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PAPER

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Data-driven modeling of impedance biosensors: a subspace approach

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Abstract

A data-driven scheme for modeling electrical impedance in biosensors is presented by a subspace method working with the singular value decomposition of structured voltage and current data. Contrary to the classical electrical impedance spectroscopy (EIS) methods, our scheme uses simple instrumentation, works in time-domain, provides fast results, and does not require semi-empirical assumptions to retrieve structured models from data. We show how data-driven models exhibit a close relationship with lumped-element circuits, encoding dielectric and conductive properties detected by the sensor in the range from 10 kHz up to 10 MHz. Performance results are shown for calibration networks and two case studies: (i) a buffer solution, and (ii) a biological cell suspension. Finally, the viability of the scheme is discussed when compared with the classical EIS method.

Keywords: biosensors, subspace methods, impedance spectroscopy, data-driven models, signal processing

(Some figures may appear in colour only in the online journal)

1. Introduction

The analysis of biological media through biosensors and electrical impedance spectroscopy (EIS) is, nowadays, an attractive label-free method for understanding bio-electrical processes and characterizing the frequency dependence of dielectric and conductive phenomena [1, 2]. Owing to their high sensitivity and versatility, impedance biosensors are ubiquitous in several applications to electrically characterize biological media such as tissues [3], bacteria [4], cells [5, 6] and nucleic acids [7], to mention only a few. Once EIS is experimentally determined, its analysis involves data fitting to a user-defined lumped-parameter equivalent circuit model (ECM) [8]. This traditional workflow has some inherent limitations, mainly, the choice of a correct model structure able to capture the impedance dynamics and to provide physical

meaning to the parameters. To this end, one should have prior knowledge (PK) of the sample under study to select an adequate model, otherwise, either over- or under-fitting issues may limit the accuracy of the method [9].

Currently, several investigations have contributed with novel techniques to circumvent inherent limitations in EIS measurement and modeling. For instance, in [10] the authors implement a data-analytic modeling approach, able to decode electrical impedance measurements of different cell lines by using a polynomial regression. An impedance modeling method based on a genetic algorithm is presented in [11] to identify the most suitable model by solving an optimization problem. For speeding-up estimation results, in [12] a quadratic interpolation retrieves the parameters of a lumped-parameter network using embedded hardware. Also, in [13] introduces learning-based models to quantitatively relate the impedance parameters with bacterial concentrations at high reliability. More recently, [14] presents an impedance-based method for sensing biological cell suspensions which

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correlates a distribution function with impedance measurements to determine the concentration. In summary, trends in advanced optimization techniques and data-driven methods offer a flexible way to circumvent the limitations of the classical biosensors models [15].

On the other hand, an ECM has structural and dynamical properties which, under some conditions, can be described by a linear-time-invariant model [16–18]. For instance, a state-space (SS) model of bioimpedance for characterizing hematocrit is presented in [19]. In [20], the authors propose an infinite-impulse-response model that relates the parameters of an ECM for bioimpedance applications. Moreover, an autoregressive model for impedance measurements in micro-scale applications was introduced in [21]. However, the dynamical point-of-view for bioimpedance measurements has been barely explored, and its applications remain limited. One of the main drawbacks is how to obtain an adequate model that, on the one hand, captures the whole dynamics of the system with enough sensitivity; and on the other, relates the model parameters with an ECM to provide physical interpretation.

Nowadays, the availability of data from impedance measurements together with advanced processing techniques pave the road to develop novel data-driven methods to construct models describing impedance data in a robust way. In this work, we introduce, to the best of our knowledge, a novel scheme to estimate a model describing the underlying dynamics of impedance-based biosensors. The proposal works around the predictor-based subspace identification (PBSID) method, which estimates from time-domain data, an SS model and a transfer function (TF) encoding the parameters of an ECM. The subspace approach has three main contributions. (i) It uses simple instrumentation based on minimal hardware design and robust signal processing techniques instead of analog circuits for signal conditioning and transformation to retrieve impedance spectra. (ii) The proposal provides fast results compared to the classical EIS since our technique operates with broadband signals and non-iterative processing methods instead of sweeping the frequency of a single sine stimulus and time-consuming procedures for data fitting. (iii) The subspace method does not require semi-empirical assumptions to retrieve structured models from data. For all cases, we show the performance and limitations of the method in calibration networks and two case studies, which are compared with the classical EIS method, to assess the viability of our proposal.

The rest of the paper is organized as follows. Section 2 introduces theoretical basis of impedance measurements and the subspace modeling method. The details of the measurement setup and the sensor are presented in section 3. Experimental results for calibration networks and two case studies are shown in section 4 along a thorough discussion. Finally, section 5 is devoted to the conclusions derived from this work.

2. Theoretical framework

2.1. Principle of impedance biosensors

2.1.1. Electrical impedance spectroscopy. An impedance-based biosensor is commonly studied in a wide frequency

Figure 1. Working principle of impedance biosensors. (a) Sketch of the impedance measurement for a SUT. (b) Generalized ECM of the impedance $Z_{ECM}(\omega)$ describing the SUT.

range using EIS as follows. The biosensor is excited with a small-amplitude alternating signal $u(t)$, which produces a linear output $y(t)$ [22], i.e. the sample does not produce overtones [23]. Typically, the former is a voltage $v(t)$, whereas the latter is the current flowing through the sample $i(t)$. By taking the discrete Fourier transform $\mathcal{F}$ of such signals, $I(\omega_i) := \mathcal{F}\{i(t)\}$ and $V(\omega_i) := \mathcal{F}\{v(t)\}$, the measured impedance spectrum is given by

$$Z(j\omega_i) := \frac{V(\omega_i)}{I(\omega_i)} = |Z|(\cos(\phi) + j\sin(\phi)), \quad (1)$$

at an arbitrary frequency ω_i , where $j = \sqrt{-1}$, $|Z|$ is the impedance magnitude, and ϕ is the phase angle between the phasors, for $i = 1, 2, \dots, N$ frequency points.

