DESIGN OF A COLE-COLE METER FOR COMPLEX IMPEDANCE MEASUREMENT OF LIVING TISSUES

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ABSTRACT
Measurement of complex impedance of biological systems is gaining wide popularity in determining the pathological and physiological status of tissues in research areas such as; body fat content, blood freshness, tissue ischemia, skin hydration, and etc. In this paper, we designed a four-probe, multi-frequency impedance meter for quick assessment of the viability of erythrocyte suspensions under storage conditions. Impedance measurements are based on magnitude-ratio and phase-difference detection principles. The system is built around a sine-wave generator, a voltage controlled current source, a phase-gain detector and a microcontroller unit. Device accuracy is checked against the HP 4284A LCR meter under different RC test loads that simulate physiological measurements. As a novelty, Cole-Cole parameters namely \( R_0 \), \( R_\infty \), \( f_c \), \( \alpha \) and the extracellular fluid and intracellular fluid resistances, \( R_e \) and \( R_i \), are directly displayed in the same device, for the ease of use.

KEY WORDS
Cole-Cole parameters, bioimpedance, magnitude-ratio and phase-difference detection.

1. Introduction

Electrical impedance is a complex quantity, which consists of a real part (resistance), and an imaginary part (reactance):

\[
\tilde{Z} = R + jX
\]

(1)

Accurate measurements of impedance of biomaterials over a broad frequency range provide valuable information about the electrical properties of tissues or organs. Some examples to bioimpedance applications are; in-vivo muscle and tissue studies, electrode skin studies, dermatological applications, drug delivery rates, pacemaker development, blood cell analysis, monitoring of viral effects on cell structure, biotechnology research, food and pharmaceuticals [1].

In a recent study, it has been shown that electrical impedance measurements can be used in the assessment of viability of red blood cells in erythrocyte suspensions under storage, as the Cole-Cole parameters are closely correlated with the physiological properties [2]. The simplest electrical model of a single cell is a combination of resistive elements and a capacitance. However, even within the same tissue, cells have slightly different shapes and structures with different time constants, producing a spread of relaxation times. Cole-Cole plots of biological tissues are depressed circles with the centre below the real axis. The intersection of the Cole-Cole plot with the real axis provides \( R_0 \) and \( R_\infty \). To account for this effect, capacitive effects of cell membranes are usually lumped in a constant phase angle impedance: \( Z_{CPA} = K(jw)^\alpha \), where \( K \) is a constant with dimensions ( \( \Omega \) sec \( \alpha \)). \( \alpha \) is an experimental parameter which has a value between 0 and 1 and shows the deviation from the pure capacitance.

The electrode-tissue interface is eliminated by using the four-electrode technique: separate pairs of electrodes are used for current injection and voltage detection [3]. Since the voltage is measured with very high input impedance, practically no current flows through the voltage detecting electrodes. Electrode polarization is avoided and the contact impedance is eliminated from the measurement [3, 4, 5].

Fig. 1 Electrical model of the biological cell. \( R_e \) extracellular fluid resistance, \( R_i \) intracellular fluid resistance and \( Z_{CPA} \) constant phase angle impedance representing the effective cell membrane capacitance.

2. Design

The Cole-Cole impedance meter (CCIM) is based on the principle of magnitude-ratio and phase-difference detection.
detection. In addition to measuring the complex impedance, Cole-Cole parameters are also obtained and displayed. Figure 2 shows the block diagram of the impedance meter.

The analyzer is built with a DDS frequency generator, a voltage controlled current source (VCCS), two high frequency instrumentation amplifiers (IA1 and IA2), a phase-gain detector (PGD) and a microcontroller unit. The results are displayed on the LCD.

The high frequency voltage generated by the DDS is transformed into a constant current of 800 μA by the VCCS. The constant current is then applied to the sample under test via symmetrical current electrodes and to the reference resistor (R).

The voltage across the sample is amplified by the instrumentation amplifier IA1. The voltage across the reference resistor is amplified by another identical instrumentation amplifier IA2. The amplified voltages are then detected by the phase-gain detector. The outputs of the PGD are connected to the microcontroller unit that performs the complex impedance calculations and derives the Cole-Cole parameters.

The current source used has two components: a sine-wave generator and a voltage controlled current source. The sine-waves are produced with DDS method that enables producing frequencies of resolution less than 1 Hz over a broad range (1 Hz – 400 MHz) [6]. Sine-waves of 1 Hz – 10 MHz are generated by the AD9835 frequency generator with a signal-to-noise ratio of 50dB min at 1 MHz output frequency. The voltage signals generated are converted into constant current signals by means of the high output impedance (63 MΩ) current generator based on the CA3280 (Figure 3).

