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Impedance Spectroscopic Analysis of the Interidigitated Flexible Sensor for Bacteria Detection

Mitar Simić, *Member, IEEE*, Tijana Kojić, Milan Radovanović, Goran M. Stojanović, *Member, IEEE*, and Hani Al-Salami

***Abstract*— In this paper we presented economical interdigitated flexible sensor on the paper substrate for bacteria detection. The impedance measurement was performed in the frequency range of 12.6 kHz–100 kHz for the suspensions with cell density of $1.5 \cdot 10^8$ CFU/mL, $1.5 \cdot 10^5$ CFU/mL and $1.5 \cdot 10^2$ CFU/mL of *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria cells, as well as for a sterile saline. Using the measured impedance data, we observed that sensor impedance magnitude increases when bacteria concentration increases. Moreover, impedance spectroscopic analysis was carried out to identify an equivalent electrical circuit for the sensor modelling. Through statistical analysis it was found that all model parameters changes according to the bacteria concentration. Additionally, obtained results showed that all fittings results in root mean square error lower than 0.076 Ω for impedance magnitude, and 0.054° for impedance phase angle. Moreover, maximum relative errors for impedance magnitude and phase angle were 0.329% and 2.898%, respectively.**

***Index Terms*— bacteria sensor, electrochemical impedance spectroscopy, electrical equivalent circuits, flexible sensors, fractional order elements.**

I. Introduction

BACTERIAL infections represent a serious threat to human [1]. Among the most common bacteria are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Clostridium difficile* and *Escherichia coli*, which can cause disease in plants and animals, as well as in humans [2]. Commonly used methods for bacteria detection are phenotypic methods (biochemical testing, use of chromogenic media) and molecular methods (hybridization-based detection, amplification methods, whole genome sequencing, and similar) [3]. However, such methods are usually time-consuming, require trained staff and have limited *in-situ* application outside the laboratory [4]. With recent technology enhancement, alternative approaches for bacteria detection and monitoring based on development and use of various biosensors [5], biofilms [6], electrochemical sensors [7], CMOS integrated bacterial sensor [8], electromagnetic sensors with microfluidic cell structure [9], electrical-optical integration [10], and image processing methods [11] were reported.

In comparison to above mentioned alternative approaches, usage of intedigitated electrode (IDE) sensors offers benefits such as economical and short development process, efficient fabrication procedures, as well as small dimensions and low power consumptions [12]. Moreover, impedance changes of IDE sensors due to the presence of parameter of interest, can be easily measured with commercial impedance analyzers (e.g. HP4194A, E4990A) but with cost effective portable embedded hardware [13], too. Because of above mentioned benefits, IDE sensors were used in applications such as blood biomarkers diagnosis [14], glucose level determination [15], ammonia detection [16], and D-serine detection [17]. Moreover, in recent related work [18], it was shown that the resonant frequency of IDE flexible sensor can be used for detection of bacteria presence in saline.

For better understanding of sensing mechanism, sensors are usually presented and analysed with equivalent electrical circuits. Structure of the used electrical circuit to model given sensor, is based on the *a priori* knowledge and understanding of the different physical phenomena (such as conduction, adsorption, polarization, etc.) existing in the analysed sen-

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sor. Equivalent electrical circuits usually consist of basic lumped electrical elements (resistors and capacitors) [19], or an empirical elements (Warburg diffusion element and constant phase element (CPE)) [20]. However, finding the mathematical expression describing sensor impedance changes as a function of measured parameter and the frequency of supplying voltage usually is not straightforward task. Quality of the proposed model is very often determined based on relative error between measured and fitted data. Moreover, correlation between changes of estimated model parameters with changes of measured values is required to ensure that applied model is appropriate.