2.1.2. Non-Faradaic (NF) impedance biosensors. In figure 1(a) we depict a simplified sketch of the working principle when measuring NF impedance in biosensors. It works in the frequency range from kHz up to tens of MHz, where dielectric effects such as interfacial and relaxation phenomena dominate [24]. Meanwhile, diffusion effects modeled by the Warburg impedance can be disregarded due to the absence of a redox couple and the relatively high-frequency range of study [25]. A working electrode (WE) provides the excitation signal $u(t)$ to the sample under test (SUT) and the sensing electrode (SE) detects the electrical response $y(t)$ of the sample. Under some conditions [26], the SUT has a specific linear impedance $Z_{SUT}(\omega)$, which depends on the angular frequency (ω_i), as well as on the physical properties of the sample, such as size, concentration, permittivity and conductivity [26].

2.1.3. Electrical circuit model (ECM). The ECM is one of the most common models to describe the response of an impedance-based biosensor [27]. A classical representation

of an ECM for NF impedance measurements is shown in figure 1(b), with an equivalent impedance $Z_{\text{ECM}}(\omega) \in \mathbb{C}$ given by

$$Z_{\text{ECM}}(\omega) = R_{\infty} + \frac{1}{j\omega C_{\text{DL}}} + \sum_{i=1}^n \frac{R_i}{1 + j\omega\tau_i}, \quad (2)$$

such that $Z_{\text{ECM}}(\omega) \approx Z_{\text{SUT}}(\omega)$. Without loss of generality, R_{∞} is the high-frequency dependent resistance, C_{DL} is the electrode-electrolyte double layer capacitance, and $\tau_i = R_i C_i$ is the i th time-constant associated to the effective capacitance C_i and resistance R_i of an i th process detected by the sensor. Albeit the impedance model (2) provides a simplistic way to describe the properties of the SUT, in practice, the capacitors C_{DL} and C_i may have a non-ideal behavior and should be reformulated as constant-phase elements (CPEs) [28] whose impedance is

$$Z_{\text{CPE}} = \frac{1}{(j\omega)^{\alpha} Q}, \quad (3)$$

where Q is the non-ideal capacitance and $\alpha \in (0, 1]$ is a fractional exponent tuning the non-ideality. Note that, if $\alpha = 1$ the CPE resembles an ideal capacitor. However, to avoid numerical complexity when dealing with fractional-order models as (3), throughout this work we assume an imperceptible contribution of CPE and use ideal capacitors instead [29]. Though this may not provide accurate fitting results as CPE do, it preserves physical meaning and yields a well-posed approximation to prove the data-driven performance of the PBSID technique.

2.2. PBSID for impedance biosensors

Let us consider that the dynamics of an electrical impedance biosensor described by the parametric model $Z_{\text{ECM}}(\omega)$, can also be modeled by the following SS model written in the predictor form [30] as

$$\mathcal{S}_p := \begin{cases} x_{k+1} &= \tilde{A}x_k + \tilde{B}z_k, \\ y_k &= Cx_k + Du_k + e_k, \end{cases} \quad (4)$$

which elements are given by $\tilde{A} = A - KC$, $\tilde{B} = [B - KD, K]$ and $z_k = [u_k^{\top}, y_k^{\top}]^{\top}$. In the above SS model, k is a discrete-time instant, $u_k \in \mathbb{R}$ and $y_k \in \mathbb{R}$ are the input and output sampled sequences of $u(t)$ and $y(t)$, respectively, $x_k \in \mathbb{R}^n$ is the predicted state and e_k is the innovation sequence. $A \in \mathbb{R}^{n \times n}$, $B \in \mathbb{R}^n$, $C \in \mathbb{R}^n$, $D \in \mathbb{R}$ and $K \in \mathbb{R}^n$ are the state, input, output, direct feed-through and Kalman gain matrices, respectively.

The problem addressed in this work is to provide a modeling scheme which (semi)-automatically estimates a SS model for impedance biosensors using the PBSID algorithm [31, 32]. The proposed approach can be summarized in the following steps.

2.2.1. Step 1: vector autoregressive with exogenous inputs (VARX) model estimation. Given an impedance measurement as shown in figure 1(a), the available set of measurements

is given by $\{(u_k, y_k) | k = 0, 1, 2, \dots, N\}$, where $u_k = u(t = k/f_s)$ and $y_k = y(t = k/f_s)$ are the discrete versions, sampled at f_s , of the input voltage and output current, respectively. Define $p \in \mathbb{N}^+$ and $f \in \mathbb{N}^+$ as the past and future data windows, respectively, with $f = p$. By iterative substituting (4) at an initial time $k = k - p$, the predicted state $x_k \in \mathbb{R}^n$ is

$$x_k = \tilde{A}^p x_{k-p} + \mathcal{K} z_{k-p}, \quad (6)$$

where $z_{k-p} = [u_{k-p,p}, y_{k-p,p}]^{\top}$ given by $u_{k-p,p} = [u_k, \dots, u_{k-p}]$ and $y_{k-p,p} = [y_{k-1}, \dots, y_{k-p}]$, and $\mathcal{K} \in \mathbb{R}^{n \times p}$ is the extended controllability matrix

$$\mathcal{K} = [\tilde{A}^{p-1}\tilde{B} \quad \tilde{A}^{p-2}\tilde{B} \quad \dots \quad \tilde{B}], \quad (7)$$

with $\tilde{A} \in \mathbb{R}^{n \times n}$, $\tilde{B} \in \mathbb{R}^n$ and p the past window. Moreover, it is known that: (i) impedance-based biosensors operate in open-loop, (ii) $Z_{\text{ECM}}(\omega)$ is stable, and (iii) $\tilde{A}^j = 0$ is nilpotent with degree p for all $j \geq p$ [33]. Then, $\tilde{A}^p \approx 0$, such that (6) simplifies as

$$x_k \approx \mathcal{K} z_{k-p}. \quad (8)$$

From (5) and (8), it follows that

$$y_k \approx \mathcal{M}_p z_{k-p} + Du_k + e_k, \quad (9)$$

where $\mathcal{M}_p \in \mathbb{R}^{(p+f)}$ is the set of Markov parameters given by

$$\mathcal{M}_p = CK = [\tilde{C}\tilde{A}^{p-1}\tilde{B} \quad \tilde{C}\tilde{A}^{p-2}\tilde{B} \quad \dots \quad \tilde{C}\tilde{B}]. \quad (10)$$

