A model of intracellular pH control

Soroush Safaei[1], Rossanna Occhipinti[2], Peter Hunter[1] and Walter Boron[2]

[1]Auckland Bioengineering Institute, University of Auckland, New Zealand
[2]Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, USA

Contributions to paper: SS and PH implemented the models from the original paper, wrote them in bond graph form and then coded them in CellML for solution with OpenCOR. RO and WB ensured that the *Physiome* models are consistent with his original equations. All four authors contributed to the writing of the paper.

Introduction

In 1976 Boron and De Weer published their landmark paper on "Intracellular pH transients in squid giant axons caused by CO_2, NH_3, and metabolic inhibitors" [1]. This paper used an experimental giant squid preparation and a mathematical model of pH buffering and the transport of protons, bicarbonate and CO_2 to establish the experimental evidence for active control of pH by a membrane proton pump. The paper reported on the consequences of exposing the axons first to elevated CO_2, then to elevated NH_4Cl, and finally to the metabolic inhibitors, cyanide, azide and dinitrophenoxide (DNP).

In the first experiment, following exposure of the cells to elevated CO_2, intracellular CO_2 rapidly equilibrates with the extracellular CO_2, and intracellular H^+ and HCO_3^- are generated from the carbonic anhydrase reaction. Since HCO_3^- rapidly leaves the cell down the now reversed $[HCO_3^-]$ electrochemical gradient (thereby increasing the drive for CO_2 entry), the accumulating H^+ results in a lowered intracellular *pH* (pH_i). The expectation was that prolonged exposure to CO_2 would cause a continued drop in pH_i, but in fact an *alkaline* drift was observed, leading to the postulate of active pumping of H^+ out of the cell at a rate that exceeds the passive shuttling by the CO_2/HCO_3^- couple. Following removal of external CO_2, intracellular CO_2 diffuses out while intracellular HCO_3^- combines with H^+ to leave the cell as CO_2. In the absence of a H^+ pump, the entire intracellular H^+ load associated with CO_2 entry would be expected to be removed. In fact pH_i was observed to overshoot its resting value by an amount consistent with the net removal of H^+ by the pump.

In the second experiment, following exposure to elevated NH_4Cl (ammonium chloride), the intracellular environment becomes alkaline as NH_3 enters and hydrates to form NH_4^+ and OH^-. Additional passive NH_4^+ entry down its electrochemical gradient opposes the NH_3 entry and slightly reduces the pH_i increase. These effects and the subsequent pH_i undershoot when the ammonium chloride is removed, are also consistent with the proposed model that includes an active proton pump.

Finally, exposure of the cells to in turn cyanide, DNP and azide resulted in intracellular acidosis, consistent with metabolic inhibition of the proton pump.

In this paper we formulate the models from the Boron and De Weer [1] paper (henceforth referred to as 'BDW') and specify the simulation using the Physiome modelling standards CellML and SED-ML in order to ensure that the model reproduces the graphs in the original paper. Note that this required an additional specification of the proton pump that was not described in the BDW paper. The curated and annotated model is made available in a form that users can run with OpenCOR[1] to understand the model and to explore the effect of parameter changes.

[1] www.opencor.ws

pH buffering by weak acids and weak bases

We begin by reviewing pH buffering by weak acids and bases, based on the work of Roos [2] (see also Boron and Boulpaep [3]). These processes are used to derive the equilibrium relations and intracellular buffering power β that are needed for the BDW model.

The dissociation of uncharged weak acid (HA) to anionic weak base (A^-) is governed by

$$HA \Leftrightarrow A^- + H^+ \quad \text{and at equilibrium:} \quad K_A = \frac{[A^-][H^+]}{[HA]} \tag{1}$$

e.g. $CO_2 + H_2O \Leftrightarrow H_2CO_3 \overset{CA}{\Leftrightarrow} HCO_3^- + H^+$, see below.

The dissociation of cationic weak acid (BH^+) to weak base (B) is governed by the reaction

$$BH^+ \Leftrightarrow B + H^+ \quad \text{and at equilibrium:} \quad K_B = \frac{[B][H^+]}{[BH^+]} \tag{2}$$

e.g. $NH_4^+ \Leftrightarrow NH_3 + H^+$, see below.

