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Laccase bioconjugate and multi-walled carbon nanotubes-based biosensor for bisphenol A analysis

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ABSTRACT

Bisphenol A (BPA) is an endocrine disruptor compound that has been detected in aquatic ecosystems. In this work, the development of an electrochemical biosensor for BPA determination based on laccase from *Trametes versicolor* is reported. A bioconjugate was optimized to maximize the biosensor electrocatalytic activity and stability, which for the first time involved the synergistic effect of this specific enzyme (6.8 UmL^{-1}), chitosan (5 $mgmL^{-1}$) and the ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate in an optimum 5:5:2 (v/v/v) proportion. This bioconjugate was deposited onto a screen-printed carbon electrode previously modified with multi-walled carbon nanotubes (MWCNTs). Nanostructuration with MWCNTs enlarged the electrocatalytic activity and surface area, thus improving the biosensor performance. The BPA electrochemical reaction follows an EC mechanism at the optimum pH value of 5.0. Linearity up to 12 μM, a sensitivity of (6.59 ± 0.04) × 10^{-2} $\mu A \mu M^{-1}$ and a detection limit of 8.4 ± 0.3 n M were obtained coupled with high reproducibility (relative standard deviations lower than 6%) and stability (87% of the initial response after one month). The developed biosensor was employed to the analysis of BPA in river water displaying appropriate accuracy (94.6–97.9%) and repeatability (3.1 to 6% relative standard deviations) proving its high potential applicability for *in situ* environmental analysis.

1. Introduction

Contaminants of emerging concern (CECs) are raising attention among worldwide scientists and regulatory authorities [1]. The presence of these chemical compounds was not earlier detected in many ecosystems or only at non relevant concentrations. Therefore, they might be affected by changes in current regulation or by passing of new laws [2]. They arrive at the different ecosystems from a wide range of sources and have potential negative effects on aquatic life and even human health. CECs encompass a variety of compounds, including pharmaceuticals and personal care products, endocrine-disrupting compounds, flame retardants, *etc.* [3]. Among them, bisphenol A (BPA) (2,2-bis(4-hydroxyphenyl) propane) is commonly used as the monomer for the industrial production of polycarbonate, epoxy resins, and other polymeric materials, with applications in beverage and food containers, such as plastic bottles or can coatings and other common articles, such as electronic devices, dental or medical materials or thermally printed papers [4]. Thus, animals and humans could be continuously exposed to BPA, since it is released into the environment during manufacturing processes or by leaching from commercial polycarbonate products. In fact, it has been detected in a wide range of matrices, both clinical (human serum, urine or muscle or liver tissues) and environmental (water, sediment, sewage, or atmospheric particles) [5]. This can result in health effects since BPA is an endocrine disruptor. The two phenol groups in its structure make BPA resemble some natural hormones, such as estradiol and diethylstilbestrol, which originate estrogenic activity [6]. It can also influence thyroid, immune and nervous system processes [7], affect human sperm quality [8] and can be related to some types of cancer [9].

BPA determination is traditionally based on highly sensitive chromatographic or mass spectrometry-based methods [5]. However, due to their long analysis times, expensive instrumentation and high reagents volumes, novel simpler, faster, and lower cost

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methodologies, such as sensors, are currently being developed. Among them, a very limited number of enzymatic electrochemical biosensors have been proposed based on the electron donation capacity of phenols to some families of enzymes [10–17]. Laccase is a copper containing oxidoreductase enzyme, commonly found in some fungi and plant species, insects, and bacteria [18]. It catalyses the oxidation of ortho- and parasubstituted polyphenols, while reducing oxygen to water and avoiding the production of toxic peroxide in the process [19].

