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ORIGINAL PAPER

Ion fluxes, transmembrane potential, and osmotic stabilization: a new dynamic electrophysiological model for eukaryotic cells

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Abstract Survival of mammalian cells is achieved by tight control of cell volume, while transmembrane potential has been known to control many cellular functions since the seminal work of Hodgkin and Huxley. Regulation of cell volume and transmembrane potential have a wide range of implications in physiology, from neurological and cardiac disorders to cancer and muscle fatigue. Therefore, understanding the relationship between transmembrane potential, ion fluxes, and cell volume regulation has become of great interest. In this paper we derive a system of differential equations that links transmembrane potential, ionic concentrations, and cell volume. In particular, we describe the dynamics of the cell within a few seconds after an osmotic stress, which cannot be done by the previous models in which either cell volume was constant or osmotic regulation instantaneous. This new model demonstrates that both membrane potential and cell volume stabilization occur within tens of seconds of changes in extracellular osmotic pressure. When the extracellular osmotic pressure is constant, the cell volume varies as a function of transmembrane potential and ion fluxes, thus providing an implicit link between transmembrane potential and cell volume. Experimental data provide results that corroborate the numerical simulations of the model in terms of time-related changes in cell volume and dynamics of the phenomena. This paper can be seen as a generalization of previous electrophysiological results, since under restrictive conditions they can be derived from our model.

Keywords Electric potential · Cell volume regulation · Ion concentration · Membrane permeability · Osmotic equilibrium

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Introduction

Cell volume and resting transmembrane potential constitute two fundamental parameters of cell life. A cell contains inside its cytoplasm substances such as DNA, amino acids, ions such as K^+ or Na^+, and sugar, which are essential to its survival. The synthetic process favors swelling of the cell (Lang et al. 1998), since the membrane permeability is very low. Without volume changes, the pressure created by the osmolarity difference between the outer medium and the cytoplasm could reach 1 atm (Armstrong 2003; Fraser and Huang 2004), which is much larger than the maximal pressure that the cell membrane can withstand. Whereas plasmalemma of bacteria are protected by a thick peptidoglycan layer that can support such pressures, eukaryotic cells have no wall and therefore must adjust their osmolarity by transferring ions and water

across their membrane. Over many years, it became clear that the osmotic stabilization of living eukaryotic cells was related to active transport of Na^+ out of and K^+ into the cell (Goldman 1943; Landahl 1948; Hodgkin and Katz 1949; Hodgkin and Horowicz 1959). These ion fluxes create a potential difference across the membrane that changes the value of the transmembrane potential. Therefore cell volume regulation and the transmembrane potential are intrinsically correlated.

Based on the extensive review of Fraser and Huang (2004, 2007), all theoretical models (to our knowledge) describing the behavior of the transmembrane potential, of the ionic fluxes, and of the cell volume consider that the water flux across the membrane is instantaneous. Therefore an osmotic equilibrium of the cell is constantly required[1] (Armstrong 2003; Endresen et al. 2000; Fraser and Huang 2004; Fraser et al. 2005). However, such a requirement does not explain the delay in the osmotic stabilization of the cell, observed in the experiments reported by Rouzaire-Dubois et al. (Rouzaire-Dubois and Dubois 2004; Dubois and Rouzaire-Dubois 2004; Rouzaire-Dubois et al. 2009) when a high osmotic stress is imposed. Actually, when the ionic fluxes across the membrane are small, the osmotic equilibrium can be considered constant, as assumed by Fraser and Huang (2004, 2007) or Armstrong (2003), for instance. However, should the cell be subjected to a large osmotic stress, then water flux across the membrane must be accounted for. In particular the above-quoted models do not describe the cell behavior within a few seconds after an osmotic stress, but only within a few minutes after, neglecting the time due to water flux. Our goal is to address these shortcomings by proposing a cell model that describes the cell dynamics from a few seconds to a few minutes after an osmotic stress.

We also aim to clarify the relationship between cell volume, transmembrane potential, and ion fluxes. More precisely, we aim to provide a new electrophysiological model of the cell in order to dynamically link the cell volume, the ionic concentrations, and the transmembrane potential by considering the water flux across the membrane. Our model is a generalization of the previous models in the sense that it describes the models of Jakobsson (1980), Armstrong (2003), Fraser and Huang (2004) or Endresen et al. (2000) under their respective restrictive hypotheses. More particularly, the model is aimed at describing precisely the cell volume and the transmembrane potential within tens of seconds after the osmotic stress. We emphasize that this description cannot be obtained by the previous models. Our goal is also to propose an explanation for the results presented by Rouzaire-Dubois et al. (Rouzaire-Dubois and Dubois 2004; Dubois and Rouzaire-Dubois 2004; Rouzaire-Dubois et al. 2009).

In order to derive our model, we consider in vitro cell cultures and more precisely spherical cells in suspension. The extracellular ionic concentrations are supposed constant, and the cell volume regulation occurs homothetically (i.e., equivalently in all directions, meaning that the cell shape does not change). These assumptions are justified for in vitro experiments, since the volume of the ambient medium is much larger than the cell volume and since no mechanical stress is imposed on the cell. Experimentally, the osmotic equilibrium is not enforced instantaneously, contrary to the assumptions of the models of Jakobsson (1980), Armstrong (2003), Fraser and Huang (2004), and Endresen et al. (2000). The main feature of the present model is the time-dependent variability of the cell volume v_i, since water molecules slowly diffuse across the cell membrane. Our model leads to an implicit dynamic link (the time derivative of the cell volume is a function of the transmembrane potential) between the cell volume, the transmembrane voltage potential, and the ionic concentrations. In the case of a spherical cell, we make this link explicit and perform numerical simulations.

Such a dynamic relationship between the cell volume and the transmembrane potential has not yet, to the best of our knowledge, been investigated. Three main results are presented in this paper: the new cell model (which takes water diffusion into account), the link between the cell volume and the transmembrane potential, and the explicit expression of the potential in terms of ionic concentrations.

For the sake of simplicity, we omit the contribution of active channels such as Na^+/K^+ pumps and voltage-dependent ionic channels. These more accurate descriptions of the ionic fluxes can be added to our model with a very slight modification and without affecting the modeling principle. Our model can already provide some insight into specific mechanisms such as regulation of muscle fatigue. Osmotically induced cell swelling has been observed during muscle contraction (Sjøgaard et al. 1985), while muscle fatigue is associated with membrane depolarization

[1] Fraser and Huang (2004, page 462) state that the volume at time $t+1$ is determined by

$$V_{c(t+1)} = V_{c(t)} \frac{J_{\mathrm{TOTAL}} A_m + \Pi_{i,(t)}}{\Pi_{e,(t)}},$$

where J_{TOTAL} is the total current at time t, A_m is the membrane area per unit volume, and $\Pi_{i,(t)}$ and $\Pi_{e,(t)}$ represent, respectively, the inner and outer osmotic pressures at t. However this method overestimates the cell volume. In Fraser et al. (2005, page 53) they adjust the predicted volume V_p by adding the influence of a solvent fraction inside the cell:

$$V_p = V_{c(t+1)}(1 - x + x\Pi_{e,(t)}/\Pi_{e,(t+1)}).$$

However these two models do not involve any time derivative of the volume, thus the volume regulation is assumed to occur instantaneously.

