Low-frequency, low-field dielectric spectroscopy of living cell suspensions

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(Received 13 February 2003; accepted 4 January 2004)

We have developed a dielectric spectroscopy technique for low-frequencies and low-electric field amplitudes. The excellent sensitivity of this method enables us to apply field amplitudes that are below the linear threshold. The dielectric constants of inorganic and organic liquids are found to be consistent with the previously reported experimental data and theoretical predictions. © *2004 American Institute of Physics.* [DOI: 10.1063/1.1649455]

I. INTRODUCTION

Dielectric spectroscopy is a powerful method which can be used to qualitatively and quantitatively study live biological systems.[1,2] A number of dielectric spectroscopy techniques have been developed[3–7] to study the response of biological systems in specific frequency ranges. A coplanar waveguide method, recently proposed by Facer, Notterman, and Sohn[8] enables measurements over a very wide spectrum (from dc to 10^{10} Hz). In this article, we present an experimental technique that specifically targets frequencies below 100 KHz. Most of the methods designed for frequencies below several kilohertz require electric fields much larger than the linear threshold of 1 V/cm,[9] or else involve other invasive processes such as chemical detection. The method presented here is sufficiently sensitive when the electric field amplitudes are smaller than the linear threshold.

The limit of very low-applied fields is important for several reasons, including the following. As was pointed out previously,[10] at low frequencies there are two major contributions to the dielectric permittivity of a living cell suspension: the reorientation of the dielectric dipoles of the individual cells and the polarization of the surface charge accumulated on the cell membrane. The surface charge accumulated on the cell membrane is directly proportional to the membrane potential. The rotational effect becomes negligible at very low-applied electric fields and, most likely, it dominates at large applied electric fields. Thus, it is only in the limit of low-applied electric fields when the dielectric permittivity is directly proportional to the membrane potential and thus only in this limit can one extract the membrane potential from the dielectric response curve.

In a previous article[11] we reported another low-frequency and low-field amplitudes dielectric spectroscopy technique using superconducting quantum interference devices, also known as SQUIDs. In that method, the electrical currents induced in the suspension by an applied electric field were measured with a SQUID, which could detect the very weak magnetic fields produced by these currents. Our previous method and the one we present here can be viewed as complementary techniques. As we will show, our method is very accurate in the range $10-10^5$ Hz, it is fast and inexpensive. The SQUID technique[11] is very accurate over the entire range from dc to 10^5 Hz but is relatively slow and expensive.

II. EXPERIMENTAL SET-UP

The experimental set-up is illustrated in Fig. 1. The SR 780 Signal Analyzer provides a sinusoidal voltage at its signal output. It also digitizes the voltage at the Channel 1 and Channel 2 inputs and takes the ratio of these two as a function of frequency. The real and imaginary parts of this ratio can be stored on a floppy for additional processing on the computer. The cell suspension is placed between two parallel planar, disk-shaped electrodes. The output voltage from the Signal Analyzer is applied to the upper electrode through resistor R_1. The bottom electrode of the cell suspension capacitor is connected to the negative input of the amplifier A_2 which holds this electrode at the ground potential. As a result, the current I that flows through the cell suspension produces a voltage drop V_1. The value of V_1 is equal to the product of I and the impedance of the cell suspension Z. The voltage $-V_2$ is equal to the product of I and the resistor R_2. Thus, the transfer function is directly related to the cell suspension impedance through: $V_2/V_1=R_2/Z$. The purpose of R_1 is to provide an upper limit for the current I as the impedance Z becomes smaller at higher frequencies. The unity gain amplifier A_1 provides buffering so that the input impedance of Channel 1 does not affect the voltage drop across the cell suspension. The values of all the components in this circuit must be accurately determined for this method to work at low frequencies. We point out that we directly measured the transfer function and not the voltage drop across the cell suspension.

A major source of error in measuring the biological impedance is the polarization that appears at the contacts between the sample and the disk-shaped electrodes. In addition, stray fields can be a problem. To minimize the effect of stray field, we use the guard ring electrode technique,[12] although the stray field effect was very weak in our case because $\varepsilon_{\text{cell}} \gg \varepsilon_0$. If a metallic electrode is immersed in a fluid or

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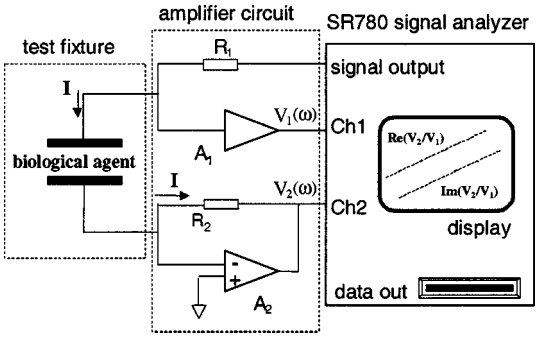

FIG. 1. Experimental set-up for measuring the dielectric permitivity and conductivity for fluids and living cell suspensions.

