brief communication

Measurement of voltage dependence of capacitance of planar bilayer lipid membrane with a patch clamp amplifier

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ABSTRACT The voltage dependence of capacitance was measured by using the setup which was almost the same as that for the study of ion channels. The coefficient which represents the voltage dependence of capacitance itself also changes as a function of the duration of voltage application if hexadecane is contained in bilayer lipid membrane (BLM). The method of Alvarez, O., and R. Latorre (1978. *Biophys. J.* 21:1–17) was extended to treat BLM with hexadecane.

INTRODUCTION

It is necessary to check the property of BLM before incorporating, or under the existence of, ion channels because the lifetimes of channels on BLM depends on the amount of solvent contained in the bilayer (e.g., Rudnev et al., 1981). Also the conductances of channels depends on the boundary potential (e.g., Bell and Miller, 1984). We describe here a method to obtain the information about the solvent in the bilayer and the boundary potential difference between the two surfaces.

As BLM has elasticity, its capacitance changes as a function of the voltage between the two surfaces due to the dielectric force (Babakov et al., 1966; White, 1970). The relation between capacitance and voltage is described by the following equation:

$$\Delta C = \alpha C_0 (V_{app} + \Delta\Psi)^2, \tag{1}$$

where C_0, ΔC, V_{app}, and α are the minimum capacitance (i.e., capacitance at $V_{app} = -\Delta\Psi$), the change in the capacitance, the externally applied voltage, and the coefficient which represent the membrane elasticity, respectively. Due to surface charges and dipoles, a boundary potential exists between the aqueous phase and the surface of BLM on each side. $\Delta\Psi$ represents the difference between the two boundary potentials (Schoch et al., 1979).

By using this relationship, several methods have been developed to measure α and $\Delta\Psi$ (Alvarez and Latorre, 1978; Carius, 1976; Cherny et al., 1980; Schoch et al., 1979). Most of these methods need special hardware. In this communication we describe a method using a patch clamp amplifier. The setup is almost the same as that for the study of ion channels. Our method is a modification of the method written in Alvarez and Latorre (1978), in which they measured the capacitance change of BLM formed with squalene which is called solvent free membrane. However, we used BLM formed with hexadecane because it is widely used for the study of channels. It is easier to measure the change in the capacitance of BLM with *n*-alkane than that of BLM with squalene because α of the former is larger than that of the latter. However, α of BLM with *n*-alkane changes as a function of the duration of voltage application whereas α of BLM with squalene is constant (Benz and Janko, 1976). We extended Alvarez and Latorre (1978) method because the meaning of α of BLM with *n*-alkane is ambiguous by their method.

EXPERIMENTAL SETUP AND MEMBRANE FORMATION

Fig. 1 shows the whole set-up. BLM was formed in a cell which consists of two aqueous compartments (1 ml in each). An Ag/AgCl/agar-gel electrode was immersed in each compartment. One electrode was connected to the amplifier via a probe, whereas the other was connected to the ground via a resistor (2 MΩ) to match the system with the capacitance compensation circuit of the patch clamp amplifier (model EPC7; List electronic, Darmstadt,West Germany). The computer (Macintosh II; Apple Computer, Inc., Cupertino, CA) had both an analogue-to-digital (A/D) and digital-to-analogue (D/A) converter (MacADIOS II; GW Instruments, Inc., Somerville, MA). Voltage steps were applied on BLM. The corresponding current signals were converted to voltage, filtered (3 kHz), and then collected. The sampling rate was 20 μs to perform input and output simultaneously.

BLM was formed in a hole (120 μm in diameter) on a Teflon thin film (25 μm thick) (Oxygen Probe Service Kit; Yellow Springs Instrument Co., Inc., Yellow Springs, OH) between the two aqueous compartments. The hole was pretreated with 0.4 μl of one percent hexadecane/

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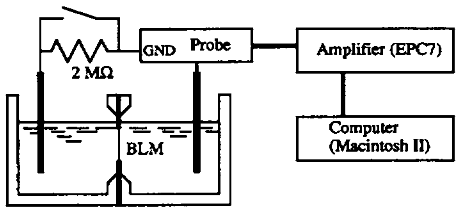

FIGURE 1 Schematic representation of the experimental setup.

