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Relating membrane potential to impedance spectroscopy

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Abstract
Non-invasive, label-free assessment of membrane potential of living cells is still a challenging task. The theory linking membrane potential to the low frequency α 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. Whereas α dispersion is related to a rather large variation exhibited by the permittivity spectrum, we report that the related decrement presented by the impedance magnitude spectrum is either extremely small, or occurs (for large cells) at very small frequencies (~mHz) explaining the lack of experimental bioimpedance data on the matter.

We stress that appropriate choice of the parameters (as revealed by the microscopic model) may enable access to membrane potential as well as to other relevant parameters when investigating living cells and charged lipid vesicles. We analyse the effect on the low frequency of the permittivity and impedance spectra of: I. Parameters pertaining to cell membrane i.e. (i) membrane potential (through the amount of the net charge on the inner side of the membrane), (ii) size of the cells/vesicles, (iii) conductivity of the membrane; II. Parameters of the extra cellular medium (viscosity and conductivity).

The applicability of the study has far reaching implications for basic (life) sciences (providing non-invasive access to the dynamics of relevant cell parameters) as well as for biosensing applications, e.g. assessment of cytotoxicity of a wide range of stimuli.

Keywords: membrane potential, diffusion effects, α dispersion, living cells, charged liposomes

Introduction

Classification of the dispersions exhibited by impedance spectra of biosystems over a wide frequency range (α, β, γ) was introduced by Schwan [1] to characterize the electric properties of biomaterials. Alpha dispersion is revealed by bioimpedance measurements in the range below 1 Hz and up to 100 kHz. Since mechanisms behind dispersions are often unclear or unknown, the dispersion grouping has been simply based upon a defined frequency range [2].

Even though Gerhard Schwarz seminal paper on low frequency, α dispersion of colloidal particles (related to displacement of counter-ions) was published five decades ago [3], assessment of living cell properties pertaining to α dispersion is still an open issue. Remarkably, such dispersion could basically be explained by various mechanisms formally described by different 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]. Moreover, a strong link to the alpha dispersion and counter-ion concepts confirms the classical theories, described for instance in the review article by Foster and Schwan [11].

Theoretical advancement providing a unitary description of both α and β dispersions related to the dielectric behavior of spherical living cells, with emphasis on the relationship between membrane potential and α dispersion was proposed by us in the early nineties [7, 8] However, to our knowledge, experimental non-invasive assessment of membrane potential using impedimetric/ dielectric assays has been not reported, as yet.

In this paper we pinpoint the limitations/constraints and suggest solutions enabling measurement of α dispersion on suspensions of living cells, or liposomes consisting of charged lipids and derivation of the related membrane potential.

We briefly introduce the theoretical model, provide the expression for suspension complex permittivity on the parameters of the microscopic model and illustrate the effect on the permittivity and impedance spectra of: I. Parameters pertaining to cell membrane i.e. (i) membrane potential (through the amount of the net charge on the inner side of the membrane), (ii) size of the cells/vesicles, (iii) conductivity of the membrane; II. Parameters of the extra cellular medium: (1) diffusion coefficient and (2) conductivity.

Within the last section of this paper, we emphasize ways and means to check the model as well as a set of parameters pertaining both to cells or vesicles under investigations and to the suspending medium fostering measurable variations of low frequency, α dispersions, exhibited by bioimpedance spectra.

Theory

Aiming to describe the dielectric behavior of a suspension of spherical living cells, we analyze the effect of a uniform ac field of angular frequency ω ($\omega = 2 \pi \nu$) and amplitude

E_0 on a suspension of shelled spherical particles (of radius R_1) with a distribution of (negative) charge, $e_0 n_{01}$, on the inner side of the shell (membrane, of thickness d) [7,8]; e_0 represents the charge of the electron. Diffusion effects are assumed both in the inner (denoted by 1) and external/ suspending medium (denoted by 3), but not in the membrane (denoted by 2). Considering σ_k as conductivity, and ε_k as permittivity within the medium k (k=1,2,3) the corresponding complex permittivity and conductivity is given by:

$$\epsilon_k^* = \frac{\sigma_k}{i\omega} + \varepsilon_k ; \sigma_k^* = i\omega \cdot \epsilon_k^* \tag{1}$$

The external medium is considered as a charge reservoir enabling positive ions (counter-ions) to migrate to the external side of the membrane and balance the negative charge distribution from the inner side (therefore, the total net charge is zero).

