**The governing equations of the red blood cell model (RCM)**

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***Incorporates minimal representation of PMg and Mg-dependent sodium pump activity[1], to explore effects of deoxy-PIEZO1-mediated Mg loss on ISC rehydration rate resulting from Na pump inhibition.***

**Introduction**

Red blood cell homeostasis addresses the subset of mechanisms that control the dynamic changes in cell volume, membrane potential, ionic composition, membrane transport and osmotic gradients in response to perturbations. The modelled system consists of a suspension of identical RBCs whose dynamic behaviour is constrained only by charge and mass conservation. The equations implement these laws following a strict computational sequence representative of the multiple interconnected processes involved. The default values set for all the model parameters have been experimentally determined, allowing model outputs to predict the homeostatic behaviour of human RBCs in physiological, pathological and experimental conditions within accuracy margins of about 5-10%.

The current text of the Governing Equations of the RCM is an updated version of the original published in 2021 **[2, 3].** The complete model code, together with a User Guide and a detailed tutorial, are available

with open access in the repository (<https://github.com/sdrogers/redcellmodeljava>).

**The initial Reference State (RS)**

The RBC reference state describes the initial condition of the system in a pump-leak balanced steady state. For compliance with initial electroneutrality and osmotic equilibrium we use a phenomenology in which the charge, nX or nX, and cell content of the global, non-haemoglobin, impermeant cell anion, QX‑, are treated as wildcard parameters in equations 3 and 8. The nx and CX- values emerging from such treatment correspond closely with the known organic and inorganic phosphate pools of metabolically normal RBCs [4]. When modifying the initial default values in the RS the wildcard parameters may change. The model automatically recalculates their value potentially changing slightly the constitutive make up of the impermeant cell anion (nX\*QX‑) in the new cell.

***Medium electroneutrality:***

**MA+(MB – MBH)+Mgluconate – (MNa+MK+2\*(MCa2++MMg2+)+Mglucamine) = 0 ….1**

Medium concentration of proton-bound buffer, MBH (HEPES, by default):

**MBH = MB\*(MH/(KB + MH)) ...................................................................................... 2**

***Intracellular electroneutrality:***

**CNa + CK + CH + 2\*CMg2+ + 2\*CCa2+ - (CA + nHb\*CHb + nX\*CX‑) = 0 ………….3**

nHb, the net charge on the haemoglobin molecule, is represented by the Cass-Dalmark equation [5],

**nHb = ɑ\*(pHi – pI) [5]……………………………………………………………..4**

where ɑ corresponds to the linear segment of the proton titration curve of Hb in intact RBCs, and pI is the pHi at the isoelectric point of haemoglobin.

In the Reference steady state the net fluxes of each of the i-transported solutes is zero, and pump-leak balance is represented by ∑Ij *= F*∑zjFj = 0, where Ij is the current carried by transporter j, *F* is the Faraday constant, zj is the net charge on each of the j-transporters, and Fj is the net flux through the j-transporter; z ≠ 0 only for electrogenic transporters.

**Medium and cell osmolarities, MOs and COs:**

**MOs = MA + MB + Mgluconate + MNa + MK + MCat + MMgt + Mglucamine ……..5**

**COs = CNa + CK + CA + CH + CMg2+ + CCa2+ + fHb\*CHb + CX‑ ………………6**

fHb is the osmotic coefficient of haemoglobin, represented with only two virial coefficients, *b* and *c*:

**fHb = 1 + *b*\*CHb + *c*\*CHb2 …………………………………………………………..7**

***Osmotic equilibrium in the reference steady state:***

**MOs = COs …………………………………………………………………………….8**

***Cytoplasmic buffering of protons, calcium and magnesium.***

Heamoglobin is the major cytoplasmic buffer for protons (eq 4) and for calcium (α-buffer in eq 9c). The main magnesium buffers are ATP and 2,3-DPG, compounds integrated within the X‑ phenomenology. Because the bound forms of Ca and Mg are contained within CX‑,they are not included as separate osmolarity contributors in eq 6, leaving only the free forms of Ca2+ and Mg2+ as osmotic contributors.