Hence, (9) corresponds to a VARX model structure, for which the optimal set $\mathcal{M}_p$ can be estimated by solving the following least-squares problem

$$\mathcal{M}_p^* = \min_{\Theta} \|Y_k - \Theta_p \mathcal{D}_{k-p,p}\|_F^2, \quad (11)$$

where $\mathcal{D}_{k-p,p} \in \mathbb{R}^{(p+f) \times (N-p)}$ is the input/output data matrix $[z_{k-1} \quad z_{k-2} \dots z_{k-p}]$, $Y_k \in \mathbb{R}^{(N-p)}$ is the predicted output vector $[y_p, \dots, y_N]$, and $\|\cdot\|_F$ denotes the Frobenius norm. Interestingly, the problem in (11) has a closed-form solution involving only algebraic operations.

2.2.2. Step 2: SS model estimation. Once the VARX was estimated, the next step is to estimate the number of states n , such that it will be possible to estimate the matrices A , B , C and D describing the dynamics of an impedance-based biosensor.

Now, we introduce the future horizon $f = p$ to define the extended observability matrix $\Gamma \in \mathbb{R}^{(f \times n)}$ as

$$\Gamma = \begin{bmatrix} C \\ C\tilde{A} \\ \vdots \\ C\tilde{A}^{f-1} \end{bmatrix}. \quad (12)$$

By multiplying the approximation in (8) by Γ , we have

$$\Gamma\mathcal{K} = \begin{bmatrix} C\tilde{A}^{p-1}\tilde{B} & C\tilde{A}^{p-2}\tilde{B} & \dots & C\tilde{B} \\ 0 & C\tilde{A}^{p-1}\tilde{B} & \ddots & C\tilde{A}\tilde{B} \\ \vdots & \ddots & \ddots & \vdots \\ 0 & \dots & 0 & C\tilde{A}^{p-1}\tilde{B} \end{bmatrix}, \quad (13)$$

which is an upper block triangular matrix. By inspection, one can approximate the matrix (13) with the optimal Markov parameters retrieved in (11). The approximated matrix $\widehat{\Gamma\mathcal{K}} \in \mathbb{R}^{p \times (p+f)}$ is given by

$$[\widehat{\Gamma\mathcal{K}}]_{ij} = \begin{cases} [\mathcal{M}_p^*]_{ij} & \text{for } i \leq j, \\ 0 & \text{for } i > j, \end{cases} \quad (14)$$

with $i = 1, \dots, p$ and $j = 1, \dots, (p+f)$. Then, it is possible to formulate the following problem

$$\Gamma\hat{X}_k = \widehat{\Gamma\mathcal{K}}\mathcal{D}_{k-p,p}, \quad (16)$$

given a matrix of state-sequences $\hat{X}_k \in \mathbb{R}^{n \times (N-p)}$ and the data matrix $\mathcal{D}_{k-p,p} \in \mathbb{R}^{(p+f) \times (N-p)}$. Thereby, the problem is to estimate the state-sequence $\hat{X}_k$ by computing the row space of $\widehat{\Gamma\mathcal{K}}\mathcal{D}_{k-p,p}$ via singular value decomposition (SVD) [34],

$$\widehat{\Gamma\mathcal{K}}\mathcal{D}_{k-p,p} = U\Sigma V^\top, \quad (17)$$

where $U \in \mathbb{R}^{p \times p}$ and $V \in \mathbb{R}^{(N-p) \times (N-p)}$ are orthonormal matrices, and $\Sigma \in \mathbb{R}^{p \times (N-p)}$ is a diagonal matrix of singular values. As the main result, SVD retrieves a vector $\tilde{\Sigma} \in \mathbb{R}^p$ containing the following useful information.

- The significant singular values of $\tilde{\Sigma}$ represent the number of states of the ECM.
- The number of states correspond to the optimal rank of the SVD [35], as well as an estimate of the ECM order, i.e. $\hat{n}$.

Such remarks allow us to avoid over-parametrization and also to reduce the SS model complexity [36]. Thus, the estimated state-sequence $\hat{X}_{k,\hat{n}}$ is straightforward computed as

$$\hat{X}_{k,\hat{n}} = \Sigma_{\hat{n}}^{1/2} V_{\hat{n}}^\top, \quad (18)$$

where $\hat{X}_{k,\hat{n}} \in \mathbb{R}^{\hat{n}}$ is the truncated SS sequence, $\Sigma_{\hat{n}} \in \mathbb{R}^{\hat{n}}$ is a diagonal matrix with the significant singular values and $V_{\hat{n}} \in \mathbb{R}^{\hat{n} \times (N-p)}$ is a matrix of significant vectors.