In this paper we present paper-based flexible IDE sensor for *Pseudomonas aeruginosa* and *Staphylococcus aureus* detection. Sensor design and fabrication procedure are discussed in details. Moreover, impedance spectroscopic analysis was carried out to identify an equivalent electrical circuit for the sensor modelling in the frequency range from 12.6 kHz to 100 kHz. Values of model parameters are estimated for reference (sterile) saline, as well as for contaminated solutions with $1.5{\cdot}10^2$ CFU/mL, $1.5{\cdot}10^5$ CFU/mL and $1.5{\cdot}10^8$ CFU/mL of *Pseudomonas aeruginosa* and *Staphylococcus aureus* cell density. Contribution of our approach is elimination of a need for wide frequency sweep (required for determination of the resonant frequency of the sensor [18]) as single-frequency impedance measurement is enough for detection of bacteria presence. Such approach reduces overall cost of the measurement device and allows integration with the sensor in portable bacteria detection device [21]. Moreover, proposed equivalent electrical circuit provide detailed analysis of sensing mechanism for bacteria detection without need for complex mathematical models presented in [22] and [23].

This paper is organized as follows. The section II describes main elements of sensor design as well as commonly used electrical circuits for IDE sensor modeling. We also present basic elements of Complex Nonlinear Least Squares (CNLS) method for parameter estimation. In the Section III, the main experimental results in sensor fabrication process are given. Moreover, the impedance spectroscopic analysis of the sensor is presented as well as parameter estimation of the proposed equivalent electric circuit using CNLS. Furthermore, sensor selectivity was discussed. The results are summarized in the Section IV. Directions of future work are identified as well.

II. Materials and Methods

A. Sensor design

The sensor was designed as an IDE structure with electrodes consisting of eight fingers. Based on our previous study [18], the general dimensions of the sensor were 17.75 mm x 7.95 mm, as it is shown in Fig. 1. The paper from company Felix Schoeller Group was used as a substrate [24], with total thickness around 210 µm. For sensing film of conductive electrodes the silver ink was chosen to be printed on the paper substrate. Paper is cost effective and mechanically flexible substrate. Furthermore, it can be rolled around different shapes (for example test tube) by means of additive manufacturing technology such as ink-jet printing. Such technology also saves materials and environment. Additionally, reported advantages of using paper as a sensing substrate are compatibility with chemicals/biochemicals [25], passive liquid transport and three-dimensional fibrous structure [26]. However, paper-based sensors have known limitations such as accuracy and sensitivity, but recent advances in fabrication and analytical techniques significantly improve their performance and application [25].

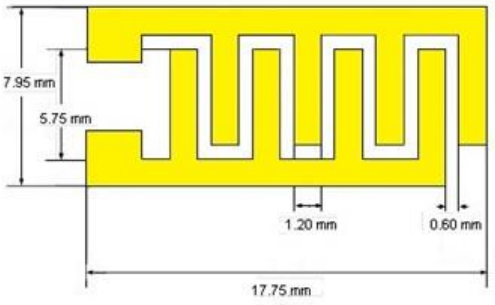

Fig. 1. The general dimensions of the fabricated IDE sensor.

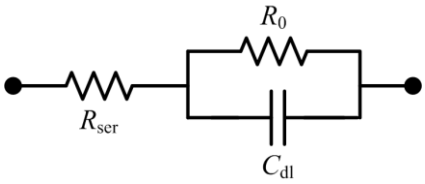

Fig. 2. Simple 2R1C model of electrochemical cell.

B. Equivalent electrical circuit for impedance spectroscopic analysis of the sensor

From a theoretical point of view, immersed IDE sensor in a solution can be treated as simple electrochemical cell. If IDE is analysed at relatively high frequencies (where mass-transport effects are minimal) it can be presented with equivalent electrical circuit known as simplified Randles model of electrochemical cell (Fig. 2) [27]. As it is consists of two resistors and one capacitor, it is also known and as 2R1C model of electrochemical cell. Elements of equivalent electrical circuit form Fig. 2 have physical interpretation as follows: R_{ser} is the solution resistance, R_0 charge transfer resistance and C_{dl} is the double-layer capacitance.

