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Linear Non Iterative Sinusoidal Fitting Algorithm for Microbial Impedance Biosensor

[1] Marco GROSSI, [1] Massimo LANZONI, [2] Roberto LAZZARINI and [1] Bruno RICCÒ

[1] Department of Electronics, Faculty of Engineering, University of Bologna, Bologna, Italy
Tel.: +39 051/2093082, fax: +39 051/2093785
[2] Carpigiani Group, Anzola Emilia, Bologna, Italy
Email: marco.grossi8@unibo.it

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Abstract: Sine wave signals are widely used in electronic applications such as digital oscilloscopes calibration, speech analysis and electrochemical sensors. In particular, impedance based microbial biosensors detect bacterial concentration by stimulating the sample with a sinusoidal test signal and measuring its electrical characteristics. Thus, for reliable microbial biosensing, fast and accurate sine wave analysis is mandatory. Many algorithms for the estimation of sinusoidal parameters exist that provide accurate estimate but are based on time consuming iterative procedures and/or need good starting values for the sine parameters. In this paper a linear non iterative algorithm based on the least squares method is presented that allows sinusoidal voltages analysis in a fast and efficient manner. The algorithm has been tested either with simulation analysis either with real impedances and the results proved to be accurate enough for reliable bacterial concentration measurement.

Keywords: Bacterial measurements, Impedance biosensors, Sine wave analysis, Least squares method, Electrical parameters estimation.

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1. Introduction

Sine wave signals are widely used in electronic applications from the amplitude and frequency modulation techniques in radio communications [1] to analog-to-digital converter (ADC) tests [2], from calibration of digital oscilloscopes [3] to control applications [4] and speech analysis and synthesis [5]. Another important field where sine wave signals play a main role is that of electrochemical sensors, where a sinusoidal test voltage is applied to the sample under test and its electrical characteristics are measured. In recent years, bacterial biosensors aimed to measure total microbial concentration as well as to detect the presence of dangerous pathogens have gained increased interest since allow fast product screening to eliminate possible threaths to consumers health. At this regard, it is estimated that each year food is responsible for 76 million cases of illnesses in the US, resulting in 325000 hospitalizations and 5000 deaths [6].

Bacterial contamination is currently measured by Standard Plate Count (SPC) technique [7]. SPC derives microbial concentration from the number of colony forming unit (cfu) grown on Petri plates filled with suitable enriching media and inoculated with serial dilution of the sample. SPC provides reliable measurements for bacterial concentration but is characterized by long response time (24-72 hours depending on the type of sample) and is essentially a laboratory based technique, requiring skilled personnel to be performed.

Research in the field of microbial biosensors has proposed a large variety of alternatives to SPC that are competitive in terms of measurement speed and are based on different transduction techniques such as impedance [8], bioluminescence [9], piezoelectricity [10] and flow citometry [11]. Such methods are characterized by lower response time than SPC (20 minutes to few hours) but they often requires complex procedures that can be performed only in laboratory based environments. The need of fast response as well as the possibility to perform measurements in-situ privileges biosensors that can be realized as portable systems or implemented in automatic form.

The sensing technique based on classic impedance microbiology [8], on the other hand, is attractive since it can detect bacterial concentration in less time than SPC (3-12 hours depending on the sample contamination) and can be easily implemented in automatic form. The authors have recently developed an embedded portable biosensor system [12], suitable for in situ measurements, that is characterized by ease of use and can be used also by non skilled operators, thus avoiding the need to ship the samples to laboratory for analysis. The impedance technique works as follows [8]: the sample under test is stored at a temperature favoring bacterial growth (generally in the range 30 °C to 42 °C) and its electrical parameters, i.e. the resistive and reactive components of the impedance Z, are measured at time intervals of 5 minutes. The electrical parameters are measured by applying a sinusoidal test signal of known amplitude and frequency to the sensor electrodes placed in direct contact with the sample and measuring the current through the electrodes. Until the bacterial concentration is lower than a critical threshold of 10^7 cfu/ml, the electrical parameters remain essentially constant at their baseline value, while, when it grows over this threshold, both the resistive and reactive components of |Z| begin to decrease. The time needed for the electrical parameters to deviate from their baseline values is called Detect Time (DT) and is known to be linearly related to the logarithm of sample bacterial concentration.

