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Impedimetric Sensors for Bacteria Detection

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Abstract

The application of electrochemical biosensors based on impedance detection has grown during the past years due to their high sensitivity and rapid response, making this technique extremely useful to detect biological interactions with biosensor platforms. This chapter is focused on the use of electrochemical impedance spectroscopy (EIS) for bacterial detection in two ways. On one hand, bacteria presence may be determined by the detection of metabolites produced by bacterial growth involving the media conductivity changes. On the other hand, faster and more selective bacterial detection may be achieved by the immobilization of bacteria on a sensor surface using biorecognition elements (antibodies, antimicrobial peptides, aptamers, etc.) and registering changes produced in the charge transfer resistance (faradic process) or interfacial impedance (nonfaradic process). Here we discuss different types of impedimetric biosensors for microbiological applications, making stress on their most important parameters, such as detection limits, detection times, selectivity, and sensitivity. The aim of the paper was to give a critical review of recent publications in the field and mark the future trends.

Keywords: Bacteria detection, impedance, biosensors, interdigitated electrode array

1. Introduction

Food- and water-borne bacterial outbreaks remain a major cause for disease and mortality throughout the world [1, 2]. The rapid detection of these pathogenic microorganisms is critical

for the prevention of these outbursts [3]. The identification and quantification of microorganisms has become a key point in biodefense, food safety, diagnostics, and drug discovery researches. The detection of pathogens and indicator microorganisms in water and food samples plays a vital role in public and environmental health. Globally, there are nearly 1.7 billion cases of diarrheal disease every year, and it is responsible for killing around 760,000 children every year (<http://www.who.int/mediacentre/factsheets/fs330/en/>).

To date, the detection and identification of pathogens rely mainly on classical culturing techniques, which require several handling steps in most cases, or on advanced “rapid” techniques in microbiology, such as biochemical kits, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR) assays [4, 5]. These methods are laborious and time consuming and lack the ability to detect microorganisms in “real time” or outside the laboratory environment [4, 6]. Over the past decade, there has been an immense effort to develop new bioassays and biosensors for the detection of food- and water-borne pathogens [7, 8]. Various biosensors for rapid identification of bacteria in food and water have been reported [2], while the most popular are optical biosensors. These biosensors offer several advantages, including speed, selectivity, sensitivity, and reproducibility of the measurement [2]. To date, the most successful optical-based biosensors are based on surface plasmon resonance (SPR) [8–10], whereby biomolecular binding events cause a change in the refractive index that is recognized by a shift in the SPR signal. However, the widespread application of these technologies for bacteria detection is limited mainly by the labor, high cost (US\$10,000–150,000), and complexity of the SPR biosensor system.

Electrochemical biosensors based on impedance technique [11] have proved to be a promising method for pathogenic bacteria detection [12, 13] due to their portability, rapidity, sensitivity, low cost, ease of miniaturization, and label-free operation, and more importantly, they can be used for on-the-spot detection. There is a lot of literature about impedance microbiology, which is based on impedance changes that occurs in culture mediums due to bacterial growth as changes in conductance, due to charged ions and compounds resulting from biological metabolism, or due to bacteria cell adhesion to the electrode surface in interfacial capacitance. It must be noted that traditional impedance microbiology is not a selective method. Some selectivity may be achieved by using selective culture mediums. However, as it is presented in this chapter, functionalization of the electrodes with high-affinity recognition elements, such as antibodies, aptamers, proteins, etc., that selectively bind target cells permit to considerably enhance the selectivity of the method. Along with this, the separation of the target cells from the rest of the sample microorganisms and their preconcentration, as discussed in the chapter “Cell Concentration Systems for Enhanced Biosensor Sensitivity” of this book, may help to reduce the detection limits and raise the selectivity of the method.

Impedance biosensors register changes in the electrical properties at their surface (either capacitance or resistance), affected by interactions between biorecognition element attached to its surface and analyte present in a sample solution. Faradic impedance measurements in the presence of a redox pair in a test solution may be performed on planar metal electrodes. However, to enhance the sensitivity of the measurements and to miniaturize the final sensor element, an impedimetric transducer with two planar interdigitated electrodes called

interdigitated electrode array (IDEA) [11, 14, 15] was introduced which consists of a series of parallel planar electrodes in which alternating electrodes are connected together, forming a set of interdigitated electrode fingers. This sensor design permits to perform a label-free detection of bacteria utilizing different biorecognition elements.

All important aspects of pathogen detection using electrochemical impedance spectrometry (EIS) will be presented in more detail in the following sections of this review.

2. EIS for bacteria detection

The electrochemical technique of impedance has been used in microbiology for detecting and quantifying bacteria during last decades. The integration of impedance technique with biosensor technology in the past few years has allowed the development of impedance biosensors, reducing assay times and detection limits.

One of the positive features of the impedance technique is its simplicity. The impedance Z is determined by applying a voltage perturbation of a sinusoidal wave of small amplitude and detecting the current response. Then impedance extends the concept of pure ohmic resistance to alternate current (AC) circuits. The impedance is the quotient of the voltage–time function $V(t)$ and the resulting current–time function $I(t)$:

$$Z(t) = \frac{V(t)}{I(t)} = \frac{V_0 \sin(2\pi ft)}{V_0 \sin(2\pi ft + \varphi)} \quad (1)$$

where V_0 and I_0 are the maximum voltage and current signals, f is the frequency, t is the time, and φ is the phase shift between the voltage–time and the current–time functions.

The impedance is a complex value because the current can differ in terms of amplitude and also a phase shift compared to the voltage–time function. Thus, impedance, as shown in Figure 1, can be described by the modulus $|Z|$ and the phase shift φ as well as the real part Z_{re} and the imaginary Z_{im} of the impedance. Therefore, for evaluating data graphically, the most popular formats are the Bode and the Nyquist plots. In the Bode plots, $\log |Z|$ and φ are represented as a function of $\log f$, while Nyquist plot data are represented as the real component of impedance (Z_{re}) on the x axis and imaginary component (Z_{im}) on the y axis [16, 17].

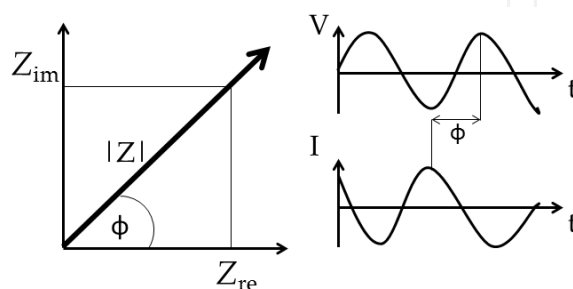


Figure 1. Complex impedance plane diagram and relation of voltage, current, and phase.

EIS studies the response of an electrochemical cell to a voltage at different frequencies. Thus, the impedance spectrum obtained allows the characterization of a complex electrode system composed of surfaces, layers, and membranes where electrical charge transfer and ion diffusion process take place. The most difficult part of the EIS is the correct interpretation of spectra that are analyzed using an equivalent circuit (EC), which consists of resistances and capacitances combined in parallel or serially, as required. Since an electrochemical cell is a complex system, an EC with components representing different physicochemical parameters and processes should be selected, reflecting the electrochemical cell's physical characteristics. However, it must be noted that typically more than one circuit model can fit obtained experimental data. Monitoring the variation of impedance elements as a function of the system properties (e.g., solution composition), it is possible to correlate total impedance changes to individual EC components and thus to confirm correct selection of the EC.

The weight with which individual EC components give their input into the total impedance depends on the applied frequency. This means that in some cases, it is possible to simplify the measurements by working in a limited range of frequencies or just one selected frequency where the relative changes of the component under interest are the largest.

Basically, for EIS performed on a metal electrode in an electrolyte solution in the presence of electroactive compounds, the elements of the EC are well known from general electrochemistry and include ohmic resistance of electrolyte (the bulk medium resistance), double-layer capacitance, charge transfer resistance, and the Warburg impedance, as is presented in Figure 2. For more complex experimental systems, additional components such as dielectric capacitor, polarization resistance, constant-phase element, interfacial impedance, coating capacitance, stray capacitance, and virtual inductors may be required to include. The measured impedance depends on all the individual contributions and distribution of this elements within the EC [12]. However, the impedimetric response in real systems is very complex, and some of the processes cannot be presented in the EC by simple (capacitor, resistance) elements. In this case, some additional EIS elements, such as constant phase element (CPE) or Warburg impedance, with known frequency response are introduced [16].

