Exact solutions of a two parameter flux model and cryobiological applications ⋆

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Abstract

Solute-solvent transmembrane flux models are used throughout biological sciences with applications in plant biology, cryobiology (transplantation and transfusion medicine), as well as circulatory and kidney physiology. Using a standard two parameter differential equation model of solute and solvent transmembrane flux described by Jacobs (1932, *J. Cell. Comp. Phys.*, 2:427), we determine the functions that describe the intracellular water volume and moles of intracellular solute for every time t and every set of initial conditions. Here we provide several novel biophysical applications of this theory to important biological problems. These include using this result to calculate the value of cell volume excursion maxima and minima along with the time at which they occur, a novel result that is of significant relevance to the addition and removal of permeating solutes during cryopreservation. We also present a methodology that produces extremely accurate sum of squares estimates when fitting data for cellular permeability parameter values. Finally, we show that this theory allows a significant increase in both accuracy and speed of finite element methods for multicellular volume simulations, which has critical clinical biophysical applications in cryosurgical approaches to cancer treatment.

Key words: cryobiology, Jacobs model, osmotic tolerance limits, mass transport, exact solution, finite element methods

⋆ This work was funded in part by the University of Missouri Comparative Medicine Center, The Cryobiology Research Institute and grants from the National Institutes of Heath (U42 RR1482, P40 RR15210, R24 RR13194, R24 RR153770, and U42 RR018877).

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[1] This work is part of a thesis to be submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Missouri-Columbia

1 Introduction

Mass transport models are used extensively throughout the biological sciences with applications ranging from plant biology and cryobiology to circulatory and kidney physiology [9,12,15,16]. The two parameter solute and solvent model developed by Jacobs [5], and the related Kedem and Katchalsky [7] model have been used for a half-century or more to model transmembrane flux in biological systems. A discussion of the similarities and differences in the two formalisms can be found in the excellent review by Kleinhans [8].

In particular, the Jacobs model has provided a simple and accurate description of solute and solvent flux using a system of ordinary differential equations [2]:

$$\begin{aligned} \dot{V}_w &= -L_p ART\Big(M_s^e + M_n^e - \delta_1 \tfrac{n_s^i}{V_w} - \delta_2 \tfrac{n_n^i}{V_w}\Big), \\ \dot{n}_s^i &= P_s A\left(M_s^e - \delta_1 \tfrac{n_s^i}{V_w}\right), \end{aligned} \tag{1}$$

where V_w and n_s^i denote the intracellular water volume and moles of an intracellular permeating solute, respectively, and the other parameters are defined in Table 1. We note that the extracellular solute concentrations are given in terms of osmolality, and the intracellular solute concentrations are given as molarity times a constant temperature dependent conversion factor, δ_1 and δ_2, to yield osmolality.

Until very recently, the use of this system was almost exclusively limited to numerical simulations—algorithms that approximate the solution of the differential equations [8]. Because of this, standard calculus techniques could not be applied to find the maximum or minimum of water and cellular volume or the times at which they occur. Additionally, algorithms for finding cell plasma membrane permeability coefficients had to either be inaccurate or very complicated and difficult to implement [13]. Furthermore, large scale calculations where hundreds of thousands of volume calculations (as with finite element models of tissue transport) are needed become exponentially computationally inefficient as the scale or accuracy is increased [10].

Recently a method for obtaining the volume maxima or minima using Eq. (1) was developed using a technique that defines an implicit relation between volume and concentration [6,19]. However the method presented in these papers loses critical time information and thus cannot be used to accurately predict when these cell volume maxima and/or minima occur. This time information is a key parameter used in the development of protocols for addition or removal of high concentrations of cryoprotective agents such as glycerol or dimethyl sulfoxide (Me_2SO) [17], in the prediction of macromolecular uptake by arteries

[2] Note that we use the mathematical convention $\dot{x} = \frac{dx}{dt}$

[15], and in kidney transport [9].

