

A Method of Resolution Improvement by the Measurement of Cell Membrane Capacitance

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Summary

When measuring cell membrane electrical capacitance in whole cell configuration using alternating currents, the resolution decreases with increasing membrane conductance and pipette resistance. Improved resolution was attained by the dual-frequency method which was modified as to control the voltage amplitude of one of the measuring frequencies. A model circuit was developed for the verification of the method. This circuit allows measurement of calibrated capacitance changes even in the range of 5 to 20 fF. Moreover, the method was applied to capacitance measurements on pancreatic exocrine acinar cells. The results of measurements on the model as well as on pancreatic acinar cells are presented. The principle can also be applied to other hardware and software methods for measuring electrical cell membrane parameters.

Key words

Patch-clamp technique – Membrane capacitance – Membrane – Conductance – Series resistance – Exocytosis – Dual-frequency method

Introduction

One of the factors which limit the resolution of measurements of cell membrane electrical parameters, (i.e. membrane capacitance (C_m) and membrane conductance (G_m)), is the ratio of the measuring current to the background current. In whole-cell measurements the noise background current consists of two main components: the noise component originating in the hardware of the measuring set-up (the i/u converter and the serial resistance of the pipette, R_s) and the electrical interferences due to the dynamics of electrical cell membrane parameters. The first component is independent of the electrical potential applied, the second one can be dependent on the potential, e.g. due to the activation or inactivation of ion channels.

The maximum potential which can be applied across the membrane is limited to values which do not significantly influence the results of the measurements. The changes of the electrical properties of the cell membrane and changes of the pipette resistance can cause a significant potential drop on the pipette

resistance, and therefore the potential across the membrane decreases. This phenomenon degrades the ratio of the signal to the background noise and thus the achievable measurement resolution.

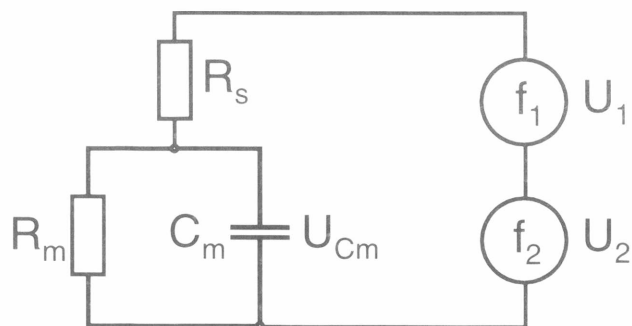


Fig. 1

Simplified arrangement of dual-frequency method. C_m = membrane capacitance; R_m = membrane resistance; R_s = serial resistance; U_1 = voltage generator of the frequency f_1 ; U_2 = voltage generator of the frequency f_2 . Parallel capacitance (not drawn) is assumed to be compensated.

Method

The following method is based on the fact that the correction of the input potential will maintain the peak-to-peak value of the potential across the cell membrane regardless of changes in G_m , R_s and C_m . The basic arrangement for using the dual-frequency method is shown in Figure 1, where the admittance A consists of the membrane resistance R_m , the (serial) pipette resistance R_s and the membrane capacitance C_m . The parallel capacitance to the network is assumed to be compensated. The membrane capacitance is given by equation 1.

$$C_m = \frac{\frac{2 \cdot [\operatorname{Re}^2(f_2) + \operatorname{Im}^2(f_2)]}{\operatorname{Im}(f_2)} - \frac{\operatorname{Re}^2(f_1) + \operatorname{Im}^2(f_1)}{\operatorname{Im}(f_1)}}{3 \cdot \omega} \quad (1)$$

with $\omega = 2f_1$, Re and Im are the real and imaginary components of the admittance for the lower (f_1) and higher (f_2) frequency. Equation 1 was derived by Rohlíček and Rohlíček (1993) and is rewritten for the frequency ratio $f_2 = 2f_1$.

Under practical conditions, the first numerator term is greater, in some cases even much greater, than the second one. This fact is evident because of the higher admittance of C_m for the higher frequency. Thus, it seems to be advantageous to correct this by enlarging only the higher frequency component. This assumption was confirmed experimentally by comparing these results with the results obtained when both frequencies were corrected.

The following equations are not valid for $R_m = 0$ (short-circuit across C_m). The potential across the membrane (U_{Cm}) is given by:

$$U_{Cm} = \frac{1}{1 + R_s/R_m} \left[U_{f_1} \cdot \frac{\sin(x + \varphi_1)}{\sqrt{1 + A^2}} + U_{f_2} \cdot \frac{\sin(k \cdot x + \varphi_2 + \psi)}{\sqrt{1 + k^2 \cdot A^2}} \right] \quad (2)$$

where: $x = \omega \cdot t$

$$A = \omega \cdot C_m \cdot R_s / (1 + R_s/R_m)$$

U_{f_1} = amplitude of the potential of frequency f_1

U_{f_2} = amplitude of the potential of frequency f_2

$$k = f_2/f_1$$

$$\varphi_1 = -\arctg A; \varphi_2 = -\arctg k \cdot A$$

ψ = phase shift between ascendant parts of the potential waveforms of U_{f_1} and U_{f_2} measured at zero-line crossing points (related to f_2).