By solving the set of equations for the potential (Poisson equation for media 1 and 3, Laplace equation for the shell and the regularity conditions), one obtains the potential in the outer medium (the origin is considered in the centre of a prototype particle) [7]:

$$\varphi(r,\theta) = -E_0 \cdot r\cos\theta + \frac{E_0 \cdot H_n}{r^2}\cos\theta \tag{2}$$

H_n comprises the entire information of the electrical and geometric parameters of the shelled particles, as revealed by expressions within Eq. 4. Notably, $3\times H_n$ represents the polarizability of the (shelled) particle. Following the Maxwell-Wagner equivalence approach, the complex permittivity of suspension is given by [7, 8]:

$$\varepsilon_{sus}^* = \varepsilon_3^* \left(1 + p \cdot \frac{3 \cdot \frac{H_n}{(R_1+d)^3}}{1 - p \cdot \frac{H_n}{(R_1+d)^3}} \right) \tag{3}$$

Where:

$$\boldsymbol{H_n} = \left[\frac{\boldsymbol{g}}{\boldsymbol{h}} \cdot \left(\sigma_2 + \boldsymbol{i}\ \omega \cdot \varepsilon_2 \varepsilon_0 (1 - \boldsymbol{Q}) + \boldsymbol{i}\ \omega \cdot \varepsilon_3 \varepsilon_0 \cdot \boldsymbol{Q} \cdot \frac{\sigma_2}{\sigma_3} \right) + i\ \omega \cdot \boldsymbol{e}_0 \cdot \boldsymbol{NN} \cdot (\boldsymbol{R}_1 + \boldsymbol{d}) \cdot (1 - \boldsymbol{Q}) - \sigma_3^* \right] /$$

$$\left[\frac{\boldsymbol{g}}{\boldsymbol{h}} \cdot \left(\sigma_2 + \boldsymbol{i}\ \omega \cdot \varepsilon_2 \varepsilon_0 (1 + 2\boldsymbol{Q}) - 2\ \boldsymbol{i}\ \omega \cdot \varepsilon_3 \varepsilon_0 \cdot \boldsymbol{Q} \cdot \frac{\sigma_2}{\sigma_3} \right) + \boldsymbol{i}\ \omega \cdot \boldsymbol{e}_0 \cdot \boldsymbol{NN} \cdot (\boldsymbol{R}_1 + \boldsymbol{d}) \cdot (1 + 2\boldsymbol{Q}) + 2\sigma_3^* \right] \cdot (\boldsymbol{R}_1 + \boldsymbol{d})^3$$

$$NN = \frac{e_0 \cdot n_{01}}{k_B T} \frac{\frac{R_1^2}{(R_1+d)^2}}{1 + i\omega \frac{(R_1+d)^2}{2 \cdot D_3}}$$

$$g = (1+2\delta)\sigma_1\sigma_1^* + 2(1-\delta)\left[i\omega \cdot \sigma_1 \varepsilon_2 \varepsilon_0 (1+S) + \sigma_2 (\sigma_1 - i\omega \cdot \varepsilon_1 \varepsilon_0 S) \right]$$

$$h = (1-\delta)\sigma_1\sigma_1^* + (2+\delta)\left[i\omega \cdot \sigma_1 \varepsilon_2 \varepsilon_0 (1+S) + \sigma_2 (\sigma_1 - i\omega \cdot \varepsilon_1 \varepsilon_0 S) \right]$$

