Correlation between the Concentration of DNA and Electrical Bioimpedance.

Angélica Hernández¹, Gerardo Ames², Virginia Sánchez³, Modesto Gómez⁴, Jennifer Sánchez⁴, Nadia M Pérez³, Itsi A Castillo⁵ and César A González⁶.

¹Universidad Politécnica de Gómez Palacio Gomez Palacio, Durango, Mexico.
Carretera El Vergel-La Torreña Km. 820, El Vergel 35120 Gómez Palacio, Dgo
²Universidad Autónoma Metropolitana-CI3M, Iztapalapa, CDMX, México.
Av. San Rafael Atlixco 186, Vicentina, Iztapalapa, 09340 Ciudad de México, CDMX.
³Instituto Politécnico Nacional-Escuela Superior de Medicina, CDMX, México.
Salvador Díaz Mirón esq. Plan de San Luis S/N, Miguel Hidalgo, Casco de Santo Tomas, 11340 Ciudad de México, CDMX
⁴Instituto Politécnico Nacional-CICS-UST, CDMX, México.
Av. de los Maestros, Santo Tomás, Miguel Hidalgo, 11340 Ciudad de México, CDMX
⁵Instituto Tecnológico de Hermosillo, Hermosillo, Sonora, México.
Av. Tecnológico 115, Sahuaro, 83170 Hermosillo, Son.

*Corresponding author: cgonzalezd@ipn.mx

Abstract. Biosensors have recently been used for the identification and genetic characterization of diseases such as Salmonella, human papilloma, and breast cancer. The aim of this study was to examine the relationship between different concentrations of Deoxyribonucleic Acid (DNA) and the corresponding values of magnitude and phase of Electrical Bioimpedance (EBI). Functions that characterize the correlation of DNA with these two parameters were derived from linear regression by means of the least squares method. According to the results, it is feasible to determine the concentration of DNA from EBI measurements (at a specific frequency) of magnitude and phase, taking into account the corresponding linear functions. The present findings indicate the feasibility of using EBI measurements of magnitude and phase as a biosensor for the detection of genes associated with genetic and infectious diseases.

1. Introduction
The latest developments in relation to sensitive and specific biosensors has created growing awareness of the possibility of applying this technology to the identification of nucleic acid. The goal is to detect genetic and infectious diseases in a rapid, simple and inexpensive manner. Deoxyribonucleic acid (DNA) biosensors and gene chips have attracted much attention because of their potential for obtaining specific DNA sequence information [1].

In recent studies, biosensors have been used for the identification and genetic characterization of diseases such as Salmonella, human papilloma, and breast cancer [2-3]. The advantage of biosensors of electrical bioimpedance (EBI) for the detection of disease is their size, speed, and capacity for label-free operation [4].

In the last two decades, many scientists in the area of chemistry and physics have come to the conclusion that DNA could function as a carrier of electronegative charge, which would give it electrical
properties linked to conductivity [5]. Moreover, such properties may be a function of the length and amount of DNA.

In recent reports, our group has proposed that induced current at a frequency in the lower part of the gamma dispersion band promotes the dipole effect of water molecules and interacts with the electronegative charge of DNA molecules. This interaction is a function of the concentration of DNA and correlates with changes in S21 transfer parameters (input-output relationship of transferred energy) associated with the volumetric electrical properties of DNA analytes [6,7]. The aim of the current contribution was to examine the correlation between the concentration of DNA and EBI parameters (magnitude and phase) as a first step in determining the technical feasibility of developing a DNA biosensor device based on EBI measurements.

2. Methods

2.1. Experimental design

Total DNA extracted from bacteria was prepared in a typical Polymerase Chain Reaction (PCR) master mix. Based on the initial DNA concentration of the sample, three other concentrations were prepared. These four samples as well as the negative control without DNA (all at a final volume of 25 μl) were subjected to EBI measurements with a wide bandwidth. For each frequency, an analysis was carried out of the possible correlation between the DNA concentration and the values for magnitude (Z) and phase of EBI.

2.2. DNA extraction and quantification

DNA was extracted from Entamoeba histolytica trophozoites and cultured axenically, then isolated by means of an organic method with a phenol-chloroform-isooamyl alcohol mixture (25:24:1) according to the manufacturer’s protocol (Invitrogen, CA, USA). The concentration of DNA was evaluated by fluorometry (Qubit 3.0, Invitrogen, CA, USA). The initial DNA concentration was 40 ng/μl, and the following serial dilutions were prepared: 1:2 (20 ng/μl), 1:5 (8 ng/μl), and 1:10 (4 ng/μl).

2.3. Preparation of the Polymerase Chain Reaction (PCR)

Five microliters of the initial DNA solution and of each dilution (1:2, 1:5, and 1:10) were added to 20 μl of PCR mix, which contained 12.5 μl of PCR master mix SYBR green (Applied Biosystems, CA, USA), 2.5 μl of each primer used in conventional PCR for E. histolytica [8], and 2.5 μl of water to give a final DNA concentration of 8 ng/μl, 4 ng/μl, 1.6 ng/μl 0.8 ng/μl. The negative control consisted of five microliters of water added to 20 μl of the PCR mix. The DNA amplification procedure was not performed because the samples in the PCR master mix solution were intended to emulate real conditions in order to assess the effects of different DNA concentrations.

2.4. Bioimpedance measurements and analysis

Typical 0.2 ml PCR tubes (Applied Biosystems, CA, USA) served as containers for the samples, being adapted with two silver needles as electrodes. EBI was measured on a ScioSpect ISX-3 system (ScioSpec Scientific Instruments Inc., Leipzig, Germany) in bipolar configuration, at the frequency range of 100Hz to 10 MHz in 128 steps logarithmically spaced, and with a potential of 250mV. The experimental setup for determining bioimpedance is shown in Figure 1. For every frequency explored, the linear correlation of the EBI parameters (Z and phase) to the concentration of DNA was analysed on the SPSS statistical software (IBM Corp., Armonk, NY, USA). Statistical significance was set at p≤0.01.

