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Short communication

Fast nonlinear region localisation for nonlinear dielectric spectroscopy of biological suspensions

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ABSTRACT

The nonlinear properties of biological suspensions have been previously presented as a bulk phenomenon without the influences of the electrodes. However, some authors have showed that the behaviour of a biological suspension is due to the nonlinear characteristics of the electrode–electrolyte interface (EEI), which is modulated by the presence of yeast cells. We have developed a method, complementary to the nonlinear dielectric spectroscopy (NLDS) which is used for the study of the behaviour of EEI with resting cell suspensions of *Saccharomyces cerevisiae*.

The method allows researchers to detect simply and quickly the voltage and frequency ranges where the metabolic activity of yeasts is detectable. This method does not replace NLDS, and aims to reduce the time during which the electrodes are exposed to corrosion by high voltages. In this paper we applied AC overpotentials (10–630 mV) with frequencies in the range from 1 to 1000 Hz. Also, we measured current harmonic distortion produced by the nonlinearity of the interface. Changes in the transfer function were observed when yeast suspension was used. Apart from the nonlinear response typical of the EEI, we also observed the biological nonlinear behaviour. The changes in the transfer functions were assessed using the overlapping index which was defined in terms of the conditional probability. The methodology was contrasted favourably with Fourier analysis. This novel strategy has the advantages of simplicity, sensitivity, reproducibility and involves basic tools such as the usual measurement of current.

1. Introduction

The nonlinear dielectric spectroscopy (NLDS) of microbiological suspensions was first proposed by Woodward and Kell (1990). They reported a biological behaviour under an applied sinusoidal electric field of less than 5 V cm^{-1} (McShea et al., 1992; Woodward and Kell, 1990, 1991a, 1991b, 1991c, 1995). They proposed a method and designed a nonlinear spectrometer for the study of the nonlinear characteristics of biological suspensions. The nonlinear spectrometer applies a sinusoidal voltage signal to a four-electrode cell through the outer electrodes and records the voltage drop between the inner electrodes. The proposed design generates two electrode–electrolyte interfaces (EEI) which have a nonlinear behaviour (Ruiz et al., 2005; Ruiz and Felice, 2007). These EEI distort the current through the cell and therefore the electric field applied is not sinusoidal. The response of the biological medium is added to the nonlinear response of the EEI. The nonlinearity of EEI was corrected with a second measuring cell, which was filled only with supernatant (without biological medium) and serves as a reference measurement. The difference between the frequency spectra of the two measurements was adopted as the nonlinear contribution of biological material. Despite the numerous attempts, the nonlinear component of EEI could not be avoided (Woodward et al., 1996, 1999, 2000). Others investigators also failed to eliminate the nonlinearity of the EEI despite using high technology systems (Nawarathna et al., 2005a, 2005b, 2006).

Treo and co-workers did measurements with an improved nonlinear dielectric spectrometer and three different electrochemical cells (Treo et al., 2005; Treo and Felice, 2009). One of them was a replica of that used by Woodward and Kell, and the other two were three- and four-electrode cells respectively. The four-electrode cell was designed to obtain an uniform electric field in the cellular suspension and low contribution of EEI due to the large area of the external electrodes. The three-electrode cell was designed to measure the impedance of EEI. They obtained variations higher than 20 dB in the third harmonic. These results indicated that the changes in the third harmonic were due to the presence of microorganisms near the EEI, and were consistent

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with Blake-Coleman's results (Blake-Coleman et al., 1994; Hutchings et al., 1994). Measurements performed with the other cells were not relevant or at least similar to Woodward and Kell's results, despite the wide range of applied electric field (0.033–30 V cm^{-1}). Interestingly, it has also been shown that the attachment of microorganisms in the EEI could produce changes in impedance measurements (Muñoz-Berbel et al., 2007, 2008a, 2008b, 2008c).

In all these studies, the harmonics in the current polarisation were analysed in frequency-domain with Fourier analysis, which is a time-consuming processing.

Moreover, the high voltages used in NLDS (above 600 mV during several minutes) led to nonlinear work zones, where the electrochemical corrosion process affects the measurement and the interpretation of results. Therefore, it is necessary to have a method which allows fast localisation of the nonlinear zones within the voltage–frequency plane to then begin a detailed harmonic analysis.

We propose a new method in the time-domain, which works complementarily with the spectrometer developed by Treo, and which aims to rapidly detect changes in the nonlinearity of the EEI. Therefore reduces the time during which the electrodes are exposed to corrosion by high voltages.

