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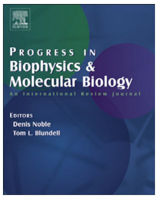

Analyses of HH and GHK equations with another perspective: Can ion adsorption also govern trans-membrane potential?

Hirohisa Tamagawa [a, *], Titus Mulembo [b], Vera Maura Fernandes de Lima [c], Wolfgang Hanke [d]

[a] *Department of Mechanical Engineering, Faculty of Engineering, Gifu University, 1-1 Yanagido Gifu, Gifu, 501-1193, Japan*
[b] *Dedan Kimathi University of Technology, PO BOX 657, 10100, NYERI, Kenya*
[c] *Centro de Biotecnologia, CNEN-IPEN/SP, Av. Lineu Prestes 2242, Campus USP, Sao Paulo, SP, 05508-000, Brazil*
[d] *Universität Hohenheim Institut für Physiologie, 230b Garbenstrasse 30, 70599, Stuttgart, Germany*

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ABSTRACT

Two mathematically distinct physiological concepts, the Goldman-Hodgkin-Katz eq. (GHK eq.) and the Hodgkin-Huxley model (HH model) were successfully associated with each other in a prior work. The previous work was performed on the following premises (i) The membrane potential is generated by ion adsorption, as opposed to the classical ion transport mechanisms, (ii) The living cell is a thermodynamically real system rather than an ideal system, and (iii) The conductance employed in the HH model is replaced by the ion activity coefficient, which is weighted with the role of conductance. Consequently, the GHK eq. was mathematically associated with the HH model through the intermediary of Boltzmann ion distribution and mass action law. To verify if our theoretical formularization could afford a physiologically, physically and chemically viable model, we performed computational analysis using the formulae (quantitative correlations between various variables) we derived in the previous work. The computational results obtained through associating the GHK eq. with the HH model validated our model and its predictions. This outcome suggests that the current prevailing physiological concepts could be expanded further, to incorporate the newly proposed mechanisms. That is, GHK eq. and HH model could be interpreted via another set of founding principles that incorporate the ubiquitous phenomena of ion-adsorption.

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* Corresponding author.

E-mail addresses: tmgwhrhs@gifu-u.ac.jp (H. Tamagawa), titus.mulembo@dkut.ac.ke (T. Mulembo), veramaura@gmail.com (V.M. Fernandes de Lima), Wolfgang.Hanke@uni-hohenheim.de (W. Hanke).

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1. Introduction

Based on sophisticated physiological theories, the GHK eq. and Hodgkin-Huxley model, have for a long while been utilized as effective tools for analyzing electrical characteristics of living cells (Gutmann and Kayzer, 1986; Ling, 1992; Jackson, 2006; Keener and Sneyd, 2008). Most physiology textbooks deal with these two theories, since they look quite complete and successful. Also, several researchers have interpreted their experimental findings and reasoned them out with these theories. However, these two models have not been mathematically associated with each other, although both have been utilized for the quantitative analysis of trans-membrane potential (TMP) characteristics. Although this discordance was pointed out almost a half-century ago (Shinagawa, 1976), the status hasn't changed till date. Even though the TMP measured or analyzed is the same, its formularization using these two theories are quite distinct. One may say that GHK eq. deals with the resting state while the HH model deals with the active state. Therefore, there need not be anything wrong with the existence of two different theories. However, it is natural to expect that these two theories can be also should be mathematically associated with each other because both deal with ionic differentials/gradients. For example, Powell and Brown suggested that action potential generation is attributed to the change of plasma membrane permeability coefficient to the mobile ions (Powell and Brown, 2021). Despite the fact that permeability coefficient is the physical chemistry-based quantity employed in the GHK eq. there have been other such reports that seek the correlation between the GHK eq. and HH model (Powell and Brown, 2021; Clay, 2009; Stiles and Gray, 2020). A way to mathematically associate the GHK eq. with the HH model was proposed in our previous work (Tamagawa et al., 2020) where it was shown that the two models could be mathematically associated with each other. This requires three premises: (i) The membrane potential is generated by ion adsorption, (ii) The living cell is a thermodynamically real system rather than an ideal system, and (iii) The ion activity coefficient is burdened with the role of conductance. Our prior work suggested that both the theories were just other expressions of the ion distribution obeying the Boltzmann distribution in a thermodynamically real system. To this end, we explored computationally if our formalization could provide physiologically conceivable results. The flow of this work is: Defining the formalization → Computational analysis → Collation/Deduction of interfaces. “Computational analysis” consists basically of (i) Deriving the quantitative (numerical) data of electrical characteristics of a living cell by following the classical theory; (ii) To check if our new theory can suffice to account for the computationally obtained data.

2. GHK eq. and Boltzmann distribution

The GHK eq. was derived using Boltzmann distribution in our previous work (Tamagawa et al., 2020). Its summary is described in this section.

2.1. Ideal system

An ideal system for investigation is modeled as a living cell consisting of immobile ions COO^- and NH_3^+, and equilibrated in a solution of a mixture of $NaCl$ and KCl in the air. The concentrations of mobile ions in this living cell model follows the Boltzmann distribution as given by Eq. (1) where z_i, e, ϕ, k and T represent ion valence, elementary charge, potential, Boltzmann constant and temperature, respectively.

$$[i]_{in} = [i]_{out}\exp\left(-\frac{z_i e\phi}{kT}\right) \tag{1}$$

$i = K^+, Na^+, H^+, Cl^-, OH^-, HCO_3^-$

Linear combination of Eq. (1) can be given by Eq. (2).

$$\sum_{i=cations} C_i[i]_{in} + \sum_{j=anions} C_j[j]_{out} = \left(\sum_{i=cations} C_i[i]_{out} + \sum_{j=anions} C_j[j]_{in}\right)\exp\left(-\frac{e\phi}{kT}\right) \tag{2}$$

$\because z_i=+1, z_j=-1$

Solving Eq. (2) with respect to ϕ, results in Eq. (3) (From here on, Eq. (3) which is based on the Boltzmann distribution of ions, is called the BD-GHK eq.).

$$\phi = -\frac{kT}{e}ln\frac{\sum_{i=cations}C_i[i]_{in} + \sum_{j=anions}C_j[j]_{out}}{\sum_{i=cations}C_i[i]_{out} + \sum_{j=anions}C_j[j]_{in}}, \ (BD - GHK\ eq.) \tag{3}$$

The commonly known GHK eq. is given by Eq. (4). It is derived using Nernst-Planck eq. (NP eq.) under the zero-current condition. From here on, Eq. (4) is called the NP-GHK eq. which is introduced in common textbooks (Keener and Sneyd, 2008). Quite Intriguingly, simply regarding C_i (C_j) of BD-GHK eq. as P_i (P_j), NP-GHK eq. is easily derived. Namely, the NP-GHK eq. is merely a linear combination of equations of Boltzmann distribution represented by Eq. (1).

$$\phi = -\frac{kT}{e}ln\frac{\sum_{i=cations}P_i[i]_{in} + \sum_{j=anions}P_j[j]_{out}}{\sum_{i=cations}P_i[i]_{out} + \sum_{j=anions}P_j[j]_{in}}, \ (NP - GHK\ eq.) \tag{4}$$

$i = K^+, Na^+, H^+, NH_3^+, j = Cl^-, OH^-, HCO_3^-, COO^-$

P_i is known as the permeability coefficient and is a uniquely determined constant, while C_i is arbitrary. Therefore, there is a contradiction between the foundations of BD-GHK eq. and NP-GHK eq. How can we settle this issue? The answer to this query is given below.