Cytoplamic Ca2+ and Mg2+ buffering have been measured with precision in intact RBCs [6-9] enabling accurate representations in the model. The total Ca and Mg content of the cells, QCa and QMg, is reported in units of mmol/(340g Hb) (or mmol/Loc**)** whereas concentrations of the free forms, CCa2+ and CMg2+, are expressed in units of mmol/Lcw, a conversion requiring translation for operational reasons in the model Equation 9a translates QCa in units of mmol/Loc to CCa in units of mmol/Lcw using:

**CCa = QCa\*( RCV/Vw) ……………………………………….. 9a**

The total calcium concentration is the sum of free and bound forms:

**CCa = CCa2+ + CCaB ……………………………………….9b**

There are two buffer systems for binding calcium in the RBC cytoplasm, α (mostly haemoglobin), and the BCa/KBCa buffer [7]. The concentration of bound calcium, CCaB, at each total calcium concentration, CCa, is represented by:

**CCaB = α\*CCa + CBCa\*(CCa2+/(CCA2+ + KBCa)) …………… 9c**

CCa2+ is solved from the implicit equation:

**CCa – CCa2+ – CCaB = 0 ……………………………………… 9d**

by the Newton-Raphson routine in the RS and at the end of the computations in each iteration cycle. The measured values of the calcium binding parameters are α = 0.30, CBCa = 0.026 mmol/Loc, and KBCa = 0.014 mM [7].

The corresponding equations for cytoplasmic magnesium buffering and CMg2+ are:

**CMg = QMg\*(RCV/Vw) ……………………………………………………. 9e**

**CMg = CMg2+ + CMgB ……………………………………………………… 9f**

**CMgB = CBMg1\*(CMg2+/(CMg2++KBMg1))+CBMg2\*(CMg2+ /(CMg2++KBMg2))+CBMg3 ..… 9g**

CMg2+ is solved from the implicit equation:

**CMg - CMg2+ - CMgB = 0 ……………………………………………………… 9h**

The measured values of the Mg bufferes [9] are: CBMg1 = 1.2 mmol/Loc, KBMg1 = 0.08 mM; CBMg2 = 7.5 mmol/Loc (15 mEq/Loc), KBMg2 = 3.6 mM; CBMg3 = 0.05 mmol/Loc. BMg1 represents ATP, BMg2 represents 2,3-DPG and miscellaneous phosphate groups, and BMg3 is an unidentified high affinity magnesium buffer.

***Effects of deoxygenation on cytoplasmic Mg2+ buffering and pHi.*** Deoxygenation increases haemoglobin binding of ATP and 2,3-DPG thus reducing their availability for buffering intracellular magnesium. CBMg1 is reduced by half and CBMg2 by 1.7 [9]. This is particularly relevant for simulating accurately the effects of changing the oxygenation condition of RBCs, a process in which changes in CMg2+ become enmeshed with effects arising from changes in the isoelectric point of haemoglobin, pI (eq 4).

*Charge balance during Mg2+ buffering changes.* By treating the Mg buffers as part of the global impermeant cell ion the osmotic balance on deoxygenation remains undisturbed. On the other hand, the change in Mg2+ charge, QMg2+, needs balancing from within the global charge on X, nX\*QX by a corresponding change in nX. Because X and Mg are treated as impermeant cytoplasmic solutes in the model, their amounts per litre original cells, QX and QMg, are set in the Reference State as constants in the system, their concentrations varying only with changes in cell volume. Therefore, from ∆nX = nXdeoxy – nXoxy and from QX-deoxy - QX-oxy = 2\*(QMg2+deoxy - QMg2+oxy), we derive nXdeoxy = nXoxy - 2\*(QMg2+deoxy - QMg2+oxy)/QX. Thus, both nXoxy and nXdeoxy become defined as constants whose values are set by the values of QMg2+ and of the wildcard parameters nX and QX- as derived from equations 3 and 8 in the Reference State. With the current default settings in the Reference State, nXoxy = -0.4352, and nXdeoxy = -0.4511, values instantly changed and reversed during deoxygenation-reoxygenation cycles.