These current problems are ameliorated with the existence of an exact solution of Eq. (1); one that expresses the water volume, V_w, and the moles of solute n_s^i, as functions of time and initial conditions. That is, given a set of initial conditions such as cell volume and surface area, intra- and extracellular concentration, etc... we would like to have a formula for water volume and moles of solute as a function of time. In this work, we present a method whereby the exact solution of Eq. (1) (and thus the exact volume and intracellular solute concentration) can be determined for all experimental time. We then apply this exact solution technique to classic cryobiological problems involving solute and solvent transport such as finding cell volume, cell water volume, and intracellular solute concentration maxima and minima, determining cell membrane permeability parameters, and improving large scale tissue transport models.

2 Methodology

2.1 A reparameterized solution to the Jacobs model

For most non-linear differential systems, it is impossible to express their solutions explicitly as a function of time and initial conditions [1]. The Jacobs model is a unique case. To our knowledge, it cannot be solved as a function of the temporal variable using traditional methods such as separation of variables or integration factors and this is likely the reason why no exact solution has emerged since its inception. Our analysis is based on a result from the theory of ordinary differential equations (ODEs): the qualitative behavior of a system of ordinary differential equations (e.g. its phase portrait, orbit structure, maxima and minima, etc.) is the same with rescaled time. In the same way that a logarithmic curve can be turned into a straight line using a logarithmic scale on one axis, we can stretch and squeeze the solution of a differential equation so that the solution appears to be linear.

For example, if a system can be written in the form

$$\dot{u}(t) = \lambda(u(t))F(u(t)), \tag{2}$$

where $\lambda : \mathbb{R}^n \to \mathbb{R}$ is a scalar valued function, the qualitative behavior is identical to that of the system

$$\dot{w}(\tau) = F(w(\tau)). \tag{3}$$

More precisely, if $u(t)$ is a solution of Eq. (2), then the function $q(\tau)$ given by

$$q(\tau) := \int_0^\tau \frac{1}{\lambda(u(s))} ds \tag{4}$$

is invertible and $w(\tau) := u(q(\tau))$. Similarly, if w is a solution of Eq. (3), then $u(t) := w(p(t))$ is a solution of Eq. (2), where $p := q^{-1}$. For a proof of this standard result, see Chicone [2].

It is convenient to rename constants as a, b, c, α, and β (see Table 2) so that Eq. (1) simplifies to

$$\begin{aligned} \dot{n}_s^i &= \beta + \alpha \tfrac{n_s^i}{V_w}, \\ \dot{V}_w &= b + a \tfrac{n_s^i}{V_w} + c \tfrac{1}{V_w}; \end{aligned}$$

or, equivalently,

$$\begin{aligned} \dot{n}_s^i &= \tfrac{1}{V_w}(\alpha n_s^i + \beta V_w), \\ \dot{V}_w &= \tfrac{1}{V_w}(a n_s^i + b V_w + c). \end{aligned} \tag{5}$$

Equation (5) is in the form of Eq. (2), where $\lambda(n_s^i(t), V_w(t)) = 1/V_w(t)$ and $F(u) = (\alpha n_s^i(t) + \beta V_w(t), a n_s^i(t) + b V_w(t) + c)$. Hence we can recover solutions of Eq. (5) from the system

$$\begin{aligned} \dot{n} &= \alpha n + \beta v, \\ \dot{v} &= a n + b v + c. \end{aligned} \tag{6}$$

The linear Eq. (6) can be solved explicitly using standard ODE techniques. In fact, the general solution is

$$n(\tau) = \frac{1}{a}\left(c_1 (r_1 - b) e^{r_1 \tau} + c_2 (r_2 - b) e^{r_2 \tau} + b\frac{c\alpha}{\gamma} - c \right), \tag{7}$$

$$v(\tau) = c_1 e^{r_1 \tau} + c_2 e^{r_2 \tau} + \frac{c\alpha}{\gamma}, \tag{8}$$

