Electrodes – the challenge in electrical characterization of biological material

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Abstract Electrodes are an important part of the impedance measurement chain but their influence is often underestimated. Electrochemical reactions or polarization effects, especially for galvanically coupled systems are sometimes completely neglected. The most important features of electrodes to be considered are the geometry with respect to the structure of the material to be tested and the electrochemistry at the electrode surface. Especially drift and corrosion effects may yield misleading results. If applicable, sophisticated electrode systems should be used in order to prevent the distorting influence of electrode polarization, for extending the useful frequency range of the electrodes or for enhancing the signal-to-noise ratio.

1. Introduction
Bio-impedance measurements for electrical characterization of cell based material use a wide range of frequencies. The conductivity of suspension medium in the presence of low cell concentration is usually measured at low frequency below 1 kHz while the behaviors of cell membranes overwhelm the electrical properties between 10 kHz and 10 MHz [1]. At much higher frequencies (1 – 20 GHz), water molecules can be detected which allows a simple access to humidity or total water content of a material. Despite at frequencies above 50 kHz inductive or capacitive coupling between electronics and material works well and at microwave frequencies antenna structures are favored, galvanically coupled electrodes dominate in most bio-impedance measurement setups.

The design of such electrodes ranges from simple plates or needles up to sophisticated systems with separated current injection and voltage monitor electrodes. Moreover, additional guard electrodes and special shape of the measurement chamber optimizes the sensitivity of electrode system, usually for unique measurement tasks. Electrodes are often designed depending on the space available for the measurement system or for highest versatility of the setup. Finite element analysis is favored for prediction of the electric field distribution. However, it does not matter whether the material under test is simulated as well or not, near reality condition are achieved only to some extend owing to the complicate electrochemistry especially of metal electrodes.

Here we introduce some of the very important issues to be taken into account for designing electrodes and for interpretation of experimental results. Although the paper is not an original contribution, it should provide some thoughts especially for scientists starting in the field of bio-impedance measurement. The basic features of impedance measurement are assumed to be known.

2. Electrode geometry
The first thing to clarify is the electrode geometry. Does the entire object fit between the electrodes or does the electrode contact a small portion of the object? Is the object microscopically small (μm-size, single cell level) or is it a macroscopic object (cm-size)? In any case, the electrode geometry should be adjusted to the geometry and the structure of the material under test. In general, parallel plate electrodes are considered to be optimal, because of the homogeneous electric field between them. However, this is not true if an irregularly shaped object is placed between the electrodes. For instance, skeletal muscle or whole organs cause distortions of the electric field between the electrodes making the interpretation of the impedance measured between the clamps tricky.

Model based data processing is advantageous for retrieving material specific properties from the electrical measurement. A simple model is a cell suspension with round shaped cells. Pauly and Schwan derived equations for calculating the intra- and extracellular conductivity as well as the cell membrane capacity from the magnitude of the impedance below and above the β-dispersion and the characteristic frequency. Although this model is used by many scientists, most of them forget about the most prominent limitations: (1) the cells are round shaped and (2) there is no interaction between them. Sometimes this model is used for cells like yeast or blood cells, departing considerably from spherical shape. In other cases, suspensions with high cell concentration above p=0.2 (cell volume fraction) are treated by using this model. Even though this is basically wrong, the departure from reality may be acceptable for practical issues.

First, one should find out what is the most important property of the material to be accessed by impedance spectroscopy [2]. As an example, detecting low cell concentration in a suspension (<10⁶ cells/ml) works best in
the low frequency region (< 10 kHz) because metabolites change the conductivity of the suspension medium. For testing the cell viability, the polarization of the whole cell becomes important. Especially in microfluidics interdigital electrodes are favored. As seen in Fig.1A, the distance between electrodes should be big enough for a field penetration sufficient for reaching a significant number of cells.

![Diagram](image)

**Fig1.** The geometry of the electrode depends on the size of the material and the property to be measured. (A) interdigital electrodes (+ and -) with a distance (> 30 µm) for deep penetration of the electric field into the material for assessment of the polarization of cells and (B) setup to foster the measurement of the medium conductivity. The tight spacing of the electrodes (< 3 µm) greatly reduces the influence of cells on the impedance spectrum.