These reactions hold on both sides of a cell membrane, with the same equilibrium constants on both sides. The neutral species (HA and B) move freely down their concentration gradients to equilibrate at equal concentration on either side of the membrane, while the charged species (A^-, BH^+ and H^+) move down their concentration gradients (within a membrane protein channel) until equilibrating with their Nernst potentials:

$$E_A = \frac{RT}{F}\ln\frac{[A^-]_o}{[A^-]_i}, \quad E_{BH} = \frac{RT}{F}\ln\frac{[BH^+]_o}{[BH^+]_i} \quad \text{and} \quad E_H = \frac{RT}{F}\ln\frac{[H^+]_o}{[H^+]_i} \tag{3}$$

When the reaction $HA \Leftrightarrow A^- + H^+$ is in equilibrium, and $[HA]_0 = [HA]_i = [HA]$,

$$K_A = \frac{[A^-]_o[H^+]_o}{[HA]} = \frac{[A^-]_i[H^+]_i}{[HA]} \quad \text{or} \quad \frac{[A^-]_o}{[A^-]_i} = \frac{[H^+]_i}{[H^+]_o}, \text{ and therefore } E_A = -E_H \tag{4}$$

Similarly, when the reaction $BH^+ \Leftrightarrow B + H^+$ is in equilibrium on both sides of the membrane, and both K_B and $[B]$ are the same on both sides (free permeation of the uncharged molecule),

$$K_B = \frac{[B][H^+]_o}{[BH^+]_o} = \frac{[B][H^+]_i}{[BH^+]_i} \quad \text{or} \quad \frac{[H^+]_o}{[H^+]_i} = \frac{[BH^+]_o}{[BH^+]_i}, \text{ and therefore } E_{BH} = E_H \tag{5}$$

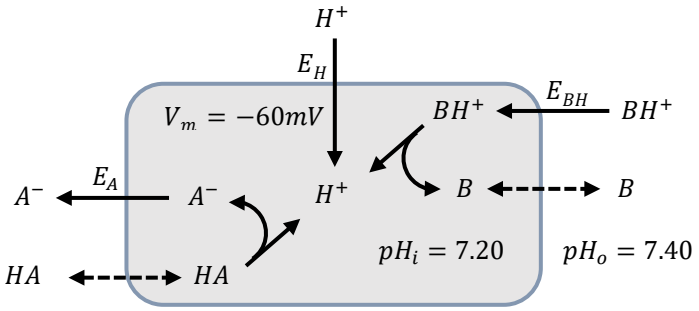

Figure 1. Solute fluxes for pH buffering by weak acids and weak bases.

The Boron and De Weer model for weak acids

The formation of bicarbonate (HCO_3^-) and protons (H^+) from CO_2 by hydration, catalysed by carbonic anhydrase (CA) within the cell is given by the reaction:

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \overset{CA}{\Leftrightarrow} HCO_3^- + H^+,$$

where the first hydration step is assumed to be instantaneous and the equilibrium coefficient is therefore $K_{CO_2} = \frac{[HCO_3^-][H^+]}{[CO_2]}$. Taking logs and using Henry's law, $[CO_2] = s.p_{CO_2}$, where s is the solubility coefficient for CO_2 and p_{CO_2} is the partial pressure of CO_2, yields the Henderson-Hasselbalch equation for the CO_2/HCO_3^- buffer system[2]:

$$pH = pK_{CO_2} + \log\frac{[HCO_3^-]}{s.p_{CO_2}}, \text{ where } pH = -\log_{10}[H^+] \text{ and } pK_{CO_2} = -\log_{10} K_{CO_2}.$$

In terms of the nomenclature above, the weak acid HA is CO_2 and its conjugate base A is HCO_3^-. The total intracellular weak acid concentration is $[TA]_i = [CO_2]_i + [HCO_3^-]_i$, and the fraction of $[TA]_i$ that remains undissociated is α. Therefore $[CO_2]_i = \alpha[TA]_i$ and $[HCO_3^-]_i = (1-\alpha)[TA]_i$. All concentrations have units *mol.m*$^{-3}$ (which is equivalent to *mM* as used in BDW).