Resuming, EIS is a very powerful tool as it permits to elucidate physical and chemical phenomena occurring in an electrochemical system, thus allowing to obtain information on changes produced by the interaction of analytes of interest, such as proteins, antibodies, or whole microorganisms, with an impedimetric sensor surface [11].

2.1. Faradic impedance

Impedimetric detection can be achieved either in a direct manner in an anylyte solution or in the presence of an additional redox probe used as a marker. In the presence of electron mediator as $\text{Fe}(\text{CN}_6)^{3-/4-}$ (ferricyanide/ferrocyanide) or $\text{Ru}(\text{NH}_3)_6^{3+/2+}$ (hexaammineruthenium III/II ions), the impedance is termed faradic impedance. The use of electron mediators requires a plentiful supply of redox species to guarantee that impedance does not become limited by the charge transfer process between electrolyte and electrode surface. In faradic impedance measurements, the main parameter is the charge transfer resistance that depends on the interface

blocking by surface products of biochemical reactions and thus may be used to measure concentration dependencies.

The behavior of simple impedance biosensors systems in faradic processes is typically interpreted by a Randles EC presented in Figure 2.

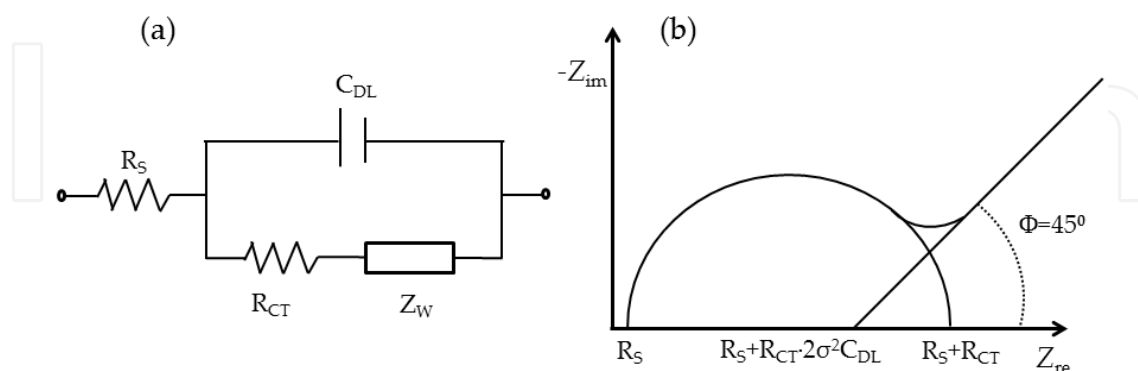


Figure 2. The Randles EC (a) and the Nyquist plot (b) of its frequency response.

The Randles EC, presented in Figure 2a, consists of solution resistance (R_s), double-layer capacitance (C_{dl}), electron transfer resistance (R_{et}) (also called charge-transfer resistance (R_{ct})), and Warburg impedance (Z_w). R_s is inserted as a series element in the EC because all the current passes through the uncompensated solution, while the parallel elements are introduced because the total current through electrodes is the sum of distinct contribution from faradic process and double-layer charging. R_s and Z_w represent bulk properties of the electrolyte and diffusion of the redox probe, while C_{dl} and R_{ct} depend on dielectric and insulating features at the electrode/electrolyte interface. The attachment of bacteria on the electrode surface would retard the interfacial electron transfer process blocking partially the surface and increase the electron transfer resistance [18].

The Nyquist plot (Figure 2b) is the best way to visualize and determinate the Randles EC elements. The semicircle observed at high frequencies corresponds to the electron transfer limited process and linear part at lower frequencies represents the diffusion limited process. The intercept of semicircle at high frequencies with the Z_{re} axis is equal to R_s , while extrapolation of semicircle to lower frequencies into another intercept with Z_{re} axis is equivalent to $R_s + R_{ct}$. The double-layer capacitance C_{dl} can be calculated from the frequency at the maximum of the semicircle. The Warburg impedance can be determined by extrapolating the 45° line observed in Figure 2 to the real axis. In some analytical applications, the Warburg impedance is often neglected by choosing a frequency range where no 45° line is observed in the Nyquist plot and bulk impedance is dominant.

2.2. Nonfaradic impedance

In the case when a redox pair is absent in the electrolyte solution, the impedance is termed nonfaradic [19] and depends on the conductivity of the supporting electrolyte and impedimetric electrode interfacial properties (interfacial capacitance or surface conductivity). Figure 3 shows the basic elements of EC in the case of nonfaradic process:

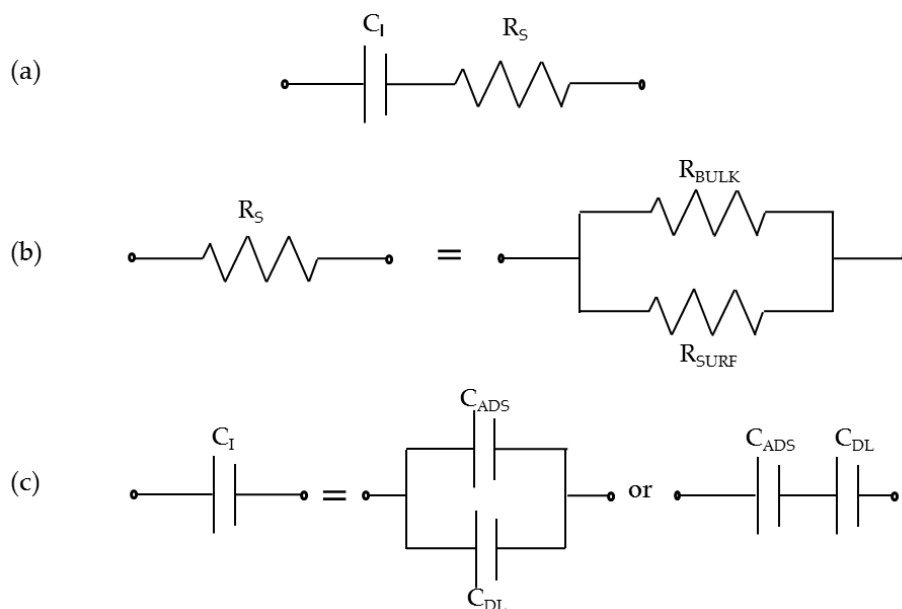


Figure 3. Typical electrical components on ECs characterizing nonfaradic impedance. (a) General circuit elements; (b) the resistance R_s in some cases may depend on bulk solution resistance and surface resistance; (c) adsorption of bacteria cells on the sensor surface results in additional capacitance that may be in parallel or in series with the electrolyte double-layer capacitance depending on the bacteria amount.

In the absence of a redox pair or if its charge transfer rate on the electrode is very slow, no faradic process occurs, and subsequent electron transfer is not produced. In these cases, the interfacial capacitance changes are often studied [20]. These capacitance changes occur when the dielectric constant or the thickness of the interfacial capacitance layer on the transducer surface change their values due to surface chemical reactions [17]. The formation of biochemical reaction products may be represented by an additional capacitor that depending on the process may be included in parallel or in series with the double-layer capacitor (Figure 3c).

It must be noted that a lot of published works refer to changes in capacitance registered by impedance spectroscopy as variations produced in the electrical double-layer capacitance. However, the double-layer capacitance, defined as an outer capacitance at the solid/liquid interface, depends basically on ionic species concentration, while interfacial capacitance depends on the presence of adsorbed species or interfacial layer formation on the electrode surface. On the other hand, R_s , which represents the solution resistance in the case of parallel electrode arrangement, may be constituted as a parallel combination of solution bulk resistance and surface resistance in the case of in-plane electrodes, for example, IDEAs [14].

All these show once again the importance of accurate interpretation of impedance data that should be based on a correct EC choice with the components that unambiguously reflect real physicochemical processes at the electrode surface.

The use of different impedimetric sensors designs, the advances in microfabrication technologies resulting in miniaturization and integration of sensors into a chip format, and better understanding of biochemical interfacial phenomena helping the analysis of impedance components using ECs should help us to improve the biosensor detection systems serving to reduce the assay time and improve the bacteria detection limits [18].

