

All natural sciences frequently use mathematics as a valuable tool, but the application of mathematics in biology is different than in physics or chemistry. Imagine a teacher of mathematics who explained to students a way to resolve the quadratic equation, but in the next lesson presented another method with another result, concluding “Both ways are probably correct, choose which you like.” This is hard to imagine, is it not? Nevertheless, such a thing seems acceptable in biology. The resting potential across a cell membrane is probably the most fundamentally important neurophysiological parameter, and yet it can be determined by two different methods - the Goldman-Hodgkin-Katz (GHK) equation and the Chord conductance (CC) equation - that give two different results. Some books do not see a problem here, advising to accept the fact that “the reversal potential is model dependent” (Keener, Sneyd, *Mathematical Physiology*, 1998). But the peaceful coexistence of two equations that give different answers to the same question is inconvenient, and the trouble is usually resolved by choosing one (in most cases – GHK) and ignoring another (consequently CC). With this approach the base principles of the theories that produced GHK and CC are often forgotten and concepts are mixed. An illustrative example of such attitude is presented in Wikipedia’s article about the membrane potential ([https://en.wikipedia.org/wiki/Membrane\\_potential](https://en.wikipedia.org/wiki/Membrane_potential)). At the end of chapter 1 (Physical basis) the equivalent electrical circuit is introduced. It would not be difficult with help of Ohm’s law to derive the equation to calculate the membrane potential in the circuit, which would happen to be the CC equation; instead Wikipedia offers (incorrectly) the GHK equation as the solution of the circuit.

Even if GHK and CC are based on different principles, it would not automatically mean that results *must* be different. Take for example the Nernst equation that describes the potential necessary to balance the transmembrane concentration gradient of a certain ion (accordingly, the potential is also called equilibrium potential). The Nernst equation can be derived from solely thermodynamic consideration, but it also arises in relation to ionic fluxes in the electro-diffusional model, which serves as a fundament for the GHK equation. However, when GHK predicts the resting potential in a system of several ions, the result is different from the prediction of the electrical CC model, where the ionic equilibrium potentials (Nernst potentials) are the batteries that generate the current in the circuit.

The **Em Calculator**, available for download on this webpage, is designed to promptly compare results of calculations by GHK and CC performed with the same initial parameters, showing additionally the corresponding relative ionic currents and the equilibrium potentials. To begin comparison, start the calculator and with no changes in its input windows press the “Calculate” button. The resulting resting potentials (Em) are quite different: -40 mV by CC and about -16.6 mV by GHK. The resting potential of several ions is the zero-current potential, when the sum of all individual ionic currents = 0. In case of CC it is easy to see that  $(E_{Na} - E_m) g_{Na} + (E_K - E_m) g_K + (E_{Cl} - E_m) g_{Cl} = 0$ . Indeed,  $(60 - (-40)) + (-120 - (-40)) + (-60 - (-40)) = 0$ . GHK predicts a different Em, but it is also a zero-current potential, since the ionic currents in the framework of electro-diffusion are calculated differently than in the CC model:

$$P_{Na} ([Na^+]_o - [Na^+]_i e^{V}) + P_K ([K^+]_o - [Na^+]_i e^{V}) + P_{Cl} ([Cl^-]_i - [Cl^-]_o e^{V}) = 0$$

where  $V' = Em F/RT$  (for more about the calculations, see *About Em Calculator* on this website). Certainly, not only  $Em$ , but also the ionic currents have to be “model dependent.”

To evaluate the abilities of these two models, let us analyze the replacement of  $Na^+$  with  $K^+$  inside a cell by the  $Na^+/K^+$ -ATPase. To start, define  $[Na^+]_o = [Na^+]_i = 145$  mM,  $[K^+]_o = [K^+]_i = 5$  mM, make the cell membrane equally permeable to the cations ( $K^+$  and  $Na^+$  permeability/conductance = 1), and exclude  $Cl^-$  from consideration ( $Cl^-$  permeability/conductance = 0). With these conditions, all currents and voltages are 0. Now begin to replace  $[Na^+]_i$  with  $[K^+]_i$ . As it was shown (Dmitriev, A.V., Dmitriev, A.A, and Linsenmeier, R.A. The logic of ionic homeostasis: cations are for voltage, but not for volume, on this page),  $K^+$  replaces an equal amount of  $Na^+$  regardless of the cation conductances and the stoichiometry of the pump, so  $[Na^+]_i + [K^+]_i$  is constant. According to the GHK equation, which in this case reduces to