The solution of equation 2 for maximum peak to peak potential (U_{PP}) on the capacitance C_m is:

$$U_{PP} = U_{Cm}(X_1) - U_{Cm}(X_2) \quad (3)$$

where X_1 is the abscissa of the positive maximum and X_2 is the abscissa of the negative maximum.

The maximum value of the potential U_{PP} is obtained for $R_s = 0$. The magnitude of U_{PP} depends on the ratio of U_{f_1}/U_{f_2} and on their phase shift ψ . With respect to the phase shift, U_{PP} has a minimum at $\psi = 90^\circ$ (related to f_2).

To obtain the same U_{PP} as for $R_s > 0$ as well as for finite values of R_m , the potential U_{f_2} should be enlarged by a multiplicative correction factor F_{Corr} given by:

$$F_{Corr} = \sqrt{1 + k^2 \cdot A^2} \cdot \frac{U_{max} \cdot (1 + \frac{R_s}{R_m}) - \sqrt{\frac{1}{1 + A^2}} \cdot [\sin(x_1 + \varphi_1) - \sin(x_2 + \varphi_1)]}{\sin(k \cdot x_1 + \varphi_2 + \psi) - \sin(k \cdot x_2 + \varphi_2 + \psi)} \quad (4)$$

Where $U_{max} = U_{PP}$ for $R_s = 0$. The coordinates of both maxima of U_{PP} are given by:

$$\frac{1}{\sqrt{1 + A^2}} \cdot \cos(x + \varphi_1) + \frac{k \cdot F_{Corr}}{\sqrt{1 + k^2 \cdot A^2}} \cdot \cos(k \cdot x + \varphi_2 + \psi) = 0 \quad (5)$$

The nonlinear equation 5 can be solved by the iteration method (Ralston 1965, Forsythe *et al.* 1977). At the beginning F_{Corr} is taken to be equal to one.

Equation 5 has four roots. We obtain four values of the potential U_{Cm} by substituting these roots into equation 2. The potential U_{PP} is given by the greatest difference between these values which are then substituted into equation 4. The solution of this equation gives the first approximation of the factor F_{Corr} . Repetition of this process yields a more accurate approximation. No more than two iterations are necessary to obtain the factor F_{Corr} with an error smaller than 1 % in the range of interest (i.e. $C_m = 5\text{--}30$ pF, $R_s = 2\text{--}20$ M Ω , $R_m = 50\text{--}1000$ M Ω).

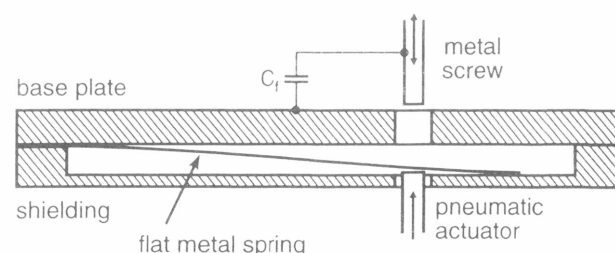


Fig. 2

Detail of a part of the model circuit used to produce defined capacitance changes of 5 to 20 fF. A well defined capacitance change between the screw and the base plate originates when the spring is pressed towards the base plate.

The resolution improvement was verified by using a slightly adapted apparatus described by Rohlíček and Schmid (1994). The correction factor was introduced by means of two software-controlled electronic step potentiometers. The first one is inserted in the sinus-generator for f_2 and enlarges the amplitude

of the potential of f_2 . The second one attenuates the signal at the input of the synchronous detectors for f_2 proportionally to the enlargement of the voltage amplitude of f_2 set by the first step potentiometer.

A model circuit was used in order to differentiate the changes of the signal to background ratio. This circuit consists of a parallel combination of a resistor (100 M Ω) and a fixed capacitance (15 pF) as well as a serial resistor (15 M Ω). These values were chosen as they closely match the values obtained by measurements on hormone-stimulated pancreatic acinar cells. Figure 2 shows a detail of the 15 pF capacitor. The metallic base plate (3 mm thick) connected with the ground electrical potential has an opening (diameter 4.5 mm) which can be covered on its

underside with a metallic flat spring. A screw (M3), connected with the other electrode of the fix capacitor C_f , is positioned in the centre of the opening so that it is partially shielded from the spring by the walls of the opening. Thus a small and well defined capacitance change takes place by pressing the spring towards the base plate. This capacitance change can be set in the range of 5 to 20 fF by adjusting the distance between the screw and the base plate. To avoid any influence by driving of the spring on the measuring circuit, the flat spring is electrically shielded and pneumatically actuated. The model circuit was mechanically designed so as to minimize the stray capacities. The capacitance changes were estimated by averaging the results of several hundred measuring cycles.