3. Impedimetric detection of metabolites produced by bacterial cells

Metabolism refers to all the biochemical reactions that occur in a cell or organism. By metabolic pathways, bacteria convert large molecules, such as polysaccharides, lipids, nucleic acids, and proteins, into smaller units as monosaccharides, fatty acids, nucleotides, and amino acids, respectively, to release energy. Consequently, this conversion of large organic substrate molecules in the medium into charged, small and more mobile ionic metabolites, which can include lactic acid, acetic acid, carbon dioxide, ammonia, bicarbonate, and urea, results in a change of the ionic composition of the growth media. In this way, these changes can be measured and related to bacterial concentration for determination of microbial growth.

Different electrochemical transduction techniques have been used for the detection of products of microbial metabolism. Amperometric technique has been reported by the use of mediators [21], which are reduced by the microorganism as a consequence of substrate metabolism; however, no examples of direct metabolite detection are found in the literature, probably due to the electroactive interference produced by the sample matrix, which can cause the transducer to generate a false current reading [22]. Potentiometric methods have been developed to detect changes resulting from metabolite accumulation of hydrogen ions [23]. Nevertheless, these electrochemical methods show some disadvantages such as insufficient sensitivity, selectivity, and sample matrix effect [22]. In addition, these methods require the use of a reference electrode, which complicates the system miniaturization and prevents its use in a small volume samples. Among different electrochemical techniques, the most extended transduction method is based on measurements of electrical impedance changes in the medium resulting from the bacterial growth.

The correlation between microbial growth and impedance was first defined by Stewart in 1899 [24]. However, it was starting from 1970s when much attention and efforts were put in this research [25–30] to monitor bacterial activity detecting changes in electrical impedance caused by growing bacterial culture. Impedance technique was shown to be useful for the estimation of microbial biomass [31], detection of microbial metabolism, and determination of the physiological state of bacteria [32–37]. The advantages of this approach are high sensitivity, relative simplicity, and comparatively low cost of the required experimental equipment [25]. In 1992, the impedance method was approved by the Association of Official Analytical Chemists (AOAC) International as a first action method for screening *Salmonella* in food samples [38]. Finally, in 1996, AOAC approved it as a final action method for the detection of *Salmonella* in food [39].

Impedance microbiology is one of the most successful of all the recently introduced rapid methods. Several analytical systems have been developed for bacteria detection, such as Bactometer (Bio Merieux, Nuertingen, Germany), Malthus systems (Malthus Instruments Ltd., Crawley, UK), rapid automated bacterial impedance technique (RABIT) (Don Whitley Scientific Ltd., Shipley, UK), and BacTrac (Sy-Lab, Purkersdorf, Austria) [18, 40–42]. They have been validated against other conventional methods, such as the most probable number method (MPN) [36] or microbial colony counts [43], showing a sensitivity comparable to these standard methods. Existing commercial instruments are widely used for different applications. For

example, all these systems have been reported to detect and make quantitative estimations and differentiation of bacteria, such as *Escherichia coli* or *Salmonella*, among others typically found in food [36, 44–48]. They have been also shown to be useful for the evaluation of different mediums for selective bacterial growth [47, 49, 50]. The use of impedance technique for bacteria determination is summarized in Table 1.

Target Microorganism	Growth Medium	Electrodes (Measure frequency)	Detection Limit	Detection Time (h)	Ref.
Enterobacteriaceae Family	BHI ¹ + 0.1% yeast extract	-(8-Channel Mathus-Meter Bactometer 32)	10 ⁴ cell·cm ⁻²	8-9	[37]
-	BHI ¹	Gold plated and stainless-steel (Bactometer, 2kHz)	10 ⁵ cell·mL ⁻¹	2,6	[28]
<i>E. coli</i>	TSB ²	Stainless steel (Bactometer, 2kHz)	10 ⁵ cell·mL ⁻¹	5-6	[29]
<i>Listeria innocua</i>	Tris-Gly ³ buffer + dextrose	Interdigitated platinum electrodes (11.43 kHz)	10 ⁵ -10 ⁷ cell·mL ⁻¹	2	[51]
<i>Listeria innocua</i> <i>Listeria monocytogenes</i> <i>E.coli</i>	Tris-Gly ³ buffer + dextrose	Interdigitated platinum electrodes (11.43 kHz)	10 ⁷ -10 ⁸ cell·mL ⁻¹	2	[52]
Coliforms (<i>E.coli</i>)	SM ⁴	-(Bactometer, 2kHz)	10 ⁴ cell·mL ⁻¹	5	[42]
<i>E.coli</i>	YPLT ⁵	Interdigitated electrodes	8-8·10 ⁸ cell·mL ⁻¹	14.7-0.8	[53]
<i>Bacillus lactis</i>	SM ⁴	-(Bac Trac)	10 ⁸ cell·mL ⁻¹	6	[48]

¹ Brain Heart Infusion broth; ² Trypticase Soy Broth; ³ Buffer Tris-glicine; ⁴ Specific Medium; ⁵ Low conductivity Yeast-Peptone-Lactose-TMAO medium

Table 1. The use of impedance technique for bacteria determination

Impedance microbiological techniques can be used to monitor bacteria viability during growth. Since only live bacteria cells present metabolic activity and are able to produce changes in the conductivity of the medium, impedance microbiology is used for differentiating live

and dead cells [51–53]. Kinetics monitoring may give additional information since the impedance growth curves under different conditions are found to be characteristic for different bacteria species. Most applications of the traditional microbiological impedance technique for the detection of bacteria were reviewed by Silley and Forsythe in 1996 [41] and Wawerla et al. in 1999 [54].

Impedance changes associated with metabolic activity of microbial cells are often expressed as the ratio of the reference impedance (medium without bacteria) to the sum of the reference and the sample, resulting in the parameter known as normalized impedance change (NIC):

$$\text{NIC}(\%) = \frac{Z_{\text{REF}}}{Z_{\text{REF}} + Z_{\text{SAMPLE}}}, \quad (2)$$

which is related to microbial growth.

The typical impedance growth curve is presented in Figure 4, where the measured impedance values are graphically plotted in relation to the incubation times.

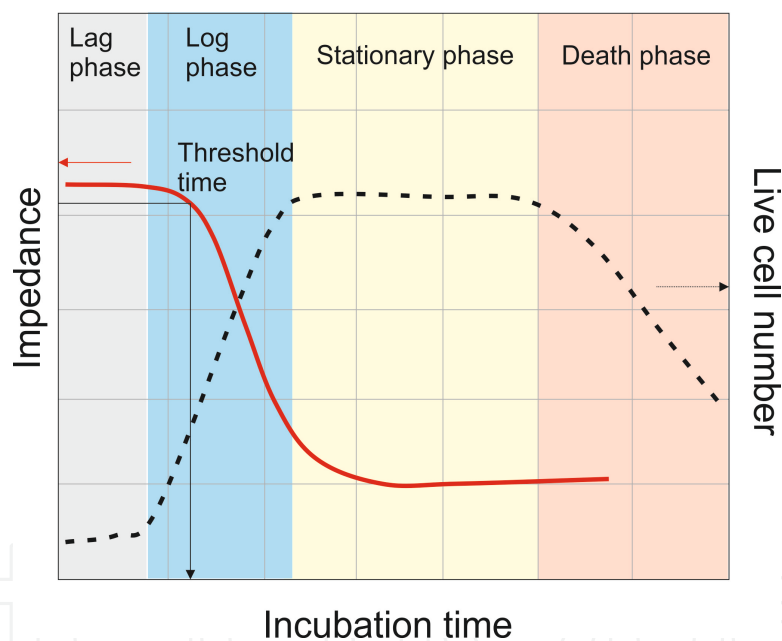


Figure 4. Microbial impedance growth curve with typical bacterial growth phases showing impedance changes (solid line) and live cell number (dashed line) in time.

To provide detectable changes in the measured impedance, a minimum concentration of microorganisms is needed in the medium. However, these microorganisms replicate and in time reach numbers sufficient to cause a detectable impedance change. This concentration of bacterial cells is defined as a threshold concentration. Thus, the threshold concentration, also called *detection limit*, refers to the lowest concentration of microorganisms that must be present to detect the change measured by impedance. The threshold concentration depends, in part, on how the detectable impedance change is defined [28], which is normally referred to the changes occurring in a control sample of a sterile broth (see Eq. (2)).

The time required for the organisms to grow to threshold concentrations is called *detection time*. The detection time depends on three main parameters:

- the initial concentration of microorganisms;
- lag phase (the initial period in which cellular metabolism is accelerated, cells are increasing in size, but the bacteria are not able to replicate);
- the generation time of the population of microorganisms (time it takes bacterial to double its concentration)

From this, it follows that if a population of organisms has similar generation times growing in a concrete medium, the detection time can be used to estimate initial concentrations [28, 29].