where $r_1 := 1/2\left(\rho - \sqrt{\rho^2 + 4\gamma}\right)$, $r_2 := 1/2\left(\rho + \sqrt{\rho^2 + 4\gamma}\right)$, $\rho := (\alpha + b)$, $\gamma := (a\beta - \alpha b)$, and c_1 and c_2 are arbitrary constants. If we specify $n_s^i(0)$ and $V_w(0)$, we have

$$\begin{aligned} c_1 &= \frac{2bc\alpha - c r_2 \alpha + \gamma(c + a n_s^i(0) + b V_w(0) - r_2 V_w(0))}{\gamma(r_1 - r_2)}, \\ c_2 &= -\frac{2bc\alpha - c r_1 \alpha + \gamma(c + a n_s^i(0) + b V_w(0) - r_1 V_w(0))}{\gamma(r_1 - r_2)}. \end{aligned}$$

Thus, the equation for total cell volume can be written as

$$\begin{aligned} V_{total}(\tau) &= V_w + n_s^i \overline{V} + V_b V_{iso} \\ &= c_1 \left(\frac{\overline{V}}{a}(r_1 - b) + 1 \right) e^{r_1 \tau} + c_2 \left(\frac{\overline{V}}{a}(r_2 - b) + 1 \right) e^{r_2 \tau} \\ &\quad + (\frac{\overline{V}}{a} b - 1) \frac{c\alpha}{\gamma} - \frac{\overline{V}}{a} c + V_b V_{iso}. \end{aligned} \tag{9}$$

We can glean some information about the exponents r_1 and r_2. For example, since our linear Eq. (6) always has an asymptotically stable rest point in physiologic conditions (data not shown), we will assume that r_1 and r_2 are both negative (see Strogatz [14]). We also note that

$$\frac{c\alpha}{\gamma} = \frac{M_n^i(0)}{M_n^e} V_w(0). \tag{10}$$

This is in agreement with the negative sign of r_1 and r_2, since, as time progresses, the first two terms of Eq. 8 go to zero, leaving $c\alpha/\gamma$ equal to the ratio of initial intracellular and extracellular concentrations. Hence,

$$\lim_{t \to \infty} v(0) - v(t) = c_1 + c_2. \tag{11}$$

Note that if the non-permeating solute concentration is constant—a common situation in cryobiology, then $\lim_{\tau \to \infty} v(\tau) = v(0)$ implies $c_1 = -c_2$.

Similarly, the exponential terms of the solution $n(\tau)$ go to zero with time. Hence, we have that

$$\lim_{\tau \to \infty} n(0) - n(\tau) = \frac{1}{a}(c_1(r_1 - b) + c_2(r_2 - b)). \tag{12}$$

For Eq. (5), we have $\lambda(n, v) = -1/v$, thus, using Eq. (4), if $q(0) = 0$, then we have

$$\begin{aligned} q(t) &= \int_0^t \left(c_1 e^{r_1 s} + c_2 e^{r_2 s} + \frac{\alpha c}{\gamma} \right) ds, \\ &= \frac{c_1 \left(e^{r_1 t} - 1 \right)}{r_1} + \frac{c_2 \left(e^{r_2 t} - 1 \right)}{r_2} + \frac{\alpha c}{\gamma} t. \end{aligned} \tag{13}$$

See Fig. 1 for a plot of $q(t)$ for typical values of L_p and P_s, where v and n are defined in Eqs. 7 and 8. Thus, the desired exact solution of Eq. (5) is given by

$$\begin{aligned} V_w(t) &= v(q^{-1}(t)), \\ n_s^i(t) &= n(q^{-1}(t)). \end{aligned} \tag{14}$$

2.2 The inverse of q

So far we have an exact solution for the Jacobs model and the function that transforms reparametrized time back to real time. We are now interested in finding the function that transforms real time to the transformed time so that we can use the exact solution methods to analyze experimental data.

We know that the time transform function q is invertible (please see Chicone [2]), and we have an explicit equation for $V_w(t)$ and $n_s^i(t)$ (Eq. (14)). The next challenge lies in finding $q^{-1}(t)$. Define $p(t) := q^{-1}(t)$.