The accuracy in estimating the electrical parameters influences the accuracy of the measured DT and thus its correlation with the bacterial concentration determined by SPC. An algorithm for efficient analysis of sinusoidal signals and accurate parameters estimation is thus mandatory for a reliable application of the impedance technique.

Sinusoidal signal in its more general form can be expressed with four parameters (offset, amplitude, frequency and phase). Different algorithms have been proposed to estimate the sine wave parameters, based on different techniques such as Discrete Fourier Transform (DFT) [13], least squares (LS) fitting [14], quadrature delay estimator (QDE) [15] and maximum likelihood estimator (MLE) [16]. Of such algorithms, those based on least squares fitting are attractive and most of them use iterative procedures to solve the non linear equations. The IEEE Standard 1057 (IEEE-STD-1057) [17] provides algorithms for fitting the four-parameters sine wave signal to noisy discrete time observations. However, as pointed out in [14], sine wave parameters estimation is intrinsically non linear only in the frequency variable, while the other three parameters can be estimated by Linear Least Squares algorithm (LLS)

[17] if the value of frequency is known. Thus, an alternative technique is to estimate the frequency with nonparametric frequency estimators and then determine the remaining three variables by LLS, solving a linear system of three equations.

Iterative procedures generally provide good accuracy but convergence can be an issue. The same algorithm can lead to different results with different choices for starting values and stop criteria.

Inaccurate start values for the iterating procedure can make the algorithm to converge to a local minimum of the error function thus failing to provide an accurate parameter estimate. Low values of signal to noise ratio (SNR) can make the situation even worse.

In this paper we present a linear algorithm for four parameters sine wave fitting that performs parameters estimate without iterative procedures. The algorithm performance has been tested with numerical simulations as well as real sinusoidal voltage signals.

2. Impedance Technique for Bacterial Concentration Measurement

The system composed of a couple of electrodes and the liquid sample under test can be modeled with the electrical circuit presented in Fig. 1 (a), where R_i and C_i represent the resistance and capacitance of the electrode-electrolyte interface, while R_m and C_m are the medium resistive and capacitive components. When the frequency of the applied test signal is relatively low (< 1 MHz), the medium capacitance is negligible, thus the electrical circuit can be simplified, as shown in Fig. 1 (b), as the series of a resistance (R_s) and a capacitance (C_s).

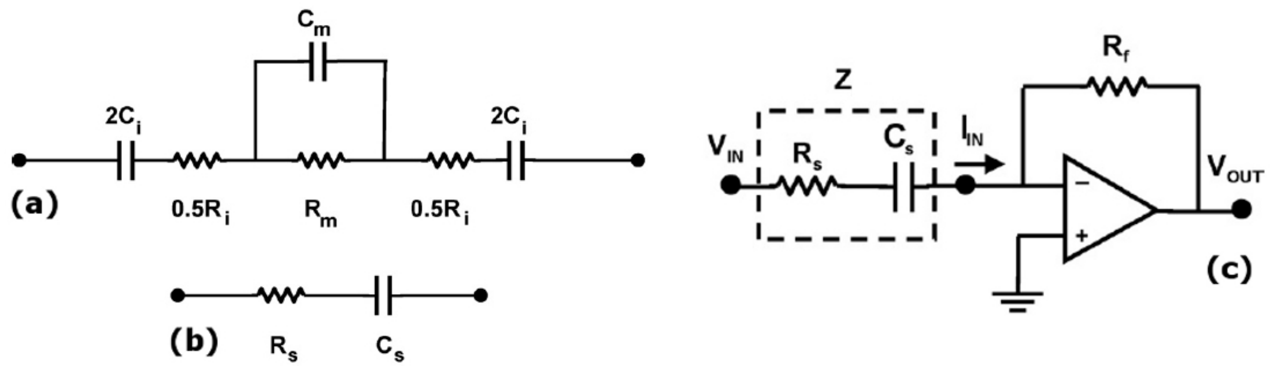

Fig. 1. Electrical model for the system composed of electrodes and the liquid media in direct contact (a). Simplified electrical model when the test signal frequency is relatively low (< 1 MHz) (b). Circuit used to measure the current through the electrodes when the test signal is applied (c).