The double-layer capacitance is formed on the interface between electrode and solution [27]. C_{dl} depends of dielectric permittivity of the double-layer (c_{dl}), the area of the electrodes (S), and the thickness of the double-layer (d). Thus, the double-layer capacitance can be calculated as:

$$C_{dl} = c_{dl}\frac{S}{d}. \quad (1)$$

The complex impedance $\underline{Z}$ of electrical network presented in Fig. 2 at some angular frequency ω [rad/s] can be expressed as follows:

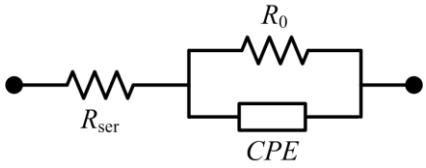

Fig. 3. Model of electrochemical cell with included CPE instead of capacitor.

$$\underline{Z}(\omega) = Ze^{j\varphi} = R_{ser} + \frac{1}{\frac{1}{R_0} + j\omega C_{dl}} \quad (2)$$

where Z denotes impedance magnitude, and φ is phase angle of impedance.

However, reported experimental work showed that double-layer usually cannot be adequately represented as an ideal capacitor [28]. Such electrochemical system is then modelled with a constant phase element (CPE) instead the ideal capacitor, as it is shown in Fig. 3.

The complex impedance of CPE, in general case, can be calculated as:

$$\underline{Z}_{CPE}(\omega) = \frac{1}{C(j\omega)^{\alpha}} \quad (3)$$

where C and α are frequency independent parameters: C is capacitance, while α takes values between 0 and 1 and presents an exponent tuning the non-ideality. If α=1, CPE resembles a capacitor, while for α=0.5 CPE presents a Warburg impedance.

The complex impedance of electrical network presented in Fig. 3 is:

$$\underline{Z}(\omega) = R_{ser} + \frac{1}{\frac{1}{R_0} + C_{dl}(j\omega)^{\alpha}}. \quad (4)$$

C. Complex Nonlinear Least Squares method for parameter estimation

Parameter estimation is required for obtaining quantitative information from equivalent electrical circuits of analyzed sensor. Classical parameter estimation methods are based on least-squares fitting [29], but non-iterative approaches are reported for some simple models [30], [31]. Nonlinear Least Squares is a data fitting method for finding the values of model parameters to minimize the squared difference between measured and estimated data. In the case of complex-valued input, it is also called Complex Nonlinear Least Squares Method (CNLS). Commonly used algorithms are Gauss–Newton, Levenberg–Marquardt and trust-region [32].

III. Experimental

A. Sensor fabrication

Sensor electrodes were printed on the paper substrate with the Dimatix deposition material printer - DMP3000 [33], located in a ISO 5 / ISO 7 class clean room. Commercially available silver ink was used for fabrication [34]. Before printing, paper substrate was located on stand that was heated to 50°C. After printing structure was heated to 120°C for 30 min.

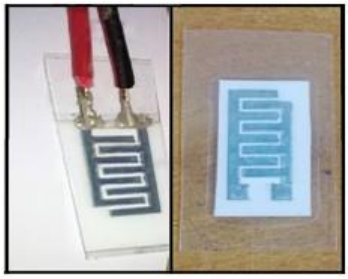

Fig. 4. Fabricated sensor on paper substrate.

Fig. 5. Laboratory measurement setup.

Next step in sensor fabrication was plastic foil lamination on 150°C to cover silver ink electrodes and paper substrate. Lamination with plastic foil of thickness 80 µm was done to prevent impurities which could affect the measurements, as well as to prevent oxidation and ageing degradation of fabricated sensors. In the same time, space between fingers of interdigitated electrode structure was opened in order to enable interaction with studied medium and to provide sensing effect. Total thickness of sensor after fabrication was 369 µm. Finally, ing, paper substrate was located on stand that was heated to

Suspensions with density of 0.5 McFarland (MCF), which

the sensor was medically sterilized. The fabricated sensor after and before adding contacts was presented in Fig. 4.