Metabolic activity measurements can be performed in either direct or indirect ways. In direct measurements, impedance electrodes immersed in the growth medium detect changes of the bacterial metabolism taking place in the bulk of a growth media. Indirect technique, however, detects CO₂ produced by microorganisms [51]. In this indirect impedance technique, the CO₂ produced due to bacteria biological activity reacts with potassium hydroxide (KOH) solution in a separate chamber. The formation of carbonates causes decrease in the solution conductivity. This technique was first described by Owens et al. [55], and it has been adapted for rapid automated bacterial impedance apparatus in other works [32]. Using this approach, a recent work of Johnson et al. [56] studies the viability of indirect impedance method using a commercial system to study microbial growth in complex food matrices. The ability of the system to detect different microorganisms in different food matrices was clearly demonstrated [56].

In direct measurements, the impedance changes may be produced by two primary sources: microbial metabolism, which alter the conductivity of the medium, and electrode interfacial impedance, changes in the surface properties of the electrodes affecting the capacitance of the electrode/electrolyte interface due to bacteria presence [26, 29, 57]. Thus, the growth of microorganisms usually results in an increase in both conductance and capacitance, causing a decrease in impedance [58]. To account for this, the detection of microbial metabolism by impedance systems is typically conducted by measuring relative or absolute changes in impedance at different frequencies at regular time intervals during the growth of bacteria at a given temperature. From the frequency dependence of the impedance using an appropriate EC, the system conductance/resistance and capacitance may be determined.

Different studies have analyzed the relationship between microbial growth and relative changes in both the capacitive and the resistive parts of impedance showing that both components are indicative of bacterial growth. In low conductivity media, the change in the conductance of the media clearly correlates with bacterial growth, whereas in more conductive media, the relative changes in conductance are smaller in comparison to impedance changes caused by polarization interfacial capacitance, the effect that can also be useful for monitoring bacterial growth [59].

As it was noted, both components can be studied separately by measuring impedance in a different frequency ranges. Various works have demonstrated the predominance at low frequency of electrode surface impedance, while impedance at high frequencies is associated with media conductance effect [60, 61]. However, the frequency range in which certain EC

components give the main input into the overall impedance may vary depending on the dimensions of the electrodes and their separation. The differentiation of the impedance effects into electrode/electrolyte interfacial capacitance and medium resistance changes at different frequencies has led to the development of impedance-splitting methods for the detection of bacteria [18].

However, in most of publications on the impedance microbiology, only the conductance of the medium is measured. In this case, impedance always decreases with time, indicating that the microorganisms are consuming growth media substrates of low conductivity metabolizing them into ionic products of higher conductivity [59].

Thus, altogether, changes registered in the microbiological impedance are dependent on bacteria species, the number of microorganisms and properties of the medium in which they are growing in, the frequency of the applied signal, the surface properties and geometry of the measuring electrodes, and the temperature [31].

Since impedance microbiology relies on determining the changes in electrical impedance of a culture medium resulting from the bacterial growth, it depends largely on the design of the growth medium since the culture medium not only supports the bacteria growth but also provides high, noninterfering signals to the overall impedance or its components. Therefore, medium, besides providing the optimum growth, activity, products yield, and morphology of the microorganisms, should contain substrates with low conductivity contribution to the overall conductivity of the medium. In this case, the sensitivity of the impedance detection strongly depends on designing an appropriate culture medium [62]. For this reason, in recent years, much of the research in the field has been directed to find or design selective media with low conductivity [49, 52, 62]. Another important feature of the culture media is its selectivity that gives the main priority for growing only for specific bacteria. In the case of nonselective detection, growth media changes in impedance may be induced by the growth of different bacteria, not only the target microorganism one wants to determine. To solve the problem, selectivity can be provided by using specific culture media designed for certain microbial type by using specific inhibitors such as antibiotics [47].

Recently, Lopez Rodriguez and co-workers [62] designed a specific medium to impedance monitoring of *Streptomyces* strain M7. The importance of this bacterium is its capacity to grow in the presence of organochlorine pesticides used by them as a carbon source and using the amino acid asparagine as a nitrogen source. Thus, the presence of these pesticides makes this medium specific for bacteria growth. On the other hand, by monitoring *Streptomyces* bacteria growth, it is possible to determine the presence of these pesticides and quantify them. Hence, this system based on impedimetric biosensor has a potential use to detect these dangerous compounds. Since amino acids frequently used in culture media interfere in impedance determinations, the work presented an optimized design regarding the culture medium capabilities on the impedance response of *Streptomyces* M7 activity. Finally, a specific medium was designed using $(\text{NH}_4)_2\text{SO}_4$ instead of asparagine as nitrogen source [62].

Much attention has also been paid to miniaturization of impedimetric sensor systems by using microelectronics lithographic techniques to fabricate microelectrodes in order to improve sensitivities or add functionalities. Interdigitated array microelectrodes has been demonstrat-

ed as a promise in impedance measurements for monitoring the growth of bacteria since they present advantages in terms of the fast establishment of the steady-state signal, the increased signal-to-noise ratio, and the use of small sample solution volumes [63, 64].

To enhance and facilitate the impedance sensing, microfluidic flow cells can be added to the interdigitated microelectrodes to achieve a fully integrated microchip. This brings different benefits such as high-detection sensitivity, small volume handling, low contamination during bacterial growth, ability to concentrate cells, and rapid detection of small number of cells [64].

With the aim to study whether impedance measurements in the microscale could provide information about the metabolic activity of bacteria, Gomez *et al.* described a microfabricated biochip with integrated fluidic paths and electrodes for impedance spectroscopy of nanoliter volumes of bacterial suspensions [65]. Later experiments analyzed the use of this microsystem to detect metabolic activity of small concentrations of different bacteria (*Listeria* and *E. coli*) in two different media (Luria Bertani broth with high ionic content and a low-conductivity medium denominated Tris–Gly–Dext). Thus, they demonstrated the capacity of the system to detect viability of small numbers (around 10^5 cfu mL⁻¹, resulting in about five cells in the 5.27 nL chamber of the chip) in a couple of hours [52].

Varshney *et al.* [64] reported double interdigitated array microelectrodes-based flow system to detect viable cells of *E. coli* O157:H7 selecting a frequency of 1 MHz to monitor the change in the impedance values. They attribute the change in impedance to a decrease in the resistance of the medium due to an increased concentration of highly charged ions corresponding to the growth of the bacterium in a low conductive medium. Thus, they found that the system may be successfully employed for the *E. coli* O157:H7 detection in a range from 8.0 to $8.2 \cdot 10^8$ cfu mL⁻¹ after 14.7 and 0.8 h of cultivation time, respectively.

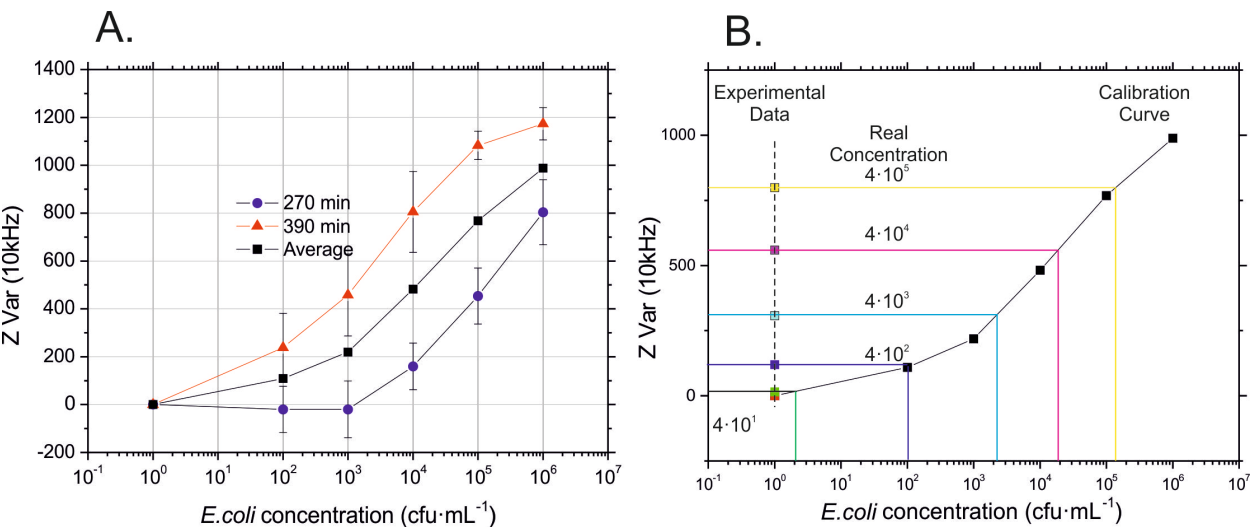


Figure 5. (A) Calibration curves showing changes in sensor impedance at different concentrations of *E. coli* measured at a fixed frequency of 10 kHz in a growth medium at 270 (●) and 390 (▲) min, and the average of these two calibrations (■). (B) Determination of *E. coli* concentration in milk samples after 6 h of incubation. Black squares represent calibration plot used to determine *E. coli* in spiked milk samples. Squares on the left side are the results of impedance changes measured in samples with “unknown” *E. coli* concentration.