cellular suspension, a boundary potential appears between the electrode and the fluid or suspension. This potential appears due to the fact that positive and negative ions are attracted to the negative and positive electrode surfaces, respectively. Like charged ions are repelled at the respective electrode surfaces. In addition electron-transfer reaction can appear at the electrode surface. The polarization effect becomes very strong when measurements are carried out at low frequencies, as in our case. To correct this effect, we used the electrode distance variation technique.[8] This technique is based on the fact that the polarization impedance is the same for different distances between the capacitor plates. The measured impedance Z^O is actually the sum between the sample impedance Z^S and the unknown polarization impedance Z^P. The following general equations apply for two different electrode positions:[12]

$$Z_1^S + Z^P = Z_1^O$$
$$Z_2^S + Z^P = Z_2^O . \quad (1)$$

By subtraction

$$Z_1^S - Z_2^S = Z_1^O - Z_2^O , \quad (2)$$

which is independent of the polarization impedance.

We model the impedance of the cell suspension as a resistor $R = d/\sigma A$ in parallel with a capacitor $C = \varepsilon A/d$, where σ and ε are the conductivity and dielectric permittivity of the cell suspension and A and d represent the surface area of the two disk electrodes and the distance between them. We can then write

$$Z_1^O - Z_1^O = \frac{d_1 - d_2}{(\sigma + i\omega\varepsilon)A},$$

which leads to the dispersion curves

$$\sigma(\omega) = \mathrm{Re}\frac{d_1 - d_2}{A(Z_1^O - Z_2^O)}, \quad \varepsilon(\omega) = \mathrm{Im}\frac{d_1 - d_2}{\omega A(Z_1^O - Z_2^O)}. \quad (3)$$

For each distance, the impedance is extracted from the measured transfer function.

III. TEST EXPERIMENTS

The experimental set-up was first tested by replacing the electrode set up with electronic components like resistors and capacitors. The measured values agreed very well with their published values. A second test was to measure the dielectric permittivities of different substances and to compare them with the standard values. The results are shown in Fig. 2 for toluene (standard value $\varepsilon_r = 2.3$), glycol (standard value $\varepsilon_r = 40$), and water (standard value $\varepsilon_r = 78$). The continuous lines represent the dispersion curves obtained without compensating for the polarization effect. It can be seen that, above 1 KHz, the values we obtained agree remarkably with the standard ones. However, below 1 kHz, the polarization effects increase substantially with decreasing frequency. Theoretical predictions by Cirkel, van der Ploeg, and Koper[13] suggest that, as a result of the polarization effects, the dispersion curves should have a frequency dependence given by $\omega^{-3/2}$. We find that the low-frequency behavior of our experimental dispersion curves is very close to the predicted $\omega^{-3/2}$ dependency. A linear fit at low frequencies of the $\log \varepsilon_r$ versus $\log \omega$ provide the following exponents: 1.3 for glycol and 1.6 for water, which are close to the predicted value of 1.5. This strongly indicates that the dispersion at very low frequencies is indeed dominated by the polarization effects. For toluene however, the polarization effect seems to be almost negligible. The dotted lines in Fig. 2 represent the dispersion curves obtained after correcting for the polarization effect by using the electrode distance variation technique. We considered three distances between the electrode plates: 10, 11, and 12 mm. The applied electric voltage amplitude was 0.3 V. Combining any two of these three distances we obtain the same dispersion curve shown in Fig. 2 by the dotted lines. We obtained the same results by applying 0.1 V over the same distances for the same substances. It can be seen that this technique has effectively removed the po-

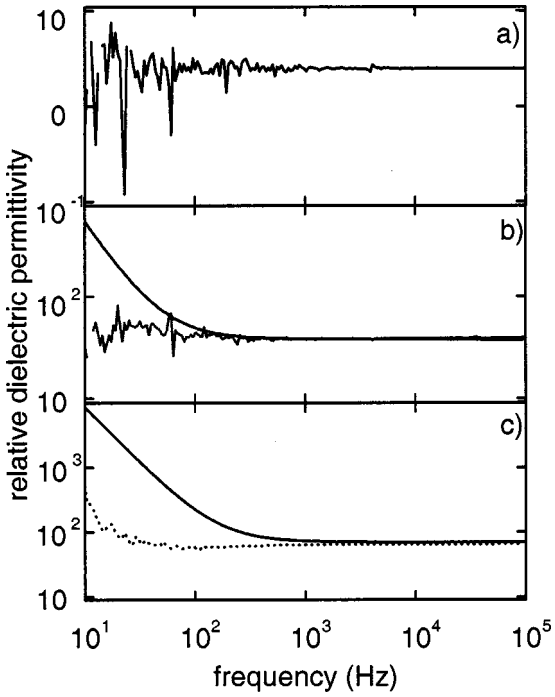

FIG. 2. Dielectric permitivity vs frequency for: (a) toluen; (b) glycol; and (c) water. The solid lines represent the dielectric values measured without compensating for the polarization effect while the dotted lines represent the dielectric values after removing the polarization effect.