2.5. Linear regression analysis

The functions that characterize the correlation of the two EBI parameters with different concentrations of DNA were found by means of linear regression. At the frequency with the most significant EBI-DNA correlation, the EBI values for Z and phase were considered independently to establish the corresponding linear equations by using the least squares method described by Espinosa et al. (2016) [9].
Where:

\[ y = a + bx \]  \hspace{1cm} (1)

\[ a = \frac{\Sigma y - b\Sigma x}{n} \]  \hspace{1cm} (2)

\[ b = \frac{n\Sigma xy - \Sigma x\Sigma y}{n\Sigma x^2 - (\Sigma x)^2} \]  \hspace{1cm} (3)

\( n \) = number of data values.

Figure 1. The experimental setup for measuring EBI in DNA samples.

3. Results

3.1. Bioimpedance findings
The spectra of bioimpedance measurements for Z and phase are illustrated in Figure 2. With greater concentrations of DNA, there was an increase in Z and a decrease in phase at a specific bandwidth. Significant correlations \((p<0.01)\) were found below 5MHz for magnitude and above 300KHz for phase, as can be appreciated in the graphs.
As a first approach to linear regression analysis, bioimpedance data at the frequency of 1MHz were selected to examine the possibility of a significant correlation of the concentration of DNA with Z and phase. For every sample (each with a known DNA concentration), the impedance values are presented in Table 1.

**Figure 2.** Spectra of EBI measurements for magnitude (Z) (above) and phase (below) made at different concentrations of total DNA (1:1= 8 ng/μl, 1:2= 4 ng/μl, 1:5= 1.6 ng/μl, 1:10= 0.8 ng/μl, and Negative= no DNA). *p≤0.01.

**Table 1.** For samples with different known concentrations of DNA, the experimental values for magnitude (Z) and phase of EBI (at 1MHz) is shown.

| EBI measurement | Sample | DNA [ng/ul] | Impedance freq. (1MHz) | Units | p<0.01 |
|-----------------|--------|-------------|------------------------|-------|--------|
| Z               | DNA 1:1| 8           | 971.001                | ohms  |        |
| Z               | DNA 1:2| 4           | 1055.750               | Ohms  | -0.99  |
| Z               | DNA 1:5| 1.6         | 1119.794               | Ohms  |        |
| Z               | DNA 1:10| 0.8        | 1166.459               | Ohms  |        |
| Phase           | DNA 1:1| 8           | -11.081                | degrees |        |
| Phase           | DNA 1:2| 4           | -12.396                | degrees |        |
| Phase           | DNA 1:5| 1.6         | -13.316                | degrees | 0.982  |
| Phase           | DNA 1:10| 0.8       | -14.217                | degrees |        |
| Phase           | Negative| 0         | -14.531                | Degrees |        |
3.2. Linear regression

With linear regression analysis, functions were found that can characterize the correlation between the concentration of DNA and the two parameters of EBI.

\[
\text{Z: } y_Z = -0.036x + 43.252 \tag{4}
\]

\[
\text{Phase: } y_{\text{Phase}} = 2.255x + 32.441 \tag{5}
\]

![Figure 3](image-url) Scatter plot of the concentration of DNA versus the experimental EBI measurements for Z (above) and phase (below) at 1MHz (blue squares). The calculated values (black dots) were established by linear regression with eqs. 4 and 5 (for \(y_Z\) and \(y_{\text{Phase}}\) respectively).

4. Discussion

Induced currents at frequencies centred in the lower part of the gamma band dispersion promote a dipole relaxation effect on a water molecule and interact with the electronegative charge of a DNA molecule. This is the biophysical basis of the present EBI measurements. Such interaction, being a function of the concentration of DNA, may correlate with changes in volumetric bioimpedance and therefore be instrumental for the detection of DNA.

Additionally, a PCR mixture contains Mg\(^{2+}\), which serves as the essential polymerase enzyme cofactor [10]. As aforementioned, the DNA molecule has a high charge density due to the electronegative charge located on its phosphate backbone. In a conventional PCR mixture, Mg\(^{2+}\) is attracted to the DNA molecule by the electronegative phosphate backbone, leaving less free molecules of Mg\(^{2+}\) in the solution. Consequently, the solution becomes less conductive [11]. However, an electropositive dye, SYBR green, was herein added to the PCR mixture [12] and was probably intercalating in the DNA molecule. This would inhibit the condensation of Mg\(^{2+}\) on the phosphate backbone, thus resulting in more free Mg\(^{2+}\) ions in the solution, a phenomenon that could possibly...
explain the current finding of a negative proportional correlation of the concentration of DNA with the magnitude of impedance.

Based on the plot of equations 5 and 6 shown in Figure 3, a significant correlation was found in the samples between the concentration of DNA and the EBI values of Z and phase at 1MHz. Hence, a linear relationship exists between the electrochemical interaction with DNA and the biophysical effect measured as Z and phase at a specific bandwidth, which will enable the determination of the concentration of DNA in a sample by the use EBI. A more accurate approximation to the relation between EBI measurements and the concentration of DNA would require the recognition of a greater complexity by considering the number of nitrogen base pairs and the number of data values. Additionally, it is necessary to apply non-linear correlation algorithms. Nevertheless, such an analysis is not within the scope of the current contribution.

5. Conclusions
According to the present results, there is a linear correlation of the concentration of DNA with the magnitude and phase of EBI measurements at a specific frequency. This will likely allow for the determination of the DNA content of a sample by EBI measurements.

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