Once the state-sequence, as well as the output and input signals are known, the system matrices A, B, C, D and K are estimated by finding the optimal solution, in a least-squares sense, of the following linear problems

$$\hat{\mathcal{Q}} = \min_{\mathcal{Q}} \|\hat{X}_{k,\hat{n}} - \mathcal{Q}\mathcal{P}\|_F^2, \quad (19)$$

$$\hat{\mathcal{R}} = \min_{\mathcal{R}} \|Y_k - \mathcal{R}\mathcal{T}\|_F^2, \quad (20)$$

where $\mathcal{Q} = [\tilde{A} \ \tilde{B}]$, $\mathcal{P} = [\hat{X}_{k,\hat{n}} \ \mathcal{D}_{k-p,p}]^\top$, $\mathcal{R} = [C \ D]$ and $\mathcal{T} = [\hat{X}_{k,\hat{n}} \ u_{k-p,p}]^\top$. Once $\hat{\mathcal{Q}}$ and $\hat{\mathcal{R}}$ are estimated, the system matrices are straightforward given by

$$\hat{\hat{A}} = \hat{A} + \hat{K}\hat{C}, \quad (21)$$

$$\hat{\hat{B}} = [\hat{B} - \hat{K}\hat{D} \ \hat{K}], \quad (22)$$

and introducing the notation $\{i\}$ for indexing the i th column block of a matrix, we have that $\hat{\mathcal{R}}\{1\} = \hat{C}$, $\hat{\mathcal{R}}\{2\} = \hat{D}$, $\hat{\hat{B}}\{1\} = \hat{B} - \hat{K}\hat{D}$, $\hat{\hat{B}}\{2\} = \hat{K}$, such that $\hat{\hat{B}} = \hat{\hat{B}}\{1\} + \hat{K}\hat{D}$ and $\hat{\hat{A}} = \hat{A} + \hat{K}\hat{C}$. As a result, we can obtain a minimal realization of a SS model describing the dynamics of impedance-based biosensors.

2.2.3. Step 3: TF approximation. The estimated SS model relates the ECM of figure 1(b) by considering that the system states are the voltage drops in each R-C pair, for $i = 1, \dots, n$, and in the double-layer capacitance, such that $x_k = [v_1 \ \dots \ v_n \ v_{DL}]^\top$. Thereby, with the estimates $\hat{A}$, $\hat{B}$, $\hat{C}$ and $\hat{D}$, the PBSID-associated TF is $\hat{H}_{\text{PBSID}}(q) = \hat{C}(qI - \hat{A})^{-1}\hat{B} + \hat{D}$, where I the identity matrix and q is the forward shift operator. The TF $\hat{H}_{\text{PBSID}}(q)$ is then converted to its continuous-time version, $\hat{H}_c(s)$, by a bilinear transformation [37], such that $\hat{H}_c(s)$ encodes the parameters of an ECM (see figure 1(b)) as

$$\hat{H}_c(s; \theta) = \hat{d} + \frac{\hat{b}_{DL}}{s} + \sum_{i=1}^{\hat{n}} \frac{\hat{b}_i}{s + \hat{a}_i}, \quad (23)$$

with $\theta = [\hat{b}_1 \ \hat{b}_2 \ \dots \ \hat{b}_n \ \hat{a}_1 \ \hat{a}_2 \ \dots \ \hat{a}_n \ \hat{b}_{DL} \ \hat{d}]$, whose elements are given by equating (2) and (23) as follows

$$\hat{d} = R_\infty, \quad \hat{b}_{DL} = \frac{1}{C_{DL}}, \quad \hat{b}_i = \frac{1}{C_i}, \quad \hat{a}_i = \frac{1}{\tau_i}, \quad \frac{\hat{b}_i}{\hat{a}_i} = R_i. \quad (24)$$

Hence, one is able to estimate the circuit parameter by simple algebraic manipulations PBSID-based model and it does not require iterative methods for data fitting as in classical EIS [38].

2.2.4. Step 4: frequency response computation. As the last step, the frequency response (FR) of the model (23) is computed. Let consider $s = j\omega_k$, where ω_k is the k th frequency point of the FR such that an impedance spectrum $Z_{\text{PBSID}}(j\omega_k)$ can be computed as

$$Z_{\text{PBSID}}(j\omega_k) = \hat{H}_c(j\omega_k) \approx \hat{C}(j\omega_k I - \hat{A})^{-1}\hat{B} + \hat{D}, \quad (25)$$

for $\omega_k = \{\omega_1, \omega_2, \dots, \omega_F\}$ a set of excited frequencies within the band of interest. Interestingly, by using the FR, one is able

Figure 2. Schematic diagram of the measurement device for measuring input/output signals from impedance biosensors.

to numerically obtain an EIS-like description, thus avoiding large measurement times, bench-top instruments and semi-empirical procedures [39], and consequently, the estimated model has both time- and frequency-domain interpretation.

3. Experimental

3.1. Measurement device

The schematic diagram of the measurement device is shown in figure 2 and fully described in [21]. It is based on a custom-designed data acquisition system based on a XC7Z010 system-on-chip alongside a dedicated analog-front-end (AFE). An Artix-7 field-programmable gate array (FPGA) operates for signal generation and acquisition, whereas the AFE performs operations for signal conditioning and conversion. The input sequence u_k is generated by an AD9717 14-bit digital to analog converter (DAC) at a sampling rate $f_{sDAC} = 20$ MHz of an arbitrary waveform generator (AWG) module within the FPGA. The analog output $v(t)$ is applied to the WE of the sensor, which detects the impedance $Z_{SUT}(\omega)$. On the other hand, a low-noise transimpedance amplifier (TIA), built around an AD8066 chip, monitors the current $i(t)$ flowing through the sensor at the SE via the feedback impedance $Z_f = R_f / C_f$. The output of the TIA is then proportional to the current as $v_o(t) = i(t) \cdot Z_f$. Afterward, the output from the AFE is synchronously digitized by the digital module with an AD9246 14-bit analog to digital converter operating at a sampling rate $f_{sADC} = 100$ MHz to produce the output sequence y_k . Once the signals u_k and y_k are collected into a register (REG), the FPGA streams them to a 32-bit dual-core ARM Cortex-A9 processor. This latter runs an application for transferring the the input/output sequences to a personal computer where the PBSID algorithm is implemented.

