

Bioimpedance Measurements and the Electroporation Phenomenon

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Abstract:

Bioimpedance measurements are used to determine physiological aspects of biological tissues. On the other hand, the electroporation phenomenon causes a variation in the electrical properties of tissue, so it is possible use bioimpedance measurement with the aim of monitor the electroporation phenomenon in real time. The objective of this article is present the basic concepts required to understand bioimpedance measurements and the utility of these for detecting the electroporation effects.

1 Introduction

Bioimpedance measurement is an emerging tool in the field of biomedical engineering. It consists in studying the passive electrical properties of biological materials to indirectly determine certain physiological aspects. These measurements usually are employed as a method for monitoring physiological variations. This monitoring method presents three main advantages. First, it is a simple technique that can be applied with just two electrode setup. Also it requires low-cost instrumentation and is able to monitoring in real time.

The bioimpedance applications are continuously rising. Currently this kind of measurements are, for example, used in cell culture count applications, to estimate blood volume (plethysmography), to detect the breathing (pneumography), to detect ischemia (restriction in blood supply) in tissue or to determine the amount of fat present in the human body.

Recently it has been proposed that bioimpedance measurements can provide real time feedback on the outcome of the electroporation treatments. There is currently no alternative on-line system to easily determine the effects that electrical pulses cause in cells, so there, it exists a certain degree of uncertainty after applying electroporation techniques.

1.1 Impedance and electrical passive properties

The term impedance (Z) describes the opposition that one element offers to the circulation of alternating current. This value is represented as a complex number which expresses the relationship between the measured voltage (V) and the current flow (I).

The impedance measurements in a material depend on both material properties (conductivity σ and permittivity ϵ) and the geometry setup used during the measurements. The impedance of a material can be transformed into electrical properties of the material by applying a scale factor called cell constant (K) which reflects the dependence on the geometry used in the measurements.

For example suppose that, using flat parallel electrodes, is desired to determine the electrical properties of a piece of material with an area (A) and certain length (d) (figure 1).

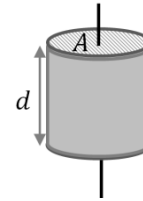


Figure 1 : Measurement cell example.

If the material is purely resistive, its impedance (Z_R) will be determined by the conductivity (σ) and the geometry.

$$Z_R = \frac{d}{\sigma A} \quad (1)$$

On the other hand, if the material is purely capacitive its impedance will be determined by the frequency (f) and the capacitance (C) (equation 2). Notice that capacitance value depends on relative permittivity (ϵ_r), the constant permittivity of the vacuum (ϵ_0) and the geometry (equation 3).

$$Z_C = \frac{-j}{2\pi f C} \quad (2)$$

$$C = \epsilon_r \epsilon_0 \frac{A}{d} \quad (3)$$

The geometry dependence of the impedance can be represented by the cell constant (K). For this specific example this value depends on area (A) and length of the material (d).

$$K = \frac{A}{d} \quad (4)$$

Using the equations presented before, it is possible to obtain a more general impedance expression, also valid for composed materials (equation 5).

$$Z = \frac{1}{K(\sigma + j2\pi f \epsilon_r \epsilon_0)} \quad (5)$$

In the field of impedance measurements, it is usual to work in terms of admittance (Y) corresponding to the inverse of impedance.

$$Y = K(\sigma + j2\pi f \epsilon_r \epsilon_0) \quad (6)$$

According to the previous formula, and knowing the cell constant value of the setup, the conductivity and the relative permittivity for each frequency can be obtained from the measured impedance, therefore electrical properties of the material can be determined.

2 Bioimpedance

2.1 Equivalent circuits and models

Biological materials are composed essentially of water and ions (electrolyte). This solution can be found inside the cells (intracellular media) and outside the cells (extracellular media). The most abundant species in the extracellular medium are sodium (Na^+) and chloride (Cl^-) and in the intracellular medium is potassium (K^+). Applying an electrical field these ions flow generating ionic currents. These ions are able to move quite freely in water, for frequencies between 100 Hz and few MHz, and it can be assumed a purely electrical resistive behavior both for intracellular and extracellular media. On the other hand, the cell membrane prevents the movement of the ions and, therefore, it behaves as an insulator between two conductive elements which is electrically equivalent to the behavior of a capacitance.