To find a *formula* for $p(t)$ in terms of $q(t)$ and its derivatives only, we can use the Lagrange-Burman reversion formula [3] to obtain the power series representation

$$p(t) = \sum_{n=1}^{\infty} \frac{t^n}{n!} \left[\frac{d^{n-1}}{dt^{n-1}} \left(\frac{t}{q(t)} \right)^n_{t=0} \right]. \tag{15}$$

In other words, in the Taylor series

$$p(t) = \sum_{n=1}^{\infty} p^{(n)}(0) \frac{t^n}{n!}, \tag{16}$$

the derivatives are given by

$$p^{(n)}(0) = \frac{d^{n-1}}{dt^{n-1}} \left(\frac{t}{q(t)} \right)^n_{t=0}.$$

In practice, it is easier to determine the Taylor coefficients by a recursion formula derived from the chain rule. Since $p(q(t)) = t$, taking the derivative gives $p'(q(t))q'(t) = 1$. Dividing by $q'(t)$ gives $p'(q(t)) = 1/q'(t)$. But since $q(0) = p(0) = 0$, we have that $p'(0) = 1/q'(0)$. The recursive formula

$$p^{(n)}(q(t)) = \frac{\left[p^{(n-1)}(q(t)) \right]'}{q'(t)} \tag{17}$$

gives $p^{(n)}(t)$ for $n \geq 2$. Evaluating at $t = 0$, we see that the derivatives of $q(t)$ at zero are $q^{(m)}(0) = r_1^m c_1 + r_2^m c_2$ for $m > 1$. Thus, this calculation is straightforward, and can be done using an algebraic processor such as Mathematica® (Wolfram Research, Champaign, IL). In this case, the inverse of q is given by the Taylor series of Eq. (16) where $p^{(1)}(0) = q'(0)^{-1}$ and $p^{(n)}(0)$, for $n \geq 2$, is given recursively by Eq. (17). While the power series representation of $p(t)$ has a nonzero radius of convergence, the difficult problem of determining this radius is beyond the scope of this work.

From a more practical standpoint, $p(t)$ can be evaluated very efficiently using numerical methods. Given t, we simply approximate the root τ of the function

$q(\tau) = t$ numerically. Efficiency gains can be made by noting the high degree of linearity of the $q(\tau)$ function—especially as time increases—and using this information to optimize the algorithm. For example, with one line of code in Mathematica®, we can calculate the inverse of q for all data points.

3 Results and Discussion

3.1 Finding cell volume and intracellular solute concentration maxima and minima

The extrema of cell volume excursion, cell water volume, and intracellular solute concentration are of great interest in a number of fields. For example, cells may lyse if their volume exceeds physiological limits, and irreparable damage may occur if the cells shrink below physiological limits [4,11]. This is especially relevant in the field of cryobiology, where cryoprotective agents such as glycerol cause the cell to shrink upon addition and swell upon removal.

Using $q(\tau)$ and its inverse $p(t)$ we have an invertible map between the original time space and our new time-transformed space (τ-space) (see Fig. 2). Because we have an exact solution that does not involve derivatives, we can use standard calculus techniques to derive information from our equations. For example, a common use for the exact solution—the one addressed in [6,19]—is the determination of the maxima and minima of cell volume excursion and/or chemical concentration. The advantage of our approach is that we can also determine the time at which the maxima and minima of total cell volume, cell water volume, and moles of intracellular solute occur.