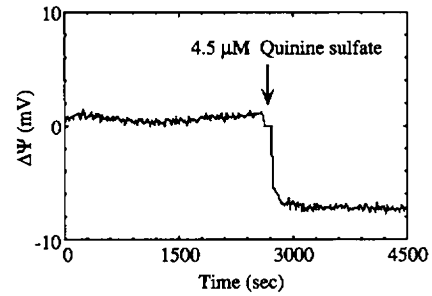

FIGURE 2 Time course of the difference of boundary potentials. At the time pointed by an arrow, 4.5 μM quinine sulfate was added to the side connected to the ground and stirred for a minute. During injection of quinine or stirring, measurement was suspended. Data was plotted in every 15 s. The aqueous solution contained 100 mM NaCl, 1 mM EDTA, 5 mM MES/NaOH (pH 5.8). Measurement conditions: $T_1 = 2$ ms, $T_2 = 2$ ms, $T_3 = 100$ ms, $T_4 = 0$ ms, $m = 24$, $n = 20$, $\Delta V = 23.8$ mV, $\{V_i\} = \{\pm 47.5, \pm 57.0, \pm 66.5, \pm 76.0, \pm 85.5, \pm 95.0, \pm 104.5, \pm 114.0, \pm 123.5, \pm 133.0 \text{ mV}\}$.

hexane (volume/volume) in each side. Monolayer of azolectin (Type IVS; Sigma Chemical Co., St. Louis, MO) was formed by dropping 10 μl of one percent lipid/hexane solution (weight/volume) to each compartment filled with aqueous solution whose level was under the hole. Then BLM was formed by raising monolayers above the hole (Montal and Mueller, 1972).

MEASUREMENTS

To calculate $\Delta\Psi$ and α in Eq. 1, various voltages are applied to BLM and the corresponding capacitances are measured. In brief, capacitances are calculated from the integral of the transient current ensuing the blip voltage. As the change in the capacitance is very small ($\Delta C/C_0$ was 0.2–1.0% at ±150 mV), some techniques are required to gain accuracy. Only to measure ΔC, set the virtual membrane capacitance to 0 pF by using the capacitance compensation circuit of the patch-clamp amplifier (Alvarez and Latorre, 1978). Although exact compensation is not required. Actually it is difficult to attain perfect compensation because the capacitance of BLM slightly changes as time passes. Though the shape of the transient currents are modified (averaged) by the filter, their integrals are held constant. In addition, the resistor between the electrode and the ground also flatten the current. By these techniques, current signal is able to be collected under the high gain amplifier mode. This minimizes the quantization error of the A/D converter while the time required for signal recording is increased. (The existence of a resistor is undesirable because the faster change in α cannot be detected and the noise on current increases, however, it is indispensable for capacitance compensation.)

We introduce here snapshot measurement by which $\Delta\Psi$ and α are calculated instantly. Continuous measurement as seen in Fig. 2 is performed by doing snapshot measurement cyclically. During the snapshot measurement, n units of voltage sequences are applied and ensuing current sequences are collected (Fig. 3). A voltage sequence is separated by four subsequences, which are composed of pedestal steps and blip pulses. The number of blip pulses per subsequence is denoted as m. T_1 and T_2 are required to wait the decay of transient current caused by a pedestal step and blip pulse, respectively. Period T_3 and T_4 are required to turn the membrane capacitance back to that before the next subsequence application. V_{base} is fixed during the snapshot measurement. If V_{base} is fixed to $-\Delta\Psi$, maximum accuracy is obtained. However $\Delta\Psi$ is calculated as a result of the measurement, rough estimation is required before the measurement. For example, in the continuous measurement, V_{base} is fixed to $-\Delta\Psi$ predicted in the previous cycle. From the standpoint of signal-to-noise ratio (S/N), the values of V_i should be selected as large as possible, that is, slightly below the breakdown voltage of the membrane. However, the set of V_i should be dispersed at the expense of the S/N ratio to avoid the

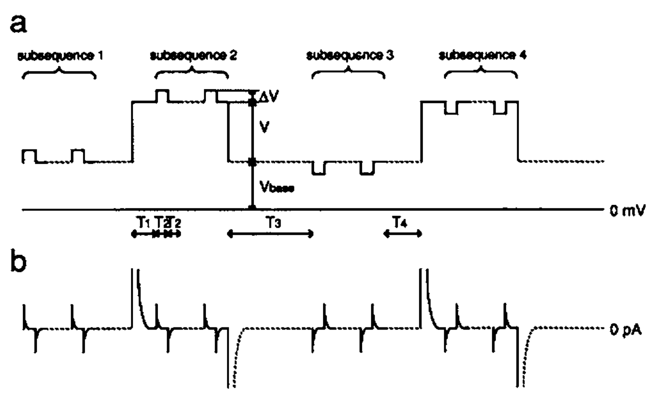

FIGURE 3 Scheme of (a) a unit of voltage sequence and (b) a corresponding current sequence.

distorted result because the snapshot measurement is sensitive to the nonlinear property of the circuit. In addition, the set of V_i should include symmetric elements centering at 0 mV.