The total concentrations of the carboxylic group, $[Carbo]$, and the amino group, $[Amino]$, contained in the system are given by Eq. (5) and Eq. (6), respectively, because of the preservation of their total quantity.

$$[Carbo] = [COO^-]_{in} + \sum_{i=cations}[COOi]_{in} \tag{5}$$

$i = K^+, Na^+, H^+, NH_3^+$

$$[Amino] = [NH_3^+]_{in} + \sum_{j=anions} [NH_3 j]_{in} \tag{6}$$

j = Cl⁻, OH⁻, HCO₃⁻, COO⁻

The law of mass action for the carboxylic group and the amino group are given by Eq. (7) and Eq. (8), respectively, where K_p^q ($p = i, j$; $q = C, A$) is a binding constant. It follows that Eq. (9) is derived.

$$K_i^C = \frac{[COOi]_{in}}{[COO^-]_{in}[i]_{in}} \tag{7}$$

$$K_j^A = \frac{[NH_3 j]_{in}}{[NH_3^+]_{in}[j]_{in}} \tag{8}$$

$$K_{NH_3}^C = K_{COO}^A \tag{9}$$

Use of Eq. (5) and Eq. (7) results in Eq. (10).

$$[Carbo] = [COO^-]_{in}\left(1 + \sum_{i=cations} K_i^C [i]_{in}\right) \tag{10}$$

$i = K^+, Na^+, H^+, NH_3^+$

Use of Eq. (6) and Eq. (8) results in Eq. (11).

$$[Amino] = [NH_3^+]_{in}\left(1 + \sum_{i=anions} K_j^A [j]_{in}\right) \tag{11}$$

$j = Cl^-, OH^-, HCO_3^-, COO^-$

Eqs. (10) and (11) are arranged into Eqs. (12) and (13), respectively.

$$\frac{[Carbo]}{[COO^-]_{in}} - 1 = \sum_{i=cations} K_i^C [i]_{in} \tag{12}$$

$$\frac{[Amino]}{[NH_3^+]_{in}} - 1 = \sum_{j=anions} K_j^A [j]_{in} \tag{13}$$

The RHS of Eqs. (12) and (13) can be arranged into Eqs. (14) and (15), respectively, using Eq. (1).

$$\frac{[Carbo]}{[COO^-]_{in}} - 1 = \sum_{i'=cations} K_{i'}^C [i']_{out} e^{-e\phi/kT} + K_{NH_3}^C [NH_3^+]_{in} \tag{14}$$

$i' = K^+, Na^+, H^+$

$$\frac{[Amino]}{[NH_3^+]_{in}} - 1 = \sum_{j'=anions} K_{j'}^A [j']_{out} e^{+e\phi/kT} + K_{COO}^A [COO^-]_{in} \tag{15}$$

$j' = Cl^-, OH^-, HCO_3^-$

The RHS of both Eqs. (12) and (14) are the same. Hence, Eq. (16) is derived.

$$\sum_{i'=cations} K_{i'}^C [i']_{in} = \sum_{i'=cations} K_{i'}^C [i']_{out} e^{-e\phi/kT} \tag{16}$$

$i' = K^+, Na^+, H^+$

The RHS of both Eqs. (13) and (15) are the same. Hence, Eq. (17) is derived.

$$\sum_{j'=anions} K_{j'}^A [j']_{out} = \sum_{j'=anions} K_{j'}^A [j']_{in} e^{-e\phi/kT} \tag{17}$$

$j' = Cl^-, OH^-, HCO_3^-$

The use of Eqs. (16) and (17) results in Eq. (18), which is identical to the NP-GHK eq. So, if P_i in Eq. of 4 is replaced by the binding constant, Eq. (18), which is identical to NP-GHK eq., is theoretically valid. From here on, Eq. (18), which K_p^q is incorporated in, is called the K-GHK eq.

$$\phi = -\frac{kT}{e} ln \frac{\sum_{i'=cations} K_{i'}^C [i']_{in} + \sum_{j'=anions} K_{j'}^A [j']_{out}}{\sum_{i'=cations} K_{i'}^C [i']_{out} + \sum_{j'=anions} K_{j'}^A [j']_{in}}, \quad (K-GHK\ eq.) \tag{18}$$

$i' = K^+, Na^+, H^+, j' = Cl^-, OH^-, HCO_3^-$

As described, the actual P_i can only take on a unique value. Such a unique value of P_i is usually not experimentally measured but rather estimated. Therefore, P_i is not a trustworthy physical quantity. If C_i takes K_p^q (or P_i is replaced by K_p^q), it is a trustworthy enough value. Therefore, it is more plausible that the unique value is the binding constant, K_p^q, rather than the permeability constant, P_i. Hence, we need to change the view of the broadly accepted NP-GHK eq. The GHK eq. should not be built on the phenomenon of transmembrane ion transport but rather on the phenomenon of ion binding (in other words, ion adsorption). Equations (16) and (17) are derived using the mass preservation of the carboxylic groups and the amino groups. Therefore, K-GHK eq., Eq. (18), is derived using the scientifically sound two equations, Eqs. (16) and (17). K-GHK eq. is identical to BD-GHK eq. Hence, K-GHK eq. is thermodynamically trustworthy enough. But still it cannot be ruled out that C_i of Eq. (3) can be arbitrarily determined as suggested by the BD-GHK eq., while the actual P_i of the NP-GHK eq. can only be uniquely determined. It must be primary because the actual system in focus is in the thermodynamically real system and not in the thermodynamically ideal system as explained below.