In the development of enzymatic biosensors, the key step is the enzyme immobilization. It should keep the protein conformation to assure the catalytic activity. Enzyme immobilization can be achieved by covalent bonding [20], physical adsorption [21] or entrapment in a polymeric matrix [22]. In this sense, chitosan, a natural non-toxic hydrophilic polysaccharide, has been extensively employed as a biomolecule entrapment agent because of the reactive amino and hydroxyl functional groups, which provide it with excellent properties, such as exceptional capability to form films, mechanical strength, biocompatibility and low cost [23–26]. Despite its many advantages, the use of chitosan in electrochemical biosensors is conditioned by its insulating nature, which could hamper the electron transfer process. This limitation can be overcome by incorporating conductive materials, such as metallic nanoparticles [23,26], carbon nanomaterials [27,28], ionic liquids [29] or by a combination of them [24,25,30,31] in the bioconjugate. Ionic liquids are molten salts at room temperatures constituted by bulky organic cations and small anions [32]. They possess many interesting properties for the development of electrochemical biosensors, such as wide potential window, good conductivity and electrochemical stability or low toxicity [33]. Moreover, ionic liquids show good biocompatibility and can help immobilize biomolecules on the electrode surface [34]. Among them, 1-butyl-3-methylimidazolium tetrafluoroborate ($BMIMBF_4$) has been used in the development of enzymatic electrochemical biosensors [35–38]. However, as far as we know, $BMIMBF_4$ was not yet explored for the development of laccase-based biosensor for BPA. It was only used in the detection of the pesticide methomyl using platinum nanoparticles and montmorillonite in carbon paste electrode [39] and in the detection of catechol using LBL assemblies of chitosan/ionic liquid/phthalocyanine [40].

The use of nanomaterials, such as multi-walled carbon nanotubes (MWCNTs), has improved the performance of enzymatic biosensors in terms of sensitivity, detection limit, stability, and other analytical parameters [41]. In particular, for electrochemical biosensors, the presence of MWCNTs increases the electron transfer kinetics due to their intrinsic high conductive properties [42–44]. Furthermore, nanostructuration of the electrode surface offers a larger surface area, which can help to adsorb analyte species or other modifiers [45,46]. In this work, MWCNTs were employed as screen printed carbon electrode (SPCE) modifiers as the initial step in the development of a sensitive and reliable electrochemical BPA biosensor based on laccase, without disregard the sustainability inherent to this type of electrochemical sensors [47]. Subsequently, and for the first-time concerning laccase-based biosensors, the enzyme was included in a homogeneous bioconjugate composed of chitosan and the ionic liquid $BMIMBF_4$ in order to increase its electrocatalytic activity and stability. The developed biosensor was successfully tested in river water samples.

2. Materials and methods

2.1. Reagents and solutions

Polyphenol oxidase laccase enzyme from *T. versicolor* (EC 1.10.3.2; 0.53 U/mg), chitosan from shrimp shells, $BMIMBF_4$, 4-aminophenol (4AMP), catechol and BPA were purchased from Sigma-Aldrich (Steinhein, Germany), whereas 2-aminophenol was acquired from Acros Organics (Belgium), phenol from Merck (Germany) and 4-nitrophenol from Riedel-de-Haën (Germany). Chitosan solution was daily prepared by dissolving 5 mg of chitosan in 1 mL of 3% (v/v) acetic acid and sonicating for 30 min. A 100 mM BPA stock solution in ethanol was also daily prepared by dissolution of 3.65 mg BPA in 160 μL ethanol (99.8%). Working BPA solutions were prepared by diluting the appropriate stock solution volume to 1.0 mL in 0.04 M Britton-Robinson buffer (BRB) pH 5.0.

Multiwalled carboxylic-functionalized carbon nanotubes synthesized by the catalytic chemical vapor deposition method, were also obtained from Sigma-Aldrich (Steinhein, Germany); they are 1.5 μm long, with an average diameter of 9.5 nm and a functionalization degree of 8%. A stock 1 $mgmL^{-1}$ MWCNTs dispersion was prepared in absolute ethanol (99.8%) by sonication for 20 min before each use.

All other reagents used in this work were of analytical grade. Milli-Q ultrapure water (ρ = 18 $M\Omega cm^{-1}$) (Millipore, Molsheim, France) was used throughout this work.

2.2. Instrumentation and conditions

Electrochemical measurements were performed using a potentiostat–galvanostat, AUTOLAB model PGSTAT12 (Metrohm, The Netherlands) controlled by the Model Nova v.11.2 software. SPCEs from DropSens Metrohm (ref. DRP-110, Spain) were used. Each SPCE is composed of carbon-based both working and counter electrodes and a silver pseudo-reference electrode. An SPE connector (DropSens Metrohm) was employed to connect the SPCEs. All the electrochemical assays were executed in 0.04 M pH 5.0 BRB at room temperature. Before each measurement, the electrode was immersed in the solution for one minute in order to have time for the enzymatic reaction to be developed. Square-wave voltammetry (SWV) parameters were optimized to maximize the sensitivity of the electrochemical measurements. The highest peak currents were obtained for a 1 Hz frequency, a 40 mV amplitude and a 5 mV potential step. Electrochemical impedance spectroscopy (EIS) experiments were carried out using a 10 mM $K_3[Fe(CN)_6]$ / 10 mM $K_4[Fe(CN)_6]$ in 0.1 M KCl solution, applying an amplitude modulation of 5 mV in a frequency range from 10^5 to 5×10^{-3} Hz. EIS results were analyzed using the software package FRA 4.9 (Eco Chemie, The Netherlands).