This can be done by setting the derivative of the solutions in τ-space equal to zero. For example, maxima and minima of the water volume are given by the solution of

$$v'(\tau) = c_1 r_1 e^{r_1 \tau} + c_2 r_2 e^{r_2 \tau} = 0. \tag{18}$$

To determine τ, we multiply both sides of Eq. (18) by $e^{-r_1 \tau}$ and rearrange the resulting equation to get

$$e^{(r_2 - r_1)\tau} = -\frac{c_1 r_1}{c_2 r_2}. \tag{19}$$

Because we have assumed that r_1 and r_2 are both negative, we know that $c_1 c_2 < 0$. Therefore, the term on the right-hand side of Eq. (19) is positive and we can take the logarithm of both sides to obtain the solution

$$\tau_{\text{water}} = \frac{1}{r_2 - r_1} \ln\left(-\frac{c_1 r_1}{c_2 r_2}\right). \tag{20}$$

Thus, we have established an explicit solution for the time (τ_{water}) when the maximum or minimum of intracellular water volume occurs. The corresponding physical time is simply $t = q(\tau_{\text{water}})$, given by Eq.(13).

We can repeat this technique for total cell volume using Eq. (9), or for intracellular permeating solute content using Eq. (7) yielding the equations

$$\tau_{\text{total}} = \frac{1}{r_2 - r_1} \ln\left(-\frac{c_1 r_1 \left(r_1 - b + a/\overline{V}\right)}{c_2 r_2 \left(r_2 - b + a/\overline{V}\right)} \right), \tag{21}$$

$$\tau_{\text{solute}} = \frac{1}{r_2 - r_1} \ln\left(-\frac{c_1 r_1 (r_1 - b)}{c_2 r_2 (r_2 - b)} \right), \tag{22}$$

where τ_{total} and τ_{solute} are the τ-space times for the maximum or minimum of the total cell volume and the number of moles of intracellular permeating solute, respectively. These formulas are valid only if the argument of the logarithm is positive, which is the case for our test values. Again, we can calculate the original time using the q transform function. To illustrate the use of the exact solution in practice we choose typical values for our parameters (as in Table 1) and use Eq. (9) to get

$$V_{total}(\tau) = 86.76e^{-6079.18\tau} - 130.569e^{-279.536\tau} + 1043.81. \tag{23}$$

A plot of cell volume versus time for both a numerically integrated solution and the new, exact, solution with both time transport functions can be seen in Fig. 2. In fact, one of the immediate advantages of this exact solution technique is that a plot can be made quickly and easily—even with a graphing calculator—to see the dynamics of the curve without the necessity of complex software. Additionally, a calculator can easily be programmed to give the cell volume maxima and minima and the times at which they occur—a significant advantage at the bench top.

A numerical calculation of the minima of both of these plots yields the minima found by calculating τ from Eq. (20): a minimal volume of 934.29 μm^3. Using the exact solution, we find that this volume occurs at $\tau = 0.000531$. We now use our $q(\tau)$ equation (Eq.(13)) to convert back to real time. In this case, $q(0.000531) = 0.277935$ minutes, which agrees with the time obtained from the numerically integrated solution.

3.2 Curve fitting

The accuracy of the Jacobs model is dependent upon the accuracy of the parameters in the model. The hydraulic conductivity L_p and solute permeability P_s coefficients control the rate at which water and solute enter the cell. To

determine these coefficients, cell volume is typically measured as a function of time while cells are exposed to media containing a permeating solute. The resulting volume versus time data are then fit using the model while varying the parameters L_p and P_s. Because the model has until now only yielded a numerical solution, developing a curve fitting algorithm has been quite difficult. Early investigators were able to fit data only by making simplifying assumptions [13]. In recent years, as computer software and processing power has improved, this range has been extended, but curve fitting has been relegated to complicated software such as MLAB® (Civilized Software, Inc., Bethesda, MD), Mathematica®, or other specialty software, and there is still a trade off between accuracy and speed.