*Effects of change in the isoelectric point of haemoglobin on cell pH, pHi.* Hb is assumed to be in a oxy-state by default, the most frequent experimental condition. Deoxygenation of Hb (Deoxy) changes its pI(0oC) from 7.2 to 7.5. The model automatically adjusts the actual pI change for the temperature of the experiment. The pI shifts during oxy-deoxy transitions cause sudden changes in the protonization condition of Hb with secondary changes in pHi, changes which the model predicts with verified accuracy [10-12]. Electroneutrality preservation during oxy-deoxy transitions requires constancy of nHb values (eq 4) when pI changes, from which the compensatory changes in pHi can be derived according to [11]:

On deoxygenation:

**pHideoxy = pHioxy + pIdeoxy - pIoxy ………………………………………………………….. 4a**

On reoxygenation:

**pHioxy = pHideoxy + pIoxy - pIdeoxy ………………………………………………………….. 4b**

**The Dynamic State.**

A first requirement at the start of simulations is to define the relative volume occupied by cells in the cell suspension system, the cell volume fraction, CVF. Perturbations alter the flux of transported solutes and water across the plasma membrane of the cell thus initiating a cascade of downstream changes in the compositions of cell and suspending medium. It is therefore important to start by listing the membrane transport component of the cell and of the equations describing their basic kinetic properties.

***Flux equations of the model,* Fi and Fj**

The substrates of the RBC membrane transporters are Na, K, A, H, Ca, Mg and water, the “i” in Fi. The sign-convention applied in the equations is for positive net fluxes into the cell (influx) and for negative net fluxes into the medium (efflux). The name convention adopted here for the transport of substrate X by the different membrane transporters is as follows: FPX = pump-mediated flux of X, with P = NaP for the Na/K pump or CaP for the calcium pump (PMCA); FGX = X-flux through electrodiffusional channel defined with constant field kinetics; FXA = electroneutral carrier-mediated cotransport of cation X and anion A defined with low-saturation kinetics; FzX = electrodiffusional flux of X through PIEZO1 channel; FCoX = electroneutral cotransport of X mediated by the Na:K:2Cl symport, of minimal expression and activity in human RBCs; FA23X = electroneutral M2+:2H+ exchange flux through the divalent cation ionophore A23187, the only exogenous membrane transporter included in the model; Fw= water flux mediated mainly by aquaporins and partly by partition diffusion through the plasma membrane.

***Flux pathways for each transported substrate, Fi:***

**FNa = FNaPNa + FGNa + FNaA + FCoNa + FzNa …………..10a**

**FK = FNaPK + FGK + FKA + FKGardos + FCoK + FzK ……………….10b**

**FA = FGA + FHA + FNaA + FKA + FzA + 2\*FCoA ………………….10c**

**FH = FGH + FHA + FCaPH + FA23H …………………………………10d**

**FCa = FCaPCa + FGCa + FzCa + FA23Ca ……………………………….10e**

**FMg = FGMg + FA23Mg …………………………………………………………10f**

**Fw = Pw\*(COs – MOs) ………………………………………………….10g**

There are no data on PIEZO1-mediated Mg2+ fluxes in RBCs. Although PzMg most certainly has a small finite value, FzMg is likely to be very small under the usually low electrochemical Mg2+ gradients across the RBC membrane. With this level of uncertainty, FzMg was not included in the current model version.

***Kinetic descriptions of individual transporters***

Certain transporter kinetics are reported in the equations with the default numerical values used for dissociation and rate constants in the model, based on well established values in the literature and on the good semi-quantitative fits to experimental data provided in the past [13-15].

***Na/K pump mediated fluxes of Na and K (f = forward; r = reverse)* [16, 17]**

We redefine the forward sodium flux through the sodium pump to introduce its CMg2+-dependence as found by Flatman[1]:

**FNaPNaf = -FNaPmaxf\*(CMg2+/(0.04 + CMg2+))\*…(**

**..\*(CNa/(CNa + 0.2\*(1 + CK/8.3)))3 \*(MK/(MK + 0.1\*(1 + MNa/18)))2 ….11a**

**FNaPmaxf should now be derived in the RS from the values set for FNaPNaf, CMg2+, CNa and MK using eq 11a, as part of the steady-state equations (1-8).**

