Revealing membrane potential by advanced impedance spectroscopy: theoretical and experimental aspects

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Abstract. In spite of recent advancement of novel optical and electrical techniques, availability of non-invasive, label-free methods to assess membrane potential of living cells is still an open issue. The theory linking membrane potential to the low frequency $\alpha$ dispersion exhibited by suspensions of spherical shelled particles (presenting a net charge distribution on the inner side of the shell) has been pioneered in our previous studies with emphasis on the permittivity spectra. We now report on both theoretical and experimental aspects showing that whereas $\alpha$ dispersion is related to a rather large variation exhibited by the permittivity spectrum the decrement presented by impedance magnitude spectrum is either extremely small, or occurs (for large cells) at very low frequencies (~mHz) explaining the lack of experimental bioimpedance data on the matter. Based on the microscopic model we indicate that an appropriate design of the experiment may enable access to membrane potential as well as to other relevant parameters when investigating living cells and charged lipid vesicles. We discuss the effect on the low frequency of permittivity and impedance spectra of: I. Parameters pertaining to cell membrane i.e. (i) membrane potential, (ii) size of the cells/vesicles, (iii) conductivity; II. Conductivity of the outer medium. A novel measuring set-up has recently been developed within the International Centre of Biodynamics allowing for sensitive low frequency (~10mHz) four point (bio)impedance assays. Its capability to test theoretical predictions is reported as well. The far reaching implications of this study applicability for life sciences (non-invasive access to the dynamics of relevant cell parameters) as well as for biosensing applications, e.g. assess the cytotoxicity of a wide range of stimuli, will be outlined.

1. Introduction
The dispersions exhibited by impedance spectra of biosystems over a wide frequency range ($\alpha$, $\beta$, $\gamma$), as introduced by Schwan [1], enable thorough characterization of the electrical properties of biomaterials. Since mechanisms behind dispersions are often unclear or unknown, the dispersion grouping has been simply sorted according to the frequency range [2] with alpha dispersion revealed by bio-impedance spectra in the range below 1 Hz and up to 100 kHz. Even though Gerhard Schwarz seminal paper on $\alpha$ dispersion of colloidal particles (related to displacement of counter-ions) was published five decades ago [3], assessment of living cell properties pertaining to $\alpha$ dispersion is still an open issue. Remarkably, such dispersion could basically be explained by different mechanisms described by microscopic models of cell systems, as the ones pioneered by us based either on shape effects (e.g. exhibited by clusters of interconnected cells [4, 5, 6]), or on displacement of counter-ions [7, 8]. Notably, studies relying on the same approaches (and deriving similar results) continue to be published [9, 10].
We now pinpoint the limitations/constraints and suggest solutions enabling measurement of $\alpha$ dispersion on suspensions of living cells, or liposomes consisting in charged lipids and derivation of the related membrane potential. Membrane potential is related to the charge asymmetry across membrane involving the charge distribution on the inner side of the membrane and the counter-ions on the external side which ensure cell/particle electrical neutrality. The effect of the charges on the inner side was implicitly considered, but not the effect of the fixed external ones. We present the expression of the complex permittivity of a suspension of spherical cells and the related effect on the permittivity and impedance spectra of: I. parameters pertaining to cell membrane i.e. (i) membrane potential, (ii) size of the cells/vesicles, (iii) conductivity of the membrane; II. conductivity of the outer medium (diffusion effect was discussed in [11]). The last section discusses ways to check the theory and the role of parameters pertaining both to cells or vesicles under investigation, and to suspending medium fostering measurable variations of low frequency, $\alpha$ dispersion exhibited by impedance spectra.

2. The model and numerical simulations
Aiming to describe the dielectric behavior of a suspension of spherical living cell, we analyze the effect of a uniform AC field of angular frequency $\omega$ ($\omega = 2 \pi f$) and amplitude $E_0$ on a suspension of shelled spherical particles (of radius $R_i$) with a distribution of (negative) charge, $e_0$, on the inner side of the shell (membrane), of thickness $d$ [7,8]. External medium acts as a charge reservoir enabling counter-ions to migrate to the external side of the membrane to balance the charge from the inner side. Diffusion effects are assumed both in the inner and external/ suspending medium, but not in the membrane. Considering $\sigma_k$ as conductivity, and $\omega_k$ as permittivity within medium $k (k = 1, 2, 3)$ the corresponding complex permittivity and conductivity is $\varepsilon_k = \varepsilon_k + \sigma_k(i\omega)$; $\sigma_k = i\omega \varepsilon_k$. Solving the set of equations for potential one obtains the potential in the outer medium, [7]:

$$\varphi(r,\theta) = -E_y r \cos \theta + \frac{E_y - R H}{\rho^2} \cos \theta$$