From equation 1 above,

$$\alpha = \frac{[CO_2]_i}{[CO_2]_i + [HCO_3^-]_i} = \frac{[H]_i}{[H]_i + K_A}, \quad (6)$$

where K_A is the acid dissociation constant (i.e. $K_A = \frac{[HCO_3^-]_i[H^+]_i}{[CO_2]_i}$).

Note that the equilibrium relation outside the cell provides the external bicarbonate concentration:

$$[HCO_3^-]_o = \frac{K_A \cdot [CO_2]_o}{[H^+]_o}. \quad (7)$$

The rate of change of $[TA]_i$ is given by the sum of the influx of CO_2 and A:

$$\frac{d[TA]_i}{dt} = \rho\left(M_{CO_2} + M_{HCO_3^-}\right). \quad (8)$$

where ρ (*m*$^{-1}$) is the area to volume ratio for the cell and converts the transmembrane flux per unit area (in units of *mol.m*$^{-2}$*.s*$^{-1}$) to a rate of change per unit cell volume (*mol.m*$^{-3}$*.s*$^{-1}$ or *mM.s*$^{-1}$).

The rate of increase of intracellular proton concentration $\frac{dQ}{dt}$ (*mol.m*$^{-3}$*.s*$^{-1}$) is therefore

$$\frac{dQ}{dt} = \rho\left[(1-\alpha)M_{CO_2} - \alpha M_{HCO_3^-} - M_{H^+}\right] \quad (9)$$

where M_H is the sum of intracellular consumption and active extrusion.

To calculate the rate of change of free protons, BDW use the definition of buffering power $\beta = \frac{dQ}{dpH}$ (*mol.m*$^{-3}$) which, with $2.303pH = -ln[H^+]_i$, or $2.303\frac{dpH}{dt} = -\frac{1}{[H^+]_i}\frac{d[H^+]_i}{dt}$, gives

$$\frac{d[H^+]_i}{dt} = -2.303[H^+]_i.\frac{dpH}{dt} = -\frac{2.303[H^+]_i}{\beta}.\frac{dQ}{dt}$$

or, with equation 9,

$$\frac{d[H^+]_i}{dt} = -\frac{2.303[H^+]_i}{\beta}\rho\left[(1-\alpha)M_{CO_2} - \alpha M_{HCO_3^-} - M_{H^+}\right]. \quad (10)$$

The three fluxes (with units of *mol.m*$^{-2}$*.s*$^{-1}$) are

[2] For arterial blood pK_{CO_2}≈6.1, $[HCO_3^-]$ ≈24*mM*, *s*=0.0346*mM/mmHg* and p_{CO_2}= 40*mmHg*, giving pH ≈ 7.34.

(i) $$M_{CO_2} = P_{CO_2}([CO_2]_o - [CO_2]_i), \quad (11)$$

where P_{CO_2} ($m.s^{-1}$) is the membrane permeability to an uncharged weak acid (note that this reflects the combination of permeation and hydration of CO_2),

(ii) $$M_{HCO_3^-} = P_{HCO_3^-} \frac{V_m F}{RT} . \frac{[HCO_3^-]_o - [HCO_3^-]_i \epsilon}{1-\epsilon}, \quad (12)$$

where $P_{HCO_3^-}$ ($m.s^{-1}$) is the membrane permeability to the charged conjugate base (HCO_3^-) and[3] $\epsilon = e^{-\frac{V_m F}{RT}}$, and the proton pump, modelled with a proton pumping rate k ($m.s^{-1}$), and

(iii) $$M_H = \begin{cases} k([H^+]_i - [H^+]_{lim}) & if\ pH_i < pH_{lim} \\ 0 & otherwise \end{cases} . \quad (13)$$

Note that $k[H^+]_{lim}$ is the basal rate at which protons are pumped out of the cell[4]. Finally, recall that

$$[CO_2]_i = \alpha[TA]_i, \quad [HCO_3^-]_i = (1-\alpha)[TA]_i \quad \text{and} \quad \alpha = \frac{[H^+]_i}{[H^+]_i + K_A}. \quad (14)$$

The parameter values specified in BDW are: ρ = 8000 m^{-1} (based on a cylindrical cell of diameter 0.25 *mm*), $\beta = -26$ $mol.m^{-3}$, P_{CO_2} = 6.10^{-5} $m.s^{-1}$, $P_{HCO_3^-}$ = 5.10^{-9} $m.s^{-1}$, K_A =10^{-3} $mol.m^{-3}$, and k > 6.10^{-3} $m.s^{-1}$.