Approximately, the standard deviation of calculated $\Delta\Psi$ is in proportion to $1/\sqrt{nm}$. Also $\Delta\Psi$ is calculated more accurately when α is large. To increase n times m in a given time for the snapshot measurement, T_3 and T_4 should be minimized. Practically, T_4 can be omitted if $\Delta V \ll V_i$ and all calculations should be performed during period T_3 if possible. Because no data is collected during T_1, minimize n and maximize m as a compensation. Because α increases as a function of the duration of voltage application, m should be large. On the other hand, the application of high voltage for a long period cause membrane disruption (Chizmadzhev and Abidor, 1980), m should not be too large.

Calculations are done as follows. Let ΔQ^j_1, $\Delta Q^j_2, \ldots, \Delta Q^j_4$ be the accumulated charges by the blip voltages in each subsequence. They are calculated as

$$\Delta Q^j_k = \frac{1}{2}\left\{\int_{T_1+(2j-2)T_2}^{T_1+(2j-1)T_2} I_k(t)\,dt - \int_{T_1+(2j-1)T_2}^{T_1+2jT_2} I_k(t)\,dt\right\}$$
$$(j = 1, 2, \ldots, m;\ k = 1, 2, \ldots, 4), \quad (2)$$

where j and k are the blip number in a subsequence and the subsequence number, respectively. Define ΔC^j as

$$\Delta C^j = C(V_{base} + \Delta\Psi + V_i) - C(V_{base} + \Delta\Psi), \quad (3)$$

where $C(V)$ represents the capacitance under voltage V. ΔC^j are calculated by the next formula (Appendix A):

$$k\Delta C^j = \frac{-\Delta Q^j_1 + \Delta Q^j_2 + \Delta Q^j_3 - \Delta Q^j_4}{\Delta V}, \quad (4)$$

where k is a constant which spans from two to six. If α changes much slower than T_2 then $k = 2$, and if α changes much faster than T_2 then $k = 6$. As the changing rate of α is unknown before the measurement, we cannot separate k and ΔC^j at this stage.

By using Eq. 4, errors caused by the following reasons can be cancelled out (Appendix B): E1, membrane conductance (assume that the membrane conductance is constant); E2, leak current parallel to the membrane; E3, Output offset error of the amplifier (i.e., zero current is not measured as zero); E4, remaining capacitive current generated by the pedestal step, if T_1 is not long enough.

By using the least-squares method, $\Delta\Psi^j$ and α^j for each j are calculated fom the set of V_i and ΔC^j_i as

$$\Delta\Psi^j = \frac{1}{2}\,\frac{\sum_{i=1}^{n} V_i^4 \sum_{i=1}^{n} V_i k\Delta C^j_i - \sum_{i=1}^{n} V_i^3 \sum_{i=1}^{n} V_i^2 k\Delta C^j_i}{\sum_{i=1}^{n} V_i^2 \sum_{i=1}^{n} V_i^2 k\Delta C^j_i - \sum_{i=1}^{n} V_i^3 \sum_{i=1}^{n} V_i k\Delta C^j_i} - V_{base} \quad (5)$$

$$k\alpha^j = \frac{1}{C_0}\,\frac{\sum_{i=1}^{n} V_i^2 \sum_{i=1}^{n} V_i^2 k\Delta C^j_i - \sum_{i=1}^{n} V_i^3 \sum_{i=1}^{n} V_i k\Delta C^j_i}{\sum_{i=1}^{n} V_i^2 \sum_{i=1}^{n} V_i^4 - \sum_{i=1}^{n} V_i^3 \sum_{i=1}^{n} V_i^3}. \quad (6)$$

RESULTS AND DISCUSSION

Fig. 4 shows the change in the capacitance as a function of the applied voltage. Though α increased as a function of the duration of voltage application, the shape of the curves were always almost parabolic.