$$Em = \frac{RT}{F} \ln \frac{[Na^+]_o + [K^+]_o}{[Na^+]_i + [K^+]_i} ,$$

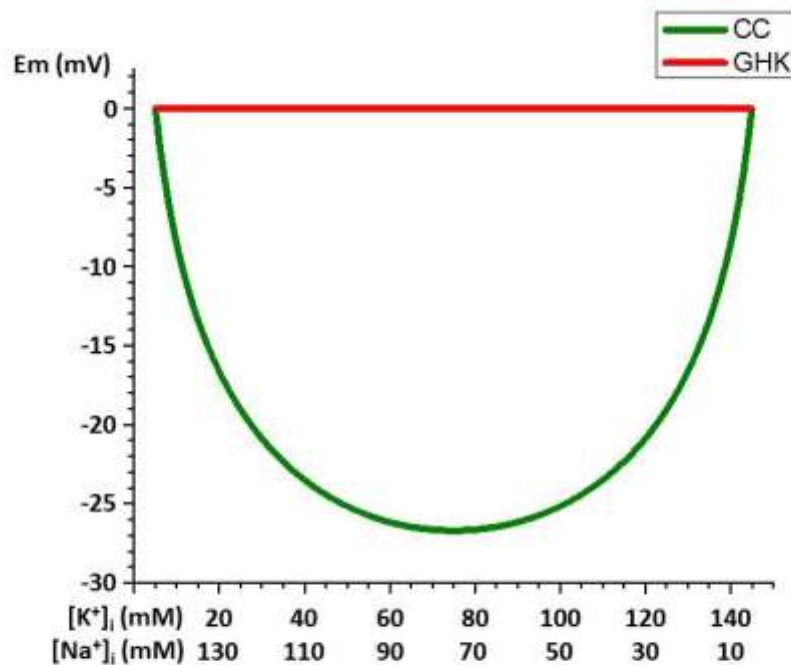
the resting potential ( $Em$ ) will always be 0, because the expression under the logarithm will always be 1 ( $[Na^+]_o + [K^+]_o = [Na^+]_i + [K^+]_i$ ). For example, in the *Em Calculator* decrease  $[Na^+]_i$  by 5 mM to 140 mM, increase  $[K^+]_i$  by 5 mM to 10 mM and calculate.  $Em$  by GHK is 0. This seems wrong, because  $[K^+]_i$  was doubled and that resulted in a Nernst equilibrium potential for  $K^+ = -18.062$  mV, but relative changes of  $[Na^+]_i$  were less than 3.5 % of the initial values, and the corresponding Nernst equilibrium potential for  $Na^+$  was less than 1mV. It is natural to expect that if two equally permeant ions had different equilibrium potentials, the resting potential should be between them, right in the middle:

$$E_m = (E_{Na} + E_K) / 2, \text{ i.e. } (-18.062 + 0.914)/2 = -8.574 \text{ mV.}$$

This is exactly the result of calculation with CC equation:

$E_m = (g_{Na} E_{Na} + g_K E_K) / (g_{Na} + g_K)$ , which with these conditions ( $g_{Na} = g_K = 1$ ) simplifies into the equation above.

During additional replacements, each combination of  $[Na^+]_i$  and  $[K^+]_i$  is associated with new combination of  $E_{Na}$  and  $E_K$  and, according to CC equation (and also common sense), with a new  $E_m$  (see figure below). But for GHK  $E_m$  is always 0, since this equation is not capable of distinguishing between  $Na^+$  and  $K^+$  when the membrane is equally permeable to them.



The failure of the GHK equation to correctly determine  $E_m$  in the special case described above raises a suspicion that it also might be inaccurate in other

conditions. In general, as formulated by B. Hille (Ion channels of excitable membranes, 1992) the reversal potential “is a weighted mean of all the Nernst potentials” which is exactly what the CC equation is and exactly what the GHK equation is not (ironically, Hille said this in respect to GHK).

There are some additional considerations that favor CC over GHK. For instance, it is relatively easy to experimentally measure electrical conductance that is used in CC. A sample evaluation of  $g_{Na}$ ,  $g_K$  and  $g_{Cl}$  in an intact retinal neuron is presented in an article on this webpage (Dmitriev et al. 2012). GHK uses permeability, which can also be determined experimentally, but not as straightforwardly as the electrical conductance.

It should be remembered that the resting potential reflects a steady, but not equilibrium, state and that state must be supported by active pumping of  $Na^+$  out and  $K^+$  into the cell by the  $Na^+/K^+$ -ATPase. But the pump is electrogenic (3  $Na^+$  for 2  $K^+$ ) and directly influences  $E_m$ . The contribution of the electrogenic  $Na^+/K^+$ -pump in  $E_m$  creates a considerable challenge for GHK (see for example Armstrong, 2003), but is easily resolved in the framework of CC. The pump hyperpolarizes  $E_m$ , shifting it closer to  $E_K$ ; as a result, in the resting state the ratio of  $Na^+$  and  $K^+$  fluxes follows the stoichiometry of the pump, i.e. = 3/2. Accordingly, the resting potential in the case of a membrane permeable to  $Na^+$  and  $K^+$ , and also having a pump, will be:

$$E_m = (2g_{Na} E_{Na} + 3g_K E_K) / (2g_{Na} + 3g_K)$$

Other ions ( $Cl^-$ ,  $Ca^{++}$ ) can be added on the basis of the same principle of a weighted mean of the Nernst potentials.

Finally,  $E_m$  predicted by CC (but not GHK) was always identical to  $E_m$  calculated in our recent computational study (Dmitriev, A.V., Dmitriev, A.A, and Linsenmeier, R.A. The logic of ionic homeostasis: cations are for voltage, but not for volume, 2019) in which the method of determining  $E_m$  was independent of both CC and GHK. In that work, transmembrane fluxes of ions were calculated in accordance with their thermodynamic driving forces, then the electrical charge inside the cell associated with the fluxes was computed, and finally,  $E_m$  was

determined as the recharging of the cell membrane capacitance. This approach permits painless incorporation of the Na<sup>+</sup>/K<sup>+</sup>-ATPase and other ionic cotransporters into the cell model. The fact that Em calculated with this “charge-difference” method was always in agreement with CC added more doubt to the accuracy of the GHK equation.

Obviously, this commentary is not the first to question the GHK equation. Years ago, papers that offered quantitative arguments in favor of CC were published ([F.F. Offner, 1991](#); [B. Tomicki, 1999](#)). Nevertheless, the dominance of GHK remains unchallenged, and the existence of CC is still generally unnoticed. We would like to attract attention to this discrepancy and possibly start a discussion about it. It would be useful to gain some insight as to what advantage researchers see in using GHK (except of tradition). Comments will be greatly appreciated.