$$\delta = \left(\frac{R_1}{R_1 + d} \right)^3 \tag{4}$$

$$S = \frac{\sigma_1 \cdot (G_1 R_1 - Tanh\ G_1 R_1)}{i\omega \cdot \varepsilon_1 \varepsilon_0 \cdot \left[(G_1 R_1)^2 \cdot Tanh\ G_1 R_1 - 2(G_1 R_1 - Tanh\ G_1 R_1) \right]}$$

$$Tanh\ G_1 R_1 \approx 1 \Rightarrow S = \frac{\sigma_1 \cdot (G_1 R_1 - 1)}{i\omega \cdot \varepsilon_1 \varepsilon_0 \cdot \left[(G_1 R_1)^2 - 2(G_1 R_1 - 1) \right]}$$

$$Q = \frac{\sigma_3}{i\omega \cdot \varepsilon_3 \varepsilon_0} \cdot \frac{1 + G_3 \cdot (R_1 + d)}{G_3^2 \cdot (R_1 + d)^2 + 2 \cdot (1 + G_3 \cdot (R_1 + d))}$$

$$G_k = \sqrt{\frac{\sigma_k^*}{D_k \varepsilon_k \varepsilon_0}} ; k = 1,3$$

p denotes volume concentration of the suspended particles, T the absolute temperature, and k_B Boltzmann constant.

Impedance spectra are derived based on ε_{sus}^* using:

$$Z_{sus}^* = \frac{GF}{i\omega \cdot \varepsilon_{sus}^*} \tag{5}$$

where GF represents the Geometric Factor of the measurement chamber.

Essentially, a suspension of shelled particles with a distribution of charges on the inner side, exhibits two types of dispersions:

1) α, described by counter-ions displacement. When no field is applied (i.e., at equilibrium) the dipole moment of the system (shelled particle and counter-ions) is zero. When applying the AC field, the counter-ions move along the external side of the membrane, their distribution changes and therefore the dipole moment of the system becomes non-negligible. By increasing the angular frequency of the AC field, the capacity of counter-ions to follow the field is gradually exceeded and therefore dispersion occurs usually at frequencies lower (hence the notation α) than the ones due to interfacial polarization (denoted by β). Notably, the dispersion described by this mechanism originates from the net charge asymmetry across the shell, directly related to the potential difference i.e., the membrane potential [7].

2) β, described by the Maxwell-Wagner interfacial polarization.

Effect of cell membrane parameters on the permittivity and impedance spectra of a suspension of living cells

The surface charge distribution n_{01} is related both to the membrane potential $\Delta\varphi_0 = -\frac{d \cdot R_1}{\varepsilon_2 \varepsilon_0 (d + R_1)} \cdot e_0 n_{01}$ and to the suspension complex permittivity ε^*_{sus} via H_n, (Eq. 3) through the term NN (Eq. 4).

We consider a suspension of shelled particles with $R_1 \sim 2$ μm (p = 0.06, $\sigma_1 \sim 0.2$ S/m, $\sigma_3 \sim 0.377$ S/m, $\varepsilon_2 \sim 12$, $\sigma_2 = 10^{-6}$ S/m, d ~ 10^{-2}μm, $D_1 \sim 2\times10^{-10}$ m^2/s, $D_3 \sim 2\times10^{-9}$ m^2/s) with membrane potential -150 mV, -75 mV and 0 V respectively (fig 1 A, B and C).

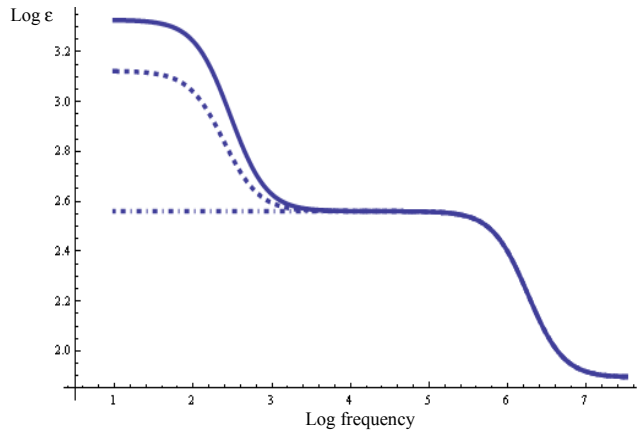

Fig. 1A: Permittivity spectra: $\Delta\varphi_0$ = -150 mV (solid), $\Delta\varphi_0$ = -75 mV (dashed), $\Delta\varphi_0 \sim 0$ V (dot-dashed line)