B. Sensor characterization using impedance spectroscopy

Impedance analyzer (HP4194A) was used to measure the impedance of the sensors submerged in the solution with different concentrations of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Measurements were performed at 8 frequencies in the range from 12.6 kHz to 100 kHz. Even the paper-based sensors are usually intended to be disposable, that means to perform measurement and to be thrown away, we performed three consecutive repeats and the measured results were reproducible. This reproducibility is allowed by the construction of the sensor, where electrodes are covered by foil and only space between electrodes was opened, and in this way the deteriation of paper through exposing in liquid environment was reduced.

Impedance analyzer was connected to PC for setup configuration and data acquisition. Laboratory measurement setup, as it is shown in Fig. 5.

TABLE I
MEASURED SENSOR IMPEDANCE MAGNITUDE (Z) AND PHASE ANGLE (φ) FOR DIFFERENT CONCENTRATIONS OF *Pseudomonas aeruginosa*.

	$1.5{\cdot}10^8$ CFU/mL		$1.5{\cdot}10^5$ CFU/mL		$1.5{\cdot}10^2$ CFU/mL		Sterile saline	
f [Hz]	Z [Ω]	φ [°]	Z [Ω]	φ [°]	Z [Ω]	φ [°]	Z [Ω]	φ [°]
12599.75	56.74	-4.21	54.85	-3.81	54.13	-4.02	54.06	-3.98
25099.50	55.21	-3.40	53.64	-3.03	52.82	-3.23	52.77	-3.22
37599.25	54.41	-3.05	52.98	-2.74	52.15	-2.92	52.09	-2.91
50099.00	53.93	-2.76	52.59	-2.51	51.74	-2.69	51.67	-2.68
62598.75	53.60	-2.54	52.31	-2.34	51.44	-2.54	51.37	-2.51
75098.50	53.36	-2.36	52.09	-2.21	51.21	-2.41	51.14	-2.39
87598.25	53.16	-2.22	51.91	-2.10	51.01	-2.31	50.96	-2.28
100098.00	53.01	-2.10	51.76	-2.02	50.86	-2.22	50.80	-2.19

TABLE II
MEASURED SENSOR IMPEDANCE MAGNITUDE (Z) AND PHASE ANGLE (φ) FOR DIFFERENT CONCENTRATIONS OF *Staphylococcus aureus*.

	$1.5{\cdot}10^8$ CFU/mL		$1.5{\cdot}10^5$ CFU/mL		$1.5{\cdot}10^2$ CFU/mL		Sterile saline	
f [Hz]	Z [Ω]	φ [°]	Z [Ω]	φ [°]	Z [Ω]	φ [°]	Z [Ω]	φ [°]
12599.75	60.55	-3.78	58.37	-3.73	55.76	-3.77	54.06	-3.98
25099.50	59.06	-3.17	57.01	-3.17	54.58	-3.03	52.77	-3.22
37599.25	58.22	-2.93	56.22	-2.94	53.89	-2.76	52.09	-2.91
50099.00	57.71	-2.71	55.74	-2.73	53.48	-2.52	51.67	-2.68
62598.75	57.35	-2.54	55.39	-2.59	53.19	-2.37	51.37	-2.51
75098.50	57.08	-2.41	55.12	-2.47	52.96	-2.25	51.14	-2.39
87598.25	56.86	-2.29	54.90	-2.36	52.78	-2.15	50.96	-2.28
100098.00	56.68	-2.19	54.71	-2.27	52.63	-2.06	50.80	-2.19

TABLE III
SENSOR SENSITIVITIES FOR DIFFERENT CONCENTRATIONS OF *Pseudomonas aeruginosa*: $1.5{\cdot}10^2$ CFU/mL ($\boldsymbol{SZ_1}$**[%]**), $1.5{\cdot}10^5$ CFU/mL ($\boldsymbol{SZ_2}$**[%]**) AND $1.5{\cdot}10^8$ CFU/mL ($\boldsymbol{SZ_3}$**[%]**).

f [Hz]	SZ_1 [%]	SZ_2 [%]	SZ_3 [%]
12599.75	0.129	1.453	4.958
25099.50	0.105	1.655	4.632
37599.25	0.118	1.706	4.449
50099.00	0.137	1.775	4.381
62598.75	0.125	1.809	4.338
75098.50	0.125	1.836	4.326
87598.25	0.117	1.864	4.326
100098.00	0.124	1.889	4.341

