

ELECTRODE POLARIZATION COMPENSATION IN NONLINEAR DIELECTRIC SPECTROSCOPY OF BIOLOGICAL CELL SUSPENSIONS

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INTRODUCTION

The technique of nonlinear dielectric spectroscopy (NLDS) injects a sinusoidal probing current or voltage into a sample, and obtains meaningful information through the analysis of a nonlinear biological response. Work appearing in the literature has suggested that features in the nonlinear response can be correlated with metabolic activity to indirectly provide information about glucose concentration [1, 2]. This type of physiological information is important in the management of diseases such as diabetes, and as such, NLDS is of interest in the noninvasive determination of glucose concentration. Nonlinear artifacts can mask the biological response, and in this paper a method is presented to compensate for electrode polarization artifacts.

BACKGROUND

In NLDS a stimulating sinusoidal input signal is applied to a test sample in the form of an AC voltage or current through a pair of current injecting electrodes. The sample's response to this electric field is then measured as a voltage at a pair of recording electrodes. The nonlinear response is observed in the time domain as a distortion in the quasi-sinusoidal voltage at the recording electrodes, while in the frequency domain this distortion appears as distinct spectral components - the harmonics - occurring at integer multiples of the input frequency.

Research first presented in [1] indicated that the *a priori* metabolic state of yeast can be determined by examining the respective dominance of the second and third harmonic magnitudes. The harmonic characteristics are attributed to the transmembrane protein H⁺ATPase. This protein is a large dipolar molecule, capable of affecting, and being affected by a surrounding electric field [1, 3]. Unfortunately, in addition to a nonlinear biological response, the signal at the recording electrodes also contains artifacts due to the electrode-electrolyte system.

Nonlinear Electrode-Electrolyte Effects

The exhibited current-voltage (I-V) relationship of electrode-electrolyte systems under low current condi-

tions is typically considered to be linear. However, electrode processes are nonlinear in nature, and this effect can be observed at low amplitudes using high resolution measuring equipment [4]. Nonlinearity increases with current, and depends on the physical properties of the system such as electrode material and electrolyte concentration [5]. The mechanism which induces this nonlinearity is electrode polarization.

Electrode polarization can be categorized by three basic mechanisms resulting in series impedances within the system [6]. The first of these is the ohmic overpotential Z_o , which arises from the impedance of the electrolyte. The second mechanism is the concentration overpotential Z_c , which is caused by the redistribution of ions at the electrode-electrolyte interface when current flows. Finally, the third mechanism is the activation overpotential Z_a , which is the result of the activation energy barrier that must be overcome for the redox reaction at the electrode interface to proceed. These impedances can vary with current, yielding a nonlinear I-V relationship [5].

The total electrode polarization impedance, denoted by Z_p , when treated as linear is $Z_p = Z_o + Z_c + Z_a$. Both Z_c and Z_a occur at the electrode-electrolyte boundary and are therefore referred to as interfacial polarization impedance [7]. An interfacial polarization impedance term, denoted by Z_i , can be defined as $Z_i = Z_c + Z_a$. The impedance Z_i is a function of current flow across the interface and is therefore nonlinear. The impedance Z_o can be considered linear below a very high applied electric field threshold [8].

In the four electrode configuration, the recording electrodes are in series with a high impedance amplifier, resulting in negligible current flow in the recording pair. The recording electrodes are therefore assumed to not be externally polarized [8], and can be considered transparent. Accordingly, nonlinear polarization artifacts arise from the current injecting electrode pair.

MATERIALS AND METHODS

Medium and Cell Suspension

Three test cases were prepared: 1) the electrolyte medium, 2) the quiescent yeast case, and 3) the active yeast case. The electrolyte medium consisted

*This work was supported by Ontario Centres of Excellence - Communications and Information Technology Ontario Division.