2. Materials and methods

2.1. Non-linear spectrometer

The setup consisted of a central PC to synchronize the instruments and to collect the data, an electrochemical analyser Solartron SI1287, a peristaltic pump, a magnetic stirrer (Fig. 1A) and a three-electrode measurement cell (Fig. 1B) (Treo and Felice, 2009). These sinusoidal voltage signals were applied to a biological suspension contained in the cell by means of two outer electrodes (counter electrode and working electrode).

The voltages were controlled by the electrochemical analyser to provide a sinusoidal electric field (without harmonic content) between the reference electrode and working electrode. The current flowing through the cell was sampled and transformed to the frequency domain using the periodogram method with a rectangular window (Welch, 1967). The length of the signal was chosen to ensure at least five samples of separation between harmonics. The system has been completed with a reservoir with solution and a pumping system to recirculate the solution through the cell. After complete 90 s of acquisition, the stirrer and pump were turned on for 10 s. When the inhibitor of H^+-ATPase was added to suspension, the mixture was stirred and pumped for 1 or 2 s to homogenize the system. Both the pump and stirrer were controlled by PC to ensure the steady state of system during the acquisition.

2.2. Three-electrode cell

The three-electrode cell was composed by a gold working electrode (WE), a reference electrode (RE) and a hemi-spherical stainless steel counter electrode (CE). The CE (area = 150 cm^2) was designed with an area larger than the working electrode in order to minimize its impedance. It was hermetically sealed with a thin acrylic piece (external diameter = 100 mm and diameter of the central hollow = 10 mm) containing a stainless steel wire that acts as a reference electrode (Dentaurum steel, diameter = 1 mm). This piece was supported on an acrylic disk (external diameter d = 100 mm) containing to WE (diameter = 8 mm). The WE was hand-polished before each experiment with diamond past and aluminium powder, up to a final roughness of 1 μm. The CE had tubing connections to allow the flow in and out of suspension. A magnetic stirring bar was introduced inside the cell.

2.3. Microbiological preparation

In order to compare our results with those of the other authors (i.e. Treo and Woodward), we used the same biological material (e.g. *Saccharomyces cerevisiae*). The microorganism was obtained locally as a lyophilized powder and resuspended to obtain the concentration as indicated below.

Different media were used in the assay:

1. Base solution (BS): 20 mM of KH_2PO_4, 30 mM of KCl, and 1 mM of $MgCl_2$, at pH 6.5.
2. Yeast cell Suspension (CS).
3. Stimulated cell suspension (SCS).

The CS was obtained by dissolving 3.7 g of dry yeast in 75 ml of BS and stirring for 5 min with a magnetic stirrer. SCS was obtained adding 150 μL of sodium metavanadate (SMV, 1 mM, an inhibitor of H^+-ATPase) to CS (Wach and Graber, 1991).

All chemicals used were of analytical grade and the water was glass-distilled (final conductivity less than 5 μS/cm).

2.4. Measurement protocol

AC overpotentials (10–630 mV, divided into 9 logarithmic steps) with frequencies within the interval of 1–65,000 Hz (divided into 10 logarithmic steps) were applied with the Solartron system. The experiments were carried out for the three media: BS, CS and SCS.

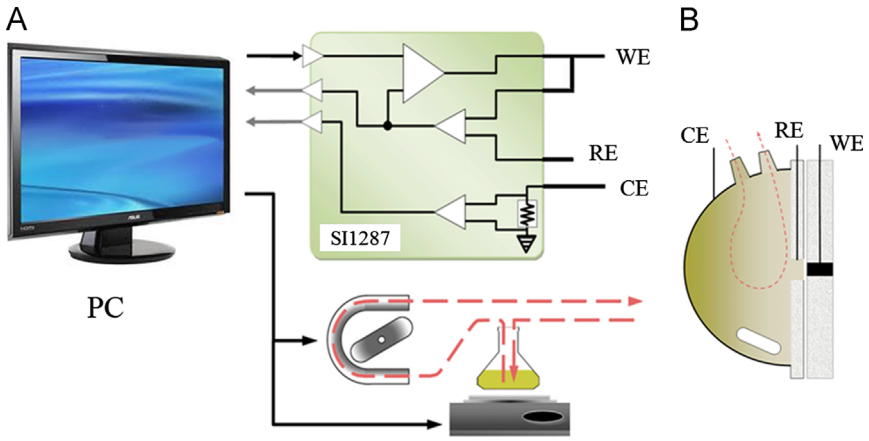

Fig. 1. Nonlinear dielectric spectrometer. (A) Equipment involved and (B) three-electrode cell. Reprinted and adapted from Treo and Felice, (2009).