In opposite, when the conductivity of the suspension medium is the property of interest, an electrode geometry like in Fig.1B increases the significance of the measurement. The electrode geometry can greatly influence the measured results for structured materials. For instance, anisotropic materials like muscle differ in their impedance spectrum between the orientation along the muscle fibers and perpendicularly to it. Inhomogeneous materials exhibit different spectrum with respect to the measurement site. Large electrodes will be used for averaged property throughout the material while for assessment of the substructure, small electrodes either scanning electrodes or electrode arrays are required. Monopolar electrodes, i.e. usually small electrodes (µm-mm) with a large counter electrode (several cm²) in a distant location are used for the assessment of the impedance in the immediate vicinity of the electrode itself. They are not suitable for the measurement of the impedance of large volumes. The reason is a drastic increase of the field strength at the electrode with reduced electrode dimension. Since the impedance is the ratio of voltage and current, a high electric field strength means a high voltage drop over a fixed distance which yields a high impedance for a given current. This means that most information about electrical material properties is got from regions with the highest field strength, here the immediate vicinity of small electrodes.

Despite of some applications where simple calibration to a biological quantity is sufficient, impedance measurements should be comparable and quantitatively interpretable. A simple measurement assesses the impedance between the electrodes. Besides the resistivity, $\rho$, also the geometry of the electrode determines the impedance: $Z = \rho \frac{l}{A} = \rho k$ where $l$ is the distance between a pair of planar, parallel electrodes and $A$ the surface area. The quotient $l/A$ is usually lumped to a geometry factor $k$. Since for most electrode configurations other than parallel plate electrodes it is complicate or impossible to calculate distance and surface area, an experimental calibration procedure is required. Briefly, the electrode is immersed in a material with known resistivity (or conductivity, $\sigma = 1/\rho$) and the geometry factor is then calculated as $k = Z\sigma$ where $Z$ is the measured impedance.

### 3. Electrode polarization

A metal in contact with electrolyte causes electrochemical reactions. Positively charged metal ions enter the solution leaving the negatively charged electrons behind. This creates a force backwards, keeping the metal ions at the metal surface or even discharging them. Thus, a current exists in both directions, for charging ions (anodic oxidation) and for discharging them (cathodic reduction). Under equilibrium condition both currents are equal resulting in zero net current. This equilibrium will be distorted by an external electric field, yielding a measurable current depending on the electrolyte and the electrode material. It is described in a lumped quantity, the exchange current density. It can range over more than 10 orders of magnitude, making an electrode polarizable or not. For instance gold in physiological electrolyte at room temperature exhibits an exchange current density on the order of 0.1-2 mA / cm². A higher current density charges the electrode interface, yielding a layer with immobilized ions which acts as capacitor. The real current through the electrode is the Faraday
current. A diffuse layer, called Gouy-Chapman-layer, extends into the bulk of the electrolyte. Due to the rate limiting step of ionic transport by diffusion, its resistance increases with decreasing frequency. A typical locus diagram of a metal electrode is shown in Fig. 3. The line at the right side arises from the diffusive contribution of the Gouy-Chapman-layer (Warburg impedance) while the half circle at the left side is typical for a resistor (Faraday contribution + Warburg impedance) with a parallel capacity (capacity of the electric double layer).

![Figure 2](image1.png)

**Fig.2** (A) Locus diagram (imaginary part $Z''$ vs. real part $Z'$) of a typical metal electrode and (B) equivalent circuit ($R_c$ – resistor for Faraday contribution of electrode impedance, $C_{dl}$ – capacity of the electric double layer, $Z_W$ – Warburg impedance, $R_{bulk}$ – bulk resistance of the electrolyte)

Since the variability of the electrode impedance is considerably high, it becomes a serious source for measurement errors and misinterpretation [3]. Moreover, as seen in Fig.2, especially at low frequencies, its magnitude becomes very high (up to MΩ), often exceeding this of the material under test. Using the two electrode interface as in Fig.3A, means, that the measured impedance is $Z_{MUT}$ (impedance of the material under test) plus twice the electrode impedance $Z_{dl}$. In such case, the measured impedance mostly depends on the electrode behavior.

