO_{3,e}^- + Lac_e^-}$$

Substituting in the electroneutrality equations yields:

$$r = \frac{Na_i^+ + K_i^+ + 2Mg_i^{2+} + (z_{Hb} Hb) + (z_{23DPG} 23DPG) + (z_{Phos,i} Phos_i)}{Na_e^+ + K_e^+ + 2(Ca_e^{2+} + Mg_e^{2+}) + (z_{Alb} Alb) + (z_{Phos,e} Phos_e) - 2S} \quad (3)$$

This is the third equation used to solve for the unknowns.

(Equations 32-36 in J&P)

Osmotic pressure balance

The osmotic pressure balance says that the osmotic pressures inside and outside the cell must be equal:

$$i = e$$

where,

$$i = \phi_i RT(Na_i^+ + K_i^+ + Mg_i^{2+} + Cl_i^- + HCO_{3,i}^- + Lac_i^- + 23DPG + Phos_i) + \phi_{Hb} RT(Hb) + RT(n)$$

$$e = \phi_e RT(Na_e^+ + K_e^+ + Mg_e^{2+} + Ca_e^{2+} + Cl_e^- + HCO_{3,e}^- + Lac_e^- + Alb + Phos_e + S_e)$$

Hence,

$$\phi_i (Na_i^+ + K_i^+ + Mg_i^{2+} + Cl_i^- + HCO_{3,i}^- + Lac_i^- + 23DPG + Phos_i) + \phi_{Hb} Hb + (n)$$

$$= \phi_e (Na_e^+ + K_e^+ + Mg_e^{2+} + Ca_e^{2+} + Cl_e^- + HCO_{3,e}^- + Lac_e^- + Alb + Phos_e + S_e) \quad (4)$$

This equation completes the set of four steady state equations used to solve for the four unknowns (r, ϕ_i , Na_e , and K_e).

(Based on Equations 22-23 in J&P)

Solving the OBEN model numerically

The goal now is to solve the 4 simultaneous equations for the 4 unknowns. The 2 steady state Na^+ and K^+ balance equations along with the electroneutrality and osmotic

balance equations can all be written solely in terms of the 4 unknowns (r , i , Na_e , and K_e) and the given parameter constants. The solutions can be found using Mathematica[®]'s **FindRoot** command. Once the variables have been solved for, all the other model values can be determined, such as the pump flux, the current internal Na^+ concentration, etc.

Tables of Numerical Values for the Constants in the Joshi and Palsson OBEN Model

Initial Component Concentrations	Internal (mM)	External (mM)
Na ⁺	10	140
K ⁺	135	10
Mg ²⁺	2.5	1.5
Ca ²⁺	~0	2.5
Cl ⁻	78	107
HCO ₃ ⁻	16	25
Lac ⁻	1.2	1.2
Phosphates	2.5	2
Sulfates	N/A	2
Albumin	1.05	N/A
23DPG	5	N/A
Hemoglobin	7.3	N/A
Δn	20	N/A
ATP	1.5	N/A

Na, K Leak Constants		
Parameter	Na	K
k _x	7.055x10 ⁻³ /hr	6.349x10 ⁻³ /hr
V _{m,x}	2.816 mM/hr	3.115 mM/hr
K _{m,x}	21.0 mM	4.0 mM

Na/K Pump Constants	
V _m	2.318 mM/hr
K _{pump,ATP}	0.040 mM
B ₁	0.0617 mM
B ₂	0.1328 mM
B ₃	6.2672 mM
k ₂ /k ₁	0.0082 mM
k ₃ /k ₁	0.0501 mM
	0.7114

Variable charge constants		
Hemoglobin	m _{Hb}	-10 eq/mol Hb/pH
	pI _{T=0}	7.2
	T	37C
Albumin	m _{Alb}	-6.65
	pK _{Alb}	4.8
23DPG	pK ₁	7.37
	pK ₂	6.78
Phosphates	pK _p	6.1

Other Necessary Constants	
pH _e	7.4
e	0.93
i	0.93
Hematocrit	0.45

Numerical Results for the OBEN Model

The following results were obtained using the Mathematica[®] program to vary a parameter, such as external pH, to see its effects on the overall model results.