2.2. Real system

Eq. (19) is given by introducing the activity coefficient, γ_α^h ($\alpha = in, out$; h = ions) to Eq. (1) (see Eq. (20)) (Lewis and Randall, 1961).

$$\gamma_{in}^i [i]_{in} = \gamma_{out}^i [i]_{out} \exp\left(-\frac{z_i e\phi}{kT}\right) \tag{19}$$

$i = K^+, Na^+, H^+, Cl^-, OH^-, HCO_3^-$

$$[h]_\alpha \rightarrow \gamma_\alpha^h [h]_\alpha \tag{20}$$

Eq. (18) is transformed into Eq. (21) using Eq. (20). Eq. (21) is the K-GHK eq. for the thermodyncamically real system where the definition of a_α^h is given by Eq. (22) where Eq. (22) is valid even when $h = NH_3^+$ and COO^-. From here on, Eq. (21) is called the RK-GHK eq.

$$\phi = -\frac{kT}{e} ln \frac{\sum_{i'=cations} K_{i'}^C a_{in}^{i'} + \sum_{j'=anions} K_{j'}^A a_{out}^{j'}}{\sum_{i'=cations} K_{i'}^C a_{out}^{i'} + \sum_{j'=anions} K_{j'}^A a_{in}^{j'}}, \quad (RK-GHK\ eq.) \tag{21}$$

$i' = K^+, Na^+, H^+, j' = Cl^-, OH^-, HCO_3^-$

$$\gamma_\alpha^h [h]_\alpha \equiv a_\alpha^h,\ h = \text{all ions including } NH_3^+ \text{ and } COO^-. \tag{22}$$

Eq. (21) is transformed into Eq. (23).

$$\sum_{i'=cations} K_{i'}^{C} a_{in}^{i'} + \sum_{j'=anions} K_{j'}^{A} a_{out}^{j'} = \left(\sum_{i'=cations} K_{i'}^{C} a_{out}^{i'} + \sum_{j'=anions} K_{j'}^{A} a_{in}^{j'} \right) \exp\left(-\frac{e\phi}{kT} \right) \tag{23}$$

Eq. (23) is further transformed into Eq. (24). This equation represents the Boltzmann distribution of ions in a thermodynamically real system.

$$\begin{aligned} &K_h^H a_{in}^h = K_h^H a_{out}^h \exp\left(-\frac{z_h e\varphi}{kT} \right) \ (H = C,\ A) \\ &\rightleftharpoons a_{in}^h = a_{out}^h \exp\left(-\frac{z_h e\varphi}{kT} \right) \end{aligned} \tag{24}$$

$h = K^+, Na^+, H^+, Cl^-, OH^-, HCO_3^-$

Now, the RK-GHK eq. is more explicitly represented as given by Eq. (25). Even the same species of mobile ions bear individual different activity coefficients γ_α^h depending on where they stay, that is, either in the cell-interior ($\alpha = in$) or in the cell-exterior ($\alpha = out$). Therefore, the activity of the individual ions are different from one another even if the ionic species is the same, unless their locations are the same as for each other. On the other hand, the NP-GHK eq. does not bear such a facet. Since the RK-GHK eq. is the thermodynamically correct GHK eq., K_p^q can take arbitrary value. But the broadly utilized GHK eq. is the NP-GHK eq., and the numerical value of P_i is forcibly selected. Therefore, P_i can be only uniquely determined.

$$\phi = -\frac{kT}{e} ln \frac{\sum_{i'=cations} K_{i'}^{C} \gamma_{in}^{i'} [i']_{in} + \sum_{j'=anions} K_{j'}^{A} \gamma_{out}^{j'} [j']_{out}}{\sum_{i'=cations} K_{i'}^{C} \gamma_{out}^{i'} [i']_{out} + \sum_{j'=anions} K_{j'}^{A} \gamma_{in}^{j'} [j']_{in}},\ (RK - GHK\ eq.) \tag{25}$$

$i' = K^+, Na^+, H^+, j' = Cl^-, OH^-, HCO_3^-$

To sum up, the RK-GHK eq. must reflect the characteristics of a thermodynamically real system better than the NP-GHK eq. Four different coefficients, $K_{i'}^{C}\gamma_{in}^{i'}$, $K_{i'}^{C}\gamma_{out}^{i'}$, $K_{j'}^{A}\gamma_{in}^{j'}$and $K_{j'}^{A}\gamma_{out}^{j'}$ are needed for the RK-GHK eq., while the NP-GHK eq. takes only two coefficients P_i and P_j. Nevertheless, the NP-GHK eq. is forcibly used to analyze the thermodynamically real system. Namely, the right equation which has to employ four coefficients is used by forcibly employing only two coefficients. Such unjustifiable approximation results in the actual fact that P_i (P_j) can only take a unique numerical value rather than an arbitrary value.

[Outcome 1] GHK type equation, RK-GHK eq., can be derived from the fundamental thermodynamics concept, Boltzmann distribution Eq. (9).

3. Associating HH eq. with GHK eq

The mathematical relationship between the GHK eq. and the HH eq. (HH model) shown in our previous work is summarized (Tamagawa et al., 2020). The resting potential and the action potential in the context of our formularization are further explained.

3.1. Mathematical relationship between GHK eq. and HH eq

From now on, the Hodgkin-Huxley model is analyzed along with the RK-GHK eq. The ordinary textbook Hodgkin-Huxley eq. is given by Eq. (26) and Eq. (27) in accordance with the cell state (resting or active) where g_h^s (s: r = resting, a = active) represents conductance (Keener and Sneyd, 2008) and E_h is given by Eq. (28). Virtually Eq. (28) is Nernst eq. From here on, Eqs. (26) and (27) are called the r-HH eq. and the a-HH eq., respectively.

Usually, in the HH model, Cl⁻ is not taken into consideration but the corresponding role is played by the "Leak Current", though not always (Ermentrout and H, 2010; Wei et al., 2020). So, in this work, we view the role of Cl⁻ to be the same as the role by the "Leak Current". Following the ordinary textbook description, the ions other than K⁺, Na⁺ and Cl⁻ are not taken into consideration as mobile ions in our cell model system, though those neglected ions are taken into consideration in the previous section.

$$\sum_{h=all\ ions} g_h^r (\phi_r - E_h) = 0,\ \ (r - HH\ eq.) \tag{26}$$

$h = K^+, Na^+, Cl^-$

[a textbook HH model equation]

$$\sum_h g_h^a(t)[\phi_a - E_h] - I(t) + C\frac{d\phi_a}{dt} = 0, (a - HH\ eq.) \tag{27}$$

$h = K^+, Na^+, Cl^-$

[a textbook HH model equation]

$$E_h \equiv -\frac{kT}{z_h e} ln \frac{[h]_{in}}{[h]_{out}},\ h\ =\ K^+,\ Na^+,\ Cl^- \tag{28}$$

[a textbook Nernst eq.]