For the high input impedance requirement of the four-electrode bioimpedance analyzer, we used the AD8130 differential to single ended, low noise and high input impedance (1 MΩ differential) amplifier with a common-mode rejection ratio of 50 dB at 1 MHz. Figure 4 shows the basic gain circuit, where \( V_{out} = V_{in}(1+R_G/R_P) \).

The phase-gain detector is the most important part of the bioimpedance analyzer. It is based on the IC AD8302, which is a fully integrated system for measuring gain-loss and phase difference in various applications. Phase and gain detections by using IC have several advantages compared to many other methods because of its rapidity in measurements and simplicity of design [7]. We used the ATmega16, (Atmel Co.) low power 8-bit microcontroller in our design. It is the key component of the system because of its leading functions on other components of the device and its features used for performing the necessary operations. The ATmega16 in our system fulfills the following functions:

- adjustment of the frequency and phase of the sine-waves generated by the frequency generator by communicating with the AD9835,
ADC operation in order to obtain the binary numbers that are used in the calculations of the unknown impedance,
- controlling the LCD unit to display the Cole-Cole parameters.

The microcontroller code is written on “CodeVisionAVR” C compiler and the microcontroller is programmed through “AVRStudio 4” software.

3. Results

3.1 Impedance Measurements

The (CCIM) is programmed to measure complex impedances at 10 discrete frequencies between 100 kHz and 1 MHz. The measured data are evaluated for calculating the Cole-Cole parameters, namely R_0, R_∞, f_c and α. The reproducibility of results is checked with pure resistors (100Ω-1kΩ). When compared with the LCR meter readings, the errors are less than 0.1%, with maximum coefficient of variation of 0.7%. Phase shifts of the system are obtained with pure resistors (Figure 5).

Different R1, R2 and C combinations in Figure 6 are measured using the (CCIM) (Table 1). Results with R_1 = 100Ω, R_2 = 330Ω and C = 1nF are given in Figure 7.

The deviations observed in the imaginary components are thought to be caused by inaccurate detection of the phase differences between the current and voltage signals, which may be due to many factors affecting the measurements such as inadequate instrumentation, the characteristics of the circuit or the stray capacitances of the cables [8]. α shows the deviation from pure capacitance (α = 0; real capacitance) and f_c, is the characteristic frequency at which the imaginary part is maximum.

In order to check the accuracy of these parameters, LCR meter measurements are fitted into Cole-Cole plots in the MATLAB environment. Table 1 shows the results with those displayed in the (CCIM) for comparison (Figure 8).

3.2 The Cole-Cole Parameters

The (CCIM) is able to calculate the four Cole-Cole parameters R_0, R_∞, f_c and α. R_∞ represents the impedance as the reactance of cell membrane approaches zero at infinite frequency and equals R_1 [2, 8, 9, 10]. R_0 is given by (R_1+R_2).

Table 1 Cole-Cole parameters with RC circuit.

<table>
<thead>
<tr>
<th>RC (Ω)</th>
<th>Theoretical calculation</th>
<th>(CCIM) Readings</th>
<th>Errors</th>
<th>MATLAB Fits</th>
<th>Errors</th>
</tr>
</thead>
<tbody>
<tr>
<td>R_0 (Ω)</td>
<td>430Ω</td>
<td>428.29Ω</td>
<td>-0.4%</td>
<td>424.7Ω</td>
<td>-1.23%</td>
</tr>
<tr>
<td>R_∞ (Ω)</td>
<td>100Ω</td>
<td>91.75Ω</td>
<td>-8.25%</td>
<td>98.66Ω</td>
<td>-1.33%</td>
</tr>
<tr>
<td>α</td>
<td>0</td>
<td>0.012</td>
<td>1.2%</td>
<td>0.009</td>
<td>0.9%</td>
</tr>
<tr>
<td>f_c</td>
<td>482.53 kHz</td>
<td>490.81 kHz</td>
<td>1.72%</td>
<td>485.69 kHz</td>
<td>-1.04%</td>
</tr>
</tbody>
</table>

Fig. 5 Phase shifts of the system.

Fig. 6 The RC test circuit.

Fig. 7 Cole-Cole Diagrams.
4. Conclusion

Cole-Cole parameters displayed by the (CCIM) are in close agreement with the theoretical and the reference LCR meter findings. The performance of the (CCIM) relies on the performance of the phase-gain detector chip AD8302, and may be upgraded by developing the software of the system due to the characteristics of the phase-gain detector. Complex electrical measurements and computation of Cole-Cole parameters are performed and displayed in a single unit. The Cole-Cole impedance meter is planned to be used on erythrocyte suspensions for quickly predicting the blood quality under different storage conditions.

References