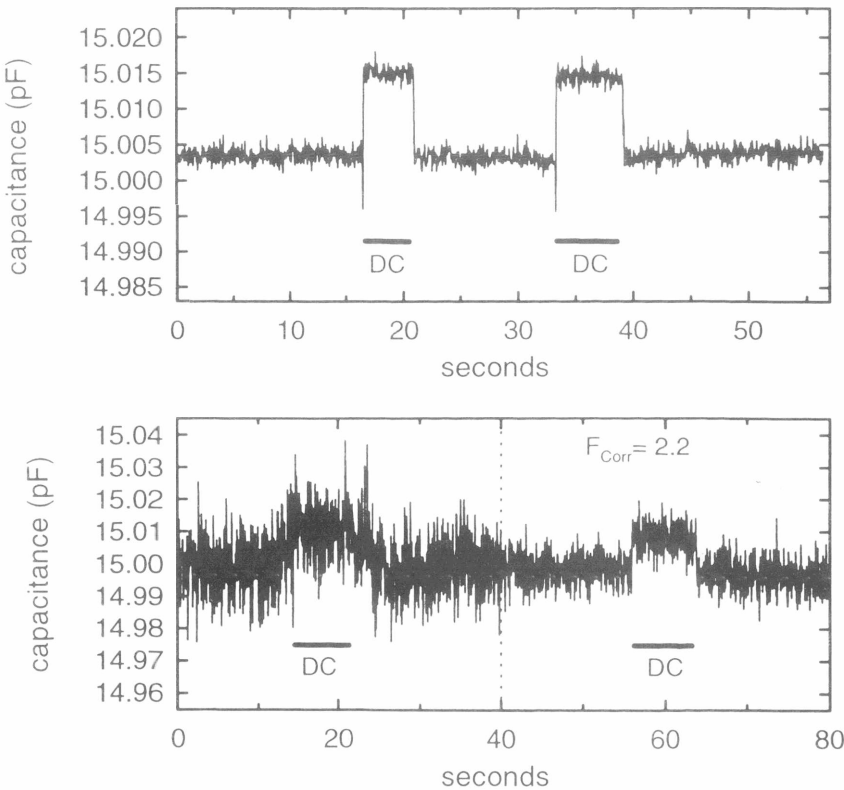


Fig. 3
Capacitance measurement on a model circuit consisting of either a 15 pF capacitor (upper trace) or a 15 pF capacitor in parallel with a 100 M Ω resistor and a series resistor of 15 M Ω (lower trace). The mechanically induced increases (DC) of the capacitance were 11.4 fF. $f_1 = 488.28$ Hz, $f_2 = 976.56$ Hz. The presented traces were obtained by sample interval equal to $1/f_1$ (2.046 ms) and the output potentials were integrated at the same interval. No averaging was used. Voltage correction by enlargement of the sine wave potential f_2 was performed in the right part of the lower panel ($F_{\text{Corr}} = 2.2$).

Results and Discussion

Figure 3 (upper panel) shows a capacitance trace recorded by the dual-frequency method using only the capacitor of model circuit ($R_s = 0$, $G_m = 0$). Small changes of the capacity ($\Delta C = 11.4$ fF) were induced at the indicated bars. When $R_s = 15$ M Ω and $G_m = 10$ nS, the noise of the C_m recording was much higher (lower panel). The result of the voltage correction of f_2 with $F_{\text{Corr}} = 2.2$ is shown in the right part of the lower panel in comparison to the C_m recording without voltage correction in the left-hand part. In the presented experiments, the frequencies of the measuring potentials were set to 488.28 Hz (f_1) and 976.56 Hz (f_2). Comparative measurements under the

same conditions at other frequencies in the range of 200 to 900 Hz (f_1) have shown a very flat optimum for resolution near 500 Hz.

Figure 4 demonstrates the effect of the voltage correction of f_2 on a C_m trace obtained from a pancreatic acinar cell. EGTA was omitted from the pipette solution to reduce the intracellular calcium buffer capacity. Within five minutes after establishing the whole cell configuration, activation of calcium-dependent chloride and nonselective cation channels caused an increase in the membrane conductance to 14.5 nS. The series resistance was 12.2 M Ω . Figure 4 shows a C_m trace without and with voltage correction ($F_{\text{Corr}} = 1.8$).