3.1.1. Identification input signal. The PBSID method described in section 2.2, as other identification methods, must

Figure 3. PRBS excitation for impedance biosensors.

(a) Time-domain signal of order $m = 10$ and length $M = 1023$ samples. (b) Frequency-domain magnitude spectrum in the range from 19.5 kHz up to 10 MHz.

work under persistence of excitation (PE) [40] to excite all the modes of the system to provide consistent estimates of the model parameters. Even though in classical EIS, a sine wave is used as the excitation signal since it easy to generate, it does not satisfies the PE condition. Thereby, we use a pseudo random binary signal (PRBS) as the input signal $v(t)$ for the impedance biosensor.

In this work, the spectral content of the PRBS is band-limited in the frequency range of 10^4 – 10^7 MHz, to minimize the low-frequency diffusion and double-layer effects [41], and for accessing conductive and dielectric phenomena such as interfacial and relaxation processes [42]. Following this rationale, we designed a PRBS of order $m = 10$ with 1023 samples exciting the band from $f_{min} = f_{sDAC} / 2^m = 19.5$ kHz to $f_{max} = f_{sDAC} / 2 = 10$ MHz, determined by the DAC sampling frequency $f_{sDAC} = 20$ MHz. Figure 3(a) shows the designed PRBS in the time-domain, with an amplitude switching between ± 10 mV to work within the impedance linear region. The frequency-domain representation of the PRBS is shown in figure 3(b) showing a flat magnitude spectrum covering the frequency range of interest [43].

Figure 4. Sketch of the impedance sensor with interdigitated structure (not-to-scale).

3.1.2. Impedance sensor. The sensing element for bio-impedance applications is shown in figure 4. The sensor has a pair of gold micron-sized interdigital electrodes (IDEs) fabricated over a glass substrate with $10 \times 6.5 \times 0.4$ mm dimensions. In this work, the IDE-based biosensor comprises 100 bands of $W = 10$ μm wide, separated by a distance $G = 10$ μm , with a band length of $L = 1600$ μm . The experimental arrangement considers a polymeric chamber covering the IDE sensitive area and bonded to the substrate, meanwhile the contact pads of the electrodes are exposed to connect them to the measurement system as shown in figure 2. The plastic chamber allows to better control the experiments for working with a sample volume of 10 μl dropped over the IDE surface using a pipette.

4. Results and discussion

We present the results for two calibration networks with different dynamics to illustrate the versatility of our proposal. Moreover, the subspace modeling approach is applied in two experimental studies: a buffer solution and a biological cell culture. We test the performance of the PBSID scheme in the following scenarios. Firstly, the time-domain estimation accuracy is evaluated by the variance-accounted-for (VAF) given by

$$\text{VAF}_k = \left\{ 1 - \frac{\text{var}(y - \hat{y}_{\text{PBSID}})}{\text{var}(y)} \right\} \cdot 100\%, \quad (26)$$

where $\hat{y}_{\text{PBSID}}$ and y are the estimated and the measured output sequences, respectively, and $\text{var}(\cdot)$ denotes the variance. On the other hand, we obtain the FR of the PBSID-based model to compare it with the ideal impedance, for the calibration cases, and with classical EIS model, for the practical study cases. Similarly to (26), we use the frequency-domain VAF to determine the accuracy as

$$\text{VAF}_\omega = \left\{ 1 - \frac{\text{var}(Z - Z_{\text{PBSID}})}{\text{var}(Z)} \right\} \cdot 100\%, \quad (27)$$

with Z the ideal or true impedance, and Z_{PBSID} the impedance model retrieved by the subspace scheme. Note that (27) should

be evaluated for the impedance magnitude and phase, separately. Finally, to assess the parametric estimation performance we compute the deviation among the retrieved parameters as follows

$$\delta_p = \left| \frac{\theta_T - \theta_{\text{PBSID}}}{\theta_T} \right| \cdot 100\%, \quad (28)$$

where θ_T corresponds to the true parameter given by the exact value of the calibration network or by the classical EIS method, meanwhile θ_{PBSID} is the estimated parameter with the PBSID scheme.

4.1. Calibration networks

The proposed PBSID scheme was calibrated using two electrical networks constructed with passive discrete components with $\pm 1\%$ and $\pm 5\%$ tolerance, for resistors and capacitors, respectively. The true value of such a components was measured with a UNI-TTM UT612 commercial LCR meter (error $< 1\%$). For these experiments, we considered $R = 100$ repetitions to account for measurement variability. The time-domain VAF_k was computed for each repetition and then averaged over R . Then, the model parameters of the PBSID-based TF in (23) were compared with the nominal values to test the accuracy of our proposal. Ultimately, the frequency-domain VAF_ω was computed using the mean impedance spectra for the model in (25) and the ideal impedance in (2).

4.1.1. Single dispersion circuit. To test the subspace-based modeling, we start with simplest case, namely a single dispersion circuit with one time-constant [12]. The calibration network has the structure shown in the inset of figure 5(a), whose components have the values θ_T listed in table 1. In figure 5(a), we show the SVD plot for ten singular values, where only the first is significant due to its magnitude is, on average, four orders greater than that of the other singular values. Thus, the magnitude gap between the singular values 1 and 2 means that $\hat{n} = 1$ corresponds to the model order estimate. By inspection of the circuit structure, the first-order model has sense, owing to the network has one pole provided by the time-constant $\tau_1 = R_1 C_1$. As a result, the time-domain accuracy of the PBSID-based model was $\text{VAF}_k = 99.5\%$ for the estimated output sequence. On the other hand, table 1 summarizes the results for the single dispersion circuit, including the PBSID estimates θ_{PBSID} alongside their uncertainty s_p , as well as the deviation δ_p among the mean estimates by PBSID and the true values θ_T . Results show that the worst case was for the resistor R_∞ with an error of 2.25% which could be related to the high-frequency artifacts; meanwhile, for the other three parameters, the error was less than 0.30%, thus indicating enough accuracy of the data-driven model. As the last step, figure 5(b) shows the FR of both the ideal impedance Z_{ECM} (continuous line) and the FR of the PBSID model Z_{PBSID} (dashed line). The frequency-domain accuracy was determined with a VAF_ω of 99.6% for the magnitude and 99.4% for the phase, with a maximum error of ≈ 4.5 Ω and $\approx 0.63^\circ$, respectively. This test verifies that the data-driven modeling

Figure 5. Subspace modeling of a single-dispersion calibration network. (a) Singular value plot showing the estimated model order $\hat{n} = 1$ using the PBSID-approach. Inset: structure of the first-order measured circuit. (b) FR magnitude and phase spectra in the range from 10 kHz up to 10 MHz.