The sample electrical parameters are measured with the circuit represented in Fig. 1 (c). A sinusoidal voltage test signal $V_{in}(t)=V_{Min}\sin(\omega t)=V_{Min}\sin(2\pi ft)$ (with V_{Min} values in the order of 50−120 mV and f from 50 to 200 Hz) is applied to the sensor electrodes (in direct contact with the sample) and the output voltage $V_{out}(t)=V_{Mout}\sin(\omega t+\varphi)=-R_f I_{in}(t)$ linearly related to the current through the electrodes is measured. It is:

$$Z = R_s + \frac{1}{j\omega C_s} = -R_f \times \frac{V_{in}(j\omega)}{V_{out}(j\omega)}$$

Thus:

$$R_s = \frac{V_{Min}}{V_{Mout}} R_f \cos(\varphi)$$

and

$$C_s = \frac{1}{\omega R_f} \frac{V_{Mout}}{V_{Min}} \frac{1}{\sin(\varphi)}$$

The resistive and capacitive components of Z can be worked out by measuring the sinusoidal signals parameters V_{Min}, V_{Mout}, φ and ω. Since for a reliable determination of DT (and thus of the microbial concentration) the electrical parameters must be determined with high accuracy (errors lower than 0.25%), the choice of an algorithm for sine wave analysis that is accurate enough and not too time consuming is mandatory in the application of the impedance technique.

3. Algorithm for Sine Wave Analysis

Given for the general four parameters sine wave signal the equation $y(t)=V_{dc}+V_{ac}\sin(\omega t+\varphi)$ we consider the following functions:

$$I(t) = \int_0^t y(\tau)d\tau = \int_0^t \left(V_{dc} + V_{ac}\sin(\omega\tau + \varphi)\right)d\tau = V_{dc}t + \frac{V_{ac}}{\omega}\cos(\varphi) - \frac{V_{ac}}{\omega}\cos(\omega t + \varphi)$$

And

$$M(t) = \int_0^t I(\tau)d\tau = \int_0^t \left(V_{dc}\tau + \frac{V_{ac}}{\omega}\cos(\varphi) - \frac{V_{ac}}{\omega}\cos(\omega\tau + \varphi)\right)d\tau =$$
$$= V_{dc}\frac{t^2}{2} + \frac{V_{ac}}{\omega}t\cos(\varphi) - \frac{V_{ac}}{\omega^2}\sin(\omega t + \varphi) + \frac{V_{ac}}{\omega^2}\sin(\varphi)$$

Thus:

$$M(t) = V_{dc}\frac{t^2}{2} + \frac{V_{ac}}{\omega}t\cos(\varphi) + \frac{V_{ac}}{\omega^2}\sin(\varphi) + \frac{V_{dc}}{\omega^2} - \frac{y(t)}{\omega^2}$$

The equation can be written as

$$K_1 t^2 + K_2 t + K_3 - K_4 M(t) - y(t) = 0$$

$$K_1 = \frac{V_{dc}\omega^2}{2},\ K_2 = V_{ac}\omega\cos(\varphi),\ K_3 = V_{dc} + V_{ac}\sin(\varphi),\ K_4 = \omega^2$$

Defining T_s the sampling period, N the number of samples and $y_n=y(nT_s)$ the sampled sine wave signal, the discrete time counterparts of I(t) and M(t) can be written as

$$I_n = I(nT_s) = \sum_{j=0}^{n} L_j(nT_s)y_j$$

and

$$M_n = M(nT_s) = \sum_{j=0}^{n} L_j(nT_s) I_j$$

where L_j is the interpolating polynomial used for discrete time integration. The equations are verified for n ranging from 0 to N-1. The sine wave parameters are then estimated in the least squares sense with the minimization of the cost function:

$$E = \sum_{n=0}^{N-1} \left(K_1 n^2 T_s^{\,2} + K_2 n T_s + K_3 - K_4 M_n - y_n \right)^2$$