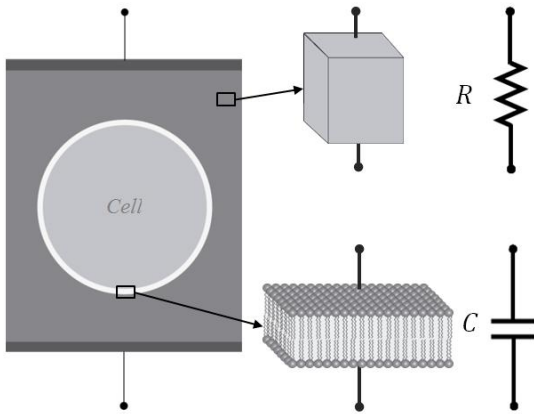


Figure 2 : Electric equivalences for a volume of electrolyte and a segment of cell membrane.

When discretizing a cell in suspension with these electrical elements, one can obtain an electronic circuit capable of predicting the electrical behavior of the cell. Circuit theory allows concentrating all the elements in a simplified circuit (figure 3). It consists of a resistance representing the behavior of the extracellular medium (R_{ext}) in parallel with the series combination of a capacitance (C_{mem}) and a resistance (R_{int}), representing the cell membrane and the intracellular medium respectively.

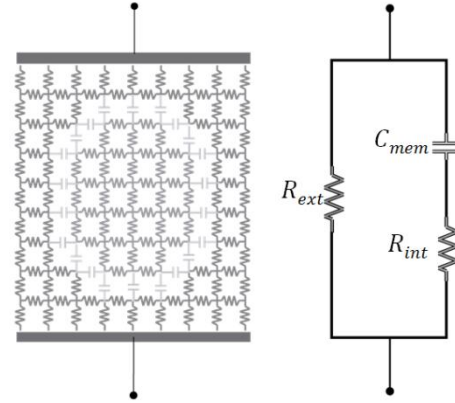


Figure 3 : Electrical model for cell as seen from the electrodes

Once the electrical model for a single cell is explained, it can be extracted a behavioral representation for a whole tissue by modeling it as an interconnection of multiple cellular models. Thereby it is obtained again an electrical circuit which can be simplified with the same structure as the cell model. There is again a representation for the extracellular medium (R_e), cell membrane (C_m) and intracellular environment (R_i).

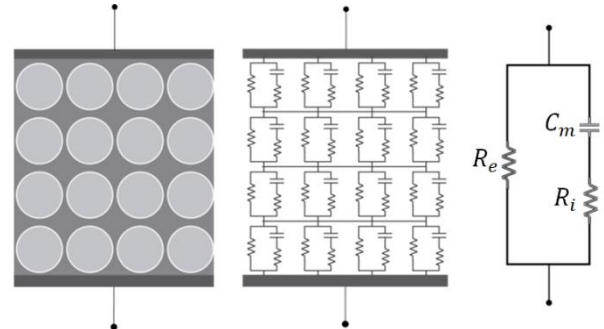


Figure 4 : Electrical model for the tissue as seen from the electrodes.

A feature of most biological tissues is that, due to the capacitive behavior of the cell membranes, the electrical impedance changes with the frequency. Observing the electrical model of the tissue (figure 4) at low frequencies it can be noticed no currents are able to pass through the membrane capacitance (C_m). They only circulate through R_e resistance. That is, the currents, unable to cross the cell membrane, are limited to circulate through the extracellular medium. On the other hand, when high-frequency currents are applied, the currents can easily pass through the cell membrane so they can circulate both through the extracellular and the intracellular media.