(Juel 1986; Clausen and Nielsen 2007). Our model represents an integration of these results.

In Sect. 2, we derive the ordinary differential equations that describe the transmembrane potential in terms of ionic concentrations and cell volume. Section 3 is devoted to numerical simulations of the equations for three different stresses. In particular, we show that the model of Fraser and Huang does not describe precisely the cell swelling (or shrinking) a few seconds after the osmotic shock, but after a few minutes, our model and the model of Fraser and Huang describe the same behavior. In Sect. 4, experiments that corroborate the numerical simulations are presented, and we conclude by discussing the results.

The transmembrane potential

According to its phospholipid composition, the cell membrane is a resistive medium with capacitance C_m in units of $\mathrm{F\,m^{-2}}$. We denote by $\mathrm{G_{ions}}$ the group of ionic species. For our numerical simulations, $\mathrm{G_{ions}}$ is $\{\mathrm{Na^+}, \mathrm{Cl^-}, \mathrm{K^+}, X\}$, where X denotes the group of nonpermeant ions. We use the convention that X denotes both ions and counterions that do not cross the membrane. Inside the cell, this set is globally negatively charged on account of the $\mathrm{Na^+}$ and $\mathrm{K^+}$ excess, and the mean charge valency ${z^i}_X$ of X is chosen to ensure electrical neutrality. We note that, in the extracellular medium, the mean valency of the nonpermeant ions, which is denoted by ${z^e}_X$, depends on the solution.

Ionic channels, across which ionic exchanges occur, are distributed over the membrane. The valency of the ions S is denoted by z_S, and the constant P_S denotes the membrane permeability to the species S. In the following, R and F denote, respectively, the gas constant and the Faraday constant, and temperature is denoted by T. Table 1 summarizes the notations used in the paper.

Ion flux through passive channels

According to the literature (Armstrong 2003; DeBruin and Krassowska 1999; Endresen et al. 2000; Hodgkin and Huxley 1952), the ionic fluxes depend on the transmembrane potential V_m. We use the convention $V_m = V_i - V_o$, where the indices "i" and "o" correspond, respectively, to the inner and outer part of the membrane. The passive ion flux equation previously described in 1943 by Goldman (1943), and modified in 1949 by Hodgkin and Katz (1949), is based on a gas-law model. It is precisely described in the book of Malmivuo and Plonsey (1995), and in the article of Endresen et al. (2000) [see also Armstrong (2003)]. According to these models, the current I_S of the permeant ion S equals

Table 1 Glossary

R	Gas constant
F	Faraday constant
T	Temperature
c	Equal to $F/(2RT)$
v_i	Cell volume
$\mathcal{A}(\Gamma)$	Membrane area
C_m	Cell capacitance
V_m	Transmembrane potential
τ_w	Membrane penetrability to water
Θ_w	Equal to $RT\tau_w$
$\mathrm{G_{ions}}$	Group of all the considered ionic species
S	Specific ionic species belonging to the group $\mathrm{G_{ions}}$
$[S]_e$	Extracellular concentration of ions S
$[S]_i$	Cell concentration of ions S
${n^i}_S$	Amount of substance of ions S in the cell
P_S	Membrane permeability to ions S
z_S	Valency of S
I_S	Current due to the fluxes of ions S
g_S	Conductance for ions S
V_S	Nernst equilibrium potential of the ions S
Π_e	Total sum of outer concentrations
Π_i	Total sum of inner concentrations

$$I_S = \frac{1}{2} z_S P_S F \left([S]_i e^{z_S c V_m} - [S]_e e^{-z_S c V_m} \right),$$

where $c = F/(2RT)$. The Nernst equilibrium potential V_S of the species S is the potential such that I_S vanishes, i.e.,

$$V_S = \frac{1}{2cz_S} \log\left(\frac{[S]_e}{[S]_i} \right). \tag{1}$$

Let g_S be the membrane conductance for the species S defined by

$$g_S = P_S z_S^2 c F \sqrt{[S]_e [S]_i}. \tag{2}$$

We therefore derive

$$I_S = g_S \frac{\sinh(z_S c (V_m - V_S))}{z_S c}. \tag{3}$$

When $z_S c (V_m - V_S)$ is small compared with 1, Eq. (3) leads to the linear equation

$$I_S = g_S (V_m - V_S). \tag{4}$$

Evolution of the ionic concentrations

The global amount of ions driven by the total current of ions $S \in \mathrm{G_{ions}}$ across the membrane Γ equals

$$\frac{\mathrm{d}{n^i}_S}{\mathrm{d}t} = -\frac{\mathcal{A}(\Gamma)}{z_S F} I_S, \tag{5}$$

where $\mathcal{A}(\Gamma)$ denotes the membrane surface area.

Osmolarity

We denote by Π_e and Π_i the respective sums of the outer and inner concentrations

$$\Pi_e = \sum_{S\in G_{ions}} [S]_e, \quad \Pi_i = \sum_{S\in G_{ions}} n_S^i/v_i.$$

Since the water is incompressible, we suppose that the change of the cell volume is described by the rate of flow of water across the membrane. Let τ_w be the water membrane penetrability[2] in $s\,m^{-1}$ in SI units. We denote by Θ_w the coefficient

$$\Theta_w = RT\tau_w.$$

According to the Landahl model (1948) we obtain

$$\frac{dv_i}{dt} = -\Theta_w \mathcal{A}(\Gamma)(\Pi_e - \Pi_i). \tag{6}$$

Recently, Hernández and Cristina (1998), or Mathai et al. (2007), replaced Θ_w by the product $P_w\mathscr{V}_w$, where R_w is the water membrane permeability and $\mathscr{V}_w$ is the water partial molar volume. Therefore the coefficients τ_w and P_w are linked by the following equality:

$$\tau_w = \frac{P_w\mathscr{V}_w}{RT}.$$

Remark 1 In many models (Armstrong 2003; Endresen et al. 2000; Fraser and Huang 2004; Malmivuo and Plonsey 1995), the plasma membrane cannot support a pressure difference. Therefore osmotic equilibrium is required at any time

$$\Pi_e = \Pi_i, \text{ which leads to } v_i = \frac{1}{\Pi_e}\sum_{S\in G_{ions}} n_i^S.$$

This equality enforces the volume to change instantaneously when the osmotic equilibrium is modified. However, the cell volume is adjusted by the diffusion of water molecules across the membrane. This phenomenon takes time and is described by the model of Landahl (1948), which is based on a Fick's law-type model to describe the diffusion of water molecules through the membrane.