Minimum of E is obtained by imposing the conditions

$$\frac{\partial E}{\partial K_1} = \frac{\partial E}{\partial K_2} = \frac{\partial E}{\partial K_3} = \frac{\partial E}{\partial K_4} = 0$$

that lead to the linear system of four equations in the four variables K_1, K_2, K_3, K_4:

$$\mathbf{A}\mathbf{x} = \mathbf{b}$$

where **A** is a 4x4 matrix, **x** and **b** are column vectors with 4 entries.

$$\mathbf{A} = \begin{bmatrix} T_s^{\,2}\delta_4 & T_s\delta_3 & \delta_2 & -\gamma_6 \\ T_s^{\,2}\delta_3 & T_s\delta_2 & \delta_1 & -\gamma_4 \\ T_s^{\,2}\delta_2 & T_s\delta_1 & N & -\gamma_2 \\ T_s^{\,2}\gamma_6 & T_s\gamma_4 & \gamma_2 & -\gamma_8 \end{bmatrix}, \quad \mathbf{x} = \begin{bmatrix} K_1 \\ K_2 \\ K_3 \\ K_4 \end{bmatrix}, \quad \mathbf{b} = \begin{bmatrix} \gamma_5 \\ \gamma_3 \\ \gamma_1 \\ \gamma_7 \end{bmatrix}$$

$$\delta_1 = \sum_{n=0}^{N-1} n = \frac{N(N-1)}{2}, \quad \delta_2 = \sum_{n=0}^{N-1} n^2 = \frac{N(N-1)(2N-1)}{6}, \quad \delta_3 = \sum_{n=0}^{N-1} n^3 = \frac{N^2(N-1)^2}{4}$$

$$\delta_4 = \sum_{n=0}^{N-1} n^4 = \frac{N(N-1)}{30}\left(6N^3 - 9N^2 + N + 1\right), \quad \gamma_1 = \sum_{n=0}^{N-1} y_n, \quad \gamma_2 = \sum_{n=0}^{N-1} M_n, \quad \gamma_3 = \sum_{n=0}^{N-1} n y_n, \quad \gamma_4 = \sum_{n=0}^{N-1} n M_n$$

$$\gamma_5 = \sum_{n=0}^{N-1} n^2 y_n, \quad \gamma_6 = \sum_{n=0}^{N-1} n^2 M_n, \quad \gamma_7 = \sum_{n=0}^{N-1} y_n M_n, \quad \gamma_8 = \sum_{n=0}^{N-1} M_n^{\,2}$$

The system, linear of four equations in four variables, can be solved using numerical computing environment software such as Matlab (MathWorks). Since the equations coefficients are of very different magnitude order, solving the system with singular value decomposition (svd) can help to improve the accuracy. Once solved in the variables K_1, K_2, K_3 and K_4 the four parameters of the sine wave signal can be determined as:

$$\omega = \sqrt{K_4}, \quad V_{dc} = \frac{2K_1}{K_4}, \quad V_{ac} = \sqrt{\frac{K_2^{\,2}}{K_4} + \left(K_3 - \frac{2K_1}{K_4}\right)^2}, \quad \sin(\varphi) = \frac{1}{V_{ac}}\left(K_3 - \frac{2K_1}{K_4}\right), \quad \cos(\varphi) = \frac{1}{V_{ac}}\frac{K_2}{\sqrt{K_4}}$$

Simulations have been carried out to test the reliability of the proposed algorithm. A voltage signal of frequency 100 Hz, offset 100 mV, amplitude 300 mV and phase -45° has been simulated. White noise has been added to the sine wave, that is the signal can be modeled with the function

$y(t)=V_{dc}+V_{ac}\sin(\omega t+\varphi)+N(0,\sigma_{Noise})$ where $N(0,\sigma_{Noise})$ is a Gaussian distributed white noise pseudorandom pattern whose statistical profile is $(0,\sigma_{Noise})$, with σ_{Noise} the absolute value of the specified standard deviation. Different values for $SNR=V_{ac}^2/(2\sigma^2_{Noise})$ as well as different sampling frequencies f_s have been tested.

Simulations and parameters estimation have been carried out with LabVIEW (National Instruments, USA) programs. Each test, performed on a single period of the signal, has been repeated 1000 times and, for each parameter, mean value and standard deviation have been evaluated.