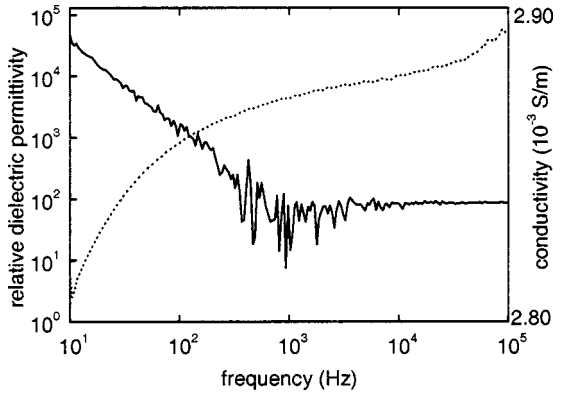

FIG. 3. Dielectric permittivity (continuous line) and conductivity (dotted line) vs frequency for fission yeast obtained after compensating for the polarization effect.

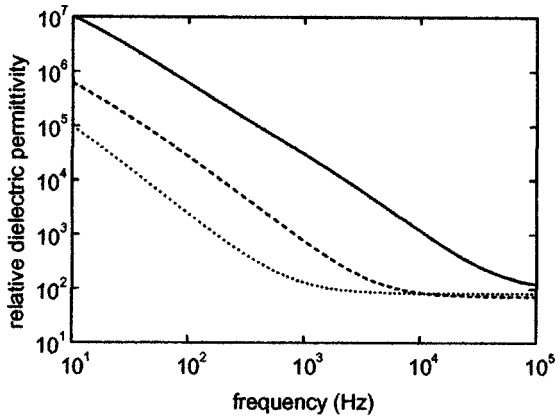

FIG. 4. Dielectric permittivity vs frequency for *E. coli* for three different concentrations: 10^{10} cells/ml (solid line), 10^{9} cells/ml (dashed line), and 10^{8} cells/ml (dotted line).

larization contribution down to 10 Hz. Both test experiments show that the proposed technique can be used to make accurate measurements of the dispersion curves at least in the range 10 Hz–100 kHz.

IV. LIVING CELL SUSPENSION EXPERIMENTS

In this section we present the experimental results for a few living cell suspensions and compare them with available data, both theoretical and experimental. First, a suspension of *Schizosaccharomyces pombe*(fission yeast) strain 972h was grown YE medium at 30 °C until the culture reached saturation. The cells were harvested by centrifugation, resuspended in sterile water, and stored at 40 °C until used. The cell suspension concentration was approximately 10^8 cells/ml. The polarization effects were eliminated by using the distance variation technique. The distances between the electrode plates were 10, 11, and 12 ml. The applied voltage was 0.3 V, leading to an electric field below the linear threshold. Figure 3 shows the dispersion curves of the dielectric permittivity and the conductance of the yeast solution. Both dispersion curves resemble the theoretically predicted features.[10] The relative dielectric permittivity of the living yeast suspension (between 10^4 and 10^5) decreases rapidly and, at higher frequencies, they flattens out approaching the relative dielectric constant of water, $\varepsilon_{\text{water}}=78$. The conductivity starts at lower values and increases as the frequency increases, flattening out at the same frequency as the dielectric permitivity.

In the next experiment we tested three solutions of *E. coli* bacteria with three different concentrations: 10^{10}, 10^9, and 10^8 cells/ml. The distances between the electrode plates used to remove the polarization contribution were 5, 6, and 7 ml, and the applied voltage amplitude was 0.3 V. Figure 4 shows the relative dielectric permittivity as a function of frequency for the three concentrations of bacteria. The dispersion curves do not show any plateau at very low frequencies. The relative dielectric permittivities decrease rapidly and, at higher frequencies, they approach the relative dielectric constant of water, $\varepsilon_{\text{water}}=78$. Similar results on *E. coli* suspensions were previously reported.[1] A ten-fold decrease in the cell concentration results in an almost ten-fold decrease in the dielectric permittivity of the suspension. This experiment shows that the large dielectric permittivity of these suspensions is due to the presence of living cells.

V. CONCLUSIONS

We have developed a dielectric spectroscopy technique for low-frequencies and low-applied electric fields amplitudes. The method has been tested on fluids with known dielectric permittivity and has been proven to be effective at frequencies ranging from 10 to 10^5 Hz and electric field amplitudes below the nonlinear threshold. The method has been used to measure the dispersion curves of the dielectric permittivity and conductivity for several living cell suspensions. The results are consistent with theoretical predictions and previously reported experimental data.

ACKNOWLEDGMENTS

This work was supported, in part, by the Texas Center for Superconductivity and Advanced Materials, by the Robert A. Welch Foundation (E-1221), and by DARPA through the Naval Surface Warfare Center Dahlgren Division (N00178-03-C-3071).

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