An equivalent electric circuit as a tool for monitoring micron sized-particles in suspension from electrical impedance measurements

A de León Hernández¹, C Sánchez Pérez¹ and R G Ramírez Chavarría²

¹Instituto de Ciencias Aplicadas y Tecnología, Universidad Nacional Autónoma de México, AP 10-186, 04510, CD MX, México
²Instituto de Ingeniería, Universidad Nacional Autónoma de México, 04510, CD MX, México

E-mail: alma.deleon@icat.unam.mx

Abstract. In this work, a microfluidic device with a three-electrode system is used to measure the electrical impedance spectrum of micron-sized particles suspended in Phosphate Buffered Saline (PBS). We use polymer particles of 15 μm and 48 μm of diameter, as they can mimic biological cells in suspension. The main purpose is to study the influence of the particle concentration on impedance parameters and to establish the basis for biological cell suspensions assays. An electric equivalent circuit model is used to describe the experimental impedance data. Results show a high dependence of electrical parameters with the mass fraction and particle size. The model considers the resistance of the bulk solution and the electrical double layer, as the two essential electrical elements. Results show a maximum error of 3% by fitting the measurements to the proposed model. This proposal could be of interest for cytotoxicity assays.

1. Introduction

Electrochemical techniques (ET) are powerful tools for biological cell assays. Some applications that use ET for in vitro studies are cell proliferation [1-2], viability [3-5], morphology [6-7], and cytotoxicity monitoring [8-10], to mention only a few. Usually, monitoring the dynamic changes induced by cell–toxicant interactions, involves the cells to be attached at the electrode surface and time-domain response assays. This type of study is time-consuming because the procedure for sample preparation must be carried out under strict protocols to avoid cross-contamination. Likewise, the effect of a drug in the cell culture is monitored by the detachment of the cells from the surface of the electrode. However, an alternative approach is the usage of ET to characterize the cells suspended in a culture medium or buffer [11], instead of growing them on the electrodes, thus providing many advantages such as faster monitoring and results in almost real-time.

The most common method to analyze EIS data is the equivalent electrical circuit model (EECM). This method pretends to describe the spectral impedance measurements through electrical elements, which in turn allow us to study the composition and structure of the media under study. The EECM is also used to relate the impedance response with the conductive and dielectric nature of an electrochemical

¹ alma.deleon@icat.unam.mx
A usual model for describing the processes at the electrochemical interface is the so-called Randles circuit. The elements of this circuit are the resistance of the medium, the double-layer capacitance, and a faradaic reaction [12].

Particularly, both electrolyte suspensions of biological cells and charged particles, exhibit a similar electrical behavior when subjected to an alternating electric field. In this study, a three-electrode EIS device is used to monitor the electrical properties of Poly (methyl methacrylate) (PMMA) particles suspensions, which are used as phantoms of biological cells samples.

We perform experiments using PMMA particles with a diameter of 15 and 48 micrometers, as these dimensions are in the range that cancer cell lines, such as MDA-MB-231, adopt when they are suspended.

EECM is proposed for PMMA suspensions using the basis of the Randles circuit. We propose the use of electrical impedance spectroscopy (EIS) alongside an EECM as a label-free method for monitoring cell death in a cell suspension. As a result, an alternative method for cytotoxicity assays is presented, which provides several advantages as fast-time response, high resolution, and simplicity.

2. Theoretical Background

Complex impedance is defined as the ratio of an oscillating electric current $I(\omega)$ applied to a sample and the drop voltage measured $V(\omega)$:

$$Z(\omega) = \frac{V(\omega)}{I(\omega)}$$

The electrical double layer (EDL) phenomenon occurs when an electrolytic solution interacts with charged particles creating a distribution of ions in the surrounding interfacial region, and also is present at the interface between a metallic electrode and the electrolyte when an electric potential is applied. The electrochemical phenomenon of the EDL is governed by the Gouy-Chapman-Stern model [11]. In this model, the EDL has two parts, an inner region (Stern layer) where the ions are strongly bounded and an outer region (diffuse layer) where they are less firmly associated [13].

EIS is a technique that can be used to characterize the polarization of charged particles and the gradient of ionic concentration around the particle surface. The EECM shown in Figure 1, is proposed to describe EDL and monitor particle size and concentration based on impedance measurements.

![Figure 1. The equivalent circuit used to analyze the EIS data of mimic biological cell suspensions.](image)

In the EECM shown in Figure 1, $R_s$ represents the solution resistance governed by the conductivity of the bulk solution, $C_d$ represents the dielectric property of the bulk solution, and finally $R_d$ and $C_{dl}$ represents the resistance and the dielectric property of the EDL in electrode/electrolyte interface and around the particles. The element $C_{dl}$ is modeled as a Constant Phase Element (CPE), which impedance is

$$Z_{CPE} = \frac{1}{Q(\omega)^n}$$

where, the exponent is $0 \leq n \leq 1$, $Q[Fs^{n-1}]$ is the CPE constant, $i = \sqrt{-1}$ is the imaginary unit and $\omega$ is the angular frequency. The case $n=1$ describes an ideal capacitor, while the case $n=0$ describes a pure resistor [13]. The equivalent impedance of the circuit shown in Figure 1 is given by:
\[ Z_{eq} = R_s + \left(\frac{1}{1 - \frac{1}{C_d \omega (R_d + \frac{1}{Q(i\omega)} \omega^n)}}\right) \]  