As revealed by fig. 1A, the permittivity spectra present a clear dependency of α dispersion on the membrane potential. However, for cells with radius ~2 μm, the spectra of impedance magnitude relative to the value at 1 kHz (impedance level prior to β dispersion) reveal (fig. 1B) very small decrements related to α dispersion, $\Delta Zr \leq 5\times10^{-3}$ % raising tough experimental constraints. The same challenge is related to phase variations in the α dispersion (fig 1C) where changes $\Delta\theta \leq 2\times10^{-3}$ degrees are emphasized.

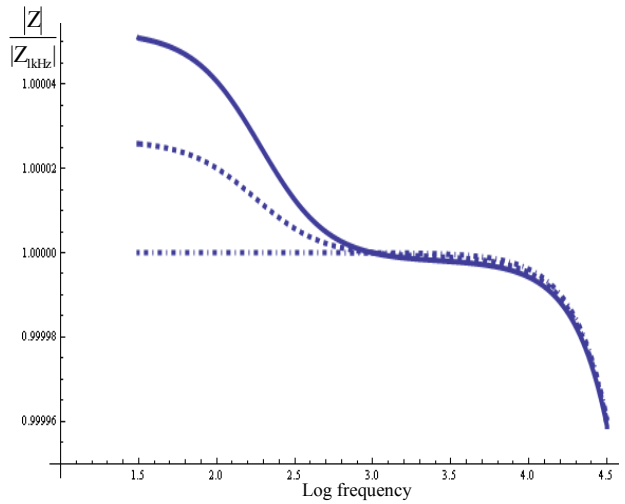

Fig. 1B: Spectra of Impedance magnitude relative to the value at 1 kHz: $\Delta\varphi_0$=-150 mV (solid), $\Delta\varphi_0$ = -75 mV (dashed), $\Delta\varphi_0 \sim 0$ V (dot-dashed line)

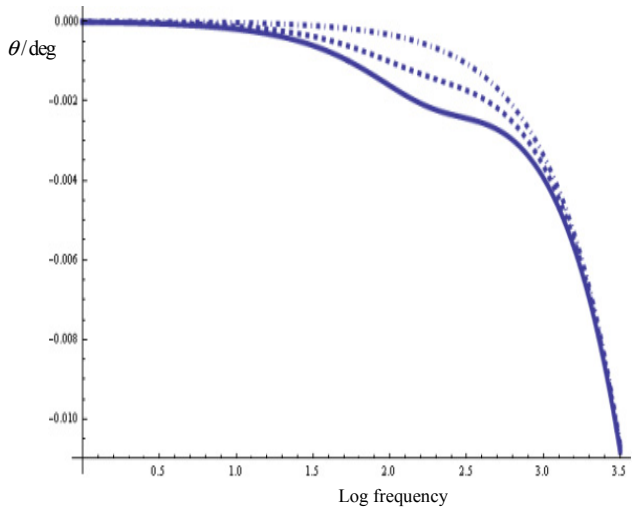

Fig. 1C: Phase Spectra: $\Delta\varphi_0$=-150 mV (solid), $\Delta\varphi_0$=-75mV(dashed), $\Delta\varphi_0 \sim 0$V(dot-dashed line)

When considering suspensions of larger cells ($R_1 \sim$ 0.5 mm), impedance variation significantly enhances, but α dispersion occurs at lower frequencies (<10 mHz) which considerably increases measurement time and experimental constraints (due to electrode polarization, probe stability etc.).