Different numerical integration techniques have been implemented to calculate I_n and M_n and the obtained results compared. In Fig. 2 the percent error on the estimated mean value for each parameter is plotted vs. f_s/f (SNR 83 dB) for four different numerical integration methods: standard Riemann (rectangular integration), Newton-Cotes formula with n=1 (trapezoidal integration), n=2 (Cavalieri-Simpson formula) and n=3 (cubic interpolating polynomial). As can be seen, standard Riemann integration produces strongly biased estimates even for high sampling rate, while Newton-Cotes formula with n=3 is the most accurate for frequency estimation but have slightly worse performance than n=1 for the other three parameters at low sampling rate. However, when f_s/f is higher than 50 the percent error is lower than 10^{-3} % for all parameters. The different numerical integration techniques produce the same results regarding the standard deviation of the measured distribution for all parameters. In the following, parameters estimate is carried out using the Newton-Cotes formula with n=3 as interpolating polynomial in numerical integration.

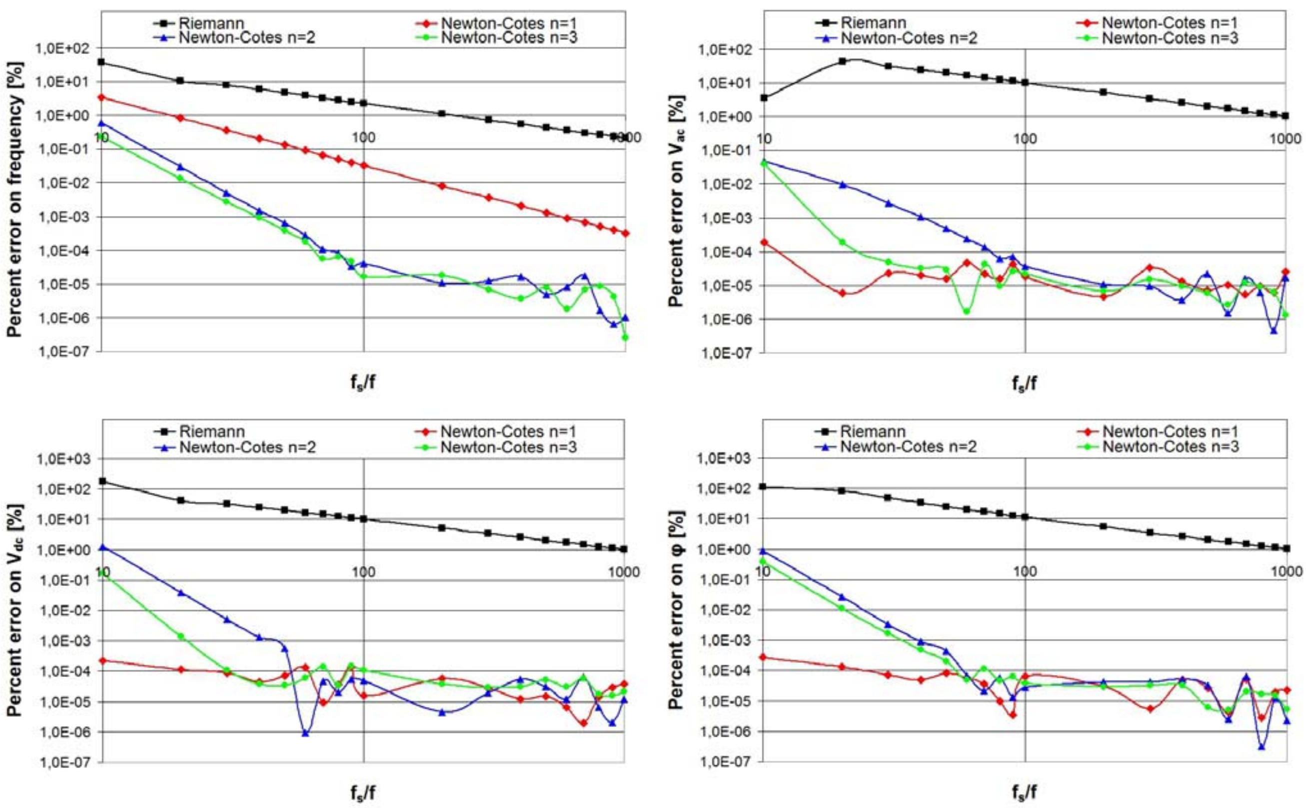

Fig. 2. Percent error on the mean value for the estimated parameters vs. f_s/f (SNR 83 dB) for four different numerical integration techniques: standard Riemann, Newton-Cotes formula with n = 1, 2, 3.