Remark 2 Note that Mathai et al. (2007) write the volume change (6) in a different way. Let R_{vol} be the ratio of the volume v_i at time t to the initial volume v_i^0, and denote by Π_i^0 the sum of the initial inner concentrations. Mathai et al. suppose that cell membrane is perfectly nonpermeant. Since no exchange occurs, inner and outer amounts of substance of the ionic species are constant. Therefore,

$$\Pi_i = \Pi_i^0/R_{vol},$$

and we deduce the Mathai et al. equation

$$\frac{dR_{vol}}{dt} = -\Theta_w \frac{\mathcal{A}(\Gamma)}{v_i^0}\left(\Pi_e - \frac{\Pi_i^0}{R_{vol}}\right). \tag{7}$$

However, we emphasize that we cannot consider the inner amounts of substance constant, since the ionic fluxes drive ions across the membrane. Therefore, equation (6) must be used. Observe that equation (6) leads to (7) by considering that no ionic flux across the membrane occurs.

Equations for the transmembrane potential

Our model links the transmembrane potential, the cell volume, and the ionic concentrations inside and outside the cell. The unknown quantities are the transmembrane potential V_m, the cell volume v_i, and the amount in the cell of substances $n^i{}_S$ of the ions $S \in G_{ions}$. We obtain a system of ordinary differential equations. This system is very similar to the models of Endresen et al. (2000) and Armstrong (2003). The main difference consists in the fact that the cell volume evolution is governed by an ordinary differential equation, while Endresen et al. and Armstrong et al. required osmotic equilibrium at any time. Using the Kirchoff law and the above equalities we derive

$$C_m\frac{dV_m}{dt} + \sum_{S\in G_{ions}} I_S = 0, \tag{8a}$$

$$\frac{dn_S^i}{dt} = -\frac{\mathcal{A}(\Gamma)}{z_S F} I_S, \tag{8b}$$

$$\frac{dv_i}{dt} = -\Theta_w \mathcal{A}(\Gamma)(\Pi_e - \Pi_i), \tag{8c}$$

where

$$\begin{cases} I_S = g_S(V_m - V_S), \quad \text{for } S \in G_{ions}, \\ [S]_i = n_S^i/v_i, \quad V_S = \frac{1}{2z_S c}\log\left([S]_e/[S]_i\right), \quad g_S = z_S^2 P_S c F\sqrt{[S]_e n_S^i/v_i}, \\ \Pi_e = \sum\limits_{S\in G_{ions}} [S]_e, \quad \Pi_i = \sum\limits_{S\in G_{ions}} [S]_i. \end{cases} \tag{8d}$$

Remark 3 (How to add the pumps fluxes and other currents?) Note that in the above equations any model of I_S can be used, for instance (3). In particular, any model of I_S describing the influence of Na/K pumps and the activity of gate voltage channels (such as sodium channels) can be added straightforwardly by replacing I_S by

$$I_S = g_S(V_m - V_S) + I_{pump} + I_{gate},$$

where I_{pump} can be described by the model of Armstrong [Eq. (4) in Armstrong (2003, page 6258)] or the complex model of Hernandez and Cristina [Eq. A1 in Hernández and Cristina (1998, page C1077)], and I_{gate} is given by the model of Endresen et al. [Appendix A in Endresen et al. (2000, page 102)], for instance.

For the sake of simplicity and to clarify our purpose, we just consider here passive fluxes across the membrane. In

[2] The parameter τ_w is a penetrability, hence it is homogeneous to the inverse of a velocity. We refer to Landalh (1948) for details.

addition it is not clear whether the pumps are activated within tens of seconds following an osmotic stress, though they are probably activated after a first cell stabilization within 1 min after the stress. The novelty of the model consists in Eq. (8c), which describes the evolution of the cell volume. Observe that the above equations involve both cell volume and membrane area. To uniquely determine the shape of the cell it is necessary to specify a law on the membrane surface. However, in the experiments, the cells in suspension are approximately spherical and therefore the geometry is entirely determined by the cell radius. The next subsection is devoted to providing the explicit link between the transmembrane potential and the cell radius.

Explicit link between the cell radius and the potential

Let us now consider spherical cells. The cell area is then $\mathcal{A}(\Gamma) = 4\pi r^2$, and Eq. (8c) becomes

$$\frac{1}{\Theta_w}\frac{\mathrm{d}r}{\mathrm{d}t} = -\left(\Pi_e - \sum_{S\in \mathrm{G}} \frac{n_S^i}{4\pi r^3/3}\right).$$

Using Eq. (8b), and by differentiation we obtain

$$\frac{1}{\Theta_w}\frac{1}{3r^2}\frac{\mathrm{d}}{\mathrm{d}t}\left(r^3\frac{\mathrm{d}r}{\mathrm{d}t}\right) = -\Pi_e\frac{\mathrm{d}r}{\mathrm{d}t} - \sum_{S\in \mathrm{G}_{\mathrm{ions}}} \frac{g_S}{z_S F}(V_m - V_S). \tag{9a}$$

The initial conditions are then

$$r|_{t=0} = r_0, \quad \left.\frac{\mathrm{d}r}{\mathrm{d}t}\right|_{t=0} = -\Theta_w(\Pi_e - \Pi_i^0). \tag{9b}$$

Denote by V_m^{rest} the resting transmembrane potential defined such that

$$\sum_{S\in \mathrm{G}_{\mathrm{ions}}} \frac{g_S}{z_S}(V_m^{\mathrm{rest}} - V_S) = 0.$$

If the cell is initially in osmotic equilibrium and if V_m equals V_m^{rest} then Eq. (9) shows that r remains at its initial value. However, a slight change in the value of V_m leads to an increase (or a decrease) of the cell radius.

Explicit formula for the transmembrane potential

It is interesting to obtain an explicit expression for the transmembrane potential in terms of the ionic concentrations. Actually, since $n_S^i = \mathrm{v}_i[S]_i$, Eqs. (8b–8a) imply

$$C_m\frac{\mathrm{d}V_m}{\mathrm{d}t} = \frac{\mathrm{v}_i}{\mathcal{A}(\Gamma)}F\sum_{S\in \mathrm{G}} z_S\left(\frac{\mathrm{d}[S]_i}{\mathrm{d}t} + \frac{[S]_i}{\mathrm{v}_i}\frac{\mathrm{d}\mathrm{v}_i}{\mathrm{d}t}\right). \tag{10}$$

In addition, assuming that the cell volume is constant, i.e., that the osmotic equilibrium is reached, we then obtain

$$C_m\frac{\mathrm{d}V_m}{\mathrm{d}t} = \frac{v_i}{\mathcal{A}(\Gamma)}F\sum_{S\in \mathrm{G}} z_S\frac{\mathrm{d}[S]_i}{\mathrm{d}t}.$$