CO_2 experiments

Here we follow the BDW experimental protocol in which a step change in extracellular p_{CO_2} from 0 to 5% (37*mmHg* or, with s = 0.0321 *mM/mmHg*, $[CO_2]_o = s.p_{CO_2}$ = 1.1877 *mM*) is applied for 2700*s* (45*mins*) at constant pH_o=7.7. The differential equations (8) and (10), with the accompanying definitions (6), (7), (11), (12), (13) and (14), are coded in CellML and solved with OpenCOR to give the plots in Figure 2.

[3] RT= 2.4777 $kJ.mol^{-1}$ at 25°C (298K), F = 96.485 $kC.mol^{-1}$ and z=1, giving RT/zF = 25.6796 *mV*.

[4] Note that equation 13 is not obvious from the original BDW paper.

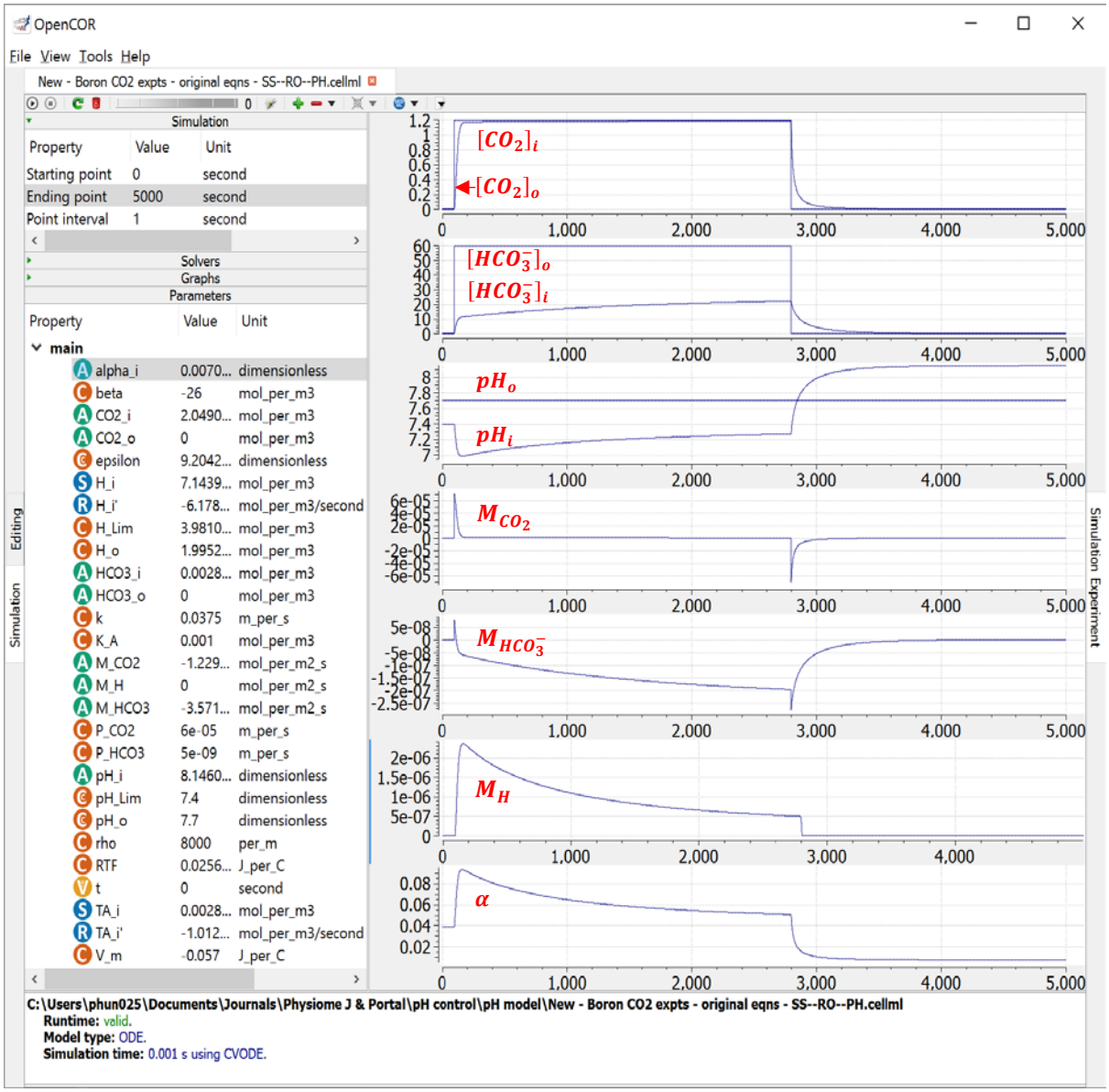

Figure 2. Solution of the BDW model during and following a 2700*s* period of externally applied CO_2. In these simulations pH_i=7.7 and $[HCO_3^-]_o$ is determined from the equilibrium with $[H^+]_o$ and CO_2 (equation 7). Note that during the plateau phase $[HCO_3^-]_i$ continues to rise while $[H^+]_i$ falls and pH_i climbs (the proton pumping rate is set to 0.0375 $m.s^{-1}$). Note also that pH_i rises higher than its starting value (about 7.4 before the CO_2 pulse and 8.15 afterwards) indicating the net extrusion of protons from the cell.

The Boron and De Weer model for weak bases

The analogous equations for a weak base $BH^+ \Leftrightarrow B + H^+$ (i.e. B is NH_3 and BH^+ is NH_4^+) are as follows:

$$\alpha_i = \frac{[NH_4^+]_i}{[NH_4^+]_i + [NH_3]_i} = \frac{[H^+]_i}{[H^+]_i + K_B}, \tag{15}$$