Figure 2 (A, B and C) reveals the dependency of α and β dispersions on membrane conductivity for all parameters as previously considered but $R_1 \sim 0.5$ mm and membrane conductivity σ_2 with 10^{-6} S/m and 10^{-5} S/m assigned values.

Figure 2A presents the permittivity spectra, whereas Figures 2 B and 2 C illustrate the spectra corresponding to impedance magnitude and phase. Notably, impedance variation related to α dispersion relative to its value at 100 Hz (impedance level prior to β dispersion), increases in this case to $\Delta Zr \sim 0.5$ %.

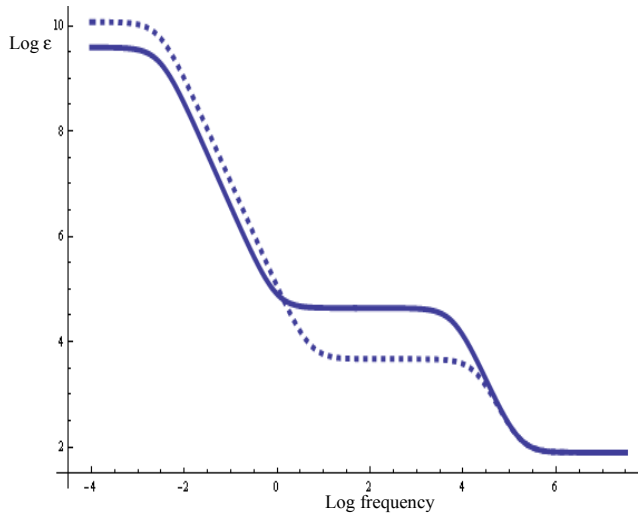

Fig.2.A: Permittivity spectra for suspensions of large cells ($R_1 \sim$ 0.5 mm), $\Delta\varphi_0$ = -150 mV; $\sigma_2 = 10^{-6}$ S/m (solid); $\sigma_2 = 10^{-5}$ S/m (dashed line)

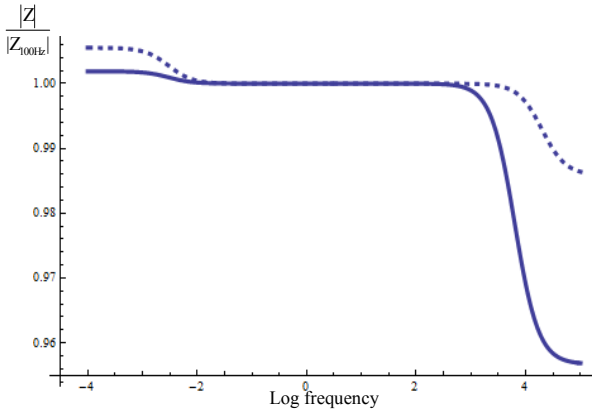

Fig. 2.B: Spectra of Impedance magnitude relative to the value at 100 Hz for suspensions of large cells (R_1 ~ 0.5 mm), $\Delta\varphi_0$ = -150 mV; $\sigma_2 = 10^{-6}$ S/m (solid line); $\sigma_2 = 10^{-5}$ S/m (dashed)

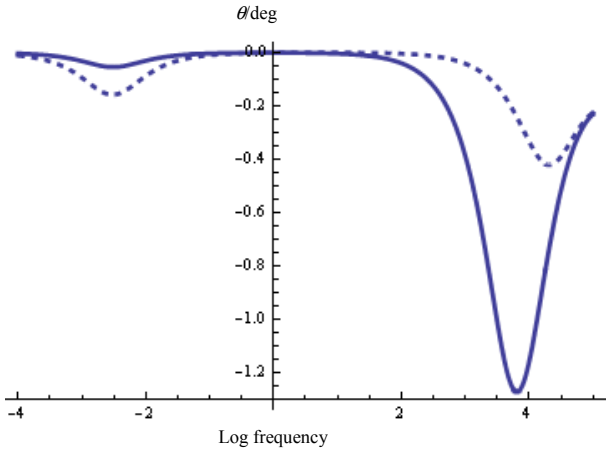

Fig. 2.C: Phase Spectra for suspensions of large cells (R_1 ~ 0.5 mm), $\Delta\varphi_0$ = -150 mV; $\sigma_2 = 10^{-6}$ S/m (solid line); $\sigma_2 = 10^{-5}$ S/m (dashed)

Effect of the outer medium parameters on the permittivity and impedance spectra of a suspension of charged vesicles (liposomes)

The dependency of α dispersion on viscosity (diffusion) and conductivity of the outer medium is described by expressions in Eq. 4.