Varying external pH

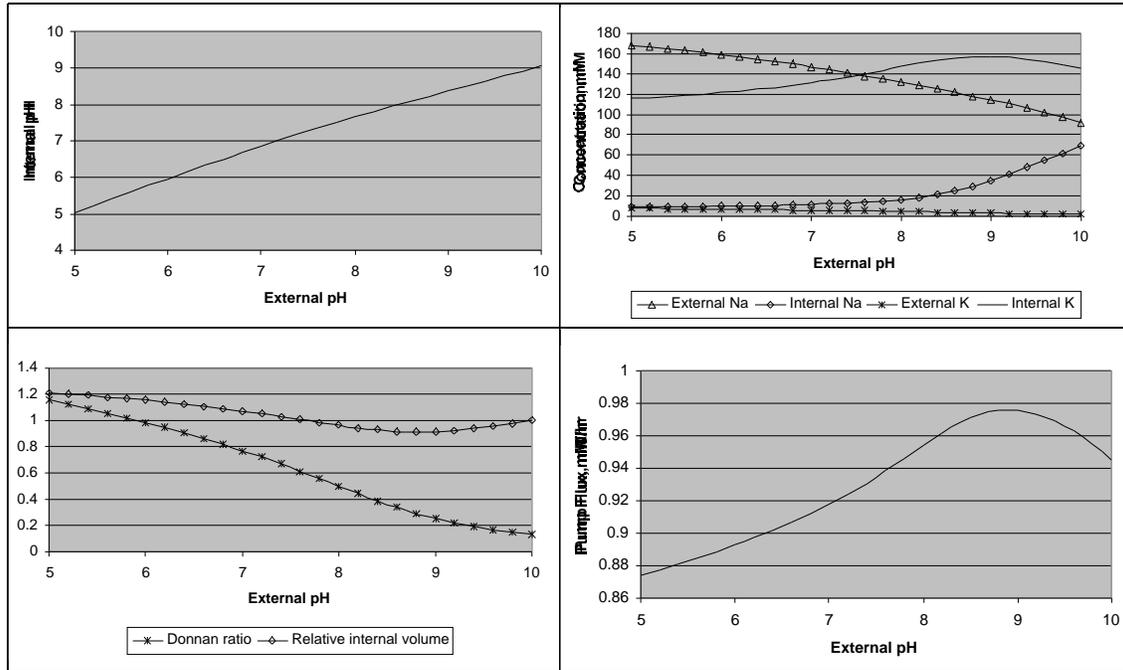


Figure 2: The results from varying external pH from 5 to 10.

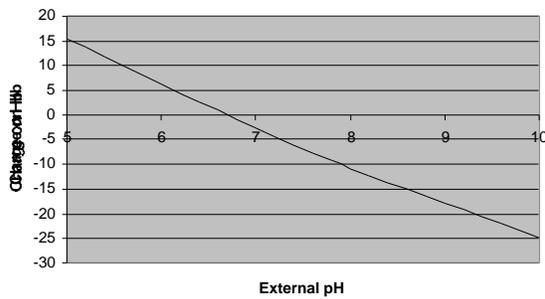


Figure 3: Increasing negative charge on hemoglobin with increasing pH

One of the most interesting aspects of these results is that at around a pH of 8, the cell is “flooded” with sodium. This flooding is due mainly to the variable charge on hemoglobin. At a pH above 8 the charge on hemoglobin becomes very negative. As the pH increases the charge becomes even more negative (see Figure 3). In order to balance this high internal negative charge, the positive sodium ion must enter the cell.

It is clear that the variable charge on hemoglobin plays a large role in the OBEN model under varying external pH conditions. This effect can be illustrated by plotting the model results using a constant charge value for hemoglobin of $z_{\text{Hb}} = -4.7$.