Fig. 5 shows the change in $k\alpha$ as a function of the duration of voltage application. This curve fits the next equation.

$$k\alpha = k_1\alpha_1[1 - \exp(-t/\tau_1)] + k_2\alpha_2[1 - \exp(-t/\tau_2)]. \quad (7)$$

The parameters are calculated as; $k_1\alpha_1 = 1.03\ V^{-2}$, $\tau_1 = 3.20$ ms, $k_2\alpha_2 = 0.46\ V^{-2}$, $\tau_2 = 24.7$ ms. As $\tau_2 \gg T_2$, k_2 can be assumed to be two. Then α_2 is $0.23\ V^{-2}$. On the other hand, τ_1 is comparative to T_2, we could not separate k_1 and α_1. All the parameters were differed membrane to membrane. Considering with the references (Benz and Janko, 1976; Leikin, 1987; Requena et al., 1975; Schoch et al., 1979; White and Chang, 1981; Wobschall, 1972),

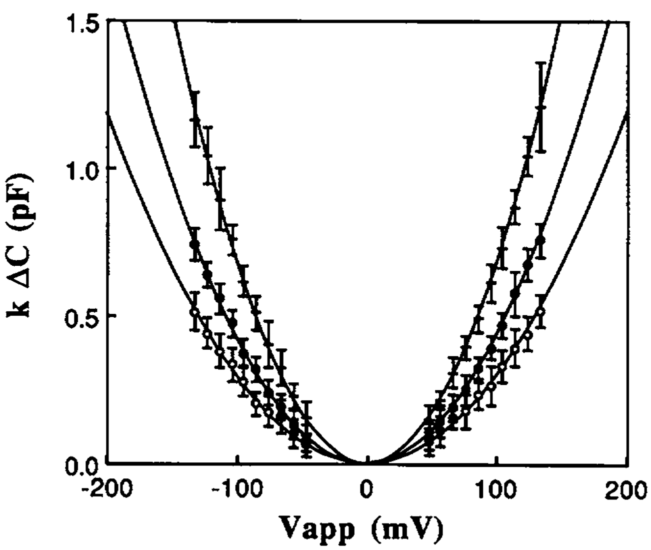

FIGURE 4 Changes in the capacitance as a function of the applied voltage. The time from the application of the pedestal voltage are (-O-) 4, (-●-) 12, and (-+-) 52 ms, respectively. The aqueous solution contained 100 mM NaCl, 1 mM EDTA, 5 mM Hepes/NaOH (pH 7.3). Measurement conditions: T_1 = 2 ms, T_2 = 2 ms, T_3 = 100 ms, T_4 = 0 ms, m = 13, n = 20, ΔV = 23.8 mV, $\{V_i\}$ = {±47.5, ±57.0, ±66.5, ±76.0, ±85.5, ±95.0, ±104.5, ±114.0, ±123.5, ±133.0 mV}. Error bars indicate the standard derivation of the 100 snapshot measurements.

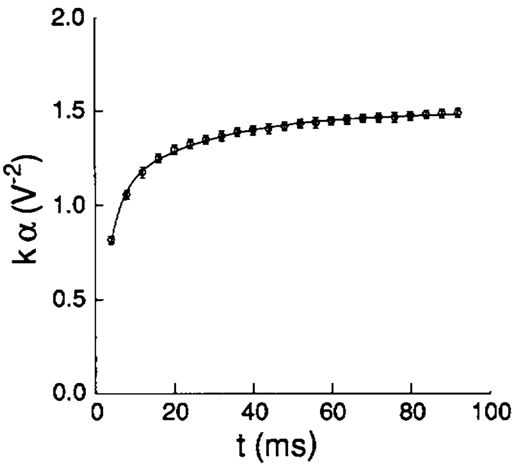

FIGURE 5 Changes in α as a function of the time from the application of the pedestal voltage. The curve fitting parameters: $k_1\alpha_1 = 1.03\ V^{-2}$, $\tau_1 = 3.20$ ms, $k_2\alpha_2 = 0.46\ V^{-2}$, $\tau_2 = 24.7$ ms. The aqueous solution contained 100 mM NaCl, 1 mM EDTA, 5 mM Hepes/NaOH (pH 7.3). Measurement conditions: $T_1 = 2$ ms, $T_2 = 2$ ms, $T_3 = 100$ ms, $T_4 = 0$ ms, $m = 23$, $n = 20$, $\Delta V = 23.8$ mV, $\{V_i\} = \{\pm 47.5, \pm 57.0, \pm 66.5, \pm 76.0, \pm 85.5, \pm 95.0, \pm 104.5, \pm 114.0, \pm 123.5, \pm 133.0$ mV$\}$. Error bars indicate the standard derivation of the 100 snapshot measurements.

the first component of Eq. 7 may probably represent the nonuniform change in membrane thickness caused by the redistribution of solvent and/or the increase in amplitude of thermal fluctuation. And the second component may represent the area expansion of the membrane at the sacrifice of annulus.