The dispersion in the estimated parameters is presented in Fig. 3, where the standard deviation is plotted vs. SNR for f_s/f 100. The algorithm performance has been compared with Cramér Rao lower bound (CRLB), i.e. the achievable lowest value of standard deviation for white Gaussian observation noise [17] and the results show that accuracy is very close to CRLB. The dispersion in the estimated parameters decreases with increasing SNR. Linear regression analysis has been carried out on the measured data and the results show that, for all estimated parameters, a 20 dB increase of SNR results in one order of magnitude decrease for the corresponding standard deviation.

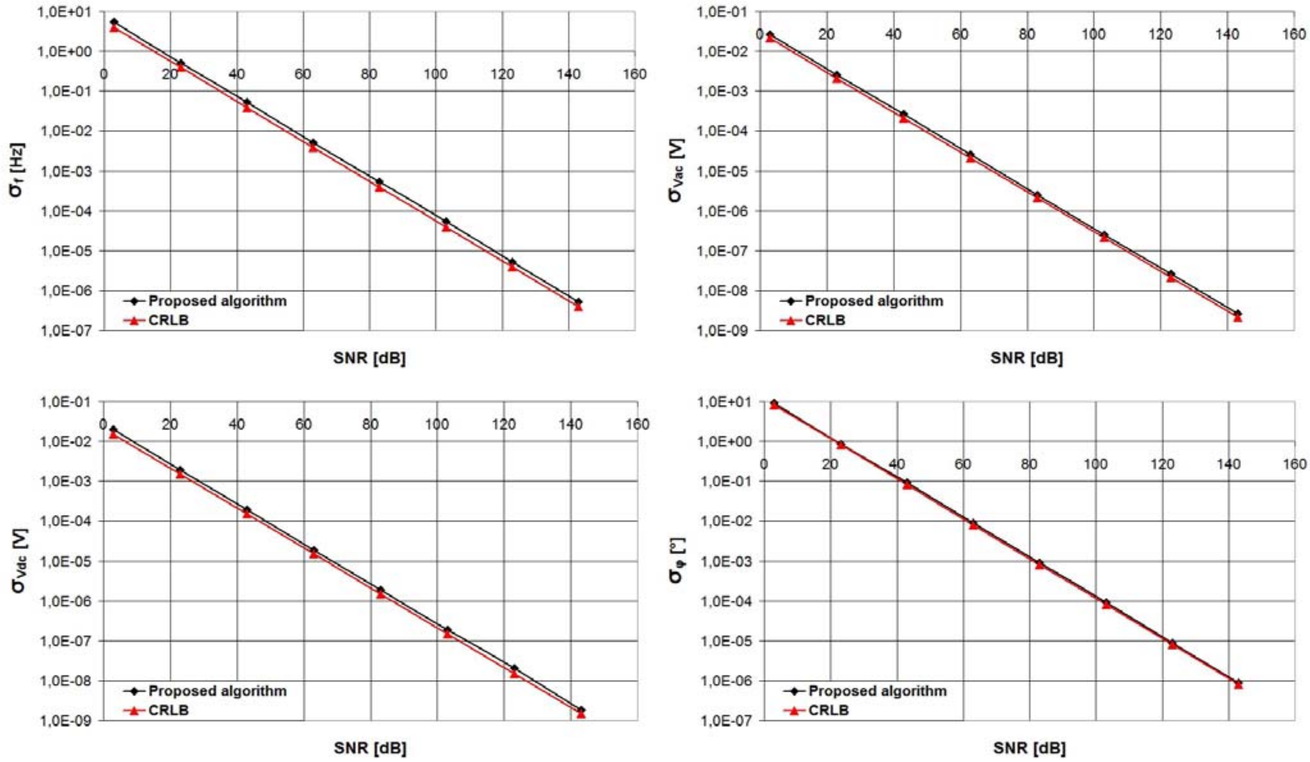

Fig. 3. Standard deviations of the measured distributions of the four parameters plotted vs. SNR (f_s/f 100) for the proposed algorithm and Cramér Rao lower bound.