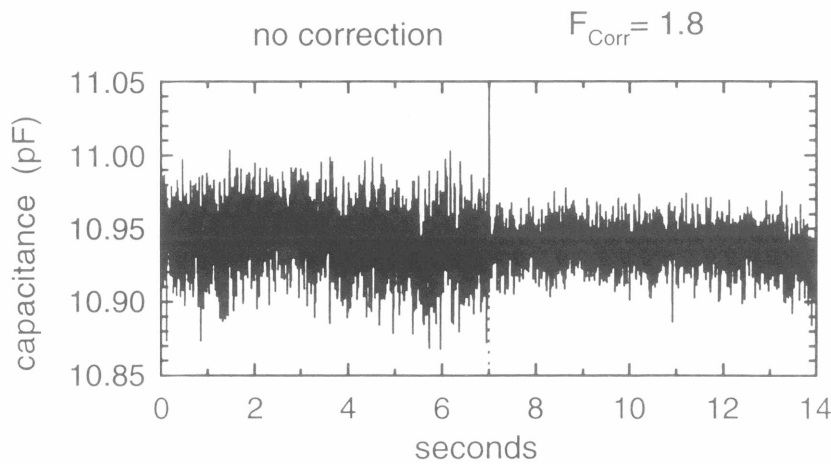


Fig. 4
Capacitance measurement on a mouse pancreatic acinar cell. The pipette solution contained 145 mM KCl, 10 mM HEPES, 2.5 mM MgATP, with no added EGTA (to avoid intracellular calcium buffering). Due to activation of calcium-dependent chloride and cation channels, the membrane conductance increased within 5 min to 14.5 nS (membrane resistance: 69 MΩ). The series resistance was 12.2 MΩ. Non-averaged data for C_m without and with voltage correction ($F_{\text{Corr}} = 1.8$) are shown (sample interval 2.046 ms).

Almost all devices applied to whole cell measurements are equipped with a compensation of the access (pipette) resistance which is accomplished by a positive feedback. Thus, a part of the output potential of the i/u transducer (which is proportional to the potential across the pipette resistance) is added to the measuring potential. There are two main drawbacks of this method. First, the compensation uses

a potential which includes the background containing noise component. In the range of the values obtained during the measurements on pancreatic cells, this signal with noise which is fed-back can be greater than the measured potential, (in extreme cases up to three times greater than the measuring potential). Second, there is danger of oscillations if R_s suddenly decreases or R_m increases.

Table 1
Some correction factors (F_{Corr}) for voltage correction of U_{I2} in dependence on cell capacitance (C_m), pipette series resistance (R_s) and cell conductance (G_m).

$R_m = 1000\text{ M}\Omega, G_m = 1\text{ nS}$					$R_m = 100\text{ M}\Omega, G_m = 10\text{ nS}$				
$R_s\text{ [M}\Omega\text{]}$					$R_s\text{ [M}\Omega\text{]}$				
C_m [pF]	5	10	15	20	5	10	15	20	
	5	1.247	1.315	1.408	1.523	1.315	1.449	1.605	1.779
	10	1.292	1.480	1.745	2.065	1.359	1.609	1.927	2.295
	20	1.459	2.026	2.772	3.631	1.523	2.142	2.925	3.806
	30	1.704	2.755	4.069	5.516	1.765	2.857	4.190	5.644

The correction factors were calculated for $f_1 = 488.28\text{ Hz}$ as described in the Methods section.

The computation of the correction factor F_{Corr} with a common PC takes several tens of milliseconds. It is therefore not possible to perform the correction in real time, because the measuring cycle is much shorter (2 ms for $f_1 = 500\text{ Hz}$). Hence we have developed an auxiliary program which computes the values of the

factor F_{Corr} in appropriate steps in the whole relevant range. These values are stored in the computer as 3D array. An array of 1000 values is sufficient to achieve better than 5 % accuracy. Access to the 3D array with the actual values of C_m , G_m and R_s allows computer-controlled real-time correction. This is particularly

important for cells which respond to external stimuli with large changes in cell membrane conductance and/or cell membrane capacitance. The array is computed before entering the main program and can be used as long as the measuring frequencies remain unchanged. The range of correction factors is divided into 8 steps at 13 % intervals. The appropriate part of

the software prevents a "jitter" change of the correction due to the noise by sufficient overlapping of the steps. A part of this array is compiled in Table 1. It is also possible to use the described correction in other hardware or software-based methods of measuring cell membrane electrical parameters (for short review see Rohlíček and Schmid 1994).

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Reprint Requests

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