where K_B is the acid dissociation constant (i.e. $K_B = \frac{[NH_3]_i[H^+]_i}{[NH_4^+]_i} = \frac{[NH_3]_o[H^+]_o}{[NH_4^+]_o}$).

The rate of change of $[TB]_i$ is given by the sum of the influx of NH_3 and NH_4^+:

$$\frac{d[TB]_i}{dt} = \rho\left(M_{NH_3} + M_{NH_4^+}\right). \tag{16}$$

Now

$$\frac{d[H]_i}{dt} = -\frac{2.303[H]_i}{\beta}\rho\left[(1-\alpha)M_{NH_4^+} - \alpha M_{NH_3} - M_H\right], \tag{17}$$

where

(i) $$M_{NH_3} = P_{NH_3}([NH_3]_o - [NH_3]_i), \tag{18}$$

(ii) $$M_{NH_4^+} = P_{BH^+}\frac{V_m F}{RT}.\frac{[NH_4^+]_o - [NH_4^+]_i\epsilon}{\epsilon - 1}, \text{ with } \epsilon = e^{-\frac{V_m F}{RT}} \tag{19}$$

(iii) $$M_H = k([H^+]_i - [H^+]_i'). \tag{20}$$

and

$$[NH_4^+]_i = \alpha_i[TB]_i, \quad [NH_3]_i = (1-\alpha_i)[TB]_i. \tag{21}$$

In this case, because $[NH_4^+]_o$ is specified we also include the dissociation to $[NH_3]_o$:

$$[NH_3]_o = \frac{1-\alpha_o}{\alpha_o}.[NH_4^+]_o, \text{ where } \alpha_o = \frac{[NH_4^+]_o}{[NH_4^+]_o + [NH_3]_o} = \frac{[H]_o}{[H]_o + K_B} \tag{22}$$

The parameter values specified in BDW are: ρ = 8000 m^{-1}, $\beta = -9$ $mol.m^{-3}$, $P_{NH_4^+}$ = 6.10^{-10} $m.s^{-1}$, P_{NH_3} = 6.10^{-5} $m.s^{-1}$, and K_B = 0.31623.10^{-3} $mol.m^{-3}$.

NH_4Cl experiments

The BDW experimental protocol in this case was a step change in extracellular NH_4Cl from 0 to 10 *mM* applied for 600*s* (10*mins*) at constant pH_o.