Table 1. Estimated parameters via PBSID modeling of a single dispersion circuit.

Parameter (units)	θ_T	$\theta_{\text{PBSID}} \pm s_p$	% δ_p
R_∞ (Ω)	221.50	216.51 ± 0.15	2.25
τ_1 (μs)	15.15	15.17 ± 0.27	0.13
C_1 (nF)	4.62	4.63 ± 0.03	0.21
R_1 (k Ω)	3.28	3.27 ± 0.04	0.30

scheme provides an adequate model order and structure, and the FR can be regarded as an EIS-like experiment.

4.1.2. Multiple dispersion circuit. The second network for calibration has a multiple dispersion structure, namely three time-constants as shown in the inset of figure 6(a). The true values of the circuit elements are listed in the second column of table 2. In figure 6(a) we show the singular value plot for the calibration network. Interestingly, the SVD plot depicts that the first three singular values are those significant since their large magnitude reflects useful information of the network dynamics; whereas, the indexes of singular values above 4 do not contribute to the circuit behavior. This rationale provides, therefore, an estimate $\hat{n} = 3$ of the model order. It can be confirmed given that the circuit corresponds to a third-order model given by the three time-constants τ_i for $i = 1, 2, 3$. Afterwards, the accuracy of the PBSID-based model was determined with a $\text{VAF}_k = 99.4\%$, thus indicating that the model well described the time-domain data. In table 2, we show the results for the parametric estimation via PBSID, and the error, % δ_p , among the mean estimates and the true values of the circuit elements. Interestingly, for all the parameters the accuracy is above 98.37%, with maxima errors of 1.17% for the capacitor C_2 and 1.63% for the time-constant τ_3 . On the other hand, figure 6(b) depicts the ideal impedance Z_{ECM} (continuous line) and the FR of the PBSID model Z_{PBSID} (dashed

line). The frequency-domain validation shows the largest error in frequencies below 10^5 Hz, leading to a VAF_ω of 99.5% and 98.7% for the magnitude and phase, respectively. Finally, this experiment verifies that the proposed PBSID scheme has enough sensitivity and resolution to identify relatively high-order models commonly encountered in bio-impedance applications [3].

4.2. Case study experiments

Once we validated the subspace approach on synthetic experiments, we modeled the impedance data of two media with interesting characteristics in biosensors field. These experiments were repeated $R = 10$ times to guarantee reproducibility and to statistically analyze the estimation results. Furthermore, we compared the FR of the data-driven model with the classical EIS, measured directly after our proposal using a single-sine sweep method in the range from 10^4 Hz up to 10^7 Hz. Finally, we fitted the measured data to the same model structure but considering CPE instead of ideal capacitors using the nonlinear least-squares method and the Levenberg–Marquardt algorithm, as in the classical EIS.

4.2.1. Phosphate buffer saline (PBS). As the first experiment, we measured PBS, an isotonic buffer commonly used in biological applications. The solution was prepared to obtain 1X PBS of pH 7.4, with conductivity $\sigma_{\text{PBS}} = 1.6 \text{ S m}^{-1}$ and relative permittivity $\epsilon_{\text{PBS}} = 78$. A droplet of 50 μl was placed over the electrodes of the sensor as shown in figure 4 and as described in section 3.

Figure 7(a) shows the SVD plot for ten values, where one can see that the singular values $\{1, 2\}$ are those significant. Hence, the magnitude gap, between the singular values 2 and 3, provides an estimate model order of $\hat{n} = 2$. Following the PBSID workflow, the accuracy of the SS second-order model

Figure 6. Subspace modeling of a multiple-dispersion calibration network. (a) Singular value plot showing the estimated model order $\hat{n} = 3$ using the PBSID-approach. Inset: structure of the third-order measured circuit. (b) FR magnitude and phase spectra in the range from 10 kHz up to 10 MHz.

Table 2. Estimated parameters via PBSID modeling of a multiple dispersion circuit.

Parameter (units)	θ_T	$\theta_{\text{PBSID}} \pm s_p$	% δ_p
R_∞ (Ω)	50.26	50.20 ± 0.18	0.12
τ_1 (ns)	3.44	3.42 ± 0.25	0.58
C_1 (pF)	10.30	10.29 ± 0.75	0.09
R_1 (Ω)	331.80	332.28 ± 2.60	0.14
τ_2 (ns)	58.47	58.46 ± 0.78	0.01
C_2 (pF)	295.60	292.14 ± 4.06	1.17
R_2 (Ω)	199.76	200.13 ± 0.04	0.18
τ_3 (μ s)	2.44	2.40 ± 0.26	1.63
C_3 (nF)	4.80	4.82 ± 0.18	0.41
R_3 (Ω)	503.17	498.80 ± 5.63	0.86

was determined with a $\text{VAF}_k = 99.1\%$. Then the SS representation was converted to the following TF

$$\hat{H}_{\text{PBS}}(s; \hat{\theta}) = \frac{\hat{b}_{\text{DL}}}{s} + \frac{\hat{b}_1}{s + \hat{a}_1}, \quad (29)$$