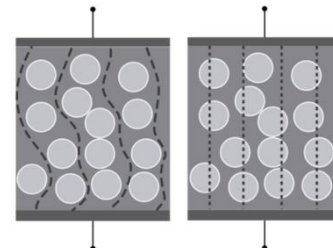


Figure 5 : (left) Current flow at low frequency in a tissue. (right) Current flow at high frequency in a tissue.

2.2 Bioimpedance representation

When impedance measurements at different frequencies are performed in biological tissue, those values can be graphically represented in different ways.

Through graphical representation of the impedance magnitude and its phase for each of the frequencies in logarithmic scale (figure 6).

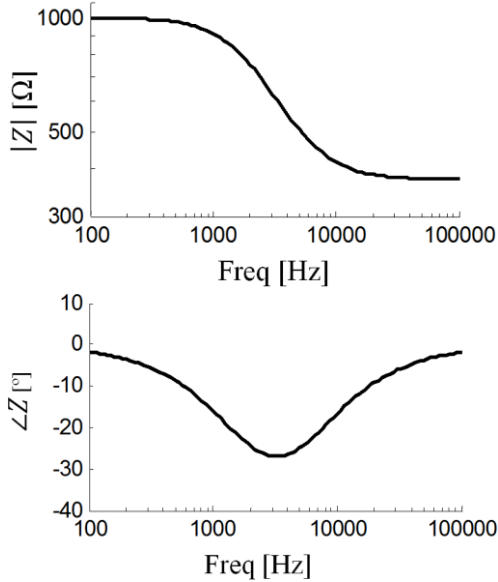


Figure 6: Impedance plot. (top) Impedance magnitude. (bottom) Phase value.

The same information can be displayed using a complex plane (Wessel diagram) where for each frequency the real part of the impedance (Resistance (R)) is plotted versus the imaginary part (Reactance (X)). The same plot type can be used to show the real part of the admittance (Conductance (G)) versus the imaginary part (Susceptance (B)).

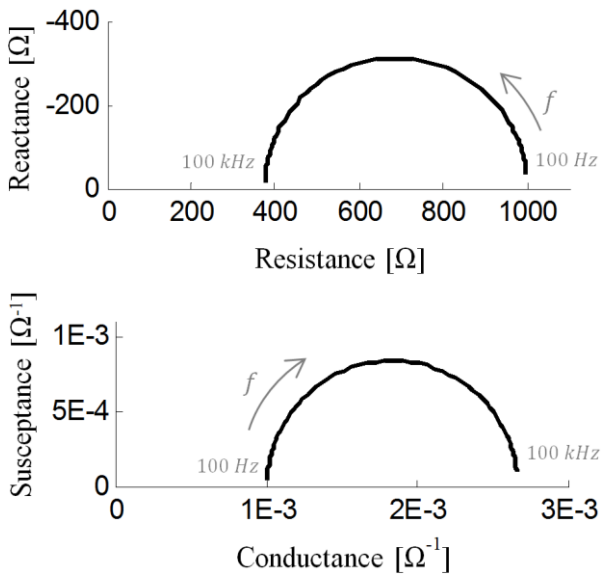


Figure 7: Wessel diagram. (top) Impedance. (bottom) Admittance.

Knowing the cell constant (K), the conductivity and the relative permittivity can be easily obtained from conductance and susceptance values.

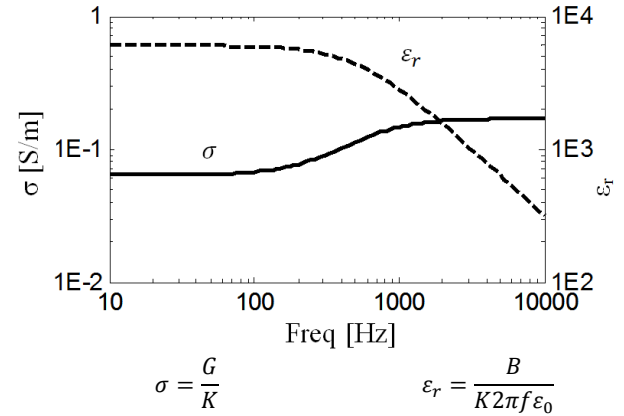


Figure 8 : Conductivity and relative permittivity plot.

