

# Considerations on electrical impedance measurements of electrolyte solutions in a four-electrode cell

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**Abstract.** A tetrapolar probe to measure the electrical properties of electrolyte solutions was implemented with gold electrodes according to the van der Pauw method. Electrical impedance spectroscopy (EIS) measurements of different concentrations of phosphate buffer saline (PBS) solution and an oral mucosal tissue sample dispersed in PBS were performed in the galvanostatic mode using a four-electrode cell (tetrapolar probe). Taking advantage of using a potentiostat/galvanostat for carrying out the electrical measurements, a simple and rapid method using a three-electrode electrochemical cell is described for: a) cleaning of electrodes, b) verification of surface chemical state of electrode material and c) choice of current supplied to electrodes for EIS measurements. Results of this research shown a depolarization effect due to the addition of oral mucosa tissue cells into the PBS solution.

## 1. Introduction

Electrical impedance spectroscopy (EIS) is a powerful method to study electrical properties of electrolytic solutions and biological tissues. Electrical properties of biological materials can be studied by two-, three- and four-electrode cells [1]. Conductivity measurements with two and three electrodes are used, however they depend on the accuracy of standards [2,3], and also they exhibit electrode polarization impedance effects. Four-electrode arrays minimize the influence of electrode polarization at low frequencies [3]. A four-electrode cell based on van der Pauw method, originally used to measure the electrical resistivity of semiconductor materials, allows obtaining an averaged reading of the impedance measurements of electrolyte solutions [4]. Furthermore, EIS is a non-invasive characterization technique that can be used to study the frequency response of the electrical impedance of biological tissues [5,6].

The electrical measurements for determining van der Pauw DC resistivity in a four-electrode cell setup require a current source and a voltmeter or electrometer. In AC measurements an impedance analyser is required. Nowadays, a growing number of laboratories possess a potentiostat/galvanostat, which is an instrument designed for a wide range of electrochemical applications: cleaning of metallic surfaces, characterization and evaluation of materials or liquid solutions, as well as determination of their thermodynamic, kinetic, electronic or electrical properties. An impedance analyser is frequently integrated to potentiostat/galvanostat, thereby expanding the possibilities of this instrument through measurement. Taking advantage of aforementioned capabilities, herein is proposed a methodology for prepare the measuring electrodes and how to set the measurement conditions.



## 2. Experimental

### 2.1. Preparation of solutions and tissue sample

Both solutions, piranha  $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$  (7:3) and 0.5M  $\text{H}_2\text{SO}_4$ , were prepared for chemical and electrochemical cleaning of gold electrodes. Electrolyte solution of phosphate buffered saline (PBS) was prepared with a final concentration of 0.680M NaCl, 0.0134M KCl, and 0.0405  $\text{Na}_2\text{HPO}_4$  adjusted at pH 7.4 with HCl (hereinafter referred to as “1 time solution”), and it was diluted to the appropriate concentration. All solutions were prepared in deionized water from a Milli-Q ultrapure water system with a resistance of 18.2M $\Omega$ . All chemical reagents used were analytical grade. Mucosa tissue cell samples were collected by rolling the cotton swab firmly on the inside of the cheek. The swab was then placed in a 100 $\mu\text{L}$  volume of 1 time PBS solution and the sample was then dislodged by shaking. The final volume of the aliquot was 500 $\mu\text{L}$  by adding 1 time PBS solution.

### 2.2. Elaboration of four-electrode cell

Four Au wires (18 kilates, 1mm of diameter and 15mm in long) were welded each one in an angle of 90° to a silver wire with the same dimensions (Figure 1). Electrodes were chemically cleaned by dipping into a piranha solution for 5 minutes, followed by rinsing with copious amounts of deionized water. Each electrode was electrochemically cleaned by cyclic voltammetry (see below). After that, electrodes were rinsed with deionized water. The cell was elaborated in Teflon as is shown in Figure 1.