Previously, we could mathematically associate the GHK eq. with the HH model by taking into consideration that the system in question is the thermodynamically real system. The previous work is summarized here (It is to be analyzed numerically in the next section.).

Eq. (24) represents Boltzmann distribution of mobile ions and it is arranged into Eq. (29).

$$Eq.\ 24 \rightarrow \gamma_{in}^h [h]_{in} = \gamma_{out}^h [h]_{out} \exp\left(-\frac{z_h e\phi}{kT} \right) \tag{29}$$

Eq. (29) is further arranged into Eq. (30) where Γ_h is defined by Eq. (31).

$$\phi = -\frac{kT}{z_h e} ln \frac{\gamma_{in}^h [h]_{in}}{\gamma_{out}^h [h]_{out}} = -\frac{kT}{z_h e} ln \frac{[h]_{in}}{[h]_{out}} - \frac{kT}{z_h e} ln \frac{\gamma_{in}^h}{\gamma_{out}^h} = E_h + \Gamma_h \tag{30}$$

$$\Gamma_h \equiv -\frac{kT}{z_h e} ln \frac{\gamma_{in}^h}{\gamma_{out}^h} \tag{31}$$

Eq. (30) is originally derived from the RK-GHK eq., that is, Eq. (30) originates from the GHK type equation, and Eq. (32) is the same as Eq. (30) but the ϕ of Eq. (30) is replaced by Φ so as to explicitly suggest that Eq. (30) is RK-GHK eq.-based rather than HH eq.-based. From here on Eq. (32) is called the RKGHK-b eq.

$$\Phi - E_h - \Gamma_h = 0,\ \ (RKGHK - b\ eq.) \tag{32}$$

[Outcome 2] Potential formula, Eq. (32), which is a function of activity coefficient is originally derived from the GHK type equation of Eq. (21).

3.2. Theoretical argument for resting potential generation

The resting potential in view of the textbook HH model is given by using r-HH eq., Eq. (26). Solving the r-HH eq. with respect to ϕ_r results in Eq. (33) (Keener and Sneyd, 2008).

$$\phi_r = \frac{\sum_h g_h^r E_h}{\sum_h g_h^r} \tag{33}$$

It was previously considered if it was possible to derive eq. (33) using the RKGHK-b eq. Then it was proven feasible as follows: Eq. (34) is a linear combination of Eq. (32).

$$\sum_h G_h[(\Phi - E_h) - \Gamma_h] = 0 \tag{34}$$

Assuming that Eq. (35), Eq. (36) and Eq. (37) hold at the resting state, then Eq. (34) can be expressed by Eq. (38).

$$G_h = g_h^r \text{ (at the resting state)} \tag{35}$$

$$\phi_r = \Phi \equiv \Phi_r \text{ (at the resting state)} \tag{36}$$

$$\Gamma_h \equiv \Gamma_h^r \text{ (at the resting state)} \tag{37}$$

$$\begin{gathered}\sum_h g_h^r(\Phi_r - E_h) - \sum_h g_h^r \Gamma_h^r = 0 \\ \left(= \sum_h g_h^r(\varphi_r - E_h) - \sum_h g_h^r \Gamma_h^r = 0 \right) \\ \rightleftharpoons \Phi_r = \frac{\sum_h g_h^r E_h - \sum_h g_h^r \Gamma_h^r}{\sum_h g_h^r}\end{gathered} \tag{38}$$

If Eq. (39) holds, then Eq. (38) becomes Eq. (40), which is exactly the same as Eq. (33). Eq. (38) is originally the linear combination of the RKGHK-b eq. Thus, we can say that both the RKGHK-b eq.-based potential and the r-HH eq.-based potential provide us with the same resting potential, and in that sense the GHK eq. and the HH model are mathematically associated with each other concerning the resting potential generation.

$$\sum_h g_h^r \Gamma_h^r = 0 \tag{39}$$

$$\Phi_r = \frac{\sum_h g_h^r E_h}{\sum_h g_h^r} \tag{40}$$

Is it possible to find Γ_h^r sufficing Eq. (39)? It is quite difficult to actually determine Γ_h^r. There are no means for experimentally determining Γ_h^r. Therefore, we have to assume that Γ_h^r behaves so as to suffice Eq. (39). This assumption may seem like a makeshift idea to the reader. But even the conductance g_h in the HH model is a sort of parameter. So far, nobody has elucidated why the conductance behaves as we know now. Nevertheless, the circuit model of the HH model is built on the unrealistic premise that the mobile ions behavior is thermodynamically ideal and that ion adsorption-desorption is negligible. Conductance g_h is defined in the textbook HH model under such unstable premises. On top of that, the HH eq. written in the orthodox physiology textbooks cannot be mathematically associated with the ordinary GHK eq. (NP-GHK eq.) as earlier described. It is a fundamentally serious physiological problem. Therefore, it is not implausible to believe that Γ_h^r suffices Eq. (39).

[Outcome 3] The condition Eq. (39) could lead to the mathematical association of the resting potential formula given by GHK type eq. with that given by HH model.

3.3. Theoretical argument of action potential generation

Here, the theoretical work on the action potential formula is presented. Introducing Eq. (41) and Eq. (42), Eq. (32) is represented here again by Eq. (43). Now, we attempt to associate the GHK type eq. with HH model as explained from now on.

$$\Phi \equiv \Phi_a \text{ (at the active state)} \tag{41}$$

$$\Gamma_h \equiv \Gamma_h^a \text{ (at the active state)} \tag{42}$$

$$\Phi_a - E_h - \Gamma_h^a = 0 \text{ (RKGHK} - \text{b eq.)} \tag{43}$$

a-HH eq. represented by Eq. (27) is the textbook HH model-based potential formula of the cell in the active state. The time-dependent behavior of the potential in the textbook HH model is attributed to the time-dependent conductance as explicitly given in Eq. (44) by $g_h^a(t)$ as well-known.

$$\sum_h g_h^a(t)[\phi_a - E_h] - I(t) + C\frac{d\phi_a}{dt} = 0 \tag{44}$$

[a textbook HH model equation]

We divide $g_h^a(t)$ into two terms on purpose so as to let $g_h^a(t)$ contains g_h^r as given by Eq. (45). Eq. (45) consists of a constant term g_h^r (conductance at the resting state) and a time-dependent term Δg_h^a (t).

$$g_h^a(t) = g_h^r + \Delta g_h^a(t) \tag{45}$$

Hence, Eq. (44) is given by Eq. (46).

