Simplified method for setting the phase angle for use in capacitance measurements in studies of exocytosis

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ABSTRACT Phase detectors (lock-in amplifiers) have been used in conjunction with patch-clamp apparatus to follow the time course of exo- and endocytotic processes. A critical step is accurate setting of the phase to separate signals due to conductance changes from those due to capacitance changes. It has been the practice to obtain this setting empirically. Analysis of the admittance, and its derivatives, of a model equivalent circuit used commonly in this field shows that the desired phase setting is just twice the phase shift of the current relative to the stimulus voltage, which is easily, accurately and quickly determined. This fact permits rapid and accurate setting of the desire phase angle for those cells to which the equivalent circuit is suitable.

INTRODUCTION

In 1982, Neher and Marty (1982) tracked exocytosis in chromaffin cells by measuring changes in cell membrane capacitance. They patched a lock-in amplifier to a patch-clamp apparatus which was processing signals from a chromaffin cell to which the pipette was sealed in the whole-cell mode. They modeled an equivalent circuit of the chromaffin cell, consisting of a series conductance, G_s, in series with a parallel membrane conductance, G, and a parallel membrane capacitance, C. The admittance, Y, is then

$$Y = G_s(G + j\omega C)/(G_s + G + j\omega C). \quad (1)$$

It is critical to the success of this method to find a phase setting at which changes in output of the lock-in amplifier due to altered conductance can be distinguished from those due to altered capacitance. This has been accomplished as follows.

Partial differentiation of Eq. 1 with respect to each of the three components of the admittance showed that arg $(\partial Y/\omega\partial C)$ is orthogonal to arg $(\partial Y/\partial G)$, and that arg $(\partial Y/\partial G_s)$ for practical purposes, under conditions used by Neher and Marty (1982), is nearly antiparallel to arg $(\partial Y/\partial G)$, within $\sim 2°$ at a frequency of ~ 1 kHz. Neher and Marty located arg $(\partial Y/\partial G)$ by making a small change in the capacitance compensation setting of the patch-clamp apparatus and adjusting the phase setting on the lock-in amplifier until the resulting signal vanished. The desired setting for estimate of capacitance was then set orthogonal to this.

In 1988, Joshi and Fernandez (1988), using the same equivalent circuit as had Neher and Marty, but using mast cells, studied the matter intensively, and concluded that a safer way to set the phase for purposes of measuring a change in membrane capacitance is to make a small change in G_s and adjust the phase setting until that change in G_s produces minimum change in the imaginary component of the output signal (the quadrature component in a dual phase analyzer). They designated this angle $\theta - 90°$, and use it to detect capacitance changes. I use θ_{Gs} to mean their θ.

There is an alternative to this empirical method of determining θ_{Gs} if the equivalent circuit is an acceptable model for the cell.

Joshi and Fernandez (1988) point out (their Eq. $2c$) that for a small change in G_s,

$$\Delta I_{Gs} = V(\partial Y/\partial G_s)\Delta G_s. \quad (2)$$

From Eq. 1,

$$\partial Y/\partial G_s = Y^2/G_s^2. \quad (3)$$

From Eqs. 2 and 3,

$$\Delta I_{Gs} = V(Y^2/G_s^2)\Delta G_s. \quad (4)$$

The same conclusion can be reached by manipulation of Eqs. $1a$, $3a$, and $2c$ of Joshi and Fernandez (1988).

From Eq. 4, it is apparent immediately that

$$\arg(\Delta I_{Gs}) = \arg(Y^2). \quad (5)$$

A proof that arg $(Y^2) = 2$ arg (Y) follows:
$Y = Y(\omega)$ is a complex variable of the general form $x + jy$. The phase shift necessary to lock-in the command sinusoidal voltage with the current output is the angle

$$\theta_Y = \arg(Y) = \tan^{-1}(y/x). \quad (6)$$

From the general expression for Y as a complex variable, find Y^2, separate real and imaginary components to obtain

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$$\arg(Y^2) = \tan^{-1}[2xy/(x^2 - y^2)]$$
$$= \tan^{-1}(2y/x)/[1 - (y^2/x^2)]$$
$$= 2\tan^{-1}(y/x). \quad (7)$$

From Eqs. 5, 6, and 7, the desired phase angle is

$$\theta_{Gs} = \arg(\Delta I_{Gs}) = 2\theta_Y. \quad (8)$$

If the model is correct, the desired angle, θ_{Gs}, is obtained easily by doubling the locked-in phase angle, θ_Y. On our apparatus (EG&G, Princeton Applied Research [Trenton, NJ] 2 phase lock-in analyzer, model 5208) the quadrature analyzer is 90° positive to the in-phase analyzer. Joshi and Fernandez (1988) (their Eq. 2) give expressions for the contribution to the change in current output, ΔI, due to a change in each of the three elements, ΔI_G, $\Delta I_{\omega C}$, and ΔI_{Gs}, and for the angles of each of the three components in complex plane:

$$\theta_G = (\text{their } \alpha) = \arg(\Delta I_{Gs}) = -2\tan^{-1}[\omega C/(G_s + G)].$$
$$\theta_{\omega C} = \arg(\Delta I_{\omega C}) = \theta_G + 90°.$$
$$\theta_{Gs} = \arg(\Delta I_{Gs}) = \theta_G + 2\tan^{-1}(\omega C/G).$$

$\theta_{\omega C}$ is the phase angle setting (their α) used by Neher and Marty (1982) to detect capacitance changes with minimum interference from conductance changes. They located that angle by rotating the slow capacity compensation pot on their patch-clamp apparatus, meanwhile searching for a phase setting on their lock-in amplifier at which the change in slow capacity compensation caused no change in lock-in amplifier output. The angle so located is θ_G. They then set their lock-in amplifier at $\theta_G + 90° = \theta_{\omega C}$.