**Figure 1.** Gold electrode and cylindrical four-electrode cell. Front view (top) and side view (bottom) of the cell.

### 2.3. Electrochemical and electrical measurements

Cyclic voltammetry measurements were performed in a conventional three-electrode cell at room temperature. Dissolved oxygen was removed from all solutions by bubbling nitrogen for 15min. The working electrode was each one of prepared gold electrodes. The counter electrode was a high purity graphite rod (99.9995%, 5mm in diameter) and an electrode Ag/AgCl/3.0M KCl placed in a Luggin capillary was used as reference. The galvanostatic mode (current constant) impedance measurements were conducted in the four-electrode cell shown in Figure 1, the way to connect electrodes to potentiostat is indicated below. EIS measurements were carried out at  $1 \times 10^{-7}$  A between 10Hz and 0.1MHz. A volume of 500 $\mu\text{L}$  of each solution was used for the PBS impedance measurements. All electrochemical and electrical measurements were performed with a potentiostat/galvanostat Autolab PGSTAT302N and NOVA 1.10.4 software.

## 3. Results and discussion

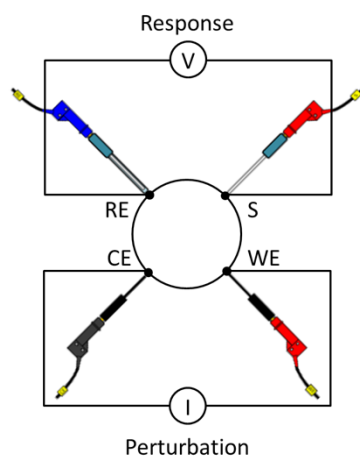
In spite of chemical cleaning of gold electrodes with piranha solution, cyclic voltammetry measurements showed a different electrochemical behaviour among electrodes. Indeed, different electrochemical reactivity evidence differences at the surface of each electrode, which could lead to thermoelectric effect during the impedance measurements. In order to avoid possible interference, oxidation-reduction cycling in 0.5M  $\text{H}_2\text{SO}_4$  was done for each electrode by scanning the potential between -0.1 and 1.6V at

a scan rate of  $0.1\text{Vs}^{-1}$ , the cycling was stopped until to obtain a reproducible and characteristic electrochemical behaviour (data not shown). According to anodic behaviour of gold in PBS solutions, it can be oxidized if the current supplied is large enough, hence, current must not exceeded about  $1 \times 10^{-7}\text{A}$  to ensure that impedance measurements are performed under linear conditions.

Impedance measurements with a set of four electrodes can minimize the errors resulting from polarization effects [2]. However, it is important to properly connect the terminals of the potentiostat to each of the four electrodes of the cell. Improper electrode connection gives incorrect results. In a four-electrode configuration, current is delivered through two drive electrodes and voltage is measured via two sensing electrode. When a potentiostat/galvanostat is used, current should be supplied through working and counter electrodes, and thereby voltage is measured at reference electrode and working electrode sense, as illustrated in Figure 2. Thus, the conductivity of electrolyte solution is obtained by measuring the impedance and the geometry of the cell, resistivity values were calculated from Equation (1) [7].

