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Dielectric Spectroscopy of Blood Cells Suspensions: Study on Geometrical Structure of Biological Cells

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***Abstract*— A promising procedure combining dielectric spectroscopy of red blood cells and an inverse application of an Effective Medium Theory (EMT) has been realized to prove the plausibility to obtain cells morphological information. This theoretical-experimental methodology could be the basis for an accurate and simple tool in diagnosis and research activity, especially when cell morphological alterations are a prime indicator of illness as in all hematopoietic pathologies.**

I. Introduction

In recent years dielectric spectroscopy has revealed as a powerful technique in different biomedical applications: biocompatibility of biomaterials, in "vitro" tissue engineering [1], hematocrit characterization [2], and tumor detection through microwave imaging (i.e. breast cancer imaging) [3], or by an "in vivo" permittivity test directly in the operating theatre [4]. Furthermore, combining such measurements of biological solutions with inverse application of Effective Medium Theory (EMT), has allowed to extract information about the biological sample, i.e, cells structure and functionality, and their variations due to external physico-chemical agents [5].

These results are of particular interest considering that morphological alterations in blood cells are a very prime indicator of hematopoietic pathologies [6]. In particular, alterations in red blood cells shape and dimension occur in the case of anemia [6], while morphological alterations at nucleus and cytosolic levels of hematopoietic progenitor cells identify and classify the differentiation block present in leukemia cells [6], [7].

Authors have recently proposed a methodology suitable to estimate a dielectric model for single liposomes, in order to obtain the EM field distribution at microscopic cellular level for bioelectromagnetic interaction studies [8]. The mentioned procedure presents two parts: first, the application of a very accurate technique for complex dielectric constant measurement of the liposome solution and second, the dielectric parameters estimation of liposome membrane through a proper EMT formulation on the measured data.

In this work, the dielectric measurement technique previously described is applied to blood cells solutions, and their geometric structures is estimated by an appropriate EMT formulation. This procedure is proposed as the basis for accurate and simple tools aiding in straightforward diagnosis of the cited pathologies.

Specifically, two types of erythrocyte solutions have been measured in order to test the feasibility of this procedure and the effectiveness of the EMT formulation adopted: in the first case, erythrocytes have been suspended in an isotonic buffer in order to preserve their typical biconcave shape; in the second case, erythrocytes have been suspended in an hypotonic buffer to induce a spherical shape.

A comparison between the two sets of measurements has been carried out and estimations have been held in order to identify the different erythrocyte eccentricity, that is known to vary from low values in the isotonic case (regular shape) to high values in the hypotonic samples (spherical shape).

The paper is organized as follows: Section II describes the preparation of erythrocyte solutions, the measurement methodology, and the EMT model adopted for the estimation procedure. In Section III experimental data are shown, and the estimated values of the extracted cell eccentricity are reported. In last section the obtained results are discussed

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drawing some conclusions.

II. Materials and Methods

A. Samples Preparation

Human blood cells were obtained from four healthy donors. The blood samples were diluted in phosphate buffer solution (PBS) and centrifuged for 10 minutes at 1000 rpm at 4 °C to separate erythrocytes from plasma and buffy coat. The red blood cells were washed twice in PBS solution, then cells were counted via manual cell count method in a counting chamber. Every sample was separated in two aliquots. One aliquot was re-suspended in the PBS buffer (*isotonic solution*) and the second aliquot was re-suspended in 63% PBS (*hypotonic solution)*. Table I reports for each donor the number of cells and the relative volume fractions calculated on the basis of the mean red blood cell volume [6].