4. Results from Real Impedances

The proposed algorithm has also been tested with real impedances realized with discrete resistors and capacitors. The sinusoidal test signal has been generated using a Tektronix AFG320 arbitrary function generator and acquired with a National Instruments USB-6211 data acquisition board featuring 16 input channels, 16 bits ADCs with a maximum sampling frequency of 250 Ksamples/s. The acquired samples are used as input to LabVIEW programs for parameters estimation. A Tektronix DPO 4032 digital phosphor oscilloscope has been used for calibration and test.

The sine wave test signal $V_{in}(t)$ of frequency 100 Hz, amplitude 100 mV, 0 V DC offset generated using the Tektronix AFG320 has been used as input for the I/V converter circuit shown in Fig. 1 (c), where a feedback resistor R_f of 1.2 kΩ has been used. Both signals $V_{in}(t)$ and $V_{out}(t)$ have been acquired and processed to estimate the signal parameters.

Three different sampling frequencies (f_s/f 50, 100 and 200) have been tested for an impedance featuring R_s = 180 Ω, C_s = 4.7 μF and an acquisition time of 150 ms (corresponding to 1.5 periods of the acquired signals). The results in Fig. 4 (where the percent dispersion of the measured parameter is presented) show that the algorithm can achieve errors lower than 0.2 % even with short time analysis. Higher sampling frequencies provides more accurate parameter estimation: a sampling frequency of

10 Ksamples/s results in a percent dispersion decrease, depending on the measured parameter, ranging from 29.74 % to 37.83 % compared to the 5 Ksamples/s case, while from 10 to 20 Ksamples/s the decrease is from 7.11 % to 23.04 %. In the following a sampling frequency of 10 Ksamples/s (f_s/f 100) has been used.