**FNaPNar = FNaPmaxr\*(CK/(CK + 8.3\*(1 + CNa/0.2)))2\*(MNa/(MNa + 18\*(1 + MK/0.1)))3 …11b**

**FNaPNa = FNaPNaf + FNaPNar ………………………………………………..11c**

**FNaPK = -FNaPNa/1.5 ………………………………………………………………..11d**

***PMCA. Calcium and proton fluxes through the calcium pump operating as an electroneutral Ca:2H exchanger [18, 19]***

**FCaPCa = -kCaP\*((CCa2+)4/((0.0002)4 + (CCa2+)4)) …………………………………12a**

**FCaPH = -2\*FCaPCa ………………………………………………………………..12b**

***Electrodiffusional fluxes of i (Na, K, Ca, Mg, H and A) through endogenous channels,* FGi *, Gardos channels,* FGGardos*, and PIEZO1 channels,* Fzi*, are represented with constant field kinetics* [20]:**

**FGi = -PGi\*(zi*F*Em/RT)\*(Ci – Mi\*exp(-zi*F*Em/RT))/(1 – exp(-zi*F*Em/RT)) …….13**

**with PGi representing the Goldmanian i-permeability in h-1 units.**

**The default RS value of PGMg should be set equal to that of Ca, PGMg = PGCa = 0.05/h**

**For this preliminary investigation using RCM, there is no need to add FzMg/PzMg**

**PGKGardos is a function of CCa2+ [21, 22] as follows:**

**PGKGardos = PKGardosMax\*((CCa2+)4/ ((KCa)4 + (CCa2+)4)) ………………………….14**

**PGCa is a function of CCa2+ and MCa2+ [22, 23] as follows:**

**PGCa = (CCa2+/(0.0002 + CCa2+))\*(MCa2+/(0.8 + MCa2+) …………………………..15**

***Low-saturation, carrier mediated flux phenomenology for electroneutral cotransporters FNaA, FKA and FHA.***

**FNaA = - kNaA\*(CNa\*CA – MNa\*MA) ……………………….16a**

**FKA = - kKA\*(CK\*CA – MK\*MA) ……………………….16b**

**FHA = - kHA\*(CH\*CA – MH\*MA) ……………………….16c**

Note that kHA, the rate constant of the H:A cotransport phenomenology representing the operation of the Jacob-Stewart mechanism (JS) is between five and six orders of magnitude faster than that of any of the other ion transporters in the membrane (see User Guide for details and references).

***Electroneutral Na:K:2A cotransport***

**FCo = -kCo\*((CNa\*CK\*CA2) – d\*(MNa\*MK\*MA2)) ……………………………17a**

**d = (CNa\*CK\*CA2)/(MNa\*MK\*MA2) ……………………………………………17b**

The CX and MX values in eq 17b are those set for the RS

**FNaCo = FKCo = FCo …………………………………………………….17c**

**FACo = 2\*FCo ……………………………………………………………..17d**

“d” is a wildcard factor introduced to set FCo = 0 only in the RS. Its value is set by the initial Na, K and A concentrations in the RS. “d” remains as a fixed-value parameter during dynamic state computations.

***Electroneutral* M2+:2H+ *exchange fluxes of Ca2+ and Mg2+ mediated by the divalent cation ionophore A23187***

The divalent cation ionophore A23187 mediates an electroneutral M2+:2H+ exchange when incorporated into cell membranes [24]. Divalent cation ionophores became essential and extensively used tools in research on calcium and magnesium function and dysfunction in RBCs [6, 8, 25, 26] and in many other cell types. To emulate experimental protocols with the use of divalent cation ionophores it became necessary to represent their transport properties in the model as an optional exogenous transporter of the RBC membrane.