B. Complex Dielectric Constant Measurement

The measure of complex dielectric constant

$$\varepsilon^*(\omega) = \varepsilon'(\omega) + i(\varepsilon''(\omega) - \frac{\sigma}{\varepsilon_0 \omega}) \quad (1)$$

has been realized on a coaxial cable sensor (properly modified [8]) combined with a vector network analyzer (VNA) on a large frequency range between 100 MHz up to 1 GHz. The adopted methodology in addition to the standard VNA calibration procedure takes full advantage of a further calibration phase using three proper standard liquids. The methodology refers to a T-shape equivalent circuit that models an interconnection network between the VNA and the coaxial cable impedance termination [9]. From reflection data measured on three calibration liquids whose dielectric functions are known and on the liquid under test (LUT), dielectric values are achieved, solving the linear equations system described in [8], [9]. An uncertainty analysis is also realized on the measured data including main sources of error such as random errors introduced by the measurement instrumentation (uncertainty on the measure of the reflection coefficient) and errors arising from inaccuracy in the dielectric data of the calibration liquids [8].

This technique is highly accurate and particularly suitable for measuring biological solutions since it needs a small sample volume during the measurement procedure.

C. Estimation with the Effective Medium Theory (EMT)

The eccentricity estimation is performed by an inverse application of the EMT. The approach based on EMT describes the macroscopic dielectric behavior of a mixture in term of the bulk properties of the constituents. For the erythrocytes in solution, frequency-dependent dielectric characteristics, erythrocytes size, shape and mutual alignment have been considered.

TABLE I
Cell Number and Calculated Volume Fraction for the Erythrocyte Solutions

Donor	Cell Number for 1 ml (manual counting method)	Calculated Volume Fraction
#1	$138*10^6$	0.0116
#2	$100*10^6$	0.011
#3	$85.5*10^6$	0.0097
#4	$63.5*10^6$	0.007

The chosen EMT formulation, able to describe geometrical and frequency-dependent properties of the inclusions, was originally derived by a Maxwell-Wagner formulation for a two-phase medium under the assumption of spherical suspended particles. Its frequency extension is due to Pauly and Schwan and it was applied to biological cells suspension in [10] taking into account the geometrical properties of the inclusions. The erythrocyte shape (both in isotonic and hypotonic solutions) is accounted as an oblate spheroid with semi-axes $a < b = c$, covered by a thin shell of thickness d. The degree of the shape modification is measured by variations in eccentricity value ($e = \frac{a}{b}$). The EMT formulation adopted has the following equation [10]:

$$\varepsilon_{mix} = \varepsilon_m \frac{1 + 2\Phi \sum_k \frac{(\varepsilon_{sk} - \varepsilon_m)}{(\varepsilon_{sk} + 2\varepsilon_m)}}{1 - \Phi \sum_k \frac{(\varepsilon_{sk} - \varepsilon_m)}{(\varepsilon_{sk} + 2\varepsilon_m)}} \quad (2)$$

where Φ is the erythrocytes volume fraction and ε_m the complex dielectric constant of the external medium.

Each cell orients randomly in the host medium, so due to its anisotropy, for the equivalent dielectric constant of the shelled ellipsoids there are three components k along the x, y, z directions:

$$\varepsilon_{sk} = \varepsilon_s \left\{ 1 + \frac{n(\varepsilon_p - \varepsilon_s)}{\varepsilon_s + (\varepsilon_p - \varepsilon_s) A_k (1 - n)} \right\} \quad (3)$$

where ε_s is the cell membrane, ε_p the cytosol, n:

$$n = \frac{(e + \frac{d}{b})(1 + \frac{d}{b})^2}{eb^2} \quad (4)$$

and A_k is the depolarization factor along the considered coordinates, for an oblate spheroid it is equal to:

$$A_y = A_z = 1/2 * (1 - A_x) \quad (5)$$

$$A_x = \frac{1}{1 - (e)^2} - \frac{e}{(1 - (e)^2)^{3/2}} \cos^{-1}(e) \quad (6)$$