It should be noted that most of the bacteria metabolic products are of acidic nature, so that the produced conductivity changes due to ionic products are accompanied by pH changes [25] of the growth media. On this effect, measurements of pH to control the bacteria growth are based [66].

Experiments performed in our research group [67] with *E. coli* in Luria Bertani (LB) bacterial growth medium supplemented with glucose performed with a miniature pH sensor–reference electrode pair showed subsequent pH changes in the pH 7–5 range with the bacteria incubation time.

In order to miniaturize the system, an IDEA [11] was used instead of the pH sensor and the reference electrode. Experimental results presented in Figure 5A show that changes of *E. coli* concentration in the growth medium provoke changes of impedance measured at a fixed frequency of 10 kHz. To avoid the undesirable effect of the bacteria attachment to the interdigitated electrode surface that might provoke additional changes in the impedance magnitude, the developed method consisted of a single measurement after a certain incubation time of *E. coli*, when a drop of the growth media was placed on a sensor surface. Impedance changes were found to depend on the number of microorganisms in a sample in the concentration range 10^2 to 10^6 cfu mL⁻¹. However, as the kinetics of the impedance changes greatly depends on the bacterial concentration, it was not possible to obtain a single calibration curve in a wide concentration range at one specifically fixed incubation time. To resolve this, a novel calibration method was proposed [67] by measuring the sensor response at 270 and 390 min of incubation and taking a mean value.

Thus, this method of measuring impedance using interdigitated microelectrodes at a fixed frequency to control the bacterial growth was used for *E. coli* determination in real samples. Our impedimetric biosensor was tested on quantification of *Escherichia coli* bacterium in milk, demonstrating the capacity of the method to detect concentrations in a range between 10^2 and 10^6 cfu mL⁻¹ with 6%–12% error margin in only 6 h (Figure 5B).

Summarizing, we may conclude that impedance microbiology, being a useful and well established bacteria control method, finds itself in a new stage of development based on the application of modern technologies oriented on a chip-based method. Advances in microfabrication are allowing the transfer of impedance microbiology to microdevices increasing signal and therefore sensitivity, minimizing sample volume, and reducing assay time [18].

4. Detection based on impedance measurements produced by surface changes of the electrodes

Most of bacteria cells are electrically charged, so due to bacterial cells, immobilization on the electrode surface of impedimetric biosensor variations in electrical impedance may be produced. The bacterial attachment also implies a reduction of the effective electrode area that may affect the charge transfer resistance in faradic impedance measurements. This means that bacterial cells attached to the sensor surface may produce variations in interfacial impedance

due to changes in surface conductivity produced by their electrical charge or the surface layer capacitance. Direct label-free impedance method of bacteria detection has gained much interest permitting to reduce substantially the detection time compared with growth-based impedance methods because this methodology is not dependent on cells replication in a culture medium or the production of metabolites [18, 68]. Different kind of electrodes, especially IDEAs, also named as interdigitated array microelectrodes (IDAM), differing in their geometry and immobilization strategies can be used as impedimetric transducers for bacteria detection.

IDEA transducers present promising advantages compared to other impedimetric biosensors as rapid detection kinetics, increase of signal-to-noise ratio, fast establishment of a steady-state response, potential low cost, and ease of miniaturization. Moreover, IDEA eliminates the requirement of a reference electrode compared to three or four electrode systems or potentiometric and amperometric devices. IDEA devices consist of a pair of comblike metal electrodes formed on a planar insulating substrate, in which a series of parallel microband electrodes are connected together by a common bus, forming a set of interdigitating electrode fingers. At present, IDEAs are widely used as impedimetric biosensors for bacterial detection [12].

Parameter designs and materials employed for electrode fabrication are important as they affect the sensitivity and operation of an IDEA. The selection of materials for electrode fabrication depends on the future application, chosen surface modification method, ionic species involved, production costs, and fabrication process. The number of electrode fingers, the spacing between each pairs, and the width, length, or height will determinate the sensitivity of the sensor [69]. Several studies show the importance of geometry for microbiological applications [70]. Bratov et al. [15] developed a transducer for biosensor applications based on a three-dimensional interdigitated electrode array (3D-IDEA) with electrode digits separated by an insulating barrier. This sensor presented considerable improvement in sensitivity compared with a standard planar IDEA design, resulting in a viable option for integrated biosensing applications.

4.1. Nonspecific immobilization on electrodes surface

The majority of detection systems in impedimetric biosensors involve a biorecognition element directly immobilized on the electrode surface to react and attach bacterial cells. However, some studies [12] pointed out that when antibodies or another biomolecules with affinity against bacteria are immobilized on the surface of electrode, the functional area of the electrode is not optimally utilized. Moreover, these biosensors show lack of reproducibility as it is difficult to repeatedly achieve the same surface density of biorecognition molecules on the sensor surface. Different strategies of the detection of bacteria without the direct immobilization of biodection molecules are discussed here.

Varshney and Li [12] suggested the use of biofunctionalized microbeads or nanoparticles as an indirect impedance measurement. The same authors developed a biosensor based on an IDEA coupled with magnetic nanoparticle–antibody conjugates for the rapid and specific detection of *E. coli* O157:H7 in ground beef samples [71]. Nanoparticles were prepared previously by immobilizing biotin-labeled polyclonal anti-*E. coli* antibodies onto streptavidin-coated magnetic nanoparticles to carry out the preselection and preconcentration of bacteria.

After the separation of bacteria immobilized on nanoparticle–antibody conjugates, they were resuspended into a low conductivity mannitol 0.1 M solution, and the concentrated sample was uniformly spread on the surface of the sensor. The frequency response of the impedance in a 10-Hz to 1-MHz range showed that the bulk resistance and the surface capacitance were responsible of the impedance changes caused by presence of *E. coli* on the surface of IDEA. The detection limit of *E. coli* O157:H7 was 7.4×10^4 in pure cultures and 8×10^5 cfu mL⁻¹ in ground beef samples, while the detection time was 35 min. The same methodology was used in a microfabricated flow cell to detect *E. coli* O157:H7 [72]. In this case, a volume of 60 nL was used, and the detection limit was as low as 1.6×10^2 and 1.3×10^3 cells in pure cultures and beef samples, respectively. This detection limit, being recalculated for a 5 mL volume, corresponds to 8.4×10^4 cfu mL⁻¹.

Recent studies of Kanayeva et al. [73] used a preconcentration technique for *Listeria monocytogenes* detection, a food-borne pathogenic bacteria. Immunomagnetic nanoparticles were functionalized with anti-*L. monocytogenes* antibodies via biotin–streptavidin bonds to capture *Listeria* in a sample during 2-h immunoreaction. To collect the complex of nanoparticles with bacteria, a magnetic separator was used and, after a washing step, *L. monocytogenes* was removed from the samples and injected in a microfluidic chip. The impedance change produced by bacteria was measured by an IDEA in the microfluidic chip in a phosphate-buffered solution. An equivalent concentration of 10^3 cfu mL⁻¹ of the original sample was detected without interferences by other bacteria as *Listeria innocua*, *E. coli* K12, *E. coli* O157:H7, *Salmonella typhimurium*, and *Staphylococcus aureus*. EC analysis indicated that impedance change was mainly produced by decrease in the medium resistance. Results obtained in milk, lettuce, and ground beef samples showed that the sample matrix effect affects the detection limit that was between 10^4 and 10^5 cfu mL⁻¹. The required detection time was around 3 h.