In albumin-free RBC suspensions, the RBC/medium partition ratio of the lipophilic ionophore A23187 is 60/1, 20 to 50% of it confined to the cell membrane [27]. The transport kinetics of the ionophore was modeled with symmetric binding (Km) and inhibitory (KI) dissociation constants for Ca2+ and Mg2+ on each membrane side, as follows:

A1 = (MCa2+/(KmCa\*(1 + MMg2+/KIMg + MCa2+))

A2 = (CCa2+/(KmCa\*(1 + CMg2+/KIMg + CCa2+))

A3 = (MMg2+/(KmMg\*(1 + MCa2+/KICa + MMg2+))

A4 = (CMg2+/(KmMg\*(1 + CCa2+/KICa + CMg2+))

Following extensive preliminary tests [28], default values of 10 mM for the four Km and KI parameter set were found to deliver excellent agreement between predicted and measured ionophore-mediated fluxes, and to ensure adequate compliance with the measured equilibrium distribution of the transported ions when ionophore-mediated net fluxes approach zero [24]: CCa2+/MCa2+ ≈ CMg2+/MMg2+ ≈ (CH+/MH+)2.

Combining the Ca2+, Mg2+ and H+ driving gradients we obtain:

B1=A1\*(CH)2 - A2\*(MH)2

B2=A3\*(CH)2 - A4\*(MH)2

The ionophore-mediated fluxes of Ca2+, Mg2+ and H+, FA23Ca, FA23Mg and FA23H, respectively, can now be computed from:

**FA23Ca = PA23\*B1 …………………………………………………………… A23-1**

**FA23Mg = PA23\*B2 ………………………………………………………………. A23-2**

**FA23H = -2\*(FA23Ca + FA23Mg) ………………………………………………… A23-3**

Where PA23 is the ionophore-mediated permeability. PA23 is a power function of the RBC ionophore concentration, PA23 = 0.22\*[I]1.45, when PA23 is expressed in units of 10-6 cm/s, and [I] in μmol/Loc [27, 29, 30]. Within the units-set in the model, numerical values of PA23 in the range 1017 to 2\*1018 offered a perfectly adequate minimalist emulation of the effects of different ionophore concentrations on the fluxes and distributions of Ca2+, Mg2+ and H+ ions in RBCs in a large variety of experimental conditions [1, 6, 28, 31-34].

**Equation sequence for the computations of dynamic states.**

Following perturbations, sustained charge conservation and electroneutrality is implemented by:

**∑ Ij = 0 …………………………………………………………………….18a**

where Ij represents the current carried by each of the j-membrane transporters. ∑Ij is therefore the fist equation that has to be solved at the start of each iteration in the computational sequence of dynamic states. Capacitative currents (Ic = C(dV/dt)) are ignored because their magnitude and time-course are orders of magnitude smaller than those of the homeostatic relevant currents. The relation between currents and fluxes, Fj, for each transporter is given by

**Ij = *F*\*zjFj ……………………………………………………………18b**

With the electrogenic flux components in the model (zj ≠ 0), ∑Ij = 0 renders:

**∑Ij = FNaPNa+FNaPK+FGNa+FGK+FGKGardos+FGA+FGCa+FGMg+FGH+FzNa+FzK+FzA+FzCa = 0 ..….18c**

**∑Ij** is a complex function of temperature, membrane potential, Em, and of the concentration of all transported and modulating substrates. With all parameters, kinetics and substrate concentrations known ∑Ij = 0 becomes an implicit equation in Em, the single unknown left, solved in each iteration with the Newton-Raphson cord approximation routine.

With Em, the new zjFj values for each of the electrodiffusional terms in eq 18c can be computed. We can now add up the absolute values of the new computed fluxes to the values of the electroneutral fluxes in the previous iteration, ∑|Fj|, to assign a new ∆t duration to each current iteration interval, as follows:

**∆t = a/(b + ∑|Fj|) ………………………………………………………………19**

The value of “a”, under user control, optimises ∆t scales for different simulations (“frequencyfactor” in the RCM); “b” is a small zero-avoidance parameter in the denominator. The advantage of this strategy over using regular iteration intervals is that by setting a constant value for the cycles per outcome (“cyclesperprint(epochs)” in the RCM) the density of data output points automatically adjusts to the overall rate of change in the system, emulating the way good experimental practice seeks to sample for data at the bench, thus optimizing comparisons between predicted and experimental results.