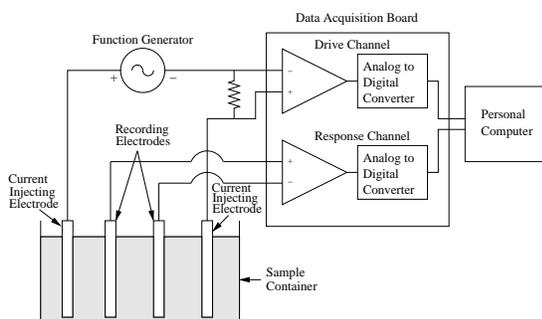


Figure 1: Nonlinear dielectric spectroscopy experimental apparatus.

of $20 \text{ mmol}\cdot\text{L}^{-1} \text{ KH}_2\text{PO}_4$, $30 \text{ mmol}\cdot\text{L}^{-1} \text{ KCl}$, and $20 \text{ mmol}\cdot\text{L}^{-1} \text{ MgCl}_2$ dissolved in distilled water. The electrolyte medium provides a suitable environment in which yeast can be either metabolically quiescent or active [1], and the ions of this salt solution are also the charge carriers responsible for current flow through the test sample. The quiescent yeast case was prepared by rehydrating freeze-dried cells (Muntions Active Brewing Yeast) at a concentration of $50 \text{ mg}\cdot\text{mL}^{-1}$ in the electrolyte medium. The active case had the same composition as the quiescent case, but also contained glucose as a metabolizable substrate, at an initial concentration of $170 \text{ mmol}\cdot\text{L}^{-1}$. Sample sizes of 2 mL were pipetted into open glass cylinders with a diameter of 16 mm .

Apparatus

A four electrode system was employed as shown in Fig. 1 under voltage clamp conditions, with the outer two electrodes supplying the perturbing current and the inner two electrodes recording the voltage. Electrodes were Ag-AgCl, prepared by chloriding 22 gauge ($640 \mu\text{m}$ dia.) silver wire (A-M Systems, 99.99% pure) in sodium hypochlorite. On-center spacing of electrodes was 2.54 mm , with a length of 14 mm immersed in the suspension. New electrodes were used for all test cases.

The sinusoidal drive input of 17 Hz at 1.5 V was supplied by a function generator (Agilent 33120A). A 470Ω resistor was placed in series with the source to enable current monitoring. The response was measured by sampling the voltage across the inner recording electrodes. Amplification, sampling and analog to digital conversion was done using a National Instruments DAQPad 6020E, with 12 bit resolution, operating at a sampling rate of 2048 Hz . The three test cases were run in parallel to obtain data simultaneously, and under the same conditions. Two second long data records were recorded every six seconds over a twelve hour period.

Signal Processing and Data Presentation

Signal processing was performed on a PC running MATLAB. The power spectral density (PSD) was estimated for each data record using Welch's averaged, modified periodogram method. Signals were windowed with a Hamming window of length 1024 samples and overlapped by 50%. The display of the resulting time-frequency data was simplified by considering only the PSD of the fundamental, and the second and third harmonics. The magnitude of the PSD at these three frequencies was plotted over time.

RESULTS

The time course harmonic magnitudes for the electrolyte medium, the quiescent yeast, and the active yeast are shown in Fig. 2. The electrolyte case is characterized by a reasonably stable harmonic pattern with the second harmonic of greater magnitude than the third, except briefly near hour 8, where a spurious peak in the second harmonic occurs. The quiescent yeast case also displays a second harmonic greater than the third, although this is reversed in the first 45 minutes. Finally, the active yeast case is not characterized by a single dominant harmonic, but instead displays three primary phases. Phase one (hours 0-1.5) and phase three (hours 5-12) are characterized by a dominant third harmonic. Phase two (hours 1.5-5) is characterized by a dominant second harmonic. Interestingly, it is in phase two that metabolic activity is also the greatest.

In comparing the quiescent and active cases, different harmonic behavior is observed. While this may be due to a nonlinear biological response, the strong non-linearity observed in the electrolyte data, in which no biological cells are present, indicates that system artifacts are also present. These system artifacts, primarily attributable to electrode polarization effects, can mask the true biological response. It is therefore necessary to compensate for polarization effects to reveal the biological response.