![Figure 3](image2.png)

**Fig.3** The principle of two (A) and four (B) electrode interface

The application of the current and measurement of voltage at separate electrodes will circumvent this problem (Fig.3B). The voltage drop at the polarized current electrodes (here outer electrodes) does not influence the measured voltage. Since the voltage monitor exhibits high impedance, no voltage drop owing to current flow through the monitor electrodes occurs. The use of monitor electrodes with equal electrochemical behavior keeps the potential difference between electrode and electrolyte equal. Therefore, it vanishes in the measured result due to the difference input of the voltage monitor [4].

![Figure 4](image3.png)

**Fig.4** useful frequency range of a comparable (A) two- and (B) four electrode interface. The diameter of the ring electrodes is 6 mm while the distance between two rings is 1 mm. Each ring is machined from stainless steel with a thickness of 1 mm (inner rings) or 2 mm (outer rings).

Often electrodes are designed without testing the frequency range of reliable measurement. A simple test for electrode configuration is the measurement of the geometry factor as function of frequency at different concentrations of the test electrolyte. The most prominent test electrolyte is KCl, a 1:1 electrolyte with almost
equal size and mobility of the ionic species K⁺ and Cl⁻. Fig. 4 gives an example for very similar electrode systems but one with two electrodes (A), the other one using a four electrode interface (B). As seen in Fig. 4B, a four electrode interface extends the useful frequency range to low frequencies by several frequency decades. Therefore, above 5 MHz a increase in k was found for the four electrode interface due to parasitic and stray capacitances. This suggests a preferred use of four electrode systems for precise measurements in the low frequency region (<10 kHz) while two electrode systems are advantageous measurements at higher frequency.

4. Non polarizable electrodes
Electrode polarization happens due to the limited exchange current density. Reversible electrodes are non-polarizable due to the electron transfer by electrochemical reaction. Typically, a metal is in equilibrium with is hardly soluble salt like silver and silver chloride. Another well-known system is Hg/HgCl (calomel). Due to the constant or determined activity of Cl⁻ ions in biological systems, Ag/AgCl – electrodes predominate non polarizable electrodes in life science. The electrochemical reaction for Ag/AgCl-electrodes is: AgCl ⇄ Ag⁺ + Cl⁻. At the anode, silver reacts with the chloride to AgCl depositing at the electrode surface while at the cathodic side AgCl is dissociated and metallic silver is deposit at the electrode. The potential difference of the Ag/AgCl-electrode and electrolyte depends only on temperature and the chloride activity and is for physiological condition on the order of -222 mV. In order to keep this potential constant, which is especially important for reference electrodes, chloridized silver wires are bathed by 3 M KCl in a separate compartment which has an ionic connection via glass frit to the material under test.

The key feature of non-polarizable electrodes is the high exchange current density. Besides redox electrodes, iridium sputtered titanium electrodes are used as non-polarizable or at least hardly polarizable electrodes.

5. Electrode noise
A critical property of electrodes is the generation of thermal noise. It is negligible for large electrodes but increases dramatically when electrodes became very small. The noise voltage within a frequency band of Δf is given by $u_n = \sqrt{4k_BT\Delta f R_{\text{electrode}}}$, $T$ is the temperature and $k_B$ the Boltzmann-constant. Since the real part of the electrode impedance (Fig.2), $R_{\text{electrode}} \propto 1/A_{\text{electrode}}$, small electrodes and especially point electrodes as often used in cell physiology exhibit a high noise level. It is even more important for electrical measurements using miniaturized electrodes since the signal level decreases with decreasing electrode surface, yielding a bad signal-to-noise ratio.

7. Conclusion
When constructing electrodes, geometry and material should be chosen with respect to the geometry of the material under test and the expected electrochemical reactions. This involves consideration with respect to aging of electrode materials as well. The simulation of electrode behavior can be only guidance. Electrodes should be tested for reliability and useful frequency range with electrolytic solutions of known conductivity. Critical problems for impedance measurements are the distinction between electrode behavior and properties of the material under test. Four electrode interfaces can avoid distortion or the measurement in the low frequency range. Electrode aging and biofouling are important issues for long lasting measurements. This raises the question for online cleaning and conditioning of electrodes.

Since electrodes are always sources of distortions while measuring impedance at biological material, both, construction and material should keep these distortions at a minimum.

8. References
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