Denoting by V_m^0 and $[S]_i^0$ the initial potential and inner concentrations, respectively, we infer the explicit formula for the potential in terms of the inner concentrations at each time t as

$$(V_m - V_m^0) = \frac{\mathrm{v}_i}{\mathcal{A}(\Gamma)}\frac{F}{C_m}\sum_{S\in \mathrm{G}} z_S([S]_i - [S]_i^0). \tag{11}$$

Define the initial potential by

$$V_m^0 = \frac{\mathrm{v}_i}{\mathcal{A}(\Gamma)}\frac{F}{C_m}\sum_{S\in \mathrm{G}} z_S[S]_i^0. \tag{12}$$

Then, the following formula, which is similar to the "charge difference" equation of Fraser and Huang, holds:

$$V_m = \frac{\mathrm{v}_i}{\mathcal{A}(\Gamma)}\frac{F}{C_m}\sum_{S\in \mathrm{G}} z_S[S]_i.$$

Remark 4 (Link with the Fraser and Huang model) In the above equality the factor

$$\mathrm{v}_i/\mathcal{A}(\Gamma)$$

ensures the dimensional consistency of the previous equality. Observe also that Fraser and Huang consider a membrane capacitance C_m^{Fraser} in units of $\mathrm{F\,m^{-3}}$, whereas the usual units for capacitance are $\mathrm{F\,m^{-2}}$, in order to obtain the consistency of their equality (1) in Fraser and Huang (2004, page 461). Therefore their capacitance depends on the cell volume and on the membrane area as

$$C_m^{\mathrm{Fraser}} = C_m\frac{\mathcal{A}(\Gamma)}{\mathrm{v}_i}.$$

It is striking that Eq. (11) involves only the inner concentrations, while the concentrations of the exterior solution are expected to have an influence on the potential. This is hidden behind the hypothesis that the outer concentrations are constant, and by supposing that equality (12) holds at the initial time. Actually, if the outer concentrations change, using the mass conservation law, we would have

$$\frac{\mathrm{d}n_S^e}{\mathrm{d}t} = -\frac{\mathrm{d}n_S^i}{\mathrm{d}t}.$$

Therefore expression (11) of the potential would be

$$(V_m - V_m^0) = \frac{F}{2C_m}\frac{\mathrm{v}_i}{\mathcal{A}(\Gamma)}\sum_{S\in \mathrm{G}} z_S\big(([S]_i - [S]_e) - ([S]_i^0 - [S]_e^0)\big), \tag{13}$$

and then supposing that

$$V_m^0 = \frac{F}{2C_m}\frac{\mathrm{v}_i}{\mathcal{A}(\Gamma)}\sum_{S\in \mathrm{G}} z_S([S]_i^0 - [S]_e^0),$$

we would have

$$V_m = \frac{F}{2C_m}\frac{\mathrm{v}_i}{\mathcal{A}(\Gamma)}\sum_{S\in \mathrm{G}} z_S([S]_i - [S]_e).$$

This formula is exactly formula (63) of Endresen et al. (2000, page 96), just replacing the factor $C_m\mathcal{A}(\Gamma)$ by the factor C. Observe that Endresen et al. consider C in units of Farads [see Table 2 of Endresen et al. (2000)], while here C_m is the usual membrane capacitance in units of $\mathrm{F\,m^{-2}}$. Therefore, our equation (10) is a generalization of the previous results of Fraser, Huang et al., and Endresen et al., and if the cell volume is constant then Eq. (13) links transmembrane potential, cell volume, and ionic concentrations.

Numerical simulations

We perform numerical simulations of problem (8) for the case of spherical cells. For all simulations, the initial radius equals 10 μm. The numerical parameters are given in Table 2.

Observe that the constant $1/C_m$ is quite large, since $C_m \approx 5 \times 10^{-2}$ SI. Therefore, a steady state is reached faster by the transmembrane potential than by the cell radius and the ionic concentrations. To solve the ordinary differential equations of the model we use the fourth-order Runge–Kutta scheme. This scheme has the advantage of being explicit (thus simple to implement) and at the same time quite stable compared with the usual Euler explicit scheme.

Effect of an osmotic stress on the transmembrane potential and the cell volume

The value of τ_w is fixed at $5 \times 10^{-13}\,\mathrm{s\,m^{-1}}$ as given by Landahl (1948), which corresponds to a water permeability P_w around $10^{-5}\,\mathrm{m\,s^{-1}}$ [see Mathai et al. (2007)]. According to Lodish et al. (1986), the inner and outer ionic concentrations for the mammalian cell in osmotic equilibrium are

$$[\mathrm{Na}]_i = 12\,\mathrm{mM}, \quad [\mathrm{K}]_i = 139\,\mathrm{mM}, \quad [\mathrm{Cl}]_i = 4\,\mathrm{mM}, \quad [X]_i = 141\,\mathrm{mM}, \tag{14a}$$

$$[\mathrm{Na}]_e = 145\,\mathrm{mM}, \quad [\mathrm{K}]_e = 4\,\mathrm{mM}, \quad [\mathrm{Cl}]_e = 116\,\mathrm{mM}, \quad [X]_e = 31\,\mathrm{mM}. \tag{14b}$$

The values of the permeant ion permeabilities and valencies given by Fraser and Huang (2004) are

$$P_\mathrm{K} = 4 \times 10^{-10}\,\mathrm{m\,s^{-1}}, \quad P_\mathrm{Na} = 0.04P_\mathrm{K}, \quad \mathrm{P_{Cl}} = 3P_\mathrm{K}, \tag{14c}$$

$$z_\mathrm{Na} = z_\mathrm{K} = 1, \quad z_\mathrm{Cl} = -1, \tag{14d}$$

and the valencies $z^i_X = -1.04, z^e_X = -1.06$ of the nonpermeant ions X ensure the electroneutrality of the inner and the outer solutions. Since we assume that the permeant ions cross the membrane through ion channels, and since the number of ion channels do not change during the cell swelling (or shrinking), it is natural to assume that the membrane permeabilities P_S are constant.

For such concentrations, the resting transmembrane potential for a cell of radius 10 μm equals −86.3 mV.[3] We imposed this value as the initial condition for the transmembrane potential.

The simulations deal with the effect of an osmotic stress on both transmembrane potential and cell volume. At the initial time $t = 0$ we change the outer osmolarity of 60 mM, and we let the system evolve until its stabilization. For the hypertonic stresses we investigate three types of osmotic stresses: first we add NaCl, then study the effect of an increase in KCl concentrations, and then do the same for nonpermeant ions X. For the hypotonic stresses, since the outer potassium concentration is low, we only investigate the hypotonic stress by decreasing the NaCl and the X osmolarities.