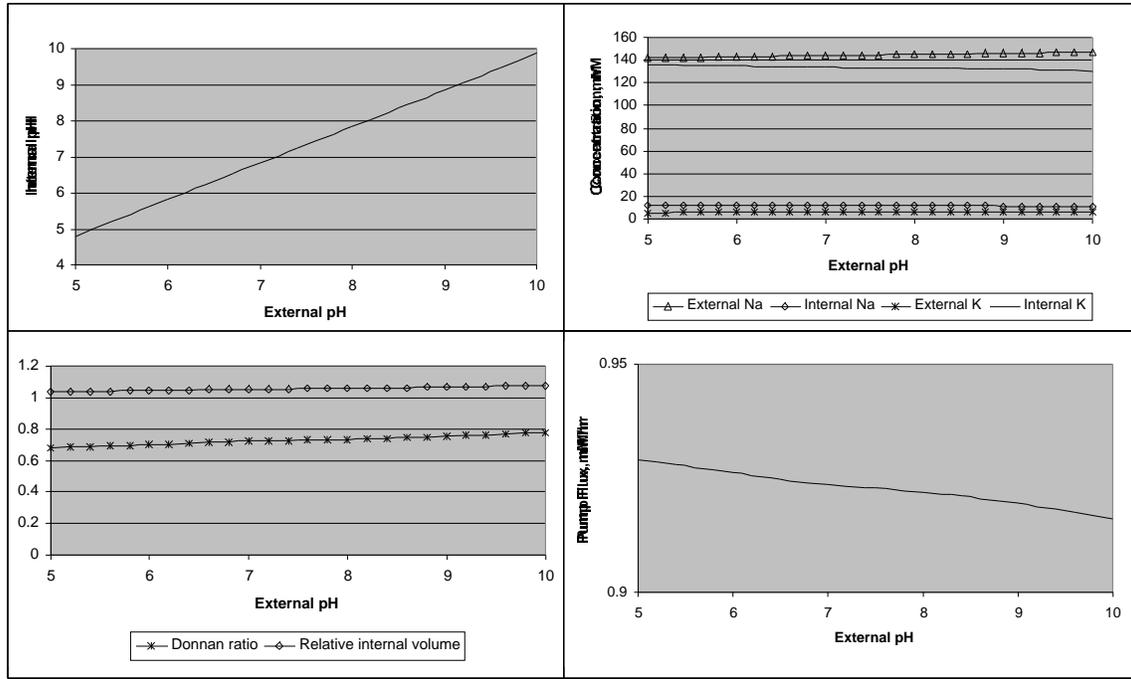


Figure 4: Results under variable external pH when the charge on hemoglobin is set to a constant value of -4.7 .

Figure 4 immediately shows that there is almost no change in the system variables (Na^+ and K^+ concentrations, Donnan ratio, cell volume, etc.) when the charge on hemoglobin is set to a constant value.

Varying external pH: Another steady state solution

Using **Findroot** to solve the four equations with four unknowns presents an interesting problem in that there is more than one set of solutions to the steady state equations. Depending on what you give Mathematica[®] as your initial guesses for the unknowns, a different steady state solution can be found. The above solutions (Figures 2-4) were results of using the following initial guesses: $r = 0.8$, $\omega_i = 1.1$, $Na_e = 140$, $K_e = 10$. However, if you change the initial guess for the relative internal volume to $\omega_i = 0.8$, the following solution is found.