(1)

Where $\theta$ and $r$ are the spherical coordinates in a system whose origin is placed at the center of the particle and the polar axis has the direction of the applied field, $H_0$ comprises the entire information on the electrical and geometric parameters of the cell prototype; $3 \times H_0$ equals particle polarizability. Following the Maxwell-Wagner equivalence approach the complex permittivity of suspension is [7,8]:

$$\varepsilon' = \varepsilon_1 + \frac{3}{1 + p} \frac{H_0}{(R_i + d)} H_1 + \frac{1 - p}{1 - p} \frac{H_0}{(R_i + d)} H_1$$

(2)

Where $p$ denotes volume concentration of the suspended particles, and:

$$NN = \frac{r_i}{k T (R_i + d)} \left( 1 + i f \left( \frac{R_i + d}{2 D_i} \right)^2 \right) ; \delta = \left( \frac{R_i}{(R_i + d)} \right)^2 ; G_k = \left( \frac{\sigma_k}{D_k \varepsilon_k} \right)^2 ; k = 1, 3$$

(3)

$$g = (1 - 2\delta) \sigma_1 \sigma_2 \left( 1 + \delta \right) \left( \frac{i \omega e_0 \sigma_1}{\sigma_1 + (1 + \delta) \sigma_2} \right) + \sigma_1 \left( \frac{1 + \delta}{\sigma_2} \sigma_2 + \frac{1}{\sigma_2 \left( 1 + \delta \right)} \right) ; h = \left( \frac{1}{\sigma_2} \sigma_2 + (1 + \delta) \frac{1}{\sigma_2 \left( 1 + \delta \right)} \right)$$

$$s = \frac{\sigma_1 \left( G_k R_i \right)^2 - 2 (G_k R_i - 1)}{i \omega e_0 \sigma_1} G_k \left( \frac{1}{G_k R_i - 1} \right) \left( \frac{1}{G_k R_i - 1} \right) \left( 1 - G_k (R_i + d) \right)^2 + 2 (1 - G_k (R_i + d))$$

$T$ represents the absolute temperature, and $k_B$ the Boltzmann constant.

2.1. Effect of cell membrane parameters on the permittivity and impedance spectra
The surface charge distribution $n_{0l}$ is related both to membrane potential and to the complex permittivity $\varepsilon'_\infty$ of the suspension via $H_0$ in equation (2) through the term $NN$, equation (3), [11].

We consider a suspension of shelled particles with $R_i \sim 2 \mu m$ ($\sigma_1 \sim 0.2 \ m^2 \cdot s^{-1}$, $\sigma_2 \sim 0.37 \ m^2 \cdot s^{-1}$, $e_0 \sim 12$, $\sigma_2 = 10^6 \ m^2 \cdot s^{-1}$, $D_1 \sim 2 \times 10^{-10} \ m^2 \cdot s^{-1}$, $D_2 \sim 2 \times 10^{-9} \ m^2 \cdot s^{-1}$, $p = 0.02$) with membrane potential: -150 mV, -75 mV and 0V respectively (figures 1 A, B). Impedance spectra are derived based on $\varepsilon'_\infty$ using:
\[ Z_{\alpha} = \text{GF} \left( \imath \omega \varepsilon_{\alpha} \right)^{-1} \]

where GF represents the Geometric Factor of the measurement chamber. Figure 1A shows the permittivity spectra and the dependency of \( \alpha \) dispersion on membrane potential. However, the spectra of impedance magnitude relative to the value at 1 kHz (the level prior to \( \beta \) dispersion) reveal (figure 1B) very small decrements \( (\Delta Z_r \leq 5 \times 10^{-3} \%) \) related to \( \alpha \) dispersion. The same challenge is met for phase variation within \( \alpha \) dispersion, \( \Delta \theta \leq 2 \times 10^{-3} \) degree, raising hard experimental constraints.