Hyperosmotic stress

In order to study an hypertonic stress, we increase the outer osmolarity by 60 mM (from 296 to 356 mM). This stress is simulated in three ways: addition of nonpermeant ions X ($[X]_e = 91\,\mathrm{mM}$), addition of NaCl ($[\mathrm{Na}]_e = 175\,\mathrm{mM}$, $[\mathrm{Cl}]_e = 146\,\mathrm{mM}$), and addition of KCl ($[K]_e = 34\,\mathrm{mM}$, $[Cl]_e = 146\,\mathrm{mM}$), while the other concentrations remain unchanged. Figure 1b shows the evolution of the cell volume for the three stresses. We observe the coincidence of the three curves, meaning that cell volume regulation is independent of the osmotic stress type.

Table 2 Numerical parameters (SI units)

R (gas constant)	$8.3\,\mathrm{J\,K^{-1}\,mol^{-1}}$
F (Faraday constant)	$310\,\mathrm{C\,mol^{-1}}$
T (temperature)	310 K
r_0 (initial cell radius)	10^{-6}m
C_m (cell capacitance)	$7 \times 10^{-2}\,\mathrm{F\,m^{-2}}$
P_K (K^+ permeability)	$4 \times 10^{-10}\,\mathrm{m\,s^{-1}}$
P_Na (Na^+ permeability)	$0.04\,P_\mathrm{K}$
P_Cl (Cl^- permeability)	$3\,P_\mathrm{K}$
P_X (permeability of nonpermeant ions)	0
z_K (K^+ valency)	1
z_Na (Na^+ valency)	1
z_Cl (Cl^- valency)	−1
$z^i{}_X$ (valency of nonpermeant ions inside the cell)	−1.04
$z^e{}_X$ (valency of nonpermeant ions outside the cell)	−1.06

[3] Observe that Fraser and Huang obtained a resting membrane potential of −88 mV [Table 2, page 466 in Fraser and Huang (2004)] for similar concentrations.

In Fig. 1a, we observe that the value of the transmembrane potential is highly dependent on the stress type. The value of the steady potential is around −90 mV when the stress is achieved by adding X ions or NaCl, whereas the potential changes dramatically and raises up to −65 mV when KCl is added. The stabilizations of both transmembrane potential and cell volume occur within a few tens of seconds for the three stresses. Figure 1a demonstrates that, unlike for the addition of NaCl, adding KCl induces a depolarization of the cell as described by Usher-Smith et al. (2006; Fig. 4a, page 235) and predicted by Fraser and Huang (2007). Note that, according to the experiments performed by Usher-Smith et al. (2006), this depolarization should induce cell swelling. However, this swelling occurs around 10 min after the addition of potassium ions as described by Usher-Smith et al. (2006; Fig. 4b, page 235), while our simulations stop 100 s after the stress, since we are interested in the dynamics of the phenomena just after the osmotic shock. We will discuss cell swelling induced by increasing the extracellular potassium concentration in Sect. 4.

Hypo-osmotic stress

We then study hypotonic stress, decreasing the outer osmolarity by 60 mM (from 296 to 236 mM). Here the stress is simulated in two ways: dilution of nonpermeant ions X and NaCl ($[X]_i = 1$ mM, $[\mathrm{Na}]_e = 130$ mM, $[\mathrm{Cl}]_e = 101$ mM), and dilution of NaCl only ($[\mathrm{Na}]_e = 115$ mM, $[\mathrm{Cl}]_e = 86$ mM), other concentrations being unchanged. The evolution of the cell volume for the two stresses is displayed in Fig. 2b. We observe that the two curves coincide, meaning that the cell volume regulation is independent of the two types of osmotic stress, similarly to the hypertonic stresses.

In Fig. 2a, we observe that the value of the transmembrane potential depends on the stress type: it stabilizes around −85 mV when the stress is produced by removal of NaCl, whereas the steady transmembrane potential is around −88 mV when KCl is removed. The cell reaches its steady state within a few tens of seconds for the two stresses as for the hyperosmotic stresses.

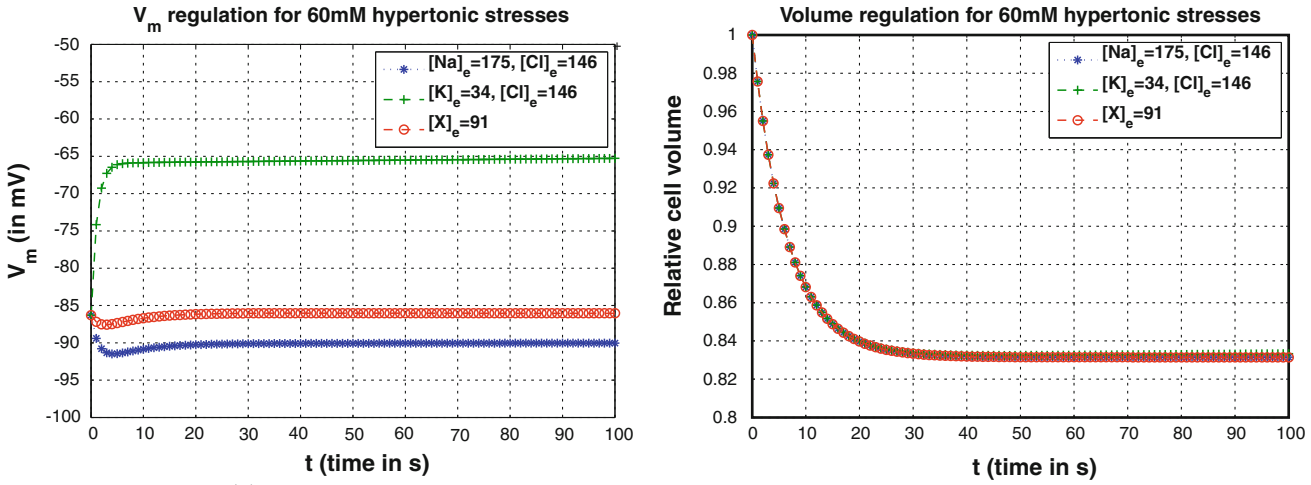
Comparison with the instantaneous model and influence of the coefficient τ_w on the cell dynamics