TABLE IV
SENSOR SENSITIVITIES FOR DIFFERENT CONCENTRATIONS OF *Staphylococcus aureus*: $1.5{\cdot}10^2$ CFU/mL ($\boldsymbol{SZ_1}$**[%]**), $1.5{\cdot}10^5$ CFU/mL ($\boldsymbol{SZ_2}$**[%]**) AND $1.5{\cdot}10^8$ CFU/mL ($\boldsymbol{SZ_3}$**[%]**).

f [Hz]	SZ_1 [%]	SZ_2 [%]	SZ_3 [%]
12599.75	3.147	7.966	12.013
25099.50	3.431	8.032	11.924
37599.25	3.449	7.935	11.759
50099.00	3.504	7.882	11.695
62598.75	3.526	7.821	11.626
75098.50	3.556	7.768	11.601
87598.25	3.572	7.727	11.577
100098.00	3.594	7.697	11.564

corresponds to $1.5{\cdot}10^8$ CFU/mL, were made in 4.5 mL of physiological saline according to the EUCAST standard [18].

Colonies of 24-hour cultures on blood agar for *Pseudomonas aeruginosa* and *Staphylococcus aureus* from HiMedia (India) were used. Diluted suspensions concentrations of 10^{-3} and 10^{-6} from initial concentration of above mentioned bacteria were prepared to provide approximate cell densities of $1.5{\cdot}10^5$ CFU/mL and $1.5{\cdot}10^2$ CFU/mL, respectively. Therefore, expected cell counts for *Pseudomonas aeruginosa* and *Staphylococcus aureus* are $6.75{\cdot}10^8$ CFU, $6.75{\cdot}10^5$ CFU and $6.75{\cdot}10^2$ CFU. All measurements were conducted in sterile conditions at the room temperature. Obtained results of impedance magnitude and phase angle changes for different concentrations of *Pseudomonas aeruginosa* and *Staphylococcus aureus* are given in Table I and Table II, respectively. We use tabular representation of data because it can be used as dataset for further analysis by other researchers in the field.

As it can be seen from Table I and Table II, impedance magnitude increases when bacteria concentration increases, while phase angle does not have the same trend of change for all frequencies. Because of that, impedance magnitude changes can be used as sensing mechanism. Such approach require initial impedance magnitude measurement of reference sterile sample, but it eliminates need for wide frequency sweep because from continuous single frequency measurement is possible to distingdish bacteria presence in the solution.

However, one the most important feature of sensors, critical for practical applications, is sensor sensitivity. In our study, sensor sensitivity S_Z describes change of sensor impedance magnitude (Z) with change of bacteria concentration, compared to the sensor impedance magnitude of sterile saline (Z_{ref}):

$$S_Z = 100\frac{Z - Z_{ref}}{Z_{ref}}[\%] \tag{5}$$

Sensor sensitivity has to be better than overall error of used measurement device. Keeping in mind that our HP4194A has declared maximum error for impedance magnitude measurement of 0.17% [35], we conducted sensitivity study of our sensor. Observed sensor impedance magnitude changes for concentrations of $1.5{\cdot}10^2$ CFU/mL (SZ_1[%]), $1.5{\cdot}10^5$ CFU/mL

Suspensions with density of 0.5 McFarland (MCF), which

50 °C. After printing, structure was heated to 120 °C for 30 min.

(SZ_2[%]) and $1.5 \cdot 10^8$ CFU/mL (SZ_3[%]) of *Pseudomonas aeruginosa* and *Staphylococcus aureus* are given in Table III and Table IV, respectively. As it can be seen, sensor sensitivity is higher than measurement error (except for $1.5 \cdot 10^2$ CFU/mL of *Pseudomonas aeruginosa*), which leads to conclusion that sensor impedance magnitude changes are related to change of bacteria concentration. From values for sensor sensitivities given in Table III and Table IV, it can be recognized a need for further analysis regarding shape of sensing electrodes as well as overall sensor dimensions with aim to increase sensor sensitivity for low concentrations of *Pseudomonas aeruginosa*.