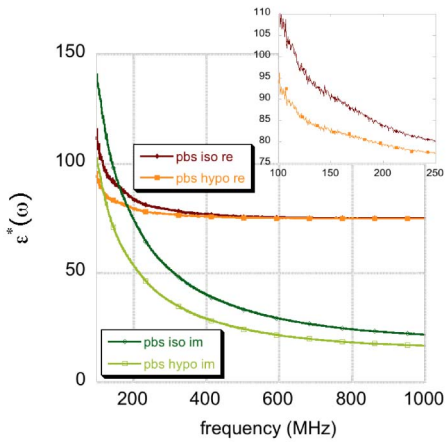

Fig. 1. PBS complex dielectric constant (real and imaginary part) frequency behavior, it is shown in the real part of the measured dielectric values the electrode polarization effect, it has been removed in the red blood cell solutions through a proper chose of the calibration liquids.

Each different medium in the mixture is characterized by a frequency-dependent complex dielectric constant based on a Debye equation in which also the conductivity terms are considered:

$$\varepsilon^*_{m,s,p} = \varepsilon^*(\omega)_{m,s,p} = \frac{(\varepsilon_{m,s,p-static} - \varepsilon_{m,s,p-\infty})}{(1 + i\omega\tau_{m,s,p})} - \frac{i\sigma_{m,s,p-dc}}{\varepsilon_0\omega} \quad (7)$$

where ω is the angular frequency, τ the single relaxation time constant, and ε_0 is the permittivity of the vacuum.

A non-linear least square-fitting algorithm (Trust Region algorithm Matlab™ release 14.07) allows the extraction of the eccentricity of the blood cells and the estimation of some geometrical values like the membrane thickness d, the cell major semi-axis b, and the evaluation of the mixture volume fraction Φ.

III. Results

All the measures were realized with VNA AGILENT E8363B provided with the calibration kit (85052-3.5 mm). For each measurement the frequency range was discretized in 1600 frequency points. Each sample was averaged on the basis of 100 times measurements. Methanol, PBS buffer, and 63% PBS solution were measured as a standard to validate the experimental procedure, in the case of non-conducting and conducting media. Red blood cells solutions were measured as biological target. All measurements were realized at room temperature of 25 °C.

The measured dielectric values for methanol fit the NBS ones [11] with a difference better than 4%; measurement uncertainty is about 3% for what concerns the real part and

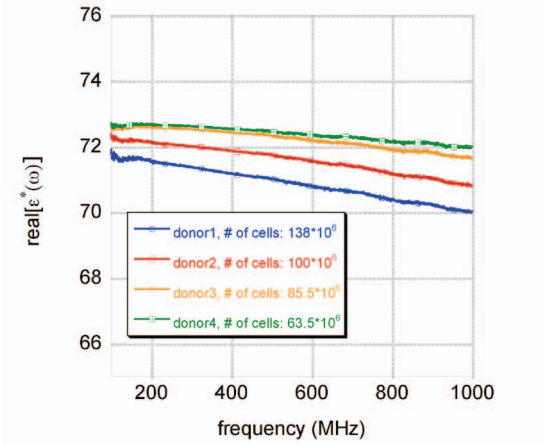

Fig. 2. Frequency behavior of the real part of the measured complex dielectric constant for isotonic solutions.

4.5% for the imaginary part of the complex dielectric constant. Fig. 1 reports the data measured for the two buffer solutions (PBS and 63 % PBS) calibrated with the standard media: air, acetone, iodine–methane [11]. The high values in the low frequency range (<300 MHz) of the real part are due to electrode polarization effect, which masks the bulk dielectric properties of the media and is typical of conductive solutions. However, such an effect is the same for both biological aggregates suspensions with small volume fraction (less than 15%) and their buffer solutions [12].

On the basis of the differential principle of the described methodology, we eliminated this phenomenon adopting PBS or 63 % PBS as one of the calibrating liquids.