where $\hat{\theta} = [\hat{b}_1 \ \hat{a}_1 \ \hat{b}_{\text{DL}}]$ is the vector of estimated coefficients, and in turn, it is related to the equivalent circuit parameters as in (24). Owing to the electrical properties of PBS and the impedance measurement effects, the TF (29) is a second-order model with an integrator modeling the double-layer capacitance, as well as one pole related to the time-constant provided by the conductive and dielectric phenomena of the electrolyte under study. Hence, it is straightforward to deduce that, the TF has the equivalent circuit structure as shown in the inset of figure 7(a). Next, we compute the FR of (29) in the range from 10 kHz up to 10 MHz according to (25). Figure 7(b) depicts the Bode plots for the experimental impedance data (dots), the EIS model (dashed line) and the FR of the PBSID model (continuous line). As expected, the PBSID well

approaches experimental data and EIS with moderate discrepancy in the low-frequency magnitude and the high-frequency phase. Also, from figure 7(b), it is possible to highlight the following observations.

- In the low-frequency range, below 10^5 Hz, the straight-line in the magnitude and the phase value $\approx 90^\circ$ are related to capacitive effect due to the electrode-electrolyte interface, which shunts the PBS conductivity.
- In the high-frequency range, above 10^5 Hz, the electrical properties of PBS become dominant given by the change in phase values, and the drastic drop in the magnitude reaching a low resistance value.

To evaluate the model performance, we computed the VAF_ω among the PBSID and EIS models, thus retrieving an accuracy of 99.3% for the magnitude and 97.1% for the phase. It confirmed that computing the FR of the PBSID-model is equivalent to the classical EIS measurement. To quantitatively assess the results, in table 3 we show the mean value of the estimated parameters with the data-driven model and their parametric uncertainty % s_p . Likewise, table 3 summarizes the values obtained by fitting EIS data to the proposed ECM, alongside with the parametric error % e_p indicating the goodness of the fit. From table 3 one can see that both methods retrieved similar for the ECM parameters. Regarding the PBSID estimates, these show consistency by keeping an uncertainty below 1.0% for all parameters. On the other hand, the EIS method retrieved a maximum fitting error of 1.62% for the capacitor C_{DL} . Although PBSID and EIS results are similar, the double layer capacitance C_{DL} showed a deviation % $\delta_p = 3.69$, which is due to the non-ideal nature modeled by a CPE. Furthermore, it is worth to notice that the ECM parameters of table 3 can be related with the impedance spectra of figure 7(b). The double layer capacitance produces

Figure 7. Subspace modeling of a phosphate buffer saline (PBS). (a) Singular value plot showing the estimated model order via PBSID. Inset: structure of the proposed equivalent circuit. (b) FR magnitude and phase spectra in the range from 10 kHz up to 10 MHz.

Table 3. Estimated ECM parameters for 1X PBS solution using the PBSID scheme and the classical EIS method.

ECM Parameter (units)	θ_{PBSID}	% s_p	θ_T	% e_p	% δ_p
C_{DL} (pF) ^a	99.2	0.71	103	0.70	3.69
C_1 (pF)	0.73	0.23	0.74	0.93	1.35
R_1 (kΩ)	7.82	0.25	7.81	0.56	0.12
τ_1 (ns)	5.78	0.26	5.77	0.44	0.17

^a For EIS C_{DL} is a CPE with $\alpha = 0.9972$ (see equation (3)).

a large impedance in the low-frequency band. Meanwhile, the resistance of PBS, R_1 , encodes its conductivity given by the flat magnitude response above 10^5 Hz. Also, the small capacitance C_1 relates the permittivity of the ionic solution as can be seen at frequencies above 10^6 Hz. With these results, we demonstrate how our method can derive a data-driven model for buffer solutions with a physical meaning.

4.2.2. Biological cells in suspension. The second experiment consisted in the measurement of a biological cell suspension to detect the underlying electrical processes. The sample was composed by cultured human keratinocyte cells of the HaCaT cell line. Briefly, the cells were cultured in Dulbecco's modified Eagles minimal essential medium. Afterwards, the cell culture was incubated at 37 °C and 5% CO_2 for 72 h. Finally, the culture was washed and resuspended in PBS to further obtain a suspension of approximately 1000 cells. A 50 μl volume of the PBS-HaCaT cells mixture was placed over the electrodes and measured as described in section 3.

Figure 8(a) shows the SVD plot for the cell suspension, where the significant singular values are at the indices $\{1, 2, 3\}$, whose magnitude is at least two orders above the others singular values. Thus, the slot in the magnitude between the singular values 3 and 4, means that useful information of the cell suspension can be described with a SS model of order $\hat{n} = 3$. In line with this finding, the accuracy of the model was

determined with a $\text{VAF}_k = 98.7\%$, such that the TF model describing the impedance of the cell suspension is

$$\hat{H}_{\text{cells}}(s; \hat{\theta}) = \frac{\hat{b}_{\text{DL}}}{s} + \frac{\hat{b}_1}{s + \hat{a}_1} + \frac{\hat{b}_2}{s + \hat{a}_2}, \quad (30)$$

with $\hat{\theta} = [\hat{b}_1 \ \hat{a}_2 \ \hat{b}_2 \ \hat{a}_1 \ \hat{b}_{\text{DL}}]$ the parameters encoding the ECM structure. The TF (30) captures the dynamics of the cell suspension as follows. The integrator $\hat{b}_{\text{DL}}/s$ models the double-layer capacitance of the electrode-PBS interface. Also, there are two first-order processes, each one modeling a resistive-capacitive effect in the sample. The first one could be related to the electrical properties of the PBS according with our previous findings; whereas, the second could be associated to the dielectric nature of the cells membrane. The ECM describing the cell suspension is shown in the inset of figure 8(a). In line with the PBSID scheme, we computed the FR of the model in (30), from 10 kHz to 10 MHz to compare it with the classical EIS method. In figure 8(b), we show the Bode plots for the experimental impedance data (dots), the EIS model (dashed line) and the FR of the PBSID model (continuous line). Therein, it is clear to see that the impedance magnitude $|Z|$ and the phase ϕ are comparable for both methods, with a maximum difference at the high-frequency for the phase values. Also, figure 8(b) confirms an adequate model structure given by the following remarks.