C. Parameter estimation using CNLS

In our tests, *trust-region-reflective* algorithm is used as implemented in MATLAB function `lsqcurvefit`. Values of algorithm parameters are presented in Table V.

The estimated values of parameters of model presented in Fig. 3 with CNLS approach for different bacteria concentrations of *Pseudomonas aeruginosa* and *Staphylococcus aureus* are presented in Table VI and Table VII, respectively. As it can be seen, increase of both bacteria concentration affects values of model parameters in the same pattern: solution

TABLE V
VALUES OF CNLS ALGORITHM PRAMETERS.

Parameter	Value
Maximum number of function evaluations	10^4
Maximum number of iteration	10^4
Termination tolerance on the function value	10^{-6}
Termination tolerance on the estimated vector	10^{-6}

TABLE VI
ESTIMATED VALUES OF MODEL PARAMETERS FOR DIFFERENT CONCENTRATIONS OF *Pseudomonas aeruginosa*.

	R_{ser} [Ω]	R_0 [Ω]	C_{dl} [F]	α
Sterile	47.264	8791.663	$3.459{\cdot}10^{-3}$	0.321
$1.5{\cdot}10^2$ CFU/mL	47.280	2869.448	$3.428{\cdot}10^{-3}$	0.322
$1.5{\cdot}10^5$ CFU/mL	48.705	286.945	$2.728{\cdot}10^{-3}$	0.348
$1.5{\cdot}10^8$ CFU/mL	50.759	28.695	$3.795{\cdot}10^{-4}$	0.504

resistance (R_{ser}) and α increases, whereas charge transfer resistance (R_0) decreases as well as double-layer capacitance C_{dl}. Changes in double-layer capacitance can be physically explained, thus they are usually analyzed and correlated with bacteria concentration changes. Bacteria presence can affect a double-layer capacitance in two ways.

The first one is an increase in double-layer capacitance as a consequence of metabolic activities during bacterial growth. During some metabolic activities, charging of uncharged (or weakly charged) substrate ions occurs. Such highly charged ions cause an increase in the permittivity of the medium. Furthermore, the thickness of the double-layer is decreased. According, to Eq. (1), with increased c_{dl} and decreased d, the double-layer capacitance increases due to bacteria presence.

The decrease in the double-layer capacitance occurs when bacteria and bacteria associated components settle in the majority of the adsorption sites of the electrode. Furthermore, electrode surface become locked, and consequently double-layer capacitance became less responsive.

The estimated values for model parameters were used for impedance calculation using Eq. (4) at the measurement frequencies. As comparison of real part (resistance $R = Z\cos\varphi$) and imaginary part (reactance $X = Z\sin\varphi$) of complex impedance is more convenient than impedance magnitude Z and phase angle φ, estimated and measured values of R and X are shown in Fig. 6 (for *Pseudomonas aeruginosa*) and Fig. 7 (for *Staphylococcus aureus*).

The quality of the proposed model was evaluated by Root Mean Square Error (RMSE) calculation for magnitude and phase angle of impedance, as it is shown in Table VIII (for *Pseudomonas aeruginosa*) and Table IX (for *Staphylococcus aureus*). As it can be seen, all fittings have RMSE value

TABLE VII
ESTIMATED VALUES OF MODEL PARAMETERS FOR DIFFERENT CONCENTRATIONS OF *Staphylococcus aureus*.