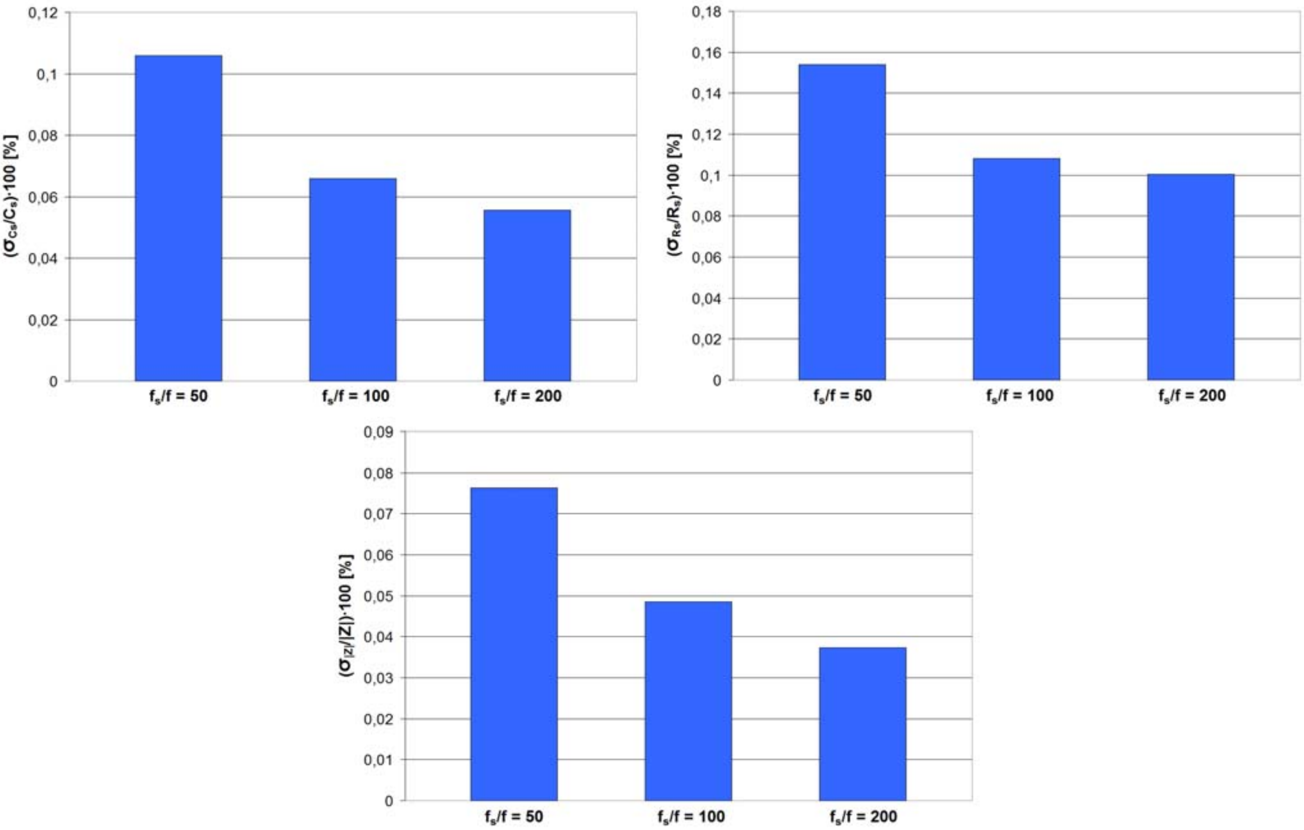

Fig. 4. Percent dispersion on the measured parameters C_s, R_s and $|Z|$ obtained with real impedance for an analysis time of 150 ms and different f_s/f values.

Different test impedances, featuring different ratios X_s/R_s, have been analyzed and the results presented in Table 1 (analysis time 150 ms). The percent dispersion on $|Z|$ is always lower than 0.2 %. For what regards R_s and C_s, an accurate estimation is achieved when the two components are of the same order of magnitude. When, on the other hand, this is not the case (III and V in Table 1), the percent error of the smaller component increases significantly (2 % for R_s in case III and 1% for C_s in case V). This situation, however, is unlikely to happen in the case of microbial biosensors based on the impedance technique, where the resistive and reactive components are of the same order of magnitude. If, however, this is the case for other types of applications, the percent error of the smaller component can be reduced to acceptable levels by averaging the estimated parameter on different signal periods. The efficiency of data averaging has been tested by estimating the smaller component in the case of averages on 4, 8 and 16 measures. The percent dispersion of the smaller component decreases of 55.4 %, 72 % and 81 % (in case III) compared to single measure when data is averaged on 4, 8 and 16 measures respectively. Regarding case V in Table 1, the corresponding reduction of the smaller component dispersion is 61 %, 79 % and 88 %. This shows that, even when the two components are not of the same order of magnitude, the smaller component can be estimated with good accuracy by averaging on less than 20 measures.

Table 1. Percent dispersion on the estimated parameters for real impedances of different values.

#	Parameters values	$(\sigma_{Cs}/C_s)\cdot 100$ [%]	$(\sigma_{Rs}/R_s)\cdot 100$ [%]	$(\sigma_{\|Z\|}/\|Z\|)\cdot 100$ [%]	X_s/R_s
I	R_s = 400 Ω , C_s = 2.2 μF	0.078	0.125	0.056	1.809
II	R_s = 180 Ω , C_s = 4.7 μF	0.065	0.108	0.048	1.882
III	R_s = 220 Ω , C_s = 0.68 μF	0.167	2.075	0.163	10.64
IV	R_s = 1500 Ω , C_s = 1 μF	0.184	0.127	0.102	1.061
V	R_s = 2700 Ω , C_s = 4.7 μF	0.929	0.090	0.093	0.125

5. Conclusions

An accurate algorithm to estimate the four sine wave parameters has been presented that is particularly suited for the analysis of low frequency signals and has been successfully implemented for the measurement of electrical parameters in an impedance based bacterial biosensor. The main advantage is that it doesn't require time consuming iterative procedures neither the knowledge of sinusoidal parameters starting values.

The results obtained with either simulations and measurements on real impedances show that it is suitable for accurate parameters estimation with low analysis time (less than two signal periods) when resistive and reactive components of the impedance are of the same order of magnitude. If this is not the case, accurate estimation can also be achieved by data averaging on multiple signal periods.

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