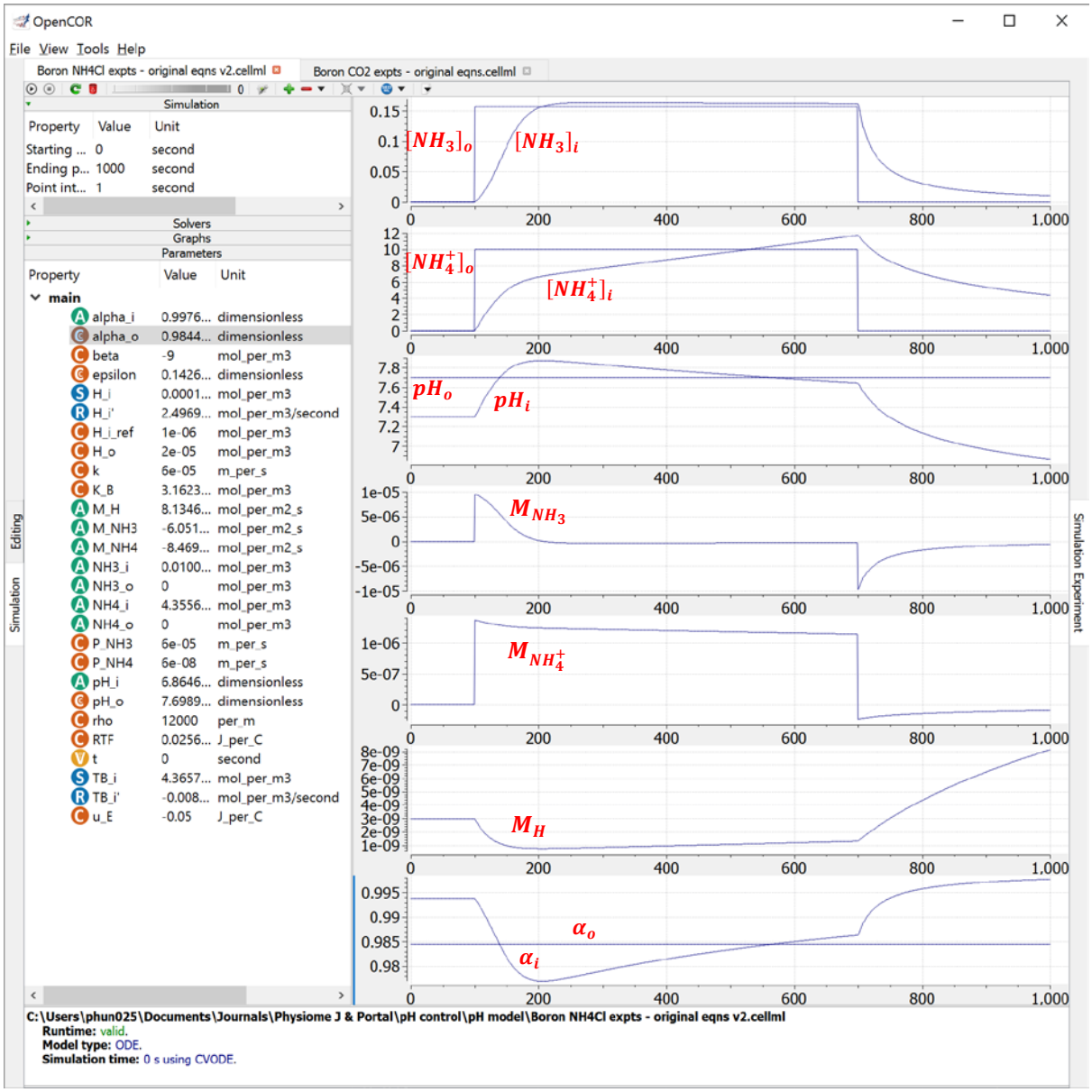

Figure 3. Solution of the BDW model during and following a 600*s* period of externally applied ammonium chloride $[NH_4^+]_o$ (= 10 *mol.m*$^{-3}$ (10*mM*)). The intracellular environment becomes alkaline as NH_3 enters (note M_{NH_3}) and hydrates to form NH_4^+ and OH^-. Additional passive NH_4^+ entry ($M_{NH_4^+}$) down its electrochemical gradient opposes the NH_3 entry and slightly reduces the *pH*$_i$ increase. When the ammonium chloride is removed $[NH_3]_i$ and $[NH_4^+]_i$ decline towards their original values but *pH*$_i$ drops well below its original value of 7.4.

References

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