A new curve fitting algorithm that does not involve numerical integration can be implemented by transforming data to the linearized space using the time transformation $p(t, L_p, P_s)$, which depends on the time and the permeability parameters L_p and P_s. We wish to minimize the sum of squares estimate:

$$SS(L_p, P_s) = \sum_{i=1}^{n} (V_{\text{total}}(t_i, L_p, P_s) - V_i)^2. \tag{24}$$

Since $p(t_i, L_p, P_s) = \tau_i$ and $V_{\text{total}}(t_i)) = v_{\text{total}}(\tau_i)$, Eq. 24 can be written in the transformed time as

$$\begin{aligned} SS(L_p, P_s) &= \textstyle\sum_{i=1}^{n} (v_{\text{total}}(p(t_i, L_p, P_s), L_p, P_s) - V_i)^2 \\ &= \textstyle\sum_{i=1}^{n} (v_{\text{total}}(\tau_i, L_p, P_s) - V_i)^2. \end{aligned}$$

The minimization of this estimate can be made using the exact solution with common software packages such as SAS® (SAS Institute, Inc., Cary, NC) or Excel® (Microsoft Corporation, Redmond, WA). An advantage of this methodology is that it will give the most precise estimates of the sum of the squared error because there is no inherent error caused by numerical integration. In many cases, this technique is also faster. On the other hand the time transform must be applied for each data point in the (L_p, P_s)-parameter space. Using the numerical inverse techniques described above, however, this transformation takes a negligible amount of time.

For example, suppose our experimental data consist of ten points over a period of ten minutes. In order to analyze this with the exact solution we only need to calculate the transform function $p(t)$ ten times to yield data analyzable with our exact solution in the linearized space, and only make ten (exact) comparisons. On the other hand, even though there are only ten points, in order to accurately calculate the volume using the numerical solution of the differential equation we must discretize our ten minute experimental time interval into a mesh fine enough to provide accurate estimates. A reasonable time-step for this system (to retain accuracy) is approximately one second.

Thus, we must perform approximately 600 calculations to obtain the volume, together with ten (non-exact) comparisons to yield our sum of squares. With many data points, this computational advantage of speed is weakened due to the increasing number of time transform calculations necessary, but the accuracy advantage will remain.

3.3 Finite element models

When modeling mass transport in organs and tissues, necessary for simulating the effects of freezing during cryosurgery, a common simulation tool is finite element analysis. This processor intensive technique models solute and solvent flux through a tissue by defining a mesh of points and generating a concentration field corresponding to these points. This field is then used to estimate transmembrane flux for cells in a region around each mesh point. Increased numbers of mesh points improve accuracy but slow computations considerably. A solute solvent flux model must be used for each group of cells surrounding the mesh points. The result of these solute solvent equations generates a new field and the state of the tissue or organ is updated as time is incremented. Current techniques use from a few hundred to hundreds of thousands of mesh points with processing time increasing significantly as the number of mesh points increases. Thus the significance of a slight improvement in the efficiency of this modeling system is amplified with the complexity of the computation. We note here that these finite element computations can be performed in the τ-space exactly and efficiently, yielding significant improvements in both computational speed and accuracy in cell volume versus time simulations.

A numerical experiment was performed in which 100,000 calculations of volume ($V_{\text{total}}(.1)$) were made for both the numeric and the exact solution of the Jacobs system (Eq. (1). A 1.2 GHz Intel Pentium 3 laptop carried out the numerical calculation in 7.771 seconds and the exact calculation more than three times faster at a time of 2.073 seconds. Thus for very large finite element grids where multiple time points are needed, the exact solution may be a significant improvement in efficiency. For example, to describe the volume flux of a relatively small tissue model containing 5000 cells (such as an islet of Langerhans) over the course of 25 minutes (the time to load islets of Langerhans with 1.5 M Me_2SO [18]) one needs at least 1500 time-steps. Thus 1500 time-steps at 5000 volume calculations each yields a total of 7.5 $\times 10^6$ calculations. On the above laptop, this calculation would take approximately ten minutes. On the other hand, using the exact solution techniques, the same calculation could be carried out with no error in 2.6 minutes. This sort of large-scale solution can be implemented to predict behavior of much more complicated systems.