We consider a suspension of charged vesicles with R_1 ~ 50 nm (p = 0.25, charge density: $e_0 n_{01} \sim 1.6\times10^{-2}$ C/m^2, σ_1 = 0.2 S/m, ε_2 = 4, $\sigma_2 = 10^{-6}$ S/m, d = 4 nm, $D_1 = 2\times10^{-10}$ m^2/s).

When assuming: $D_3 \sim 5\times10^{-9}$ m^2/s, $\sigma_3 \sim 0.377$ S/m and $\sigma_3 \sim 0.1$ S/m we find that α and β dispersions superpose: due to the relatively high value of the diffusion coefficient in the suspending medium, the α dispersion is shifted over the β dispersion.

Figures 3 A, B and C show permittivity, impedance magnitude and phase spectra respectively, emphasizing that separation of the frequency ranges of α and β dispersions for suspensions of charged liposomes is achieved by lowering the diffusion coefficient (increasing the viscosity) of the suspending medium to $D_3 = 5\times10^{-10}$ m^2/s. Also lowering the conductivity of the external medium (to σ_3 ~ 0.1 S/m) one notices an increase of α dispersion magnitude as revealed by both permittivity and impedance spectra.

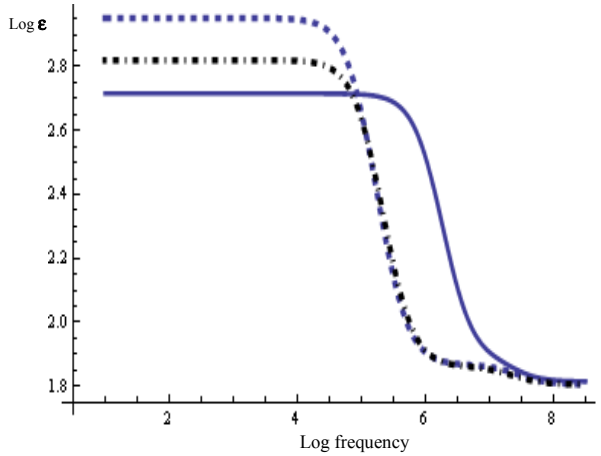

Fig. 3.A: Permittivity spectra for suspensions of charged liposomes: σ_3 = 0.377 S/m & $D_3 = 5\times10^{-9}$ m^2/s (solid); σ_3 = 0.377 S/m & $D_3 = 5\times10^{-10}$ m^2/s (dashed); σ_3 = 0.1 S/m & $D_3 = 5\times10^{-10}$ m^2/s (dot-dashed)

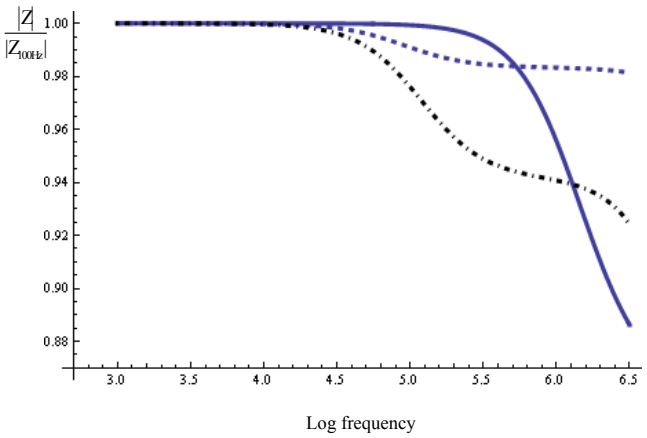

Fig. 3.B: Spectra of Impedance magnitude relative to the value at 100 Hz for suspensions of charged liposomes σ_3 = 0.377 S/m & $D_3 = 5\times10^{-9}$ m^2/s (solid); σ_3 = 0.377 S/m & $D_3 = 5\times10^{-10}$ m^2/s (dashed); σ_3 = 0.1 S/m & $D_3 = 5\times10^{-10}$ m^2/s (dot-dashed)