Fig. 2 is an example of the time course of $\Delta\Psi$. $\Delta\Psi$ is the average of $\Delta\Psi^j$ because $\Delta\Psi^j$ did not depend on j in the range of our experiments. Though the initial condition of the two aqueous phase was identical, $\Delta\Psi$ often deviated in $\sim \pm 3$ mV. The reason was perhaps due to the difference of the raising speed of the *cis* and *trans* aqueous phase level when BLM was formed. Because $k\alpha$ differed membrane to membrane, the standard deviation varied every time. Normally it was ~ 0.5 mV. In our experience, $k\alpha$ decreased, if the levels of aqueous phases were moved up and down many times before forming the membrane. Perhaps the precoated hexadecane on the Teflon thin film was wiped out.

There are some differences between the pulse sequence of our method and that of Alvarez and Latorre (1978). The duration of pulses differ, though we do not discuss it here because it merely depends on the property of hardware. In their method, $m = 1$. We extended this point to measure the change in α as a function of the duration of the voltage application. They used only subsequence one and two. With their sequence, errors caused by E1 to E3 are eliminated, whereas E4 remains. In their method, V_{base} is always fixed to zero. This point is undesirable as suggested in Chizmadzhev and Abidor (1980). In the reference (Alvarez et al., 1983), subsequence two and four were used. By using these subsequences, calculation is simplified while the S/N ratio becomes worse because only the capacitance change caused by blip instead of pedestal is detected. In addition E4 still remains.

The experimental setup was the same as that for the ion-channel experiment. It is possible to examine whether membrane is symmetric or how it is asymmetric from $\Delta\Psi$ before the incorporation of channels to BLM. It is also possible to estimate how much the membrane contain solvents from $k\alpha$. Under the existence of several gramicidin channels, we could measure both (single and multi) channel activity and capacitance change of BLM. The standard deviation of $\Delta\Psi$ and $k\alpha$ grew worse as the number of gramicidin channels increased. Currently, we do not know the applicability for other channels in the same condition written in this communication. The range of applicability can be explored if shorter *n*-alkane is used, because α increases as the length of *n*-alkane decreases. On the other hand, the thickness of BLM also increases as the length of *n*-alkane decreases (Benz and Janko, 1976). This means BLM with shorter *n*-alkane is not similar to biomembrane.

APPENDIX A

Suppose voltage V has been applied to the membrane and then ΔV is added on the voltage V. We assume two extreme cases. (*a*) First, assume that the capacitance changes much slower than T_2. Because blip pulses are applied on a pedestal step, the average voltage of pedestal step is $V + \frac{1}{2}\Delta V$. Then the change in the charge ΔQ is

$$\Delta Q = C(V + \tfrac{1}{2}\Delta V)(V + \Delta V) - C(V + \tfrac{1}{2}\Delta V)V = C(V + \tfrac{1}{2}\Delta V)\Delta V. \tag{A1}$$

Applying this equation to the sequence in Fig. 3 yields,

$$\Delta Q_1 = C_0[1 + \alpha(V_{\text{base}} + \Delta\Psi + \tfrac{1}{2}\Delta V)^2]\Delta V \tag{A2}$$

$$\Delta Q_2 = C_0[1 + \alpha(V_{\text{base}} + \Delta\Psi + V_i + \tfrac{1}{2}\Delta V)^2]\Delta V \tag{A3}$$

$$\Delta Q_3 = C_0[1 + \alpha(V_{\text{base}} + \Delta\Psi - \tfrac{1}{2}\Delta V)^2](-\Delta V) \tag{A4}$$

$$\Delta Q_4 = C_0[1 + \alpha(V_{\text{base}} + \Delta\Psi + V_i - \tfrac{1}{2}\Delta V)^2](-\Delta V). \tag{A5}$$