$$\sum_h \left(g_h^r + \Delta g_h^a(t)\right)[\phi_a - E_h] - I(t) + C\frac{d\phi_a}{dt} = 0 \text{ (same as a} - \text{HH eq.)} \tag{46}$$

Now, we attempt to attribute the time-dependence of potential in the RKGHK-b eq., Φ_a, to the time-dependence of Γ_h^a on purpose rather than to the time-dependence of conductance. Therefore, the RHGHK-b eq. is more explicitly expressed by Eq. (47).

$$\Phi_a - E_h - \Gamma_h^a(t) = 0 \text{ (RKGHK} - \text{b eq.)} \tag{47}$$

If Φ_a of GHK type eq. Eq. (47) is same as ϕ_a of HH model Eq. (46) as given by Eq. (48), it is interpreted as GHK eq. can be mathematically associated with the HH model. If Eq. (48) is valid, Φ_a of GHK type eq. Eq. (47) suffices Eq. (49).

$$\phi_a = \Phi_a \tag{48}$$

$$\sum_h \left(g_h^r + \Delta g_h^a(t)\right)[\Phi_a - E_h] - I(t) + C\frac{d\Phi_a}{dt} = 0 \text{ (same as a} - \text{HH eq.)} \tag{49}$$

Introducing the relationships given by Eq. (50) and Eq. (51), Eq. (49) can be arranged into Eq. (52) using Eq. (47) (RKGHK-b eq.). Eq. (52) is the combination of the RKGHK-b eq. and the a-HH eq. Hence, ϕ_a can be mathematically expressed by associating the RKGHK-b eq. with the a-HH eq. From here on, Eq. (52) is called the RHGHK-HH eq.

$$C = \sum_h C_h \ , \ h \ = \ K^+, \ Na^+, \ Cl^- \tag{50}$$

$$I(t) = \sum_h I_h(t)\ , \quad h \ = \ K^+,\ Na^+,\ Cl^- \tag{51}$$

$$\sum_h \left[\mathrm{g}_h^r \Phi_a(t) - \mathrm{g}_h^r E_h + \Delta \mathrm{g}_h^a(t) \Gamma_h^a(t) \right] = \sum_h \left(I_h(t) + C_h \frac{d\Phi_a}{dt} \right) \quad (\mathrm{RKGHK-HH\ eq.}) \tag{52}$$

Now, what is the condition needed for validating Eq. (52) is described as follows: Eq. (53), Eq. (54) and Eq. (55) are derived from Eq. (52) by trial and error and by considering the physical meaning of the HH model circuit. The following three conditions are sufficed as long as RKGHK eq. and HH eq. are mathematically associated with each other. Especially Eq. (55) suggests that $\Gamma_h^a(t)$ can be expressed by the function of conductance (and the current) by solving it with respect to $\Gamma_h^a(t)$. Of course, Eq. (55) does not explain what causes the time-dependent characteristics of $\Gamma_h^a(t)$ but at least suggests that it is possible to attribute the action potential behavior to the time-dependent characteristics of an ion activity rather than to the time-dependent conductance.

$$I_h(t) = I_h^C(t) + I_h^{gE}(t) \tag{53}$$

$$I_h^C(t) = C_h \frac{d\Phi_a}{dt} \tag{54}$$

$$I_h^{gE}(t) = \mathrm{g}_h^r \Phi_a(t) - \mathrm{g}_h^r E_h + \Delta \mathrm{g}_h^a(t) \Gamma_h^a(t) \tag{55}$$

[Outcome 4] We found that three conditions Eq. (53)–(55) which are needed for mathematically associating GHK type eq. with the HH model when the cell is in the active state.

4. Numerical analysis of resting and action potentials

4.1. Resting potential sufficing both GHK type eq. and HH model

Next, computational analysis was performed to see if the equations so far derived for mathematically associating the GHK type eq. with HH model could provide reasonable results. The computer program introduced in the Japanese-written textbook ref. (Yamamoto, 2019) is used for whole computation described in this section 4.1.

First, the electrical characteristics shown in Figs.1 - 4 are computationally obtained using the r-HH eq. and the a-HH eq. (both are equations within the range of ordinary textbook HH model) (Gutmann and Kayzer, 1986; Ling, 1992; Jackson, 2006; Keener and Sneyd, 2008). Hence, data shown in Figs. 1–4 are merely commonly known computational electrophysiology outcomes. For the computation, the parameters in Table 1 are employed. These parameters are from ref. (Yamamoto, 2019). Hence, the parameters are physiologically reasonable enough.

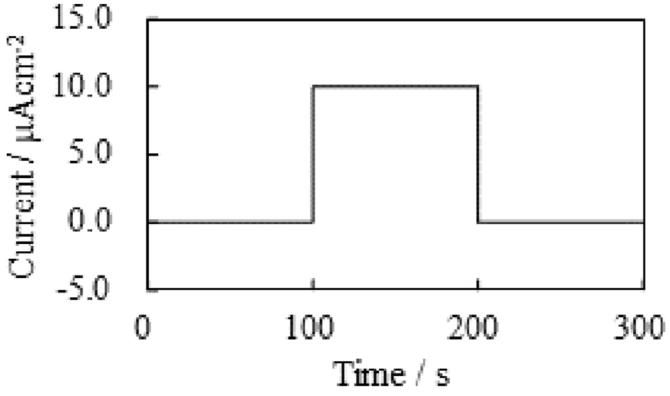

Fig. 1. Current vs. Time, This current is injected into a-HH eq. (Eq. (27)).

The injected current $I(t)$ for computing the a-HH eq. is zero or 10 $\mu\mathrm{Acm}^{-2}$ as shown in Fig. 1.

Fig. 2 shows the time-dependent behavior of ϕ_a computed using Eq. (27), and it suggests that the system in question is in the active state from $t = 100$ ms through 200 ms, and in the resting state (the injected current in Fig. 2 is zero) for the rest of the time.

The computational results based on the a-HH eq. (the textbook HH model) provides with −65 mV as the resting potential and the potential spike train as the action potential. These potential characteristics are physiologically reasonable of course since the computational results were obtained under the common theory. For a discussion to be presented later, we computationally obtained Ionic current vs. Time and it is shown in Fig. 3.

Fig. 4 shows the time-dependent behavior of $g_h^a(t)$ ($h = K^+$ and Na^+) computed using the a-HH eq., where g_{Cl}^a is constant 0.3 $mScm^{-2}$ as given in Table 1 by $\overline{\mathrm{g}_{Cl}^a}$. These computational results can provide the resting state conductance (as well as the active state conductance) and the obtained g_h^r's are as summarized in Table 2.