Advantages of using microbeads and nanoparticles are based in the separation and concentration of a specific strain of bacteria from the native sample previous to registration process, which permits to reduce the background noise caused by nontarget compounds or other bacteria. Furthermore, in some cases, the surface of the electrodes can be used multiple times because recognition elements are not attached to the sensor surface. However, the increase in number of the detection process steps from the initial sample treatment to the final bacteria detection could result in the sensitivity and reproducibility loss of a biosensor device.

In this field of detection without chemical attachment of a biorecognition element to the sensor surface, our group has recently developed a modified TaSi₂ IDEA on a SiO₂ substrate to study interactions with bacteria present in a sample solution [74]. Bacteria immobilized on the sensor affect the surface charge and produce changes in the superficial impedance. In the studied case, the sensor surface was chemically modified by a layer-by-layer method [75] with oppositely charged polyelectrolyte layers by alternating polyethyleneimine (PEI) and poly(sodium 4-styrenesulfonate) (PSS). *E. coli* ATCC 10536 was employed for sensitivity and time evaluation. Bacteria were immobilized on the IDEA with a PEI–PSS–PEI multilayer, taking into account the ability of PEI to react chemically with outer membrane compounds of gram-negative bacteria. Results obtained showed a detection limit of the sensor as low as 10^1 cfu mL⁻¹ and response time around 20 min.

Despite the satisfactory results obtained, the main drawback of this technique was the nonspecificity of this methodology because all other negatively charged particles that might be present in the sample would adhere to the sensor surface as well. Moreover, the reuse of thus fabricated sensors is very complicated due to a very strong adhesion of PEI polyelectrolyte to silicon dioxide sensor surface. However, in combination with preconcentration and separation techniques mentioned above, this device [74] may be advantageous due to its higher sensitivity.

4.2. Bacteria detection by biorecognition elements on the sensor surface

Most of the studied impedimetric biosensors have been functionalized by the immobilization of biorecognition elements on their surface. A biorecognition element is a biomolecule (antibody, protein, peptide, etc.) with specific affinity that selectively reacts with a specific target analyte [17, 76]. The detection process involves the formation of a complex between the sensing recognition biomolecule and the specific analyte (proteins, nucleic acids, antibodies, antigens, microorganisms, or whole cells). Generally, the electrical properties of the sensor surface are altered by the two components, the biorecognition element in a first phase and the specific target in a second phase.

Different strategies are used to promote the immobilization of the biorecognition element on impedimetric biosensors [7, 17, 77]:

- Bioaffinity layers (avidin–biotin system)
- Thiol containing self-assembled monolayers (SAMs) on gold
- Langmuir–Blodgett films
- Chemical grafting through silanization strategies
- Thin polymers
- Polyelectrolyte films (layer by layer)

The choice of the appropriate immobilization technique depends on the biomolecule nature, reproducibility, cost, and difficulty of immobilization. Immobilized biomolecules have to maintain their active structure, function, high sensitivity and selectivity, fast reaction kinetics, and high stability and not to be desorbed during the use of biosensor. More information on immobilization strategies may be found in a specific review [78].

Previously in Section 2, the main differences between faradic and nonfaradic impedance has been reported. In next sections, impedimetric biosensors functionalized with biorecognition elements attached on electrode surface will be discussed, taking into account whether faradic or nonfaradic processes are responsible for sensitivity.

4.2.1. Impedimetric immunosensors

Immunochemistry is a well-studied and developed area, so the implementation of this technique to different kinds of electrochemical impedimetric biosensors has been widely used

in the past years. The major advantage of immunosensors is the specificity and sensitivity of biomolecular interactions between the antibody and the antigen (from a little target molecule to bacteria). Moreover, the advances in production techniques of monoclonal antibodies, genetic engineering, and recombinant antibodies have improved binding-ability and stability on biosensor surfaces [79]. However, the main drawback of antibodies that prevents their widespread use in biosensors is the lack of stability, as many of them lose their activity quite rapidly. A few examples of reported impedimetric immunobiosensors for microbiological applications are discussed later in this section, stressing the impact of sensor geometry and electrodes configurations and their materials, antibodies, and bacteria species detected.

A high number of impedimetric immunobiosensors reported in literature are based on faradic impedance measurements. One of the earliest works on electrochemical impedance spectroscopic biosensors is by Ruan and Yang in 2002 [80], who reported an immunosensor based on a planar IDEA with indium tin-oxide (ITO) electrodes. Anti-*E. coli* antibodies were immobilized using an epoxysilane layer for chemical anchoring of antibodies to capture *E. coli* cells. Impedance was measured in the presence of a redox couple, $\text{Fe}(\text{CN})_6^{3-/4-}$, in that way attached bacteria partially block the metal electrode surface, which allows to register the increase of electron transfer resistance (R_{et}) with increasing concentration of bacteria. The biosensor could detect the *E. coli* bacteria with a detection limit of 6×10^3 cells/mL and a linear response in the R_{et} between 6×10^4 and 6×10^7 cells/mL. Lately, Yang et al. [81] developed another immunosensor for detecting the same bacteria using the same antibodies, but in this case, anti-*E. coli* O157:H7 antibodies were immobilized on an ITO interdigitated array sensor surface through hydroxyl groups of ITO electrode and carboxylic groups of antibodies. As in previous studies conducted by Ruan and colleagues [80], faradic EIS was measured using a redox probe. The R_{et} increased on the immobilization of antibodies and bacterial cells that behaved as insulators, allowing to obtain a correlation between the electron transfer resistance and bacterial concentration between 10^5 and 10^8 cfu mL⁻¹, but a detection limit of 10^6 cfu mL⁻¹ was quite high.

It should be noted that faradic impedance measurements with a redox probe do not use any possible advantages presented by IDEAs, as the charge transfer resistance is not dependent on the electrode geometry and is affected only by the total electrode area [11]. For these measurements, simple plane electrodes can be used as well.

Although *E. coli* is the most popular model bacterial system, many studies are focused on detecting other bacteria. For example, Mantzila et al. [82] developed a faradic impedimetric immunosensor for the detection of *S. typhimurium* in milk samples. Polyclonal antibodies anti-*Salmonella* were cross-linked in gold electrodes in presence of glutaraldehyde and different mixed SAMs. High selectivity was obtained in front *E. coli* bacteria in milk samples, while the detection limit for *Salmonella* was indicated at a concentration level three orders of magnitude lower than the infectious dosage that is around 10^5 cfu mL⁻¹ [83].

The most important challenge in microbial sensor development is the reduction of the detection limit and detection time. One of the latest publications [84] report *E. coli* O157:H7 bacteria immobilization on a gold electrode with anti-*E. coli* antibodies through the SAM of mercaptohexadecanoic acid. EIS was used for detecting pathogenic bacteria, while SPR was used to monitor the antibody immobilization. R_{ct} values obtained with a redox probe were used to

monitor changes produced by bacterial interaction with antibody. To our knowledge, this work reported the lowest detection limit of 2 cfu mL⁻¹.

On the other hand, other studies were focused on nonfaradic impedance measurements. Radke and co-workers [85] used an IDEA chip with gold electrodes modified by a 3-mercaptopenthyldimethylethoxysilane and a heterobifunctional cross-linker to immobilize antibodies. The reported optimum width and spacing were 3 and 4 µm, respectively. The impedance across the interdigitated electrode was measured after immersing the functionalized biosensor in a peptone solution with *E. coli* O157:H7, and the resulting impedance change caused by bacterial attachment was monitored. In this case, the impedance was measured in a frequency range of 100 Hz to 10 MHz, and main differences due to bacteria concentration were obtained at low frequencies where the impedance depends mainly on interfacial capacitance. The biosensor was able to discriminate between different concentrations from 10⁵ to 10⁷ cfu mL⁻¹ at a frequency of 1 kHz. The same electrode system being used in pure cultures and food samples (romaine lettuce) showed reduced one order of magnitude detection limit (10⁴ cfu mL⁻¹) [5].

Tan et al. [86] developed a PDMS microfluidic immunosensor integrated with specific antibodies on an alumina nanoporous membrane for the rapid detection of *S. aureus* and *E. coli* O157:H7 with EIS. In this case, antibodies were immobilized on the membrane using self-assembled (3-glycidoxypentyl)trimethoxysilane (GPMS). For bacteria detection, a frequency range from 1 to 100 Hz was applied for both bacteria, and changes produced by bacterial attachment were obtained around 100 Hz. Cross-bacteria experiments showed a high specificity of anti-*E. coli* and anti-*S. aureus* antibodies utilized, and detection within 2 h showed a detection limit of 10² cfu mL⁻¹. Thus, the combination of a microfluidic chamber and different substrates such as an alumina nanoporous membrane for the immobilization of antibodies offered new approaches for the immunodetection of bacteria.