$$\rho(\omega) = Z(\omega) \pi h / \ln 2 \quad (1)$$

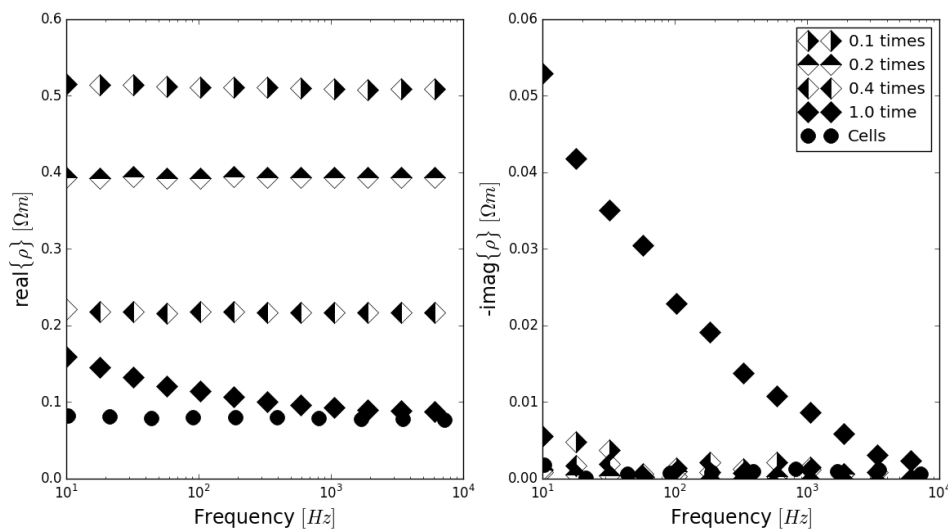
Where  $\rho(\omega)$  is the electrical resistivity ( $\omega$  is the frequency),  $Z(\omega)$  is the impedance of the measured electrolyte solution, and  $h$  is the height of the liquid filling the cylindrical cell, to be 10mm.



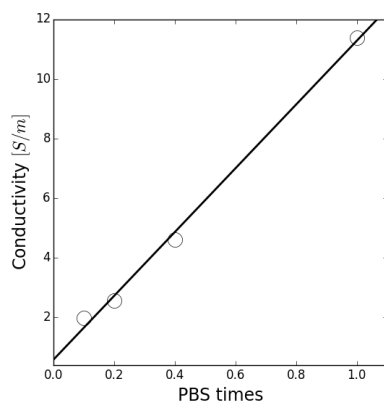
**Figure 2.** Schematic view of a tetrapolar setup (van der Pauw geometry) for resistivity measurements of electrolyte solutions whether a potentiostat/galvanostat is used. RE, reference electrode; S, working sense; CE, counter electrode; WE, working electrode. Bare Au wires are used as electrodes (see Figure 1).

Figure 3 shows the resistivity values for 1.0, 0.4, 0.2 and 0.1 times PBS over the frequency range of 10 to 105Hz. All curves show a consistent decrease in resistivity with the increase in PBS concentration. With the exception of 1 time PBS solution, the resistivity/impedance of the others solutions is constant over the entire range of frequencies measured. In the case of 1 time PBS solution, the increase of resistivity at low-frequency values could be due to the polarization effect, which occurs when there is an accumulation of ionic charge at the interface between the electrodes and electrolyte solution [8]. The calculated standard deviation over the frequency range where resistivity is approximately constant is lower than 1.03%. In spite of polarization effect at low frequencies, the high frequency data show that the conductivity (it is the reciprocal of resistivity) of PBS solutions varies linearly with concentration as would be expected for a stock solution and its successive dilution (Figure 4).

EIS measurements of an oral mucosal tissue sample dispersed in 1 time PBS solution was performed. The magnitude of the impedance obtained for a volume of  $500\mu\text{L}$  of oral tissue-PBS mixture is greater than oral tissue-free PBS solution (Figure 3). Indeed, oral mucosal tissue cells are not a simple electrolyte and ion-exchange process are needed to stabilize their membranes [8]. Surprisingly, addition of oral mucosal tissue sample to PBS depolarizes the electrodes, and hence the impedance measurement becomes constant at low frequencies. The impedance of the system gives insight into the adhesive behaviour of the cells on the electrodes.



**Figure 3.** Electrical resistivity,  $\rho$ , measurements for PBS solutions of different concentrations and mucosal tissue cell sample dispersed in 1 time PBS solution.



**Figure 4.** Electrical conductivity of PBS solutions at different concentrations. PBS times equal to 1.0 mean the PBS without dilutions and e.g. 0.2 means five dilutions of PBS.

#### 4. Conclusions

Potentiostat/galvanostat offers a host of features and benefits to establish appropriate conditions for carried out conductivity measurements in a four-electrode cell. EIS measurements can provide useful information about resistivity and conductivity of electrolytic solutions; furthermore it can apply to study ions moving through the cell membranes.

#### Acknowledgements

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