The resting potential formula Φ_r of the RKGHK-b eq. (Eq. (32)) has to be identical to the resting potential formula ϕ_r of the r-HH eq. (Eq. (33)) under the assumption of Eq. (39) as long as both GHK type eq. and HH model can be mathematically associated with each other. Under this assumption Eq. (39), Eq. (40) is derived as the resting potential formula. Plugging g_h^r's into Eq. (40) results in −65 mV which is exactly same as the potential provided by a-HH eq earlier described. Hence, the RKGHK-b eq. and the r-HH eq. are mathematically associated with each other. They are simultaneously validated even in the quantitative physiological sense under the condition of Eq. (39) which is a necessary condition leading to the mathematical association of GHK type eq. with HH model.

4.2. Action potential sufficing both GHK type eq. and HH model

Here, we focus on the action potential behavior using the computationally obtained common electrophysiological data already shown in Figs. 1–4 and Tables 1 and 2.

$\Delta g_h^a(t)$ shown in Fig. 5 was computed by Eq. (45) using the numerical values of $g_h^a(t)$ given in Fig. 4 and g_h^r given in Table 2 (*Attention*: Eq. (45) is our model not a common methodology). Since $g_h^a(t)$ and g_h^r were obtained using the a-HH eq. (27), $\Delta g_h^a(t)$ is also regarded as a-HH eq.-based quantity.

$g_h^a(t)$ governs the time-dependent behavior of $\phi_a(t)$ according to the a-HH eq. within the textbook HH model. It was earlier suggested that the RKGHK-b eq. can be mathematically associated with the HH eq. by attributing the time-dependent potential behavior to $\Gamma_h^a(t)$ rather than to $g_h^a(t)$, that is, solving Eq. (55) with respect to $\Gamma_h^a(t)$, it is possible to burden $\Gamma_h^a(t)$ with the role of conductance. Thus, the computational procedure of $\Gamma_h^a(t)$ is presented here: Eq. (55) is derived from the RKGHK-HH eq. (Eq. (52)) where RKGHK-HH eq. suffices both GHK type eq. and HH model since it is derived using both the RKGHK-b eq. (Eq. (47)) and the HH model (a-HH eq.).

Table 1
Physical quantities for computing potential.

C^a	$\overline{\mathrm{g}_K^a}^b$	$\overline{\mathrm{g}_{Na}^a}^b$	$\overline{\mathrm{g}_{Cl}^a}^b$	$E_K{}^c$	$E_{Na}{}^c$	$E_{Cl}{}^c$
1	36	120	0.3	−77	50	−54

[a] Membrane capacitance/μFcm^{-2}.
[b] Maximal conductance in the active state/$mScm^{-2}$.
[c] Equilibrium potential/mV.

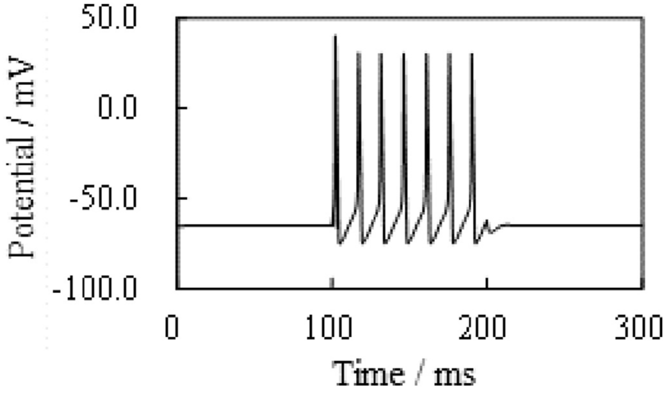

Fig. 2. Potential vs. Time computed using a-HH eq. (Eq. (27)) in case where the injected current is Fig. 1.

$I_h^{gE}(t)$ is computationally obtained earlier, and I_K, I_{Na} and I_{Cl} in Fig. 3 are $I_K^{gE}(t)$, $I_{Na}^{g}{}^{E}(\mathrm{t})$ and $I_{Cl}^{gE}(t)$, respectively. E_h is given already in Table 1. Hence, $\Gamma_h^a(t)$ can be obtained by solving Eq. (55) with respect to $\Gamma_h^a(t)$. Since Δg_{Cl}^a is zero as described in Fig. 5, $\Gamma_{Cl}^a(t)$ cannot be uniquely determined. Therefore, $\Gamma_{Cl}^a(t)$ is computed by plugging computed ϕ_a $(= \Phi_a(t))$ of Fig. 2 and E_h into RKGHK-b eq. All the computed $\Gamma_h^a(t)$ are shown in Fig. 6.

The activity coefficient ratio, $\mathrm{R}_{\Gamma h}^{a}(t)$ is defined by Eq. (56), and it is computed by plugging $\Gamma_h^a(t)$ into Eq. (56). The computed results are shown in Fig. 7. Although it is not known if the time-dependent potential behavior of the living cell can be attributed to the time-dependent behavior of $\Gamma_h^a(t)$ rather than $g_h^a(t)$, $R_{\Gamma h}^{a}(t)$ in Fig. 7 are not physiologically unreasonable values.

$$R_{\Gamma_h^a}(t) \equiv \frac{\gamma_{in}^h(t)}{\gamma_{out}^h(t)} = exp\left(-\frac{z_h e \Gamma_h^a(t)}{kT}\right) \tag{56}$$

Next, we focus on the value of E_h shown in Table 1, which is given by Eq. (28), where Eq. (28) states that E_h is a function of $[h]_{in}$ and $[h]_{out}$. It is worthwhile to see if physiologically reasonable values for $[h]_{in}$ and $[h]_{out}$ exist in this work's theoretical treatment. One Japanese-written textbook shows one example of the cell ion distribution (Miyakawa and Inoue, 2003), and it is shown in Table 3. Unfortunately, these ion concentrations do not reproduce the E_h's shown in Table 1. However, a slightly different ion concentration shown in Table 4 can. So, the ion concentration shown in Table 4 is not unreasonable physiological values.

To sum up, it is possible to computationally obtain E_h and $\Gamma_h^a(t)$, both of which can suffice both RKGHK-b eq. and a-HH eq. Therefore, it is possible to computationally reproduce the action potential profile derived from the textbook equation of a-HH eq. by the use of RKGHK-b eq. ($\Phi_a(t)$ can be obtained by plugging both E_h and $\Gamma_h^a(t)$ into Eq. (47).) So, even if the action potential generation is attributed to the time-dependent activity coefficient rather than to the time-dependent conductance, the action potential generation is reproducible. Needless to say, we confirmed that the potential profile shown in Fig. 2 is perfectly reproducible.