Figure 8. Subspace modeling of HaCaT biological cells in suspension. (a) Singular value plot showing the estimated model order via PBSID. Inset: structure of the proposed equivalent circuit. (b) FR magnitude and phase spectra in the range from 10 kHz up to 10 MHz.

Table 4. Estimated ECM parameters for HaCaT cells in suspension using the PBSID scheme and the classical EIS method.

ECM Parameter (units)	θ_{PBSID}	% s_p	θ_T	% e_p	% δ_p
C_{DL} (pF) ^a	87.9	1.09	89.8	0.49	2.12
C_1 (fF)	3.56	2.39	4.57	0.54	22.1
R_1 (kΩ)	94.2	1.58	94.1	0.20	0.10
τ_1 (ns)	0.34	1.63	0.43	0.54	12.2
C_2 (pF)	0.88	0.34	0.89	0.92	1.14
R_2 (kΩ)	69.4	0.64	69.5	0.36	0.14
τ_2 (ns)	61.1	0.64	60.5	0.98	0.99

^a For EIS, C_{DL} and C_1 are CPE with $\alpha = 0.9842$ and $\alpha_1 = 0.7721$, respectively (see equation (3)).

- At frequencies below 10^5 Hz the high magnitude and phase reflect the double-layer capacitance due to the electrode-PBS interface.
- At frequencies within 10^5 – 10^6 Hz, the influence of the PBS conductivity is more evident as the impedance magnitude is flat and the phase tends to increase.
- At frequencies above 10^6 Hz, the dielectric effect of the cell membrane is reflected by an inflection point in the magnitude and a local minimum in the phase near to 4 MHz.

Taking the VAF_ω as the metric of performance, the frequency-domain accuracy was 99.5% and 97.4% for the magnitude and phase, respectively. In table 4, we summarize the parameters retrieved by the PBSID model (θ_{PBSID}) and with the classical EIS (θ_T). For both cases, we show the performance analyses given by the parametric uncertainty % s_p for PBSID, and the parametric error % e_p for EIS fitting. Though the discrepancy % δ_p among the parameters is low for almost all cases, the capacitor C_1 and the time-constant τ_1 have the largest deviation. This is due to C_1 is not an ideal capacitor and is better to model it with a CPE. Furthermore, the ECM parameters in table 4 have a close relationship with electrical processes of the cell suspension and the FR in figure 8(b). The double layer is modeled by the capacitance C_{DL} , conducting to high impedance values at the low-frequency band. On the other

hand, the R-C parallel network, comprising R_1 and C_1 , is modeling the presence of HaCaT cells in the sample, and is identified at frequencies above 10^6 Hz. This can be regarded as the effective capacitance and resistance describing the interfacial polarization among the cellular membrane and the surrounding medium. Ultimately, the second R-C circuit, with elements R_2 and C_2 , describes the electrical properties of the buffer solution, i.e. PBS. This can be confirmed by the flat magnitude response in the intermediate frequency range, as well as by the effective value of the capacitance C_2 , influenced by the PBS permittivity ($\epsilon_{\text{PBS}} = 78$) which is three orders of magnitude greater than the HaCAT cells capacitance, C_1 . According to this experiment, we show that the PBSID scheme is able to describe the electrical behavior of a cellular suspension, and it could be extended to further studies such as for monitoring cell growth and morphology, fouling and biophysical processes, to mention only a few.

4.3. Benchmark test

For comparison purposes, we contrasted the PBSID with the rule-of-thumb EIS for impedance biosensors. To this end, we selected eight scores: the measurement time (t_{meas}), the time required for run the fitting algorithm (t_{run}), if PK of the model structure is required, the optimization method for parameter

Table 5. Benchmark score for PBSID and the classical EIS.

Method	t_{meas} (ms)	t_{run} (s)	PK	OM	$\# \theta^a$	T/F	CPE	% A
PBSID	~ 2.73	~ 0.72	No	Algebraic	$2(n)+2$	Both	No	$>90\%$
EIS	~ 12.46	~ 3.85	Yes	Iterative	$3(n)+3$	F	Yes	$>99\%$

^a n : Number of R-C circuits.

estimation, the number of parameters ($\# \theta$) to estimate, if works in time- and frequency-domain (T/F), the ability to work with CPEs, and the accuracy of the method (% A). Table 5 summarizes the performance of the PBSID and EIS methods.

From the benchmark test, one can notice that PBSID measures faster, has superior performance for signal processing, and is less computationally expensive than EIS. Though PBSID is a data-driven method working with algebraic methods for parameter estimation, its major drawback is that it cannot model CPE. This situation, indeed, currently limits the fitting accuracy of the PBSID method.

5. Conclusion

In this work, we introduced a data-driven scheme that automatically models the dynamics of impedance-based biosensors. The approach was based on the PBSID method working around the SVD of structured voltage and current signals. We showed how the subspace approach could estimate optimal ECMs without PK of the physical sample properties. The frequency-response of the derived data-driven models was comparable with classical EIS experiments from 10 kHz up to 10 MHz, thus demonstrating the versatility of our approach. Due to PBSID cannot model CPEs, the proposed approach cannot compete in accuracy with the classical EIS methods. However, the obtained accuracy is acceptable to model dielectric phenomena of biological media for frequencies above tens of kHz. We expect to improve the PBSID for modeling non-ideal circuit elements so the accuracy could be enhanced. Thereby, it will be possible to broaden the applications of the PBSID to model low-frequency phenomena, such as the Warburg impedance.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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