	R_{ser} [Ω]	R_0 [Ω]	C_{dl} [F]	α
Sterile	47.264	8791.663	$3.459{\cdot}10^{-3}$	0.321
$1.5{\cdot}10^2$ CFU/mL	49.213	879.213	$3.384{\cdot}10^{-3}$	0.325
$1.5{\cdot}10^5$ CFU/mL	50.286	87.921	$3.085{\cdot}10^{-3}$	0.310
$1.5{\cdot}10^8$ CFU/mL	54.029	21.394	$3.085{\cdot}10^{-4}$	0.506

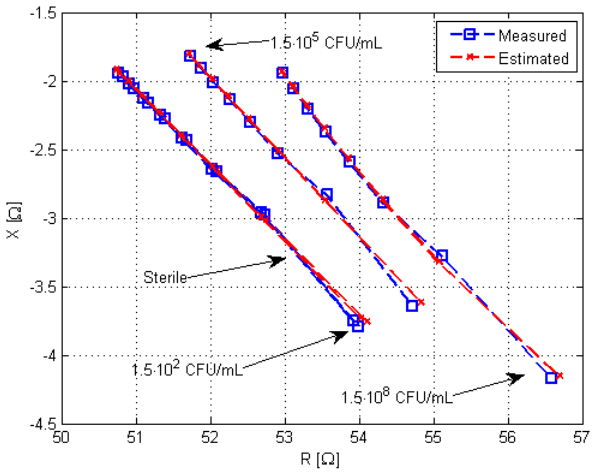

Fig. 6. Cole-Cole plots (resistance R versus reactance X) of measured and estimated sensor impedance for different concentrations of *Pseudomonas aeruginosa*.

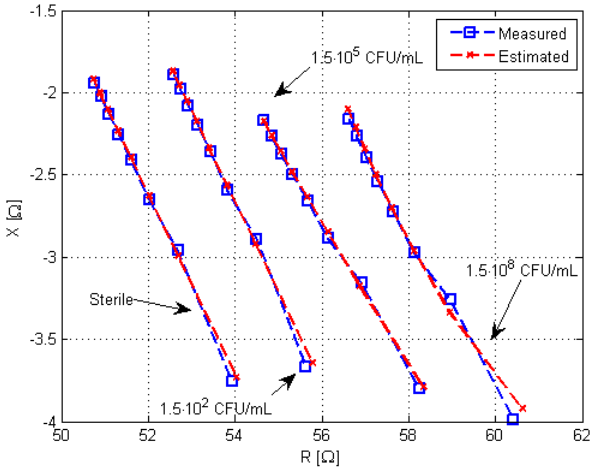

Fig. 7. Cole-Cole plots (resistance R versus reactance X) of measured and estimated sensor impedance for different concentrations of *Staphylococcus aureus*.

lower than 0.076 Ω for impedance magnitude and 0.054° for impedance phase angle.

Moreover, distributions of relative errors at different frequencies for sensor impedance magnitude ($\delta Z(f)$[%]) and phase angle ($\delta\varphi(f)$[%]) for different bacteria concentrations of *Pseudomonas aeruginosa* and *Staphylococcus aureus* can be calculated using following expressions:

$$\delta Z(f) = 100\frac{Z_{est}(f) - Z_{meas}(f)}{Z_{meas}(f)}\,[\%] \quad (6)$$

$$\delta\varphi(f) = 100\frac{\varphi_{est}(f) - \varphi_{meas}(f)}{\varphi_{meas}(f)}\,[\%] \quad (7)$$

where *meas* in subscript indicates measured values shown in Table I and Table II, while *est* in subscript indicates calculated values obtained by putting estimated values of

model parameters from Table VI and Table VII in Eq. (4).

TABLE VIII
RMSE VALUES FOR SENSOR IMPEDANCE MAGNITUDE ($RMSE_Z$ [Ω]) AND PHASE ANGLE ($RMSE_\varphi$ [°]) AT DIFFERENT CONCENTRATIONS OF *Pseudomonas aeruginosa*.

	$RMSE_Z$ [Ω]	$RMSE_\varphi$ [°]
Sterile	0.038	0.027
$1.5{\cdot}10^2$ CFU/mL	0.045	0.034
$1.5{\cdot}10^5$ CFU/mL	0.043	0.031
$1.5{\cdot}10^8$ CFU/mL	0.042	0.030

TABLE IX
RMSE VALUES FOR SENSOR IMPEDANCE MAGNITUDE ($RMSE_Z$ [Ω]) AND PHASE ANGLE ($RMSE_\varphi$ [°]) AT DIFFERENT CONCENTRATIONS OF *Staphylococcus aureus*.