Gathering (A2–A5) yields,

$$\begin{aligned} -\Delta Q_1 + \Delta Q_2 + \Delta Q_3 - \Delta Q_4 &= 2\Delta V\{C_0[1 + \alpha(V_{\text{base}} + \Delta\Psi + V_i)^2] \\ &\quad - C_0[1 + \alpha(V_{\text{base}} + \Delta\Psi)^2]\} \\ &= 2\Delta V[C(V_{\text{base}} + \Delta\Psi + V_i) - C(V_{\text{base}} + \Delta\Psi)]. \end{aligned} \tag{A6}$$

(*b*) Next, assume that the capacitance changes much faster than T_2. The change in the charge ΔQ is,

$$\Delta Q = C(V + \Delta V)(V + \Delta V) - C(V)V. \quad \text{(A7)}$$

Applying this equation to the sequence in Fig. 3 yields,

$$\Delta Q_1 = C_0[1 + \alpha(V_{base} + \Delta\Psi + \Delta V)^2] \cdot (V_{base} + \Delta\Psi + \Delta V) - C_0[1 + \alpha(V_{base} + \Delta\Psi)^2](V_{base} + \Delta\Psi) \quad \text{(A8)}$$

$$\Delta Q_2 = C_0[1 + \alpha(V_{base} + \Delta\Psi + V_i + \Delta V)^2] \cdot (V_{base} + \Delta\Psi + V_i + \Delta V) - C_0[1 + \alpha(V_{base} + \Delta\Psi + V_i)^2](V_{base} + \Delta\Psi + V_i) \quad \text{(A9)}$$

$$\Delta Q_3 = C_0[1 + \alpha(V_{base} + \Delta\Psi - \Delta V)^2] \cdot (V_{base} + \Delta\Psi - \Delta V) - C_0[1 + \alpha(V_{base} + \Delta\Psi)^2](V_{base} + \Delta\Psi) \quad \text{(A10)}$$

$$\Delta Q_4 = C_0[1 + \alpha(V_{base} + \Delta\Psi + V_i - \Delta V)^2] \cdot (V_{base} + \Delta\Psi + V_i - \Delta V) - C_0[1 + \alpha(V_{base} + \Delta\Psi + V_i)^2](V_{base} + \Delta\Psi + V_i). \quad \text{(A11)}$$

Gathering (A8–A11) yields,

$$\begin{aligned} -\Delta Q_1 + \Delta Q_2 + \Delta Q_3 - \Delta Q_4 &= 6\Delta V\{C_0[1 + \alpha(V_{base} + \Delta\Psi + V_i)^2] - C_0[1 + \alpha(V_{base} + \Delta\Psi)^2]\} \\ &= 6\Delta V[C(V_{base} + \Delta\Psi + V_i) - C(V_{base} + \Delta\Psi)]. \end{aligned} \quad \text{(A12)}$$

APPENDIX B

Let g_m, g_p, I_{offset}, and Q_{remain} be the membrane conductance, the conductance which is parallel to the membrane, virtual current which caused by the output offset error of the amplifier, and the charge generated by the pedestal step but charged after the time point T_1, respectively. Then the apparent charges which are collected by the computer are,

$$\begin{aligned} \Delta Q'_1 &= \tfrac{1}{2}\{[\Delta Q_1 + (g_m + g_p)\Delta V + I_{offset}T_2] - [-\Delta Q_1 + (g_m + g_p)\cdot 0 + I_{offset}T_2]\} \\ &= \Delta Q_1 + \tfrac{1}{2}(g_m + g_p)\Delta V \end{aligned} \quad \text{(B1)}$$

$$\begin{aligned} \Delta Q'_2 &= \tfrac{1}{2}\{[\Delta Q_2 + (g_m + g_p)(V + \Delta V) + I_{offset}T_2 + Q_{remain}] - [-\Delta Q_2 + (g_m + g_p)V + I_{offset}T_2]\} \\ &= \Delta Q_2 + \tfrac{1}{2}(g_m + g_p)\Delta V + \tfrac{1}{2}Q_{remain} \end{aligned} \quad \text{(B2)}$$

$$\begin{aligned} \Delta Q'_3 &= \tfrac{1}{2}\{[\Delta Q_3 + (g_m + g_p)(-\Delta V) + I_{offset}T_2] - [-\Delta Q_3 + (g_m + g_p)\cdot 0 + I_{offset}T_2]\} \\ &= \Delta Q_3 - \tfrac{1}{2}(g_m + g_p)\Delta V \end{aligned} \quad \text{(B3)}$$

$$\begin{aligned} \Delta Q'_4 &= \tfrac{1}{2}\{[\Delta Q_4 + (g_m + g_p)(V - \Delta V) + I_{offset}T_2 + Q_{remain}] - [-\Delta Q_4 + (g_m + g_p)V + I_{offset}T_2]\} \\ &= \Delta Q_4 - \tfrac{1}{2}(g_m + g_p)\Delta V + \tfrac{1}{2}Q_{remain}. \end{aligned} \quad \text{(B4)}$$