(3)

3. Materials and methods
The liquid–particle mixture for measurement contained PMMA particles and PBS solution. The suspensions were obtained by diluting a first sample with a mass fraction \((M_f)\) of 0.01, resulting in 5 samples with different mass fraction (0.0006, 0.00125, 0.0025, 0.005, and 0.01). Two sets of five samples were elaborated, one set with PMMA particles of 15 μm of diameter and a second set with particles of 48 μm of diameter. The suspensions were stabilized with one percent of sodium dodecyl sulfate to avoid agglomeration of the particles. Before measurements, the samples were in an ultrasonic bath for five minutes. Experiments were carried out using a microfluidic device composed by a PDMS chamber with a three-electrode sensor (DropSens™ DRP-G-MEAB555), the chamber was filled with 50 μL of sample and impedance spectrum was obtained. Sample injection and draw were performed with a syringe pump. We cleaned the chamber by pumping 200 μL of PBS at the end of each measurement.

The EIS measurement was carried using a homemade potentiostat [14], which applies an alternating excitation voltage (0.1 V) and measure the resultant current within the frequency range from 1 kHz to 1 MHz.

4. Experimental results and discussion
The measured impedance spectra for the samples are represented by the Nyquist diagrams in Figure 2 and Figure 3 (symbols). The region with a straight-line behavior corresponds to the low frequencies and is associated with the electrode-electrolyte interface due to the polarization of the electrodes. The semicircle at high frequencies describes the effects of the ionic distribution around the particles [17]. The fitting results of the impedance measurements and the model of equation (3) are shown in Figure 2 and Figure 3 (solid line).

We fitted the experimental data of the ten samples to the EECM of equation (3) by using the complex non-linear least-squares (CNLSS) method and the Levenberg-Marquardt optimization algorithm. As a result, the maximum fitting error was 3% for the ten samples. According to this, our EECM-based tool shows a twofold advantage, it has physically meaningful about the structure of the suspensions, and it is a good candidate for monitoring the concentration of the micron-size PMMA particles.
4.1. Analysis of the parameters in the equivalent circuit

From Table 1, one can observe that the four electrical components for the EECM (see Figure 1) change with the concentration of PMMA particles. The resistance of the bulk solution and the EDL decreases as particle concentration increases, and the capacitance of the solution and the EDL increases when the concentration increases.

Table 1. Fitting results for 15μm and 48μm PMMA particle suspensions using the proposed EECM.

| Mf  | PBS  | 0.0006 | 0.00125 | 0.0025 | 0.005 | 0.01 |
|-----|------|--------|---------|--------|-------|------|
| Ø [μm] | Rs [Ω] | Rd [Ω] | Cq [nF] | Cd [nF] | Q [10^-6] | Cdl [F/s] | n |
| 15 | 108.6 | 92.4 | 6.7 | 3.9 | 0.6 |
| 48 | 107.7 | 90.0 | 8.7 | 5.3 | 0.6 |
| 15 | 266.3 | 234.6 | 6.2 | 4.4 | 0.6 |
| 48 | 241.6 | 222.8 | 9.4 | 5.5 | 0.6 |

Figure 4 shows the change in solution and EDL resistance as a function of concentration for each particle size. For 15 μm particles, Rs has a linear dependence with mass fraction, whereas Rd has an exponential dependence. For 48 μm particles, both Rs and Rd have exponential dependence with mass fraction. In both cases, Rs and Rd decrease as there are more particles in suspension. Since the polarization of the electric double layer is accompanied by a gradient of ionic concentration around the particle surface, as there are more particles, the ionic distribution involves a decrease in bulk solution resistance. From the fitted model, it seems that particle size has a minor influence on low mass fraction suspensions, which means that the total particle surface produces a minor effect on the resistance; conversely, as concentration increases, the particle size has a strong influence in EDL.

Figure 5 shows the results for the EDL capacitance. For both size particles, EDL capacitance increases as the mass fraction increases, nevertheless for 48 μm particles, Q increases at a higher rate, this means that for a larger total particle surface area there is a larger accumulative effect of EDL capacitance. Since the proposed EECM for fitting the impedance spectra has a simple electrical elements configuration, the CFE combines several effects from the EDL capacitance. We can imply from the results that meanwhile there are more particles, the capacitive properties increase.
Figure 5. CPE constant as a function of mass fraction for PMMA particle suspensions with diameters of 15μm and 48μm.

For cytotoxicity assays, the most representative parameter seems to be Q. This constant could monitor the ionic distribution and polarization phenomenon caused for change in the structure of the membrane cell.

5. Conclusions
In this work, we show the usefulness of a microfluidic device composed of a PDMS chamber with a three-electrode sensor, in which, electrical impedance measurements were performed in small volumes (50μL) of micro-size PMMA particles in PBS. It was found that, by analysing the electrical impedance spectra using the equivalent electrical circuit technique, it is possible to characterize electrolytic suspensions of PMMA particles with a different mass fraction. Our findings conducted to determine that, when there are more particles, the ionic distribution involves a decrease in bulk solution resistance and the capacitive properties increases. These results point-out that through our device, it could be possible to monitor changes in concentration, establishing the bases to apply it to detect changes in the population of cells in suspension with application in toxicity assays, without the need to seed the culture on the substrate of the electrode.

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