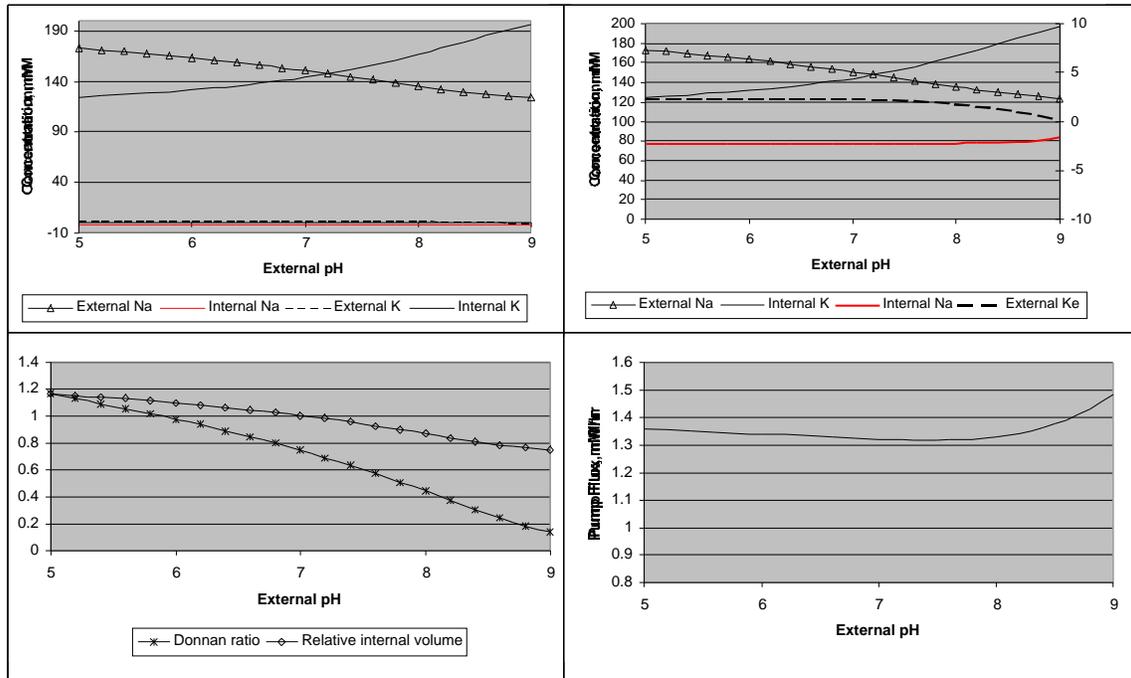


Figure 5: Alternate solutions to varying the external pH. Note this is a physiologically unrealistic state since the internal Na concentration is negative (shown in both the upper graphs). The upper-right graph is the same as the upper left but an extra scale has been added on the right hand side, corresponding to the internal Na and external K to better show the negative concentration values.

While this is a numerically valid solution to the algebraic equations, it is a physiologically unrealistic solution. In this set of results, the internal Na^+ concentration is negative.

Varying initial ATP concentration

Varying the initial ATP concentration shows the robustness of the system with regards to losing its energy source. Not until the concentration of ATP falls below around 0.2 mM (5 times the K_m value of the pump, $K_{Pump,ATP} = 0.04$) does the pump start to fail. At this point the external and internal concentrations of Na and K start to equilibrate and the internal volume shoots up to nearly double its original volume (well past the cell's bursting point).

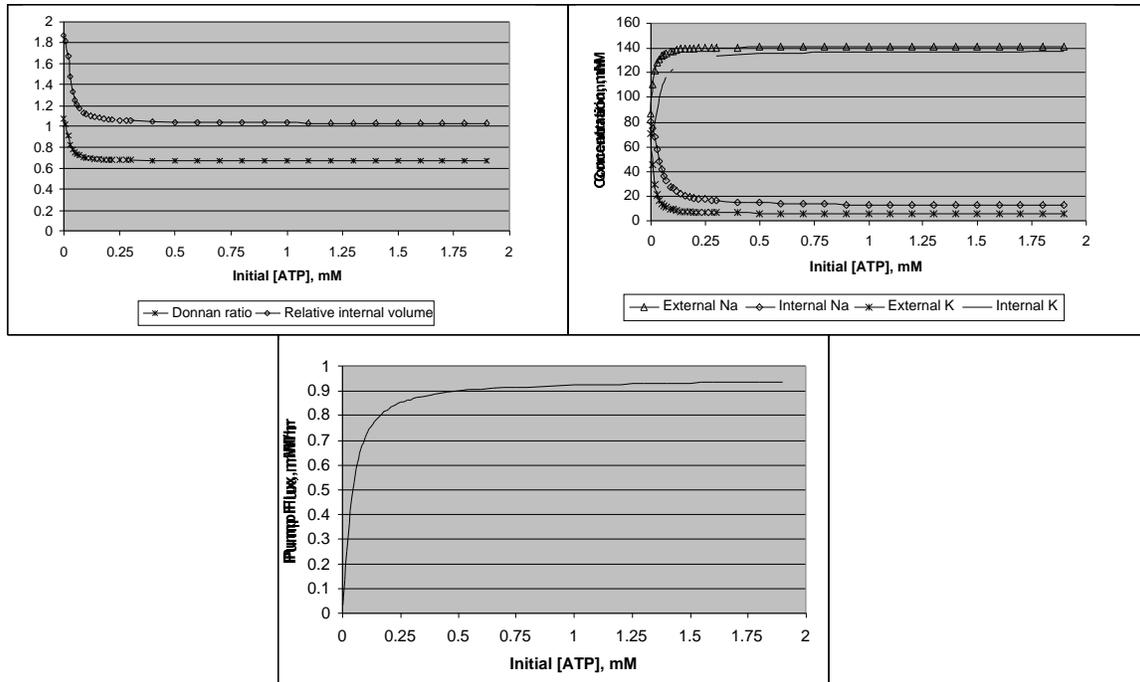


Figure 6: Varying initial ATP concentration.

Summary

The Joshi and Palsson OBEN model reduces to a set of four simultaneous algebraic equations. These equations can be solved using Mathematica[®]. In doing so one must be cognizant of peculiarities in the numerical analysis and set the **Findroot** function's convergence criteria and error tolerances appropriately. The equations have an alternate solution which is physiologically unrealistic hence one must also be careful in choosing proper initial starting solutions in the **Findroot** function.

These equations can be coupled to the dynamic equations that describe metabolic dynamics. The resulting time varying volume and fluctuations in the ATP concentration couple the two sets of equations together. This coupled ordinary differential equation and algebraic equation set describes the full red blood cell metabolism.