In this subsection we study the effect of a reduction in water penetrability τ_w on the dynamics of the phenomena, and we compare the dynamics of our model with the instantaneous model described by Fraser and Huang (2004; Eq. 5, page 462). To simulate the instantaneous model we just replace Eq. (8c) by imposing the osmotic equilibrium at any time:

$$\mathrm{v}_i = \frac{\sum_{S\in \mathrm{G}} n_S^i}{\Pi_e}. \tag{15}$$

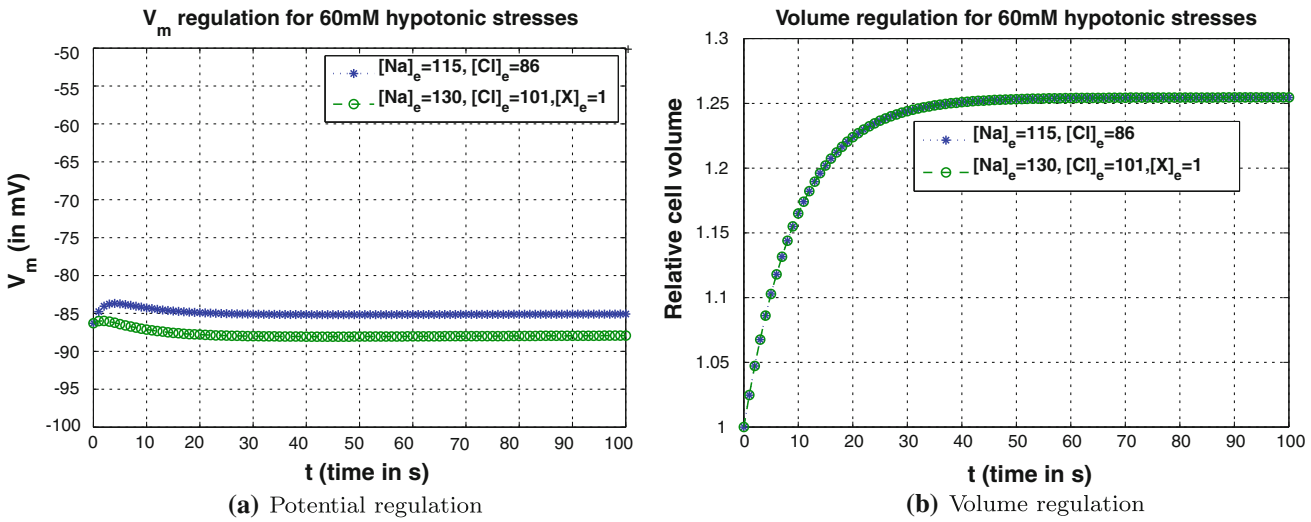
In Eq. (8c) note the difference between the coefficient τ_w and the usual coefficient of permeability, since the SI units for penetrability as described by Landahl (1948) are $\mathrm{s\,m^{-1}}$, whereas they are $\mathrm{m\,s^{-1}}$ for a permeability. The higher τ_w, the more permeant the membrane is. In accordance with Landahl (1948), the value of τ_w is around 5×10^{-13} $\mathrm{s\,m^{-1}}$. We numerically investigate the effect of a reduction of this value.

According to Fig. 3, when the membrane penetrability to water τ_w decreases from $10^{-12}\,\mathrm{s\,m^{-1}}$ to $10^{-13}\,\mathrm{s\,m^{-1}}$, a



(a) Potential regulation **(b)** Volume regulation

Fig. 1 Transmembrane potential (**a**) and cell volume (**b**) responses to a hypertonic stress: $P_e = 356$ mM, $P_i = 296$ mM. The water penetrability equals $\tau_w = 5 \times 10^{-13}\,\mathrm{s\,m^{-1}}$



(a) Potential regulation **(b)** Volume regulation

Fig. 2 Transmembrane potential (**a**) and cell volume (**b**) behaviors for hypotonic solution: $P_e = 236$ mM, $P_i = 296$ mM. The water penetrability equals $\tau_w = 5 \times 10^{-13}\,\mathrm{s\,m^{-1}}$

delay in the stabilization of the cell volume can be observed. Actually, while the stabilization occurs in about 20 s for $\tau_w = 10^{-12}\,\mathrm{s\,m^{-1}}$, the cell volume does not reach its steady state within 100 s when the value of τ_w decreases to $10^{-13}\,\mathrm{s\,m^{-1}}$. On the contrary, for the instantaneous model, the volume stabilization occurs immediately after the osmotic stress, which is not physically plausible since the water flux across the membrane is a time-consuming phenomenon, as described by the experiments of Sect. 4 (Fig. 5), and as already reported by Rouzaire-Dubois et al. experimentally (Rouzaire-Dubois and Dubois 2004; Dubois and Rouzaire-Dubois 2004; Rouzaire-Dubois et al. 2009). A decrease of τ_w, caused by alterations of the membrane phospholipids, for instance, could explain the differences in the delays observed experimentally by Rouzaire-Dubois et al. (Dubois and Rouzaire-Dubois 2004; Rouzaire-Dubois and Dubois 2004) in the cell osmotic stabilization. Moreover, let us note that the values of the steady potential given by Fig. 4 are similar (around -90 mV) for τ_w equal to $10^{-12}\,\mathrm{s\,m^{-1}}, 5 \times 10^{-13}\,\mathrm{s\,m^{-1}}$ or $10^{-13}\,\mathrm{s\,m^{-1}}$, as for the instantaneous model, meaning that a decrease of the penetrability of water does not affect the value of the transmembrane potential but the dynamics of volume regulation.

Preliminary experiments for hypertonic stresses

To validate the theoretical results we performed preliminary experiments. More precisely in this section, we aim to show that the dynamics of the experimental cell volume stabilization occurs within the time predicted by the model after osmotic stresses.

Materials and methods

Cell culture

DC-3F cells (Chinese hamster fibroblast lung cells) were grown in minimum essential medium (Invitrogen, Cergy-Pontoise, France) supplemented with 10% fetal bovine serum (Invitrogen), 500 U/ml penicillin, and 500 µg/ml streptomycin (Invitrogen), defined as complete medium. Cultures were maintained in humidified atmosphere with 5% CO_2 at 37°C. Cells were routinely passed every 2 days.