2.3 Cole-Cole model

The basic electrical model presented before (figure 4), is useful to understand the electrical behavior of the tissue for frequencies between 100 Hz and few MHz. Nevertheless, this basic model based on simple RC model does not match perfectly to the experimental measurements. In the bioimpedance field, is commonly used a little more complex model, called Cole-Cole, to describe the experimental results (equation 7).

$$Z = R_{\infty} + \frac{R_0 - R_{\infty}}{1 + (j2\pi f\tau)^{\alpha}} \quad (7)$$

This model characterizes the tissue bioimpedance using four parameters, R_{∞} is the resistance at infinite frequency (R_e/R_i in the RC model), R_0 is the resistance at frequency 0 Hz (R_e), τ is the time constant ($\tau = C \cdot (R_0 - R_{\infty})$) and α is a value below 1 (typically around 0.8). If the α value is 1 the Cole-Cole describes the basic RC model.

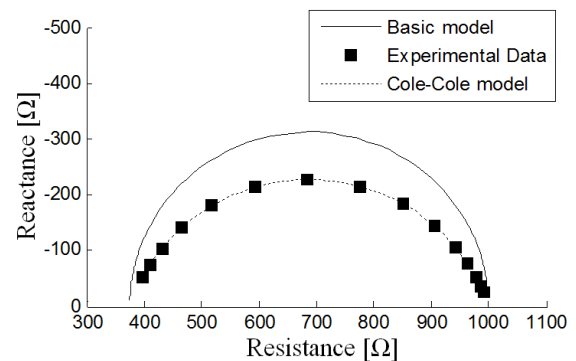


Figure 9 : Wessel diagram using Cole-Cole model.

3 Bioimpedance changes due to electroporation

Electroporation is the phenomenon in which cell membrane permeability to ions and molecules is increased by exposing the cell to short high electric

field pulses. This permeabilization of the cell membrane can be used in order to enhance the penetration of drugs, DNA molecules (gene transfection) or to destroy undesirable cells.

The phenomenon mechanism of this permeabilization still being not completely known but can be assumed that after very high electric field pulse (from 100 to 3000 V/cm) are applied, a structural change are present in the cell membrane, causing the appearance of pore shaped openings. The increased permeability of the cell membrane caused by electroporation allows the ions to pass through the membrane. This can be modeled as a resistance (R_{pore}) in parallel to the capacitance (C_m) described for a non-electroporated membrane.

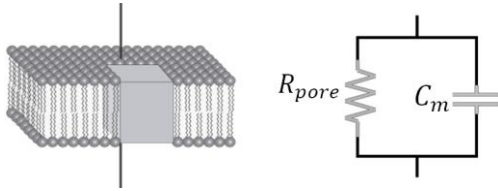


Figure 10 : (left) Representation of pore in cell membrane. (right) Electroporated membrane model.

By incorporating this new element, an electric model of the electroporated tissue can be implemented. At high frequency, the current can freely pass through the membrane, so that the electroporation effect is imperceptible. However, unlike the non-electroporated tissue, at low frequencies the current is able to flow through the intracellular medium via an alternative route generated in the membrane. That is the reason when a tissue is electroporated the impedance at low frequencies is significantly reduced while at high frequencies the value remains constant.

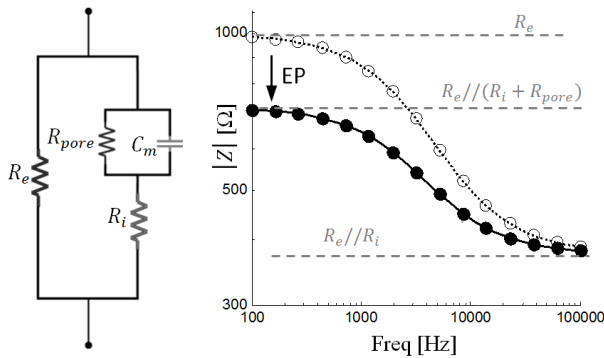


Figure 11 : (left) Model for electroporated tissue. (right) Impedance versus frequency for electroporated (●) and non electroporated tissue (○).