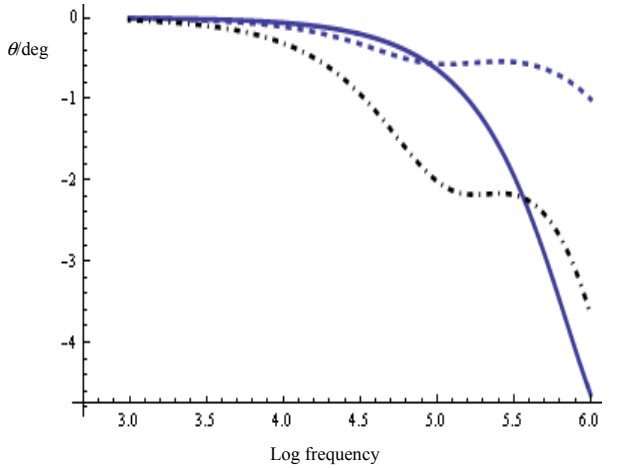

Fig. 3.C: Phase Spectra for suspension of charged liposomes σ_3 = 0.377 S/m & $D_3 = 5\times10^{-9}$ m^2/s (solid); σ_3 = 0.377 S/m & $D_3 = 5\times10^{-10}$ m^2/s (dashed); σ_3 ~ 0.1 S/m & $D_3 = 5\times10^{-10}$ m^2/s (dot-dashed)

Discussion

This study emphasises the dependency of α dispersion (related to displacement of counter-ions) on membrane potential and on several parameters pertaining to the membrane (shell) and to the suspending media, highlighting a set of experimental constraints for measuring impedance (magnitude and phase) spectra exhibiting both α and β dispersions on suspensions of living cells or charged liposomes. Aiming for increased generality to avoid spectra dependency on experimental set-up (i.e. geometric factor of the measurement chamber) the impedance magnitude spectra where plotted relative to the value at a frequency corresponding either to an almost constant value (plateau) between the α and β dispersions, or to the level of impedance magnitude prior to the α dispersion when a plateau region preceding the β dispersion cannot be defined.

Whereas the dependency on membrane potential (modeled based on a distribution of net charge on the inner side of the membrane) is clearly shown by permittivity spectra, impedance spectra reveal very tiny variations pertaining to α dispersion emphasizing the challenges to perform such measurements. 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 (< 1 mHz) posing significant experimental challenges (due to electrode polarization and specimen stability).

A slight increase of membrane conductivity is accompanied by an increase of the α dispersion as revealed by both the permittivity and impedance (magnitude and phase) spectra. Therefore, we stress on possible biosensing application of bioimpedance assays related to assessment of pore formation effect of various stimuli.

Analysis of the effects of diffusion and conductivity of the suspending medium on impedimetric (dielectric) behavior of suspensions of charged liposomes reveals that α and β dispersions superpose when assuming the parameters of a normal (physiologic like) medium. However, we show that an increase of medium viscosity (decrease of diffusion coefficient) provides a means to shift α dispersion towards lower frequencies enabling both dispersion to be distinctively appraised. Notably, the decrease of diffusion coefficient is normally accompanied by a decrease of the conductivity (providing an additional increase of impedance variation in respect to α dispersion). Nevertheless, when coping with biological cells, isotonic conditions have to be assured, therefore the limitations for lowering the conductivity of the suspending media beyond physiological values are evident.

Advances of the experimental set-up and appropriate choice of both the 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.

Acknowledgments

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