Joshi and Fernandez (1982) recommended that the phase angle chosen for capacitance detection be $\theta_Y - 90°$. Ordinarily $\theta_G + 90°$ and $\theta_{Gs} - 90°$ are nearly the same because θ_G and θ_{Gs} differ by nearly 180°. However, as Joshi and Fernandez point out, if the two angles are not close, then, for those cells in which changes in G_s are more of a problem than changes in G, one is better off using $\theta_{Gs} - 90°$. Because θ_{Gs} is just $2\theta_Y$, and θ_Y can be set automatically, it is quick and simple to locate $\theta_{Gs} - 90°$.

The procedure was tested on a model circuit. With the in-phase analyzer at $2\theta_Y = \theta_{Gs}$, 1 MΩ on the series resistance trimpot produced a change in the real component of the output current and no change in the imaginary component, which in our apparatus was analyzed automatically at $\theta_{Gs} + 90°$. We then reset the in-phase analyzer to $2(\theta_Y - 90°) = \theta_{Gs} - 180°$ so that the quadrature analyzer was at $\theta_{Gs} - 90°$. The 1 MΩ change in series resistance compensation was expressed only in the signal from the in-phase analyzer (the real component). Unfortunately in the Dagan 8900 patch-clamp apparatus (Dagan Instruments, Minneapolis, MN) the circuit labeled "slow capacity compensation" is not a pure capacity compensation; it lacks a variable capacitor; the trimpot is a variable resistor. Rotation of this pot gives a signal, a combination of ΔI_{Gs} and $\Delta I_{\omega C}$, which produces a signal from the in-phase analyzer as well as from the quadrature analyzer (the imaginary component) due to this deficiency in the Dagan 8900. Those whose patch-clamp apparatus has a calibrated variable capacitor in the slow capacity compensation circuit will not have this problem. With this method of obtaining $\theta_{Gs} - 90°$, a change in series resistance, which is the bugaboo of the whole-cell mode, has no effect on the imaginary component of the current output. Calibration of the response of the imaginary component to a change in capacitance permits use of signals from this channel to quantitate membrane capacitance changes, with no interference by changes in series resistance.

Our routine with biological material is to clamp a cell in the whole-cell mode in the usual way, low-pass filtered at 10 kHz, with no adjustment of slow capacity compensation. The lock-in amplifier is then autoset to find θ_Y in response to an 800 Hz, 5 or 10 mV_{rms} sinusoidal command input to the patch-clamp apparatus. The autoset, which locks the reference, or command, voltage input into current output from the patch-clamp apparatus, requires only a few milliseconds. At this setting, the signal from the in-phase analyzer is maximum, and the signal from the quadrature analyzer is minimum or zero. The in-phase analyzer is then set at $2(\theta_Y - 90°)$. The validity of the setting is checked by reducing series resistance compensation by 0.1 or 0.2 MΩ, verifying that this produces no signal from the quadrature analyzer. The "slow capacity compensation" pot (not calibrated in the Dagan 8900, but approximately 200 fF according to the manufacturer's circuit diagram) is rotated at low gain to confirm that the signal appears in the quadrature output of the lock-in amplifier, and to provide a crude calibration.

From time to time during the course of the experiment the phase setting is checked; slow capacity compensation is off and the lock-in amplifier autoset is activated. If there has been a change, the in-phase analyzer is reset to the new value of $2(\theta_Y - 90°)$. The procedure takes a few seconds. This is a kind of phase tracking, a term introduced by Fidler and Fernandez (1989) for their more sophisticated technique. Both the method proposed here and the method of Fidler and Fernandez have the advantage of not requiring exact cancellation of the time constant due to cell membrane components; in fact, slow capacity compensation must be off in the proposed method, so that Eq. 1 is valid. Fidler and Fernandez used the software based phase detector (Joshi and Fernandez, 1988). A software-controlled relay transiently added 1 MΩ to the series resistance, and the program searched for θ_{Gs}, about once in 10 s. $\theta_{Gs} - 90°$ is then set for measurement of $\Delta I_{\omega C}$. The method is probably faster overall than the method proposed here, and has the further advantage of permitting recalibration of the $\Delta I_{\omega C}$ signal without additional interruption of the experiment. The

method proposed here has the same accuracy in locating $\theta_{Gs-90°}$, and is as fast in locating θ_Y as the method of Fidler and Fernandez is in locating θ_{Gs}. It has the advantage of being available to those who have the hardware, but lack the systems capability of Fidler and Fernandez.

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