**Figure 1A** Permittivity spectra: \( \Delta \phi_0 = -150 \text{mV} \) (solid), \( \Delta \phi_0 = -75 \text{mV} \) (dashed), \( \Delta \phi_0 \sim 0 \text{V} \) (dotted)

**Figure 1B.** Impedance magnitude spectra relative to the value at 1 kHz: \( \Delta \phi_0 = -150 \text{mV} \) (solid), \( \Delta \phi_0 = -75 \text{mV} \) (dashed) & \( \Delta \phi_0 \sim 0 \text{V} \) (dotted)

When considering suspensions of larger cells \( (R_1 \sim 0.5 \text{ mm}, \text{as for the case of } Xenopus laevis \text{ oocytes}) \), impedance variation significantly enhances, but \( \alpha \) dispersion occurs at lower frequencies \( (<10 \text{ mHz}) \) which considerably increases measurement time and experimental constraints (due to electrode polarization, probe stability etc.). Figure 2 (A, B) reveals the dependency of \( \alpha \) and \( \beta \) dispersions on membrane conductivity for all parameters as before, but \( R_1 \sim 0.5 \text{ mm} \) \( (\Delta \phi_0 = -150 \text{mV}) \).

**Figure 2A** Permittivity spectra of oocytes, \( \sigma_2 = 10^{-6} \text{S m}^{-1} \) (solid); \( \sigma_2 = 10^{-5} \text{S m}^{-1} \) (dashed)

**Figure 2B** Impedance magnitude spectra of oocytes relative to 1kHz, \( \sigma_2 = 10^{-6} \text{S m}^{-1} \) (solid), \( \sigma_2 = 10^{-5} \text{S m}^{-1} \) (dashed)

Figure 2A presents the permittivity spectra, whereas figure 2B illustrates the spectra corresponding to impedance magnitude. Notably, impedance variation related to \( \alpha \) dispersion relative to its value at 100 Hz (level prior to \( \beta \) dispersion), increases to \( \Delta Z_r \sim 0.5% \). Therefore, we stress on the possible biosensing applications related to assessment of pore formation effect of various stimuli by impedance spectroscopy when considering large cells, or (charged) vesicles.

3. Experiments
A dedicated front end enabling low frequency (\( \geq 10 \text{mHz} \)) four point measurements using a 1260 Solartron impedance analyzer was developed in house, such as to control the low frequency polarization effects. The system was used to derive the impedance spectra of suspensions of living cells, \( Xenopus laevis \) oocytes \( (R_1 \sim 0.5 \text{mm}, \rho \sim 0.3) \) and POPG liposomes \( (R_1 \sim 5 \mu m, \rho \sim 0.1) \) as shown in figure 3, in both cases \( \sigma_2 = 0.03 \text{S/m} \). The measurement time for each spectrum spanned \( \sim 3 \) minutes. Notably, for measuring \( \alpha \) dispersion on oocytes the frequency spectrum should start at an
even lower frequency $\leq 1\text{mHz}$ (as shown in figure 2B), as for POPG liposomes, the diffusion of the outer medium $D_3$ should be decreased towards $\sim 10^{-13}\text{ m}^2/\text{s}$ [11].

4. Discussion
This study emphasises the dependency of $\alpha$ dispersion (related to displacement of counter-ions) on membrane potential and on several parameters pertaining to membrane and to suspending media, highlighting a set of experimental constraints for measuring impedance spectra exhibiting both $\alpha$ and $\beta$ dispersions in suspensions of living cells or charged liposomes. The dependency on membrane potential is clearly shown by permittivity spectra, whereas impedance spectra reveal very tiny variations pertaining to $\alpha$ dispersion. We show that larger size of the cells/vesicles is accompanied by an increased variation of the impedance; however, the dispersion shifts towards lower frequencies, a domain more challenging experimentally (due to electrode polarization and specimen stability). A slight increase of membrane conductivity is accompanied by an increase of $\alpha$ dispersion in both permittivity and impedance spectra. Thus, possible biosensing application of bioimpedance assays are related to assessment of pore formation effect of various stimuli (e.g. toxins, membrane disrupting peptides etc.).

Advances of the experimental set-up and appropriate choice of both type of cells (or liposomes) and of parameters of the suspending medium could lead to detailed interrogation and improvement of the theoretical model, as well as open new applications in biomedicine and related fields.

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