4 Conclusions

We have presented an exact solution to a system of differential equations that has been in continuous use in biology for more than 70 years in modeling solute and solvent transmembrane flux for single cells, multicellular systems and tissues. Our method has distinct advantages over traditional numerical integration techniques in both calculation time and numerical accuracy, which allow for expanded applications in optimizing CPA addition and removal and in modeling solute and solvent flux in large multicellular systems. Finally, we have presented simple formulas for the calculation of maximum and minimum cellular water volume, intracellular solute concentration, total cell volume and the times at which they occur without requiring numerical integration.

Figure Legends

Figure 1. Plot of $q(\tau)$ using the test values from Table 2. Note that the high degree of linearity allows for an efficient transformation from the original time space to the τ-space.

Figure 2. To use the exact solution we apply a time transform, $p(t)$, to take data from the physical space on the left (panel A) to the new space (τ-space) on the right (panel B). Note that the volume excursions remain unchanged, and all analysis of the transformed system will apply to the original system. To return to the original time, we use the inverse transform $q(\tau)$. In this figure we show plots of both numerically integrated and exact solutions using the appropriate transform function, e.g. the plot on the left shows an overlay of $V_{\text{numeric}}(t)$ and $V_{\text{exact}}(p(t))$, and the plot on the right shows an overlay of $V_{\text{numeric}}(q(\tau))$ and $V_{\text{exact}}(\tau)$.

Table 1
Definitions of major symbols and their test values

Symbol	Test Value	Units	Description
i, e			Superscripts (i, intracellular; e, extracellular)
n, s			Subscripts (n, non-permeating; s, permeating)
V_b	0.4	unitless	Osmotically inactive fraction
V_{iso}	1000	μm^3	Isosmotic cell volume
V_w		μm^3	Intracellular Water Volume
n_s^i		fmol	Femtomoles intracellular permeating solute
n_n^i		fmol	Femtomoles intracellular non-permeating solute
δ_1	1	osmol L mol^{-1} kg^{-1}	Osmolality conversion factor for permeating solute
δ_2	1.95	osmol L mol^{-1} kg^{-1}	Osmolality conversion factor for non-permeating solute
L_p	0.1	μm min^{-1} atm^{-1}	Hydraulic Conductivity
P_s	10	μm min^{-1}	Solute permeability coefficient
A	483.6	μm^2	Cellular surface area (assumed fixed)
R	0.08206	L atm K^{-1} mol^{-1}	Gas constant
T	295.16	Kelvin	Temperature
t		min	Time
$\overline{V}$	.0730151	L mol^{-1}	Partial molar volume of typical CPA
M_s^e	1.0	osm kg^{-1}	Extracellular permeating solute osmolality
M_n^e	0.3	osm kg^{-1}	Extracellular non-permeating solute osmolality
M_s^i	0	osm kg^{-1}	Initial intracellular permeating solute osmolality
M_n^i	0.3	osm kg^{-1}	Initial intracellular non-permeating solute osmolality

Table 2
Definitions of constants and their test values

Constant	Test Value	Parameters
a	1171.32	$L_pART\delta_1$
b	-1522.72	$-L_pART\left(M_s^e+M_n^e\right)$
c	210837	$L_pART\delta_2 n_n^i$
n_n^i	180	$M_n^i(0)V_w(0)$
α	-4836	$-P_sA\delta_1$
β	4836	$P_sAM_s^e$
γ	-1.69935 $\times 10^6$	$-\delta_1 P_s L_p A^2 RTM_n^e$
ρ	-6358.72	$-\delta_1 P_s A + L_pART\left(M_s^e+M_n^e\right)$
$2r_1$	-12158.4	$\rho-\sqrt{\rho^2+4\gamma}$
$2r_2$	-559.072	$\rho+\sqrt{\rho^2+4\gamma}$
c_1	121.178	$(2bc\alpha - cr_2\alpha + \gamma(c+ax(0)+by(0)-r_2y(0)))/\gamma(r_1-r_2)$
c_2	-121.178	$-(2bc\alpha - cr_1\alpha + \gamma(c+ax(0)+by(0)-r_1y(0)))/\gamma(r_1-r_2)$
c_3	600	$\alpha c/\gamma$