Focusing on Eq. (54), there were no means to enable us to obtain the numerical values of I_K^C, I_{Na}^C and I_{Cl}^C individually, nor for the numerical values of C_K, C_{Na} and C_{Cl}. But we can theoretically address at least Eq. (57).

$$\begin{aligned}\sum_{h=K^+,Na^+,Cl^-} I_h^C(t) &= \sum_{h=K^+,Na^+,Cl^-} C_h \frac{d\Phi_a(t)}{dt} \rightleftharpoons \frac{\sum\limits_{h=K^+,Na^+,Cl^-} I_h^C(t)}{\sum\limits_{h=K^+,Na^+,Cl^-} C_h} \\ &= \frac{d\Phi_a(t)}{dt} \xrightarrow[Eq.\ 50]{} \frac{\sum\limits_{h=K^+,Na^+,Cl^-} I_h^C(t)}{C} = \frac{d\Phi_a}{dt}\end{aligned} \tag{57}$$

$\sum\limits_{h=K^+,Na^+,Cl^-} I_h^C(t)$ can be given by Eq. (58), hence, computable using

Table 2
Resting state conductance.

g_K^r/mScm^{-2}	$g_{Na}^r/\mathrm{mScm}^{-2}$	$g_{Cl}^r/\mathrm{mScm}^{-2}$
0.367	0.011	0.300

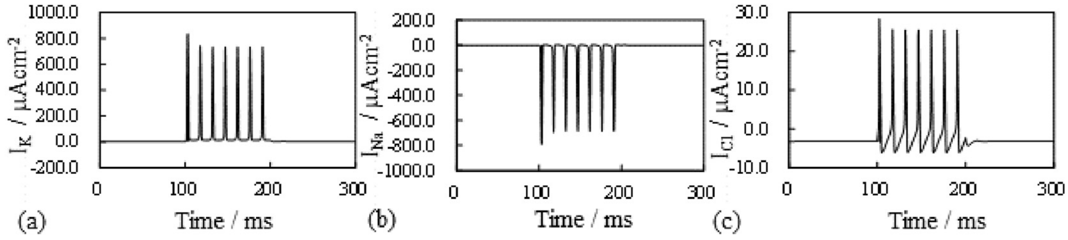

Fig. 3. Current vs. Time computed using a-HH eq. (Eq. (27)) in case where the injected current is Fig. 1 (a) K^+ current (b) Na^+ current (c) Cl^- current.

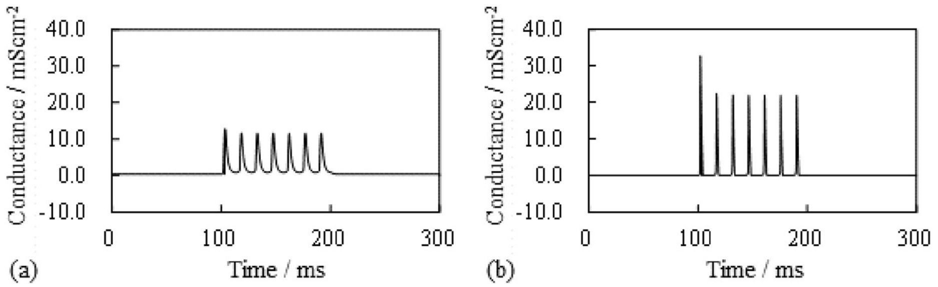

Fig. 4. Conductance vs. Time computed using the a-HH eq. (Eq. (27)) in case where the injected current is given in Fig. 1. (a) $g_K^a(t)$ (b) $g_{Na}^a(t)$ $g_{Cl}^a(t)$ is not shown here since $g_{Cl}^a(t)$ is a constant of 0.3 $mScm^{-2}$ $(=\overline{\mathrm{g}_{Cl}^{a}})$.

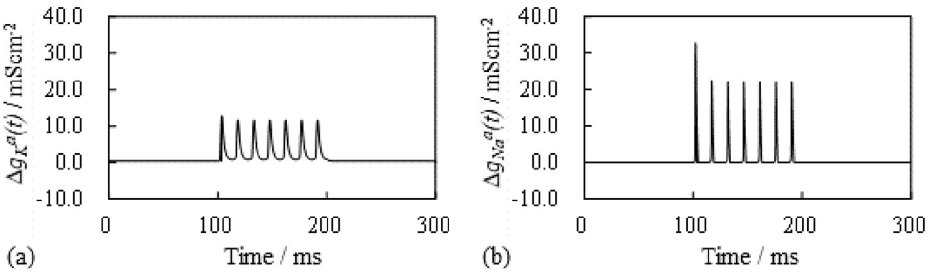

Fig. 5. $\Delta g_h^a(t)$ vs. Time, (a) $g_K^a(t)$, (b) $g_{Na}^a(t)$, $\Delta g_{Cl}^a(t)$ is not shown here since g_{Cl}^a is constant. Hence, $\Delta g_{Cl}^a(\mathrm{t}) = 0\ mScm^{-2}$.

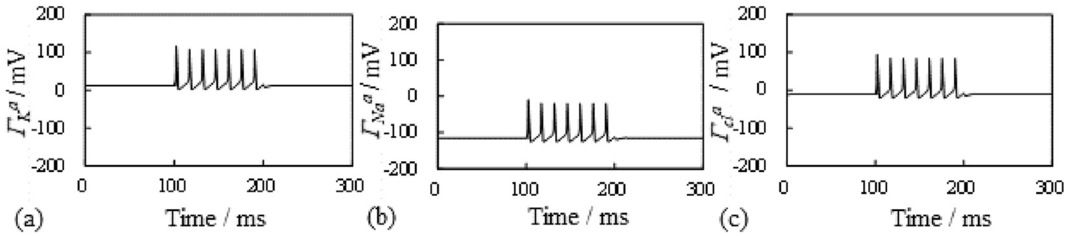

Fig. 6. $\Gamma_h^a(\mathrm{t})$ vs. Time, (a) $\Gamma_K^a(\mathrm{t})$ vs. Time, (b) $\Gamma_{Na}^a(\mathrm{t})$ vs. Time, (c) $\Gamma_{Cl}^a(\mathrm{t})$ vs. Time.