A. Dielectric Measurements: Erythrocyte Solutions

Complex dielectric constants of red blood cells in isotonic solutions are reported from all donors (Fig. 2). The good sensitivity of the technique to the different concentrations is evident, similar sensitivity is shown in the case of hypotonic ones (Fig. 3).

In hypotonic erythrocyte solutions for donors number 3 and 4 there is an increase of the real values of the dielectric constant due to the variation of the solution conductivity with respect to the case of isotonic ones; donors 1 and 2 don't evidence these variations, probably counterbalanced by a stronger erythrocyte shape variation more significant in higher volume fraction [13].

B. EMT estimation: the cell geometrical parameters

The final step towards the eccentricity estimation has been the application of equation (2) to the data just reported in Fig.s 2 and 3. Table II reports all the estimated data, it is worthwhile to notice how volume fraction values are in good agreement with the ones proposed in Table I. Furthermore the extraction of the geometric parameters as the red blood cell

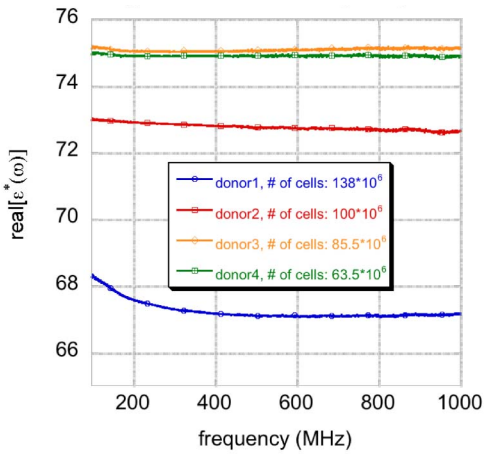

Fig. 3. Frequency behavior of the real part of the measured complex dielectric constant for hypotonic solutions.

membrane thickness and major semi-axis are in accordance with the ones proposed in literature [6].

These data prove the plausibility of the estimation procedure for a correct evaluation of cell eccentricity.

Examining the data for eccentricity, a difference of about 166 % of the mean value between the two kinds of samples is evidenced, implying a major semi-axis of 3.75 μm (mean value) for both erythrocyte shapes and a minor semi-axis of 0.28 μm and 0.75 μm for isotonic and hypotonic solutions respectively. This result confirms the possibility to obtain cells morphological information from the proposed methodology, allowing the use of the proposed procedure as an accurate and easy tool in diagnosis and research activity.

IV. Discussion and Conclusions

In this work an accurate dielectric measurement technique applied to red blood cell solutions and an estimation of cell geometric structures are proposed. This has been tested on two types of erythrocytes solutions whose eccentricity is known to vary between low values in the isotonic case (regular shape) and high values in the hypotonic samples (spherical shape). A proper formulation of the EMT taking into account cell morphological parameters is applied in inverse way to extract erythrocyte eccentricity. A comparison between eccentricity data obtained from isotonic and hypotonic solutions evidences a variation of 166 %, proving the possibility of the methodology to discriminate between different cell geometrical structures.

This procedure seems to be the basis for an accurate and simple tool in diagnosis, of cells morphological variations, as the ones related to all hematopoietic diseases. Moreover it can be utilized for monitoring different stages of cell morphology conformation, induced from external physico-chemical agents as recently proposed in [7].

TABLE II
Estimated Volume Fraction, Membrane Thickness and Major Cell Axis Dimension for the Erythrocyte Solutions

Donor	Estimated Volume Fraction	Membrane Thickness	Major Cell semi-axis Dimension
#1	0.022	8.8 nm	3.9 μm
#2	0.011	9.9 nm	3.6 μm
#3	0.011	9.5 nm	3.8 μm
#4	0.007	9.6 nm	3.7 μm

Donor	Eccentricity of Isotonic Erythrocyte Samples	Eccentricity of Hypotonic Erythrocyte Samples
#1	0.082	0.15
#2	0.078	0.24
#3	0.066	0.17
#4	0.076	0.28

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