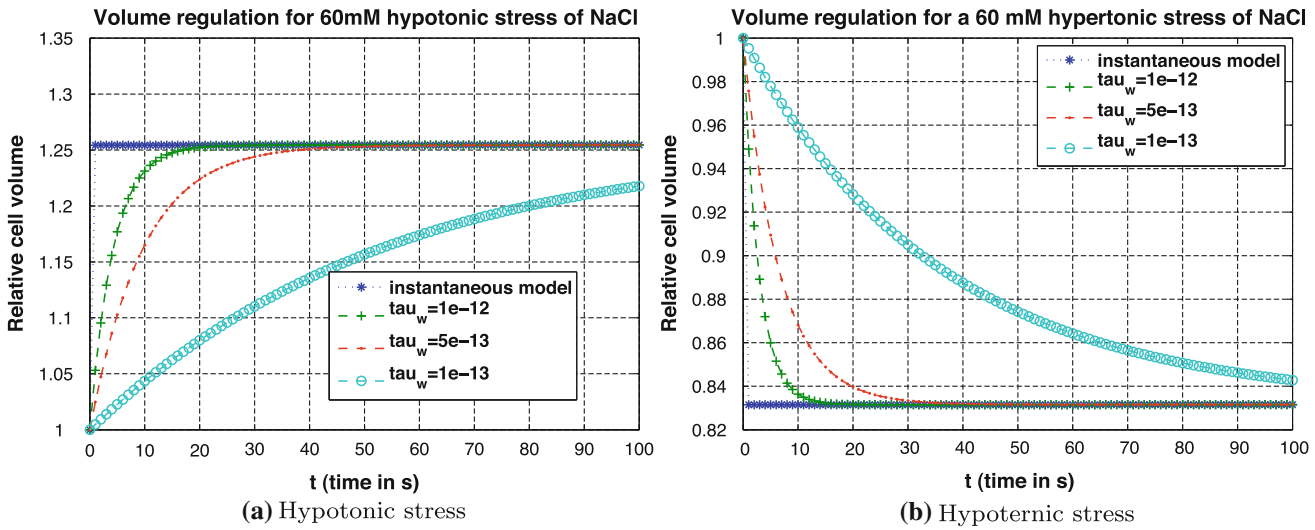
Solutions used

The solutions used were all phosphate-buffered saline (PBS) based (D-PBS 10×, Invitrogen, diluted with milli-Q water, final molarities: Na^+ −154 mM; K^+ −4 mM; Cl^- −140 mM; $H_2PO_4^-$ −1.5 mM; HPO_4^{2-} −8 mM). Hypertonic solutions were obtained by adding either NaCl or KCl in order to increase their concentration by a minimum of 25 mM and a maximum of 300 mM.

Fluorescence-activated cell sorter (FACS) experiments

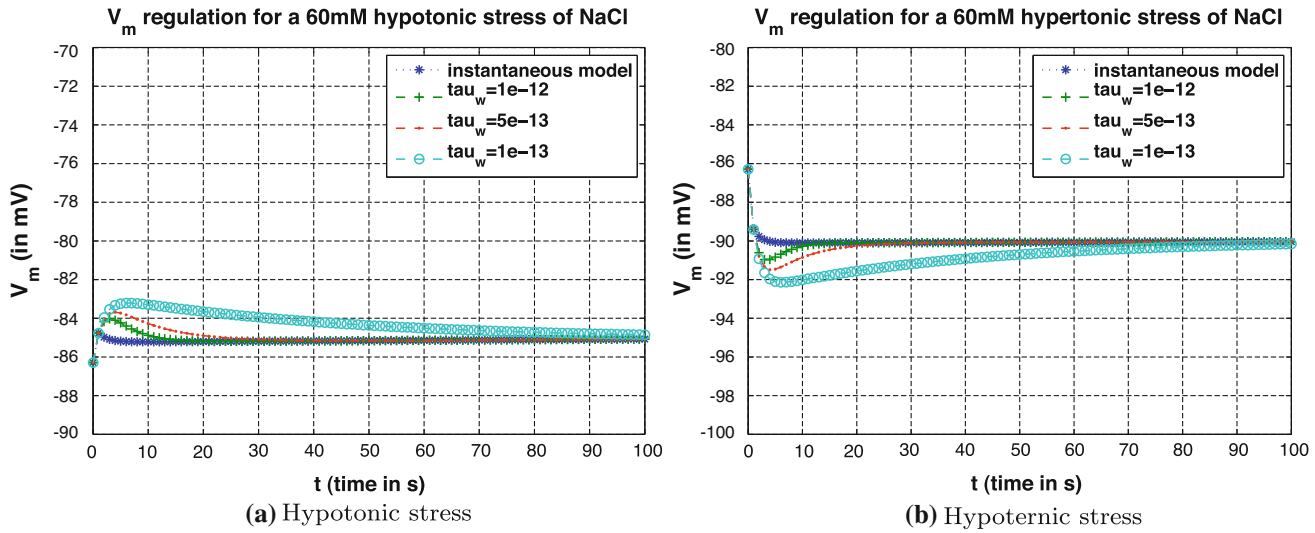
Cells were harvested by trypsin and placed in PBS solution for 15 min before any osmotic stress. The hypertonic stresses were obtained by adding the suitable volume of PBS supplemented with 300 mM NaCl or KCl to 500 µL PBS containing the cells in suspension.

In FACS analysis, the dynamic evolution of the forward scattering (FSC) was obtained starting with 800 µL of cells in suspension. Of this, 300 µL was used to obtain the initial FSC value. The tube was then removed from the FACS



(a) Hypotonic stress **(b)** Hypoternic stress

Fig. 3 Cell volume behavior for hypotonic (**a**) and hypertonic (**b**) stresses of amplitude 60 mM by changing the concentration of nonpermeant ions *X*



(a) Hypotonic stress **(b)** Hypoternic stress

Fig. 4 Transmembrane potential behaviors for hypotonic (**a**) and hypertonic (**b**) stresses of amplitude 60 mM by changing the concentration of nonpermeant ions *X*

(FACScalibur, Becton Dickinson, San Jose, USA) and replaced with another one containing the remaining 500 µL mixed with the correct volume of PBS supplemented with NaCl solution at 300 mM just before starting the stressed cells FACS acquisition. Each reported value is the average of 100–500 cells FSC acquisition. The steady volume ratios for each stress were measured and lasted 40 s, during which the FSC was stable. Approximately 50,000 cells were run through the FACS 3 min after the hypertonic stresses.

Statistical analysis of FSC measurements

In static experiments, since the cell populations did not follow a Gaussian distribution, the mean FSC for each individual experiment is the average value of all the events considered as cells. Final results are the average ± standard deviation (SD) of three individual experiments. All the calculations were performed with MATLAB® software (R2007a, The MathWorks).

Results

Dynamics of the cell volume regulation

The dynamic evolution of the FSC (as explained in the "Materials and methods" section) was recorded for the three different NaCl stresses indicated in Fig. 6. The experimental averaged data are plotted using thin lines, the

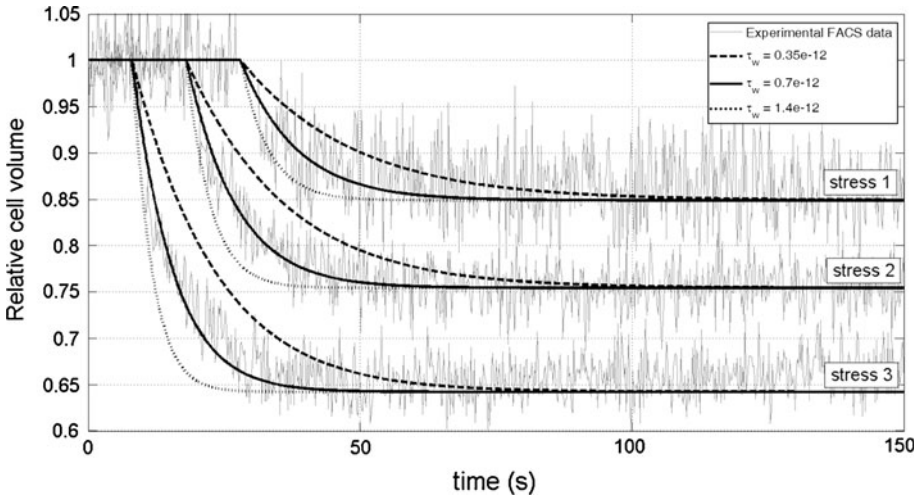


Fig. 5 Dynamic evolution of the relative cell volume for the three stresses defined in the text