With the new Fi(t) and ∆t the new Qi(t) may be computed using the values of FNa(t), FK(t), FA(t), FH(t), FCa(t) and FA23Mg(t) from equations (10a-f) as follows:

**∆QNa = FNa\*∆t ………………………………………………………20a**

**∆QK = FK\*∆t ……………………………………………………… 20b**

**∆QA = FA\*∆t ……………………………………………………… 20c**

**∆H = FH\*∆t ……………………………………………………… 20d**

**∆QCa = FCa\*∆t ……………………………………………………… 20e**

**∆QMg = (FGMg + FA23Mg)\*∆t ……………………………………………………20f**

**QNa(t) = QNa(t-∆t) + ∆QNa ……………………………………………20g**

**QK(t) = QK(t-∆t) + ∆QK ……………………………………………20h**

**QA(t) = QA(t-∆t) + ∆QA ……………………………………………20j**

**QCa(t) = QCa(t-∆t) + ∆QCa ……………………………………………20k**

**QMg(t) = QMg(t-∆t) + ∆QMg ……………………………………………20l**

∆H is a special case because ∆H adds to the only titratable proton buffer nHb\*QHb, so that:

**nHb(t)\*QHb = nHb(t-∆t)\*QHb + ∆H ……………………………….21a**

**nHb(t) = nHb(t-∆t) + ∆H/QHb ............................................................21b**

From which we can now compute the new cell pHi from eq 4 by solving for pH(t):

**pH(t) = nHb(t)/ɑ + pI …………………………………………………21c**

The new intracellular H+ concentration in molar units is:

**CH(t) = 10-pH(t) …………………………………………21d…**

With the new Qi(t), we need the new cell water volume, Vw(t) in order to compute the new cell concentrations, Ci(t) = Qi(t)/Vw(t). The water flux across the RBC membrane, Fw, is driven by the osmotic gradient across the RBC membrane (eqs 5 and 6):

**Fw(t) = Pw\*(COs(t) – MOs(t-∆t)) ………………………………………..22a**

COs(t) can be computed from the altered osmotic load resulting from the ∆Qi changes during ∆t operating on the cell volume at the start of the each iteration interval:

**COs(t) = (QNa(t) + QK(t) + Q(A)t + QCa(t) + QMg(t))/Vw(t-∆t) +**

**+ (fHb\*CHb + CX‑)(t-∆t) ……………………………22b**

The new cell water volume, Vw(t), and volume-associated variables, RCV(t), MCHC(t), Density(t) and Hct(t), can now be computed from:

**∆Vw(t) = Fw(t)\*∆t …………………………………………23a**

**Vw(t) = Vw(t-∆t) + ∆Vw ……………………………….. 23b**

**RCV(t) = 1-Vw(t=0) + Vw(t) ……………………………….23c**

**MCHC(t) = MCHC(t=0)/RCV .............................................23d**

**Density(t) = ((MCHC(t=0)/100) + Vw(t))/RCV .................. 23e**

**Hct(t) = Htc(t=0)\*RCV ……………………………………...23f**

With Vw(t) we proceed to compute next the new (t) intracellular concentrations of Na, K, A, H, Ca, Hb, and X‑:

**CNa(t) = QNa(t)/Vw(t) ………………………………… 24a**

**CK(t) = QK(t)/Vw(t) ………………………………………. 24b**

**CA(t) = QA(t)/Vw(t) ………………………………………. 24c**

**CCa(t) = QCa(t)/Vw(t) …………………………………. 24d**

**CMg(t) = QMg(t)/Vw(t) …………………………………. 24e**

**CHb(t) = QHb/Vw(t) ……………………… …………. 24f**

**CX‑(t) = QX‑/Vw(t) ………………………………………. 24g**

The new osmotic coefficient of Hb, fHb(t), can now be calculated from eq 7 and the new CHb(t):

**fHb(t) = 1 + *b*\*CHb(t) + *c*\*CHb(t)2 ………………............ 25**

***Computation of the medium concentrations at time = t.***

Medium concentration changes arise from independent solute and water transfers between cells and medium under mass conservation. At constant suspension volume, water transfers between cells and medium generate self-compensating changes in cell and medium volume fractions, CVF and (1-CVF), respectively, according to:

∆CVF + ∆(1-CVF) = 0 …………………………………………………….26a

By mass conservation, the Qi changes during ∆t, ∆Qi, are transferred to the medium, ∆Qim, so that:

∆Qim + ∆Qi = 0 .........................................................................................26b

∆Qim can be expressed in terms of Mi changes during ∆t as follows:

∆Qim = Mi(t)\*(1-CVF(t)) – Mi(t-∆t)\*(1-CVF(t-∆t)) ..................................26c

Replacing ∆Qim by -∆Qi (eq 26b) in equation 26c and solving for Mi(t), we obtain:

**Mi(t) = (Mi(t-∆t)\*(1 - CVF(t-∆t)) - ∆Qi)/(1 - CVF(t)) ……………………………. 26d**

With eq 26d we can now compute the new medium concentrations at time = t for transported solutes, eqs 27a-f, and for impermeant solutes (∆Qi = 0) whose concentration changes only because of water shifts, eqs 27g-k:

**MNa(t) = (MNa(t-∆t)\*(1-CVF(t-∆t)) - ∆QNa)/(1 – CVF(t)) ……………………… 27a**

**MK(t) = (MK(t-∆t)\*(1-CVF(t-∆t)) - ∆QK)/(1 – CVF(t)) …………………………. 27b**

**MA(t) = (MA(t-∆t)\*(1-CVF(t-∆t)) - ∆QA)/(1 – CVF(t)) …………………………. 27c**

**MCa(t) = (MCa(t-∆t)\*(1-CVF(t-∆t)) - ∆QCa)/(1 – CVF(t)) …..……………… 27d**

**MMg(t) = (MMg(t-∆t)\*(1-CVF(t-∆t)) - ∆QMg)/(1 – CVF(t)) ……....…………….. 27e**

**MBH(t) = (MBH(t-∆t)\*(1-CVF(t-∆t)) - ∆QH)/(1 – CVF(t)) …………………...... 27f**

**MB(t) = (MB(t-∆t)\*(1-CVF(t-∆t)))/(1 – CVF(t)) ………........……………........... 27g**

**Mgluconate(t) = (Mgluconate(t-∆t)\*(1-CVF(t-∆t)))/(1 – CVF(t)) ……..............… 27h**

**Mglucamine(t) = (Mglucamine(t-∆t)\*(1-CVF(t-∆t)))/(1 – CVF(t)) ……........…… 27j**

**Msucrose(t) = (Msucrose(t-∆t)\*(1-CVF(t-∆t)))/(1 – CVF(t)) …………..........…… 27k**

With MBH(t) and MB(t) from eqs 27e-f we can now compute the new medium proton concentration MH(t) by solving eq 2 for MH, so that:

**MH(t) = KB\*(MBH(t)/(MB(t) – MBH(t))) ........................................................... 28a**

With MH(t), we can now compute pHm(t), and also the proton and anion concentration ratios across the membrane, rH(t) and rA(t), respectively, critical parameters for driving the proton transport dynamics in the model ([35]; User Guide).

**pHm(t)= -log(MH(t) ......................................................................... 28b**

**rH(t) = MH(t)/CH(t) ……………………………………………… 28c**

**rA(t) = CA(t)/MA(t) ………………………………………………. 28d**

This completes the list of sequential computations within each iteration cycle of the core red cell model.

There is a substantial body of additional information implemented in the model, marginal to the core of the governing equations, information revised, filtered, and, when experimentally tested and confirmed, incorporated in the model as part of the continuous process of improving the quantitative accuracy of model predictions. The effects of temperature on transport, in addition to those incorporated within the kinetic description (constant field equation, zi*F*Em/RT, for instance) are represented by factors using the traditional Q10 phenomenology, with the option to alter the default values of 4 and 2 for active and passive transport, respectively. Most ion effects render themselves to be represented by factors modifying rate constants or dissociation constants in flux equations (e.g Na/K pump, eq 11), without altering the actual overall kinetics of the transport pathways. Because of their particular relevance to the circulatory behaviour of RBCs we only listed here explicitly the modulating effects of Ca2+ on PGGardos and on PGCa, equations 14 and 15, respectively. The complete model code is available with open access in the repository (<https://github.com/sdrogers/redcellmodeljava>).

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