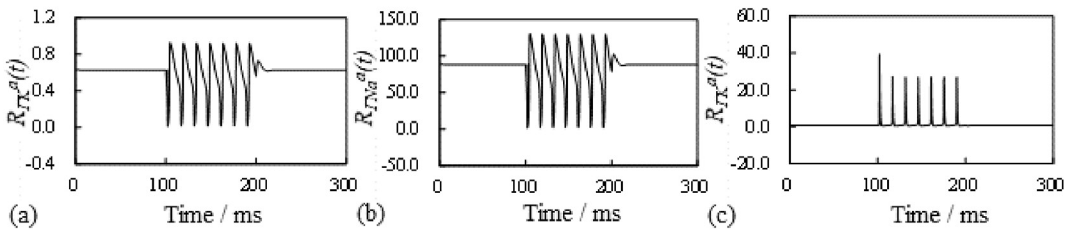

Fig. 7. $R_{\Gamma h}^a(t)$ vs. Time, (a) $R_{\Gamma K}^a(t)$ vs. Time, (b) $R_{\Gamma Na}^a(t)$ vs. Time, (c) $R_{\Gamma Cl}^a(t)$ vs. Time.

the numerical data of injected current, $I(t)$, and $I_h^{gE}(t)$ given in Fig. 1.

$$\sum_{h=K^+,Na^+,Cl^-} I_h^C(t) = I(t) - \sum_{h=K^+,Na^+,Cl^-} I_h^{gE}(t) \tag{58}$$

$d\Phi_a/dt$ can be computed using the potential data in Fig. 2 which is obtained by the a-HH eq. Now, the numerical computation of the RHS of Eq. (59) is performed. The result should be constant, since C of Eq. (59) is constant 1 ($\mu F/cm^{-2}$) as given in Table 1.

$$\begin{aligned} &\frac{\sum_{h=K^+,Na^+,Cl^-} I_h^C(t)}{C} = \frac{d\Phi_a}{dt} \\ &\rightleftharpoons C = \frac{\sum_{h=K^+,Na^+,Cl^-} I_h^C(t)}{\frac{d\Phi_a(t)}{dt}} \end{aligned} \tag{59}$$

We further arranged Eq. (59) into Eq. (60) for the computational purpose, and C is numerically computed using Eq. (60). Namely, the numerical values of $\Delta\Phi_a(t)$ and Δt from the data in Fig. 2 are used where $\sum_{h=K^+,Na^+,Cl^-} I_h^C(t)$ of Eq. (60) was computed using Eq. (58).

$$\begin{aligned} Cd\Phi_a(t) = &\left(\sum_{h=K^+,Na^+,Cl^-} I_h^C(t) \right) dt \\ &\rightleftharpoons C\Delta\Phi_a(t) = \left(\sum_{h=K^+,Na^+,Cl^-} I_h^C(t) \right) \Delta t \end{aligned} \tag{60}$$

There exist some computational difficulties: It is impossible to compute C, when $\Delta\Phi_a = 0$. Hence, such cases are eliminated from our computational results as meaningless condition. Fig. 8 shows the numerical result of C when $100\mathrm{s} \le t \le 200\mathrm{s}$ (nonzero current $I(t)$ shown in Fig. 1 is imposed on the HH circuit model). C should be constant 1 ($\mu F/cm^{-2}$) as in Table 1. However, C shown in Fig. 8 exhibits some spikes and a slight fluctuation (not a perfect constant). Such problems must arise purely due to the deviation of numerical input data of $\Delta\Phi_a$ and/or Δt from their ideal numerical data (practically which cannot be obtained). Especially, if the deviation of the numerical input data $\Delta\Phi_a$ from the ideal one is a bit large within the short time period Δt, it can result in the significant deviation of computational outcome of C from the ideal outcome 1 μF/

Table 3
One example of cell ion distribution[a].

ionic species	cell-interior/mM	cell-exterior/mM
K^+	400	20
Na^+	50	440
Cl^-	120	560

[a] Reproduced from the Ref. (Miyakawa and Inoue, 2003).

Table 4
Cell ion distribution reproducing E_h in Table 1.

ionic species	cell-interior/mM	cell-exterior/mM
K^+	400	20
Na^+	60	420
Cl^-	80	650

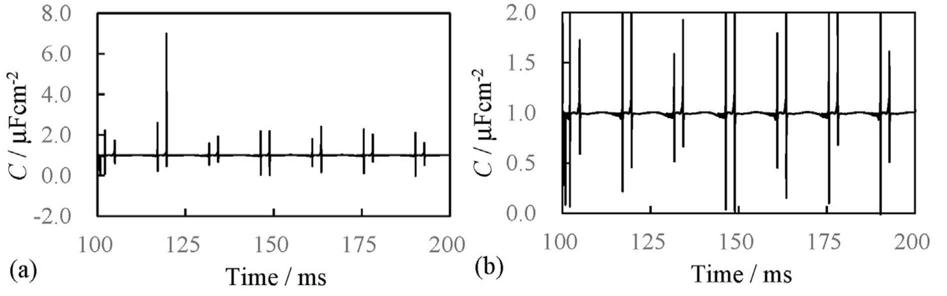

Fig. 8. Computed capacitance using Eq. (60), (a) Entire diagram, (b) when vertical axis is magnified.

cm^{-2}. But it is not inappropriate to say that Fig. 8 suggests basically $C = 1$ ($\mu F/cm^{-2}$).

Our theoretical model confirmed that the potential predicted computationally by the HH model is basically reproducible even by the GHK eq.-based equation by burdening the activity coefficient of ions with the role of conductance. Of course, it can be said that this theoretical treatment is “merely burdening the activity with the role of conductance”. However, the activity coefficient bears a thermodynamical meaning. Activity is considered as an essential factor for treating the thermodynamically real electrolytic solution system, and the activity variation is a universal phenomenon. No electrolytic solution systems behave ideally. Therefore, it is worthwhile investigating the role of ion activity for the TMP generation.

5. Conclusions

The current work demonstrates that the time-dependent TMP profile can be attributed to the time-dependent characteristics of the ion activity coefficient behavior. Also, this view could mathematically associate the GHK eq. (more precisely, RKGHK-b eq.) with the HH model. The consideration of the variance of ionic activity coefficient provides us with physiologically more relevant and less vitally deterministic approach. However, this paper's intention is not to emphasize that the textbook GHK eq. and/or HH model are/is misplaced. Rather, we point out that there are also other viable perspectives involved that need to be explored further. As suggested in this paper, it is possible to rebuild the theory of the cellular electrical characteristics with a fresh or amalgamated perspective. The benefit of this new mandate is justified by the issues we have already elaborated via our recent publications the refs. (Manoj and Tamagawa, 2021) and (Mnoj et al., 2021).

CRediT authorship contribution statement

Hirohisa Tamagawa: Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing. **Titus Mulembo:** Validation, Writing – review & editing. **Vera Maura Fernandes de Lima:** Validation, Writing – review & editing. **Wolfgang Hanke:** Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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