Considering the electric behavior of the electroporated tissue, the most sensitive frequency to detect this effect is at 0 Hz. However, for reasons showed in the following section, is not possible to measure bioimpedance at such low frequencies. In addition, larger measurement periods are required for low frequencies. To determine this low frequency impedance values is possible to, first, perform several impedance measurements at different higher frequencies (impedance spectroscopy) and use these

data to determine the Cole-Cole model parameters that best fit to the experimental measurements. Once all the parameters are extracted, is possible to determine bioimpedance at any frequency, even at 0 Hz. This approach can be used to monitor the effects of electroporation in biological tissues.

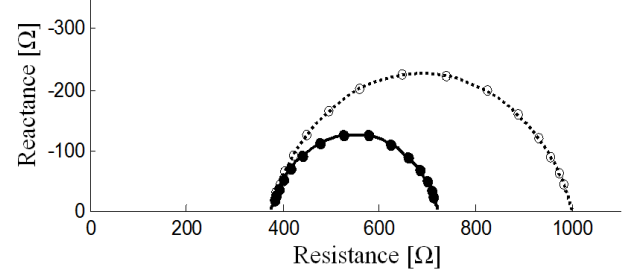


Figure 12 : Wessel diagram of impedance for electroporated (●) and non electroporated tissue (○).

4 Bioimpedance measurement systems

4.1 Electrode Model

By definition, the electrode is an electrical conductor to connect an electric circuit with a non-metallic element. Some metal electrodes, as copper, easily react with biological materials producing degradation of the electrodes and damaging the tissue. In bioimpedance measurements noble metals or stainless steel electrode materials are commonly used. The electrons cannot be directly exchanged between the electrode and the tissue. To start the direct electric conduction between the electrodes and the electrolyte, a minimum electric potential (approximately 1V) is required to produce oxidation and reduction chemical reactions. However, this is an undesired effect because such reactions would damage both electrodes and tissue. In addition, large currents could be dangerous, producing muscular stimulation and heating the tissue due to joule effect. It has to be noted that conductivity of ionic solutions shows a positive dependence on the temperature. Is estimated that the conductivity increases 2% per each °C, and therefore can affects the impedance measurements. For those reasons, low alternating currents (in the low mA range) are used in bioimpedance measurements.

The fact that no electron exchanges occurs in the electrode-electrolyte interface, any excess of charge in the electrode tends to be compensated by counter-charge in the electrolyte. This creates the so called electrical double layer that in principle can be modeled by a capacitance.

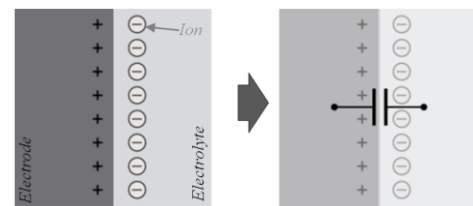


Figure 13 : (left) Electrode electrolyte interface. (right) Equivalent electric model.

This electronic equivalence is only true in cases with perfectly smooth electrode surface. At microscopic level any solid metal electrodes present some roughness producing a frequency dependence behavior. This interface impedance, called constant phase element (CPE), can be approximately modeled by equation 8. Observe that if $\beta=1$, the impedance equation is equivalent to that of a capacitance. However β value is around 0.8 that is why CPE is also known as a pseudo-capacitance.

$$Z_{CPE} = \frac{1}{(j2\pi f C_{CPE})^\beta} \quad (8)$$

This interface impedance disturbs the bioimpedance measurements, particularly at low frequencies, and must be kept as low as possible. Increasing the area or the roughness (effective area) of the electrodes, a large capacitance (C_{CPE}) could be obtained and therefore lower interface impedance.