ELECTRODE POLARIZATION COMPENSATION

The interfacial electrode polarization impedance results in a nonlinear perturbation to the biological sample. Therefore electrode polarization artifacts elicit a biological response from a nonlinear stimulus input. The compensation method, which extends earlier work presented in [9], simulates the biological response to a stimulus containing no polarization artifacts.

To compensate for electrode polarization effects two distinct data sets are considered. One data set, that of the electrolyte case, contains only the nonlinear interfacial impedance Z_i and the electrolyte impedance

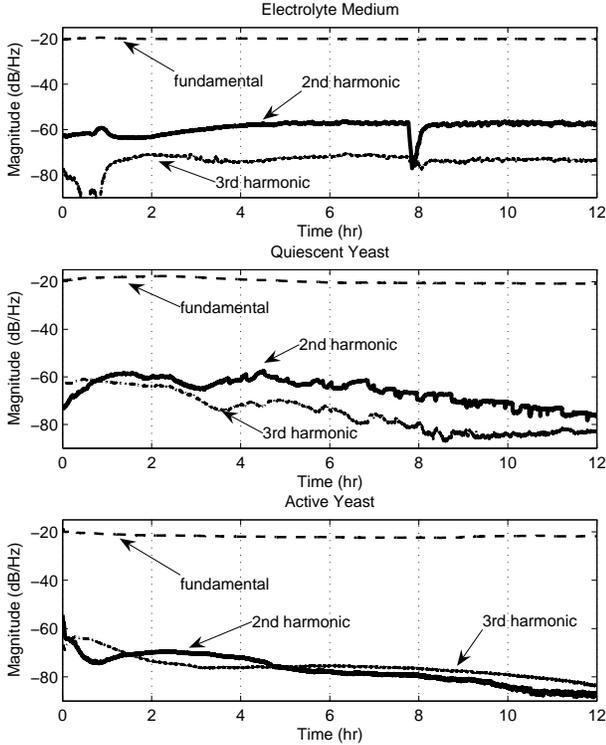


Figure 2: Experimental time-course harmonic magnitudes.

Z_o , which was found to be reasonably approximated as linear. This first data set can be considered the polarization reference. The second data set, recorded from a cell suspension, includes a contribution due to Z_i , and a biologically modified Z_o .

The first aspect of the polarization compensation method is to form a model describing the interfacial polarization artifact as observed at the recording electrodes. A pure sinusoidal input v_{in} applied across the drive electrodes is given by $v_{in}(t) = A \sin(2\pi F_{in}t)$, where A represents the input amplitude attenuated by Z_o . Implementation of the compensation method used a numerical value of A determined by measuring the peak AC amplitude of the voltage waveform at the recording electrodes.

To model the output v_p resulting from the nonlinear attenuation of the input voltage due to Z_i , a Taylor series expansion $v_p(v_{in}(t))$ about an input $v_{in}(t) = 0$ is formed:

$$v_p(v_{in}(t)) = \sum_{n=0}^{\infty} k_n v_{in}^n(t). \quad (1)$$

Equation (1) was truncated after the seventh order term by empirically determining the minimum order in which the first five harmonics were stable. The model was then fit using a nonlinear least squares method

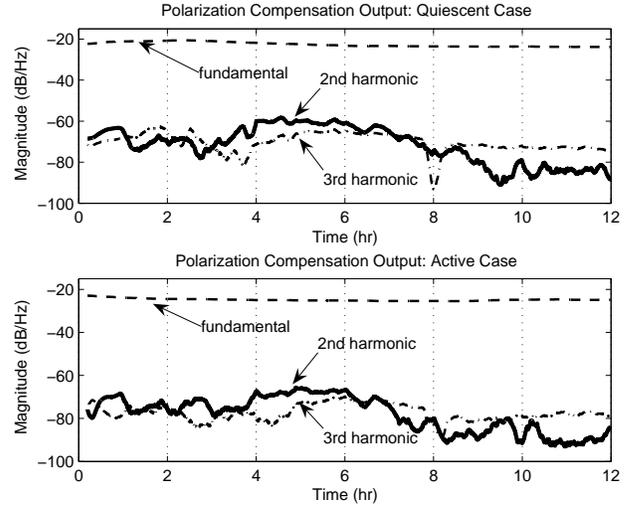


Figure 3: Compensated time-course harmonic magnitudes.

to the electrolyte data sampled at the recording electrodes. This fitting process was repeated for each data record occurring every six seconds over the twelve hour total test time. From this method, a time course of k_n coefficients were obtained to describe the effect of Z_i as observed at the recording electrodes.