One of the main problems of immunosensors is the difficulty to reuse the biosensor once bacteria are attached. Single-use disposable sensors are attractive; however, their production cost should be very low, and this typically is not the case. Hence, it is required to find some treatment to remove the sensor surface coating in order to use it several times. Dweik et al. [87] established a cleaning protocol for a biosensor based on a gold interdigitated microelectrodes for the detection of viable *E. coli* O157:H7 using anti-*E. coli* IgG antibodies. They assured that a 30-min treatment with acetone, followed by a wash with isopropanol and distilled water, and exposure to plasma for 2 min with a power of 48 W permit to restore the sensor surface to its initial state and thus to reuse each device at least for five times.

Without any doubt, antibodies are the most widely used bioreceptors in biosensor research and development. However, as it was mentioned, the main problem of the stability of antibodies after the immobilization on a sensor surface remains a challenge as well as short shelf lifetime and decrease of binding efficiency over time [19]. Furthermore, antibodies production and purification costs are an added difficulty. For this reason, there is a permanent search for other biorecognition elements as bioreceptors for biosensing.

4.2.2. Aptamers

Aptamers are short series of single-stranded DNA or RNA oligonucleotides obtained artificially via in vitro process called systematic evolution of ligands exponential enrichment

(SELEX) [88]. Aptamers have been used for biosensing applications due to their ability to bind with high selectivity to a specific target molecule. These artificial nucleic acid ligands can be generated against amino acids, proteins, drugs, and other molecules, and they can be applied for the detection of various targets molecules and even whole cells or organisms [12]. The high specificity and affinity to target molecules, the ease of synthesis, and immobilization without compromising their biological activity allows their use as biorecognition elements for bacterial detection. The majority of aptasensors are focused on the detection of protein targets, but recently appeared publications devoted to direct bacterial detection. During the SELEX process, whole microorganisms can be employed as target during aptamer synthesis because of the interesting membrane proteins bound specifically to aptamer [89]. One of principal advantages of this method of aptamer synthesis is the ability to target and specifically differentiate microbial strains without having previous knowledge of the membrane molecules or structural changes present in that particular microorganism [90]. Compared to antibodies, aptamers can be chemically modified and labeled more easily facilitating the functionalization of solid surfaces and nanoparticles and can be used in real samples, which is especially useful for environmental and food control applications. The major disadvantage is probably that DNA and RNA structures are highly sensitive to nuclease degradation, but in biosensing applications, the presence of nucleases is not very common.

The majority of assays with aptamers in impedimetric biosensing applications have been reported in terms of faradic measurements. Labib and co-workers reported impedimetric sensors for bacteria viability and typing [91, 92]. In particular, they developed DNA aptamers against *Salmonella enteritidis* pathogen and used it in a mixture of related pathogens including *S. typhimurium*, *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, and *Citrobacter freundii* to confirm specificity of the aptamers. The integration of aptamer onto an impedimetric biosensor was conducted via self-assembling onto gold nanoparticle-modified screen-printed carbon electrode. The aptasensor was incubated for 1 h in different aliquots with increasing concentration of *S. enteritidis* in phosphate-buffered saline. The binding between the target bacteria and the respective aptamers blocked the charge transfer resistance (R_{et}) from a solution-based redox probe to the electrode surface. The obtained detection limit was 600 cfu mL⁻¹, while R_{et} changes produced by other bacterial species were very low compared with *S. enteritidis* target bacteria. This work presented a significant proof of concept for the first aptamer-based impedimetric sensor for typing bacteria [92].

More recent studies has been focused on developing electrochemical impedimetric biosensors for Salmonella detection using a specific ssDNA aptamer [93]. In this case, the biosensor was based on a glassy carbon electrode modified with graphene oxide and gold nanoparticles. Nanoparticles were used for signal amplification and better biocompatibility to detect biological molecules. The modified electrode was incubated in the presence of Salmonella, and its faradic impedance was measured. The optimal incubation time was determined to be 35 min, while the detection limit obtained was as low as 3 cfu mL⁻¹. Furthermore, the specificity was also compared with different strains of bacteria as *L. monocytogenes*, *B. subtilis*, *E. coli*, *S. aureus*, or *S. pyogenes*, and changes registered after 35 min of incubation were much lower than for the *Salmonella*. The resistance value was also obtained monitoring the electron transfer between the Fe(CN)₆^{3-/4-} electrolyte solution and the electrode. Similar methodology was used

by Jia et al. [88] on the performance of an impedimetric aptasensor for *S. aureus* detection by EIS. In this case, a detection limit of 10 cfu mL^{-1} was obtained and high selectivity over other pathogens was also demonstrated.

Probably, alluding to advantages mentioned previously, the use of aptamers in biosensing by electrochemical techniques will increase in the subsequent years.

4.2.3. Antimicrobial peptides

The use of antimicrobial peptides (AMPs) as biorecognition elements for bacterial detection on impedimetric sensors has progressed in recent years. AMPs are a family of biomolecules that are crucial in the innate immune defense of many organisms that display a broad spectrum of activity against gram-negative and gram-positive bacteria. Basically, the antimicrobial activity has been attributed to their capacity to target and disrupt bacterial membranes [94, 95]. First experiments of AMPs for biosensor applications were conducted by Kulagina et al. [96, 97]. They reported two biosensor assays using magainin I as the recognition molecule in the fluorescent-based detection of *E. coli* and *Salmonella*.

First experiments with electrochemical nonfaradic impedance technique with an APM immobilized on IDEAs were done by Mannoor et al. [98] in 2010. They accentuated the high stability of AMPs in harsh environmental conditions, the durability of AMPs immobilized on sensors under natural ambient environment, and their semiselective binding nature to target cells that allows to bind a variety of pathogens [98]. In this case, the AMPs were immobilized on a gold microcapacitive electrodes via a C-terminal cysteine residue, and the biosensor was exposed to various bacteria concentrations ranging from 10^3 to 10^7 cfu mL^{-1} . The variation in impedance change at a fixed frequency of 10 Hz was observed directly proportional to the number of bacterial cells bound to the immobilized AMPs and manifested in a logarithmic increase with serially diluted bacterial concentrations. The detection limit of the device to *E. coli* was 10^3 cfu mL^{-1} (1 bacterium/ μL). Other bacterial species were tested to investigate the selectivity of AMP-functionalized microelectrodes: gram-negative pathogenic *E. coli* O157:H7, *E. coli* ATCC 35218, pathogenic *S. typhimurium*, and gram-positive pathogenic *L. monocytogenes*. The response of biosensor with magainin I was clear preferential toward pathogenic gram-negative species of *E. coli* and *Salmonella*, especially toward *E. coli* O157:H7, demonstrating interbacterial strain differentiation and maintaining recognition capabilities toward pathogenic strains of *E. coli* and *Salmonella*. This research group also demonstrated antimicrobial peptides self-assembling onto a wireless graphene nanosensor integrated on a tooth for remote monitoring of a respiration and bacteria detection in saliva [99].

Similar studies were conducted by Lillehoj et al. [100], who reported a microfluidic chip for the multiplexed detection of bacterial cells using AMPs. Peptide immobilization on the sensors was made via cysteine–gold interactions, revealing robust surface binding. Samples containing *Streptococcus mutans* and *Pseudomonas aeruginosa* were attached to the chip, and both microorganisms were detected at minimum concentrations of 10^5 cfu mL^{-1} in 25 min.

Other works used interdigitated impedimetric arrays for gram-positive bacteria detection with naturally produced AMPs from class IIa bactericins. Etayash et al. [101] used leucocin A, a

representative a class IIa bacteriocin, chemically synthesized and immobilized on interdigitated gold microelectrodes via C-terminal carboxylic acid of the peptide and free amines of a preattached thiolated linker, as antilisterial microbial peptide. In this case, the authors highlighted the narrow activity spectrum of class IIa bacteriocins with high effectiveness with which they act by receptor-mediated mechanism with the target bacterial cells. In this case, leucocin A was used for the real-time detection of *L. monocytogenes*. The detection limit was as low as 10^3 cfu mL⁻¹, which is the equivalent of 1 bacterium/ μ l. The biosensor also selectively detected *Listeria* in front of other gram-positive strains at 10^3 cfu mL⁻¹. Thus, in this work, high sensitivity and selectivity were obtained.

