LETTERS TO THE EDITOR

Comments on the Frequency Domain Analysis of Asymmetry Current in Squid Axon Membrane

Dear Sir:

I would like to contribute to the discussion about the measurement of gating currents in the frequency domain. I refer to the paper of Takashima (1978) and the comments of Taylor and Bezanilla (1979).

As pointed out by the latter two authors the crucial points in the experiments of Takashima (1978) are: (*a*) Nonlinear gating effects have a dispersion range with a central frequency of 0.8 kHz. The frequency of 1.5 kHz is at the high frequency end; therefore only small capacitance changes are to be expected. (*b*) The resolution of the admittance measurement was insufficient to detect these small capacitance changes. (*c*) It is necessary that the system be in a steady state with regard to the system parameters and to the time scale of the excitation frequency. Otherwise, the meaning of impedance has to be defined more precisely.

I would like to make some suggestions how to overcome these drawbacks. Takashima et al. (1977), Takashima (1978), and Takashima (1979) used a lock-in amplifier as oscillator and detector. Obviously they made no use of the lock-in mode. The detector operated as a tuned amplifier with adjustable bandwidth. Higher performance can be reached in the lock-in mode. Here the noise rejection is increased by using a larger time constant of the low-pass filter at the output of the lock-in amplifier. This technique is commonly used, but inadequate here, too. The lock-in amplifier is overloaded at the moment of the voltage jump, and the low-pass filter increases the time for overload recovery of the output. But this difficulty is avoided when instead of the low-pass filter a three-mode integrator (e.g., Teledyne Philbrick, Dedham, Mass.; model 4850) is used together with an appropriate timing circuit. This device is in the hold mode during the overload of the lock-in amplifier. The leading edge of the voltage pulse at time $t = 0$ triggers a delay, T_d. The integrator stays in the hold mode during the delay and is therefore not affected by an overload of the lock-in amplifier. After the delay it integrates the signal $u_L(t)$ of the output of the lock-in amplifier during the time T_i. After that, it is again in the hold mode. During integration the output of the integrator has changed by:

$$\Delta V_i = \frac{1}{\tau} \int_{T_d}^{T_d+T_i} u_L(t)\mathrm{d}t. \tag{1}$$

τ is the time constant of the integrator. The mean value of $u_L(t)$ during the integration time is:

$$\overline{u}_L = \Delta V_i \cdot \tau / T_i. \tag{2}$$

If the integration time, T_i, is a multiple of the period

$$T_e = 1/f \quad \text{of the AC excitation voltage,} \quad T_i = n \cdot T_e, \; n \text{ is a positive integer,} \tag{3}$$

$\overline{u}_L$ in Eq. 2 corresponds mathematically to a Fourier coefficient of the input signal, selected by the reference signal and the phase setting at the lock-in amplifier (Pipes and Harvill, 1970). A low-pass filter with time constant T_f—instead of the integrator—will meet the corresponding condition if $T_f \gg T_e$. In practice, n must be large enough to suppress the contribution of noise. But a large n can be achieved easily by repetition of the integration procedure during successive voltage pulses. In this it resembles signal averaging in time domain measurements. If the system is not yet in a steady state during the sampling interval, the phase correlation between the AC excitation voltage and successive

TABLE I
CONVERSION BETWEEN THE NOTATIONS OF TAYLOR AND BEZANILLA (1979) AND CARIUS (1979)

Taylor and Bezanilla	Carius
Q_T	$\alpha \mid z \mid NF$
α	$\alpha \mid z \mid$
A	$(2k_i)^{-1}$

N is the molar surface concentration of charged particles, z is their valency, F the Faraday constant, k_i is the first-order rate constant at the voltage $V = V_0$, A the corresponding time constant, and Q_T is the absolute value of the apparent charge density of the moving particles. Only four parameters, e.g., $\alpha \mid z \mid$, V_0, N, and k_i, can be determined from experiments.

voltage pulses must be at random. It is recommended to make a further integration at the resting potential of the axon in the interval between two voltage pulses. After each integration period, T_i, the output signal, V_i, of the integrator is read by a high-precision, data acquisition system of an on-line computer. The difference, u_L, from two successive integrations, one at rest and the other during a voltage pulse, is processed according to statistical standard programs. Small admittance changes up to ±5% can be calculated directly from the two out-put signals u_L at 0° and 90° phase setting of the lock-in amplifier, without rebalancing the bridge. The changes of capacitance and conductivity are linear functions of the two output signals. For calibration the bridge is disbalanced step by step by using the control knobs. I feel that this would improve the technique of measurements in the frequency domain and render it comparable with the high standard those in the time domain have reached by signal averaging. I have had good experience with this method.

Besides these technical comments I would like to offer another approach for detecting nonlinear effects in the frequency domain: the measurement of harmonics, e.g., the DC voltage dependence of the second harmonic. I reported on this method some time ago (Carius, 1976), and recently I have applied it to the translocation of hydrophobic ions through lipid bilayers (Carius, 1979). The model used there is equivalent to dipole orientation or gating charges moving between two stable states under the action of an electric field. The method applies also to more refined kinetic models for the gating mechanism (Keynes, 1975). Formulae for the generation of harmonics can be calculated in the same manner as demonstrated with the two-state model (Carius, 1979). To facilitate the application of the formulae given there to the problem of gating currents, Table I gives the conversion between the notations of Taylor and Bezanilla (1979) and Carius (1979).

I have computed the current at double frequency for the parameters given by Taylor and Bezanilla (1979): $Q_T = 1{,}000$ electronic charges/μm^2, $V_0 = -20$ mV, $A = 200$ μs, and $\alpha = 2$. At a DC voltage, $V = 0$ mV, and at an excitation frequency of $f = 1.5$ kHz the amplitude of the current is $i_{2f} \simeq 0.35$ $\mu A/cm^2$ at an AC voltage of 5 mV rms. This corresponds to a second harmonic signal of 10 μV in my experimental set-up and can easily be detected in the second harmonic lock-in mode, even when the bridge is out of balance at the fundamental frequency (Carius, 1979). An excitation voltage of 5 mV rms is appreciably higher than that used in the experiments of Takashima, but the error due to the dependence of the capacitance on the amplitude of the AC voltage is <3% at $f = 1.5$ kHz.[1] The error increases to 8% at most in the low frequency limit. The integration procedure described above (Eqs. 1–3) works in the second harmonic mode of the lock-in amplifier as well as at fundamental frequency. I hope that my suggestions will stimulate further experimental work in this field.

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