beginning of the stresses being deliberately shifted by 10 s each. The part of the curves with no change in cell volume (initial FSC) was acquired from a 300 µL cell suspension. Then, the missing part of the curves corresponds to the time required to remove the tube from the FACS and replace it with the cells just osmotically stressed. On Fig. 5 we have superimposed the theoretical volume evolution for three different values of membrane penetrability to water denoted by τ_w. The experimental data provided by FSC cannot be precisely fitted, but they display the dynamics of the volume regulation after the shock. As described by Fig. 5 we can give a good framing of τ_w, as all three stresses have their best fit for $\tau_w = 7 \times 10^{-13}\,\mathrm{s\,m^{-1}}$. Stress 2 in particular validates the dynamics of the cell volume regulation described by Eq. (8c).

Volume ratios within 3 min after the stresses

For the different hypertonic solutions, cells were analyzed using a FACS 3 min after the hypertonic stress. The mean FSC, which can be considered as a good representation of cell size, was measured, and experimental volume ratios were thus calculated. Simultaneous numerical simulations of the model were performed with the experimental values of the initial concentrations. Experimental and numerical ratios at 3 min after the stress are plotted in Fig. 6. It appears that the theoretical values are very close to the experimental data for the NaCl osmotic shocks. The numerical ratios slightly underestimate the measured ratios for the KCl hypertonic stresses. Noticeably, for the same increase in molarity, the model predicts a lower amplitude of the volume change after KCl than NaCl stress. Experimental data confirm this tendency but show a larger difference than predicted, though we have only considered passive fluxes in the model. Addition of potassium ions is known to activate Na/K pumps, leading to cell swelling.

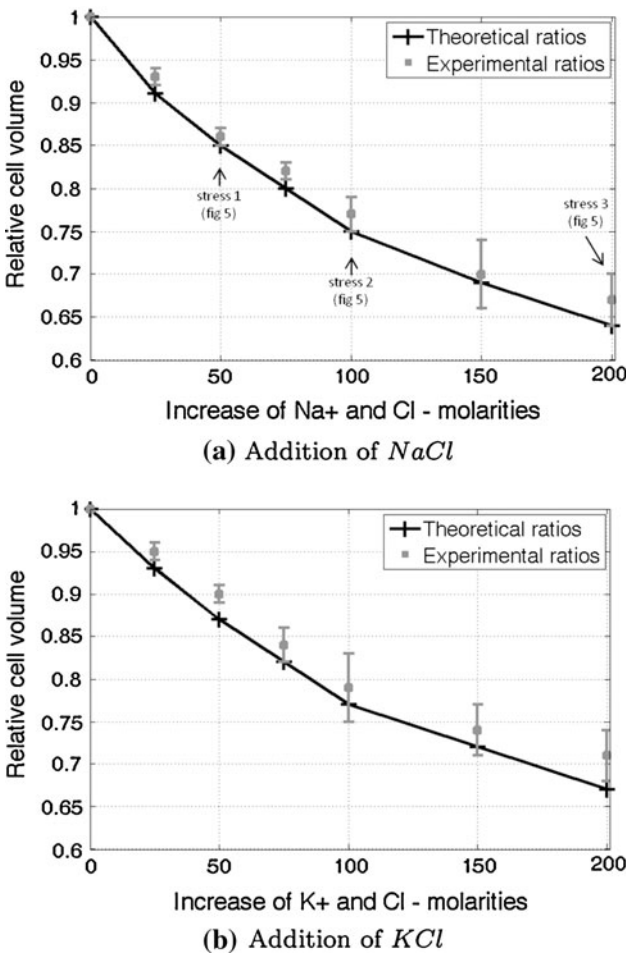


Fig. 6 Relative cell volume after stabilization for an osmotic stress of 60 mM by adding NaCl (**a**) or KCl (**b**); means ± SD of three experiments

In Remark 3, we have described how to add the fluxes associated with Na/K-ATPase. However we emphasize that, despite the simplicity of our flux model, the numerical results fit the experiments very well.

Discussion

We have proposed a complete and generic model for the transmembrane potential and cell volume regulation. This model, based on ordinary differential equations, dynamically links the membrane potential, the cell volume, and the internal and external ionic concentrations. In the case of spherical cells, we explicitly link the behavior of the cell volume to the transmembrane potential. The main insight consists in taking into account the water diffusion across the membrane due to the osmotic pressure: our model describes precisely the cell swelling (or shrinking) a few seconds after an osmotic stress, a result that cannot be obtained by the previous models. Moreover, the models presented previously by Jakobsson (1980), Armstrong (2003), Endresen et al. (2000), Fraser and Huang (2004), and Hernández and Cristina (1998) can be derived from it. More precisely, if the cell volume is constant, we recover the model of Endresen et al. (2000), and if the osmotic equilibrium is instantly reached, our model is then similar to the model of Arsmtrong (2003) and leads to the "charge difference" equation of Fraser and Huang (2004). Observe that the new model describes precisely the cell volume regulation within a few seconds after the osmotic stress, which the previous models cannot do, as shown in Fig. 3b and Fig. 5. We also emphasize that the fluxes due to pumps such as Na/K-ATPase can be easily added, but for the sake of clarity we have only considered fluxes through passive channels.

Preliminary experiments validate the model proposed in this paper. The numerical simulations fit very well with the experimental data after NaCl addition, while a larger difference appears after KCl addition. It can be noticed that the final values obtained during dynamic experiments are coherent with the ones obtained by static analysis of a large number of cells. Moreover, the time resolution of FACS seems well adapted to this kind of dynamics. Indeed, FACS is fast enough to describe quite precisely the volume evolution, the time needed to actually mix solutions in order to make the stress being the limiting factor. FACS thus appears to be an efficient tool for evaluating the volume dynamic evolution. In a next step it will be interesting to investigate if the dynamics are different depending on cell type. Indeed, the differences between KCl and NaCl stresses might be relevant to metabolic issues. Using different cell types or acting on metabolism with appropriate drugs are avenues that appear worth exploring.

Models based on instantaneous volume regulation cannot explain the delays in cell volume stabilization observed experimentally by Rouzaire-Dubois et al. (Dubois and Rouzaire-Dubois 2004; Rouzaire-Dubois et al. 2009) for the fifth generation of cells in culture submitted to a permanent osmotic stress. Comparatively, the first generation stabilizes very quickly (Dubois and Rouzaire-Dubois 2004). Our model provides a possible explanation for such delays. Alterations of the membrane lipids, increasing with each new generation of cell culture, could decrease the value of τ_w, thus leading to such results.

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