Finally, Li et al. [102] developed a novel biosensor based on faradic impedance for the detection of *E. coli* O157:H7 using a film formed of ferrocene–peptide conjugate on a gold electrode, and magainin I as antimicrobial peptide. Other bacteria as nonpathogenic *E. coli* K12, *Staphylococcus epidermidis* and *Bacillus subtilis*, were used to evaluate the selectivity of biosensors. Obtained results revealed that *E. coli* O157:H7 was preferentially selected as reported before [98]. For impedance measurements, a redox probe with 5 mM of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ was used to optimized detection and study changes produced in the charge transfer resistance (ΔR_{et}). The detection limit obtained was 10^3 cfu mL⁻¹, similar to previous studies.

Although the use of antimicrobial peptides in biosensing applications offers a robustness and stability compared to other biorecognition elements such as antibodies, the main drawback of these elements is the low or lack of specificity against different species and especially different bacterial strains. We have mentioned some examples of aptamer biosensors with more affinity for certain species than others, but for real biomedical or biosafety applications, where the identification of pathogenic bacteria causing human diseases is really important, AMPs performance remains a challenge and has to be studied more in detail.

4.2.4. Lectins

Another kind of biorecognition element described in literature is lectin, a carbohydrate-binding protein or glycoprotein produced by many organisms (from viruses and microorganisms to plants and mammals) that selectively and reversibly react with mono- and oligosaccharides, widely present on bacterial cell surface [103]. The recognition of these carbohydrates on bacteria surface can be used for the specific detection of bacteria. Carbohydrate–protein interaction is much weaker than protein–protein interaction, but these molecules are more stable and smaller than antibodies, and they can neither be denatured easily nor lose their activity [104]. Moreover, the small size of lectins allows to obtain higher densities of carbohydrate-sensing elements on a sensor surface, leading to higher sensitivity and lower nonspecific adsorption [13].

Gamella et al. [105] reported a lectin-based screen-printed gold electrode for the impedimetric detection of bacteria based on faradic impedimetric measurements. In this case, concanavalin A (ConA), a mannose- and glucose-binding lectin, was used as biorecognition element for interaction with carbohydrate of *E. coli* surface. Biotinylated ConA and *E. coli* formed a complex in solution, and after 1 h at room temperature, the complex was immobilized on the surface. Impedimetric measurements were conducted afterward in a solution of the redox probe of

$K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. The electron transfer resistance (R_{et}) varied linearly from 5×10^3 to 5×10^7 Ω . The selectivity was evaluated with different lectins and three different bacteria: *E. coli*, *S. aureus*, and *Mycobacterium phlei*, and satisfactory conclusions were achieved.

Other studies have been conducted to detect sulfate-reducing bacteria such as *Desulforibrio caledoinensis* by immobilizing ConA using an agglutination assay. Wan et al. [106] immobilized ConA onto a gold electrode using amine coupling on the surface with 11-mercaptoundecanoic acid. A redox probe with $Fe(CN)_6^{3-/4-}$ was used to obtain faradic impedance spectra, and an electron transfer resistance (R_{et}) was monitored with the increases of bacterial concentration. The system showed high sensitivity with a linear correlation in the concentration range from 1.8×10^0 to 1.8×10^7 cfu mL⁻¹.

Recent studies has been performed by our group [74] developing an impedimetric transducer based on an interdigitated electrode where ConA lectin was utilized as a biorecognition element. Nonfaradic processes were monitored through R_s changes on IDEA surface. ConA was attached on sensor surface by a layer-by-layer method through PEI–ConA interaction. *E. coli* was used as bacterial model, and similar detection limits of 10^4 cfu mL⁻¹ were obtained as reported by others [105].

Despite advantages presented by lectins as biorecognition elements for biosensing applications described previously, some drawbacks have to be mentioned. The inherent disadvantage of lectins is that several lectins can bind different carbohydrates as well as different carbohydrates can bind the same lectin [107]. These properties of lectins reduce significantly the specificity between bacterial species and especially between bacterial strains. Therefore, in bacterial detection where bacterial membrane consists of a series of different carbohydrates and lipopolysaccharides (aside from other components), the specific detection of bacterial species can produce false positive in complex samples.

4.2.5. Other biorecognition elements

As a recent alternative, the use of bacteriophages as biorecognition elements has been proposed [108, 109]. Bacteriophages are virus of bacteria that utilize bioreplicative machinery to multiply and bind selectively against outer membrane of the bacterial cell-surface proteins, lipopolysaccharides, pili, and lipoproteins. Therefore, bacteriophages can be used as biorecognition element due to additional properties such as high specificity, low-cost production, long shelf life, and thermostability during handling. Furthermore, metabolic products or intracellular components of bacteria realized by lytic action of phages can be an alternative route for biosensing.

Mejri and co-workers [110] developed a biosensor based on the use of T4 bacteriophage for *E. coli* recognition. In this case, antibodies and phages were compared for *E. coli* biosensing by using EIS. Both biorecognition elements were physisorbed on interdigitated gold microelectrodes. Measurements with phages immobilized on the surface were conducted by monitoring the variations in impedance module (ΔZ) at a fixed frequency of 233 MHz. Results showed an increase of the initial impedance after about 20–25 min, followed by an important decrease in impedance. The initial increase was attributed to the phage–bacteria recognition, while the

subsequent decrease presumably happens as consequence of bacterial lysis and release of intracellular components. In case of antibodies, only the initial increase was observed, demonstrating the lytic effect of T4 phage. Linear response was observed for *E. coli* range concentrations from 10^4 to 10^7 cfu mL⁻¹. However, no response was produced in the case of *Lactobacillus* at the same concentrations.

Other recent work conducted by Tlili et al. [111] studies a bacteriophage-impedimetric biosensor for the identification and quantification of *E. coli* with bacteriophage T4. In this case, during the lytic process, the realization of Tuf gene was amplified by a loop-mediated isothermal amplification (LAMP) method and monitored by linear sweep voltammetry (LSV) as a confirmation assay. The phage was attached on a cysteamine-modified gold electrode in the presence of 1,4-phenylene diisothiocyanate, and bacteria adhesion was monitored by changes in electron transfer resistance (ΔR_{et}) in Fe(CN)^{63-/4-} redox pair solution. Electrochemical impedance results reveal a detection limit of 800 cfu mL⁻¹ and a detection time of 15 min, while confirmation assay by LAMP assay and LSV requires 40 min. The reduction of one order of magnitude was obtained by the detection of Tuf gene.

Some drawbacks on the use of bacteriophages in biosensing applications have to be mentioned. During lytic process of bacterium, the signal on a biosensor would be lost or significantly affected due to the components released with bacterial cell disruption [112]. Moreover, some studies suggest that phages bound to the sensor lose their bacterial binding capability upon drying because their tail fibers collapse and are unavailable to bind to the bacterial host [113]. In addition, phages have relatively large sizes, which limit their biosensing applications on particular sensor where detection is limited by distance.

5. Final remarks

Electrochemical biosensors based on impedance detection each year are used more widely due to their high sensitivity and rapid response, which makes this technique extremely useful to detect biological interactions. The detection of pathogenic bacteria using impedance techniques, introduced in this chapter, is an important field that still requires further development.

The detection of bacteria by EIS may be performed in two ways: (1) by the detection of metabolites produced by bacterial growth and involving conductivity changes in the sample and (2) by bacterial detection based on the immobilization of bacteria on electrode surface through biorecognition elements (antibodies, antimicrobial peptides, aptamers, etc.), which is oriented basically on registration of changes in charge transfer resistance (faradic process) and interfacial impedance (nonfaradic process).

The first method is simpler but requires working with low conductivity media and takes longer times. The second method, especially accompanied by some preconcentration technique, may be very fast, selective, and sensitive. Nevertheless, there are still a lot of challenges to be overcome aimed on lower detection limits, shorter detection times, selectivity, and sensitivity. A great help in resolving some of these problems may arrive from using IDEAs. However,

analyzing current publications, we may note that there is poor understanding of how biochemical interactions on a sensor surface affect its electrical properties. Without clear knowledge of interfacial chemical processes and their effect on a complex interfacial impedance, it would not be possible to optimize the measurement procedures and sensor geometry, thus improving sensors performance.

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