The second part of the compensation method is to consider v_p as the perturbing input to the biological sample. Given that biologically produced harmonics were expected, a Taylor series expansion was chosen to model the biological response:

$$v_o(v_p(v_{in}(t))) = \sum_{n=0}^{\infty} m_n v_p^n(v_{in}(t)). \quad (2)$$

Equation (2) was truncated after the seventh order term and fit using a nonlinear least squares method to the yeast data sampled at the record electrodes to obtain the m_n parameters. These m_n parameters describe the biological response to the polarization dependant nonlinear perturbing electric field.

To examine the biological response to an artifact-free input, the biological response to a pure sinusoid was simulated by using the m_n parameters, and letting

$$v_p = A \sin(2\pi F_{in}t). \quad (3)$$

The resulting output v_o , shown in Fig. 3, simulates the biological response without the nonlinear polarization artifacts.

The plots of the compensation output show that from approximately hours 0 to 1 and hours 3 to 12, both the active and quiescent cases exhibit a similar pattern

in terms of the dominant harmonic. Between hours 1 and 3, the two cases display a different pattern. During this period for the quiescent case, the third harmonic magnitude is greater than the second. In this same period for the active case, the second harmonic is greater than or equal to that of the third. The period of greatest metabolic activity in the active case occurs between hours 1 and 5. Therefore in the first half of the highly metabolic period, a differing dominant harmonic is observed in the two cases.

DISCUSSION

In the uncompensated data (Fig. 2), an obvious difference in harmonic pattern is apparent when comparing the active and quiescent cases. However, this is not the case with the compensated data (Fig. 3), except between hours 1 and 3, during which time the dominant harmonic differs. This suggests that in general, polarization artifacts dominate in the uncompensated data, rather than a biological response.

When comparing the active and quiescent cases of the compensated data, only the first half of the highly active period is characterized by differing dominant harmonics. This suggests that the observed response may not be due to a transmembrane protein, but instead may be attributable to some other mechanism. One mechanism to consider is the effect of cellular bulk on the polarization layer. Similar experimentation in [10], in which hollow polymer spheres of diameter $0.4 \mu\text{m}$ were used rather than yeast cells, found that the addition of the spheres to an electrolyte medium modified the harmonic pattern.

The yeast species used in this work, *S.cerevisiae*, is a top-fermenting yeast. While metabolizing glucose, the cells move towards the surface of the test container. After reproducing, the yeast cells flocculate and drop to the bottom of the container. It is reasonable to expect that the movement of yeast cells from bottom to top and back, would cause some dynamic behavior at the electrode-electrolyte interface. As the compensation method assumes that electrode polarization artifacts are common between the electrolyte and biological cases, any change in the polarization layer caused by cell movement would be reflected in the compensation output. In future work, the effect of cellular bulk may be reduced by employing an ion-permeable membrane to restrict cellular contact with the electrodes.

Others have also examined electrode polarization effects. Schwan has modelled polarization effects using a Taylor series [11], and [7] developed this further. More recent work has seen a theoretical model based on the Butler-Volmer equation [12].

CONCLUSION

The technique of NLDS was used to examine the metabolic response of a suspension of the yeast cells to a low frequency perturbing AC signal. Nonlinear electrode polarization artifacts were found to contribute to the recorded response. To remove the masking effect of these artifacts, a compensation method was developed to isolate the biological response. The results of the compensation method showed one period where a notably different response was observed between the active and quiescent cells. Further work is required to understand the mechanism behind this difference.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the contributions of Biopeak Corp., Ottawa, Canada in this project.

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