However, when they are gathered, errors are eliminated as follows:

$$\begin{aligned} -\Delta Q'_1 + \Delta Q'_2 + \Delta Q'_3 - \Delta Q'_4 &= -[\Delta Q_1 + \tfrac{1}{2}(g_m + g_p)\Delta V] \\ &\quad + [\Delta Q_2 + \tfrac{1}{2}(g_m + g_p)\Delta V + \tfrac{1}{2}Q_{remain}] \\ &\quad + [\Delta Q_3 - \tfrac{1}{2}(g_m + g_p)\Delta V] \\ &\quad - [\Delta Q_4 - \tfrac{1}{2}(g_m + g_p)\Delta V + \tfrac{1}{2}Q_{remain}] \\ &= -\Delta Q_1 + \Delta Q_2 + \Delta Q_3 - \Delta Q_4. \end{aligned} \quad \text{(B5)}$$

REFERENCES

Alvarez, O., and R. Latorre. 1978. Voltage-dependent capacitance in lipid bilayers made from monolayers. *Biophys. J.* 21:1–17.

Alvarez, O., M. Brodwick, R. Latorre, A. McLaughlin, S. McLaughlin, and G. Szabo. 1983. Large divalent cations and electrostatic potentials adjacent to membranes. *Biophys. J.* 44:333–342.

Babakov, A. V., L. N. Ermishkin, and E. A. Liberman. 1966. Influence of electric field on the capacity of phospholipid membranes. *Nature (Lond.)*. 210:953–955.

Bell, J. E., and C. Miller. 1984. Effects of phospholipid surface charge on ion conduction in the K+ channel of sarcoplasmic reticulum. *Biophys. J.* 45:279–287.

Benz, R., and K. Janko. 1976. Voltage-induced capacitance relaxation of lipid bilayer membranes. *Biochim. Biophys. Acta.* 455:721–738.

Carius, W. 1976. Voltage dependence of bilayer membrane capacitance. *J. Colloid Interface Sci.* 57:301–307.

Cherny, V. V., V. S. Sokolov, and I. G. Abidor. 1980. Determination of surface charge of bilayer lipid membranes. *Bioelectrochem. Bioenerg.* 7:413–420.

Chizmadzhev, Yu. A., and I. G. Abidor. 1980. Bilayer lipid membranes in strong electric fields. *Bioelectrochem. Bioenerg.* 7:83–100.

Leikin, S. L. 1987. Thermal vibrations and capacitance of bilayer lipid membranes in an electric field. *Biol. Mem.* 2:1519–1543.

Montal, M., and P. Mueller. 1972. Formation of bimolecular membranes from lipid monolayers and a study of their electrical properties. *Proc. Natl. Acad. Sci. USA.* 69:3561–3566.

Requena, J., D. A. Haydon, and S. B. Hladky. 1975. Lenses and the compression of black lipid membranes by an electric field. *Biophys. J.* 15:77–81.

Rudnev, V. S., L. N. Ermishkin, L. A. Fonina, and Yu. G. Rovin. 1981. The dependence of the conductance and lifetime of gramicidin channels on the thickness and tension of lipid bilayers. *Biochim. Biophys. Acta.* 642:196–202.

Schoch, P., D. F. Sargent, and R. Schwyzer. 1979. Capacitance and conductance as tools for the measurement of asymmetric surface potentials and energy barriers of lipid bilayer membranes. *J. Membr. Biol.* 46:71–89.

White, S. H. 1970. Thickness changes in lipid bilayer membranes. *Biochim. Biophys. Acta.* 196:354–357.

White, S. H., and W. Chang. 1981. Voltage dependence of the capacitance and area of black lipid membranes. *Biophys. J.* 36:449–453.

Wobschall, D. 1972. Voltage dependence of bilayer membrane capacitance. *J. Colloid Interface Sci.* 40:417–423.