4.2 Four-electrode method

Taking into account the impedance contribution of the electrode (Z_e), if a couple of electrodes are used both inject the current and to measure the voltage drop, the final measured impedance (Z_m) is the desired impedance measurement (Z_x) plus two times the impedance of the electrodes (Z_e) (figure 14). In certain circumstances where small electrode areas or frequencies below 10 kHz are used, the parasitic impedance is sufficiently large to disturb the measurements. Because of that, an alternative measuring method, called four-electrode or tetrapolar method, is commonly used in bioimpedance. It consists on injecting the current with a couple of electrodes and the resulting voltage drop is measured with another couple of electrodes. As current does not flow across the voltage measurement electrodes, it ideally cancels the influence of the electrode impedance in the final measure.

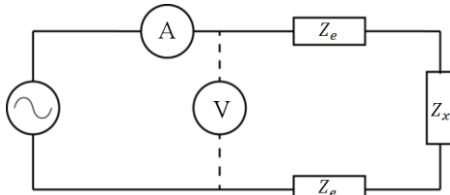


Figure 14 : Schematic concept for the two electrode method.

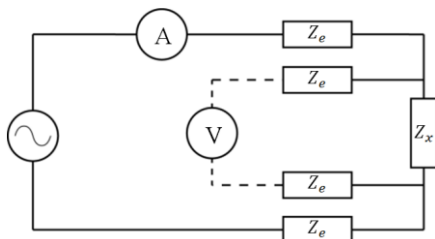


Figure 15 : Schematic concept for the four-electrodes method.

4.3 Instrumentation

One of the advantages of the bioimpedance monitoring is the relative low cost and simplicity of the electronic instrumentation. There are many methods to measure impedance. Figure 16 and figure 17 show two simplified circuits commonly used in bioimpedance measurements using two electrodes and four electrode measurement method respectively.

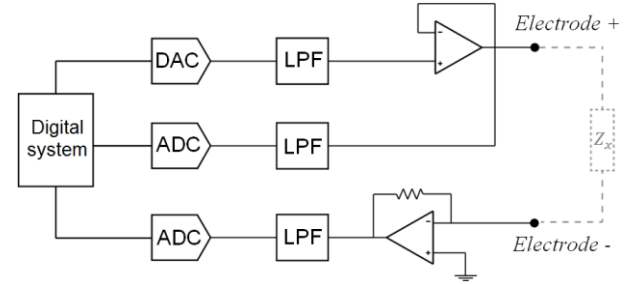


Figure 16 : Schematic representation for impedance analyzer circuit for 2 electrode measurements method.

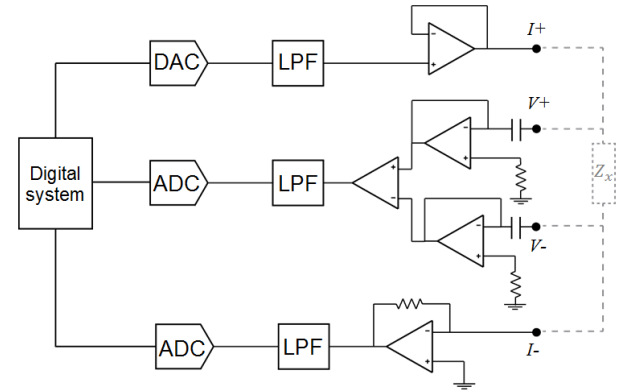


Figure 17 : Schematic representation for impedance analyzer circuit for a 4 electrode measurement method.

Recently, impedance measurement integrated circuits, specially designed for body composition determination in commercial weight scales have appeared in the market. This kind of integrated circuit, as AFE4300 from Texas Instruments or AD5933 from Analog Device, allows developing a bioimpedance measurement system with minimal implementation.

Bibliographie:

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- [2] B. Rubinsky, *Irreversible Electroporation*. Springer Verlag, 2009.