	$RMSE_Z$ [Ω]	$RMSE_\varphi$ [°]
Sterile	0.038	0.027
$1.5{\cdot}10^2$ CFU/mL	0.053	0.029
$1.5{\cdot}10^5$ CFU/mL	0.038	0.020
$1.5{\cdot}10^8$ CFU/mL	0.076	0.054

error for impedance magnitude was 0.206% at frequency f = 12599.75 Hz for bacteria concentration of $1.5{\cdot}10^5$ CFU/mL, while the biggest relative error for impedance phase angle was 1.772% at frequency f = 100098.00 Hz for bacteria concentration of $1.5{\cdot}10^2$ CFU/mL.

In case of *Staphylococcus aureus* the biggest relative error for impedance magnitude was 0.329% at frequency f = 12599.75 Hz, while the biggest relative error for impedance phase angle was 2.898% at frequency f = 100098.00 Hz, both for bacteria concentration of $1.5{\cdot}10^8$ CFU/mL.

A more detailed graphical presentation of relative errors distribution at different frequencies for sensor impedance magnitude and phase angle is provided as supplementary data of this manuscript.

D. Sensor selectivity

A paper-based flexible IDE sensors has ability to detect specific bacteria through measurement of resonant frequency as a response, because each bacteria species (and concentration) will have own fingerprint into the resonant frequency. This was shown recently in [18]. However, in this study, we contributed with analysis if paper-based flexible IDE sensors can detect bacteria presence with measurements in narrow frequency range. As it can be seen from Table I and Table II, presence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria lead to increased impedance magnitude of the sensor at each frequency in analyzed range. Additionally, observed changes of equivalent electrical circuit parameters with changes of *Pseudomonas aeruginosa* and *Staphylococcus aureus* concentrations create the same pattern, which can be used as a sensing mechanism for bacteria presence detection. Main feature of such approach is elimination of a need for wide frequency sweep (required for determination of the resonant frequency of the sensor [18]) as single-frequency impedance measurement is enough for detection of bacteria presence. Moreover, such approach reduces overall cost of the measurement device and allows integration of developed sensor in portable bacteria detection device [21].

However, simple and economical impedance measurement in narrow frequency range can be used as a part of an integrated system for specific bacteria detection as well. Bacteria selectivity may be obtained by using specific antibiotics or similar inhibitors as selective culture mediums [37]. Another approach is to use IDE sensors for fast bacteria detection in combination with much more expensive methods for specific bacteria recognition (such as monitoring the CO_2 produced due to bacteria biological activity with KOH [37], or optical biosensors based on Surface Plasmon Resonance [38]). However, the widespread application of such methods is limited by the high cost and complexity [38].

IV. CONCLUSION

In this work we presented interdigitated electodes paper-based sensor for detection of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The impedance spectroscopy analysis revealed that the complex impedance of the sensor were strongly dependent on bacteria concentration in the frequency range from 12.6 kHz to 100 kHz. The obtained complex impedance data of the sensor was fitted with equivalent circuit using CNLS. It was observed that at higher bacteria concentrations solution resistance increased, with charge transfer resistance decreasing. Constant phase element, used for modelling of the double-layer, experienced decrease in capacitance while non-dimensional fractional parameter α increased. We have also presented comparison of measured impedance and calculated impedance using estimated values of model parameters. Obtained results showed that all fittings results in root mean square error lower than 0.1 Ω, with relative errors lower than 3%.

Based on presented findings, our future studies be focused towards designing and prototyping the battery powered portable impedance measurement device suitable for integration with presented sensor for *in-situ* detection of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Moreover, we will analyse applications with different bacteria as well as different sensor substrates.

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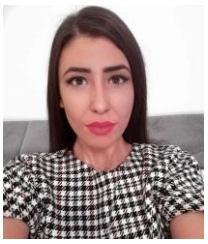

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