Scanning electron microscopy (SEM) images were obtained in a Quanta 400 FEG ESEM/EDAX Genesis X4M microscope at the Centre of Materials of the University of Porto (Portugal).

2.3. Biosensor construction

The working electrodes of SPCE were first modified by drop coating 5 μL of a 1 $mgmL^{-1}$ MWCNTs dispersion. After that, a bioconjugate was prepared by mixing 5 μL of a freshly prepared 6.8 UmL^{-1} laccase solution, 5 μL of a 5 $mgmL^{-1}$ chitosan solution and 2 μL of the $BMIMBF_4$ ionic liquid (5:5:2, v/v/v). This bioconjugate was then deposited onto the MWCNTs modified electrode. The resulting biosensing platforms (SPCE/MWCNTs/$BMIMBF_4$-Lac-Ch) were kept at 4 °C overnight to promote chitosan gelification.

2.4. Determination of BPA in river water samples

Water samples were collected from Portuguese rivers – one from Ave river and two from Douro river at Afurada and Ribeira zones (Porto Metropolitan area). For the electrochemical measurements, the samples were diluted in 0.01 M BRB pH 5.0 (300 μL to 500 μL) in order to fulfill the optimal enzymatic conditions. The

proposed biosensor performance was validated by recovery assays of fortified water samples at a 10 μM spiking level. All determinations were conducted in triplicate by the standard additions method.

3. Results and discussion

3.1. Biosensor construction

For the biosensor development, laccase, chitosan, $BMIMBF_4$ and MWCNTs were used as electrode modifiers with the aim of obtaining the best response. As can be seen in Fig. 1, in the presence of BPA an anodic peak is observed for all the electrode modifications studied. In the case of the bare electrode (SCPE, curve a), this peak can be attributed to the BPA oxidation (0.39 V). The presence of laccase on the electrode surface (SPCE/Lac, curve b) causes a 30% increase in the peak current and a 60 mV decrease in the peak potential when compared to the bare electrode. This is due to oxidation of the product originated from the enzymatic catalysis in addition to the direct oxidation of BPA at the electrode surface [18]. This can be experimentally confirmed by analysing BPA right after its deposition in the biosensor surface in comparison to its analysis after a 30 min reaction period which besides further increasing BPA peak (about 45% increase) also leads to the observation of two new peaks in the voltammogram at around + 0.09 and + 0.18 V (Fig. S1; Supplementary Material). This confirms the suitability of laccase in the development of a BPA biosensor. Enzyme stability is of great importance in the biosensor development. Thus, an entrapment biopolymer, chitosan, was employed to immobilize the laccase. The SCPE modified with the polymeric gel (SPCE/Lac-Ch, curve c) shows an almost 3-fold rise in the peak current due to the formation of a film on its surface, which prevents the leaching of the enzyme. The addition of $BMIMBF_4$ to the enzymatic bioconjugate (SPCE/$BMIMBF_4$-Lac-Ch, curve d) enhances the biosensor response since it preserves the rigid structure of the enzyme, therefore improving the stability and activity of the enzyme, and compensates the poor electrical conductivity of chitosan. $BMIMBF_4$ can contribute to charge transport by acting as a fortifying supplier of ion carriers (conductivity of 0.35 S/m at 25 °C) [48,49]. This electrochemical behavior is in accordance with those previously reported by Shangguan et al. [50] and Gomes et al [51]. Finally, in order to improve the biosensor sensitivity, the SPCE was modified by drop casting of MWCNTs, forming a first MWCNTs layer and the bioconjugate was then deposited onto it. This two-steps modification led to better results than incorporating the MWCNTs into the bioconjugate (data not shown), since the MWCNTs enlarge the electric conductivity, the electrocatalytic activity and the electroactive surface area contributing to the enzymatic bioconjugate immobilization [52]. The electrode modified with MWCNTs and the bioconjugate (SPCE/MWCNTs/$BMIMBF_4$-Lac-Ch, curve e) displays the highest current response, highlighting the importance of each component's role in the biosensor development. Thus, the proposed biosensor is composed of a first layer of MWCNTs onto which the bioconjugate containing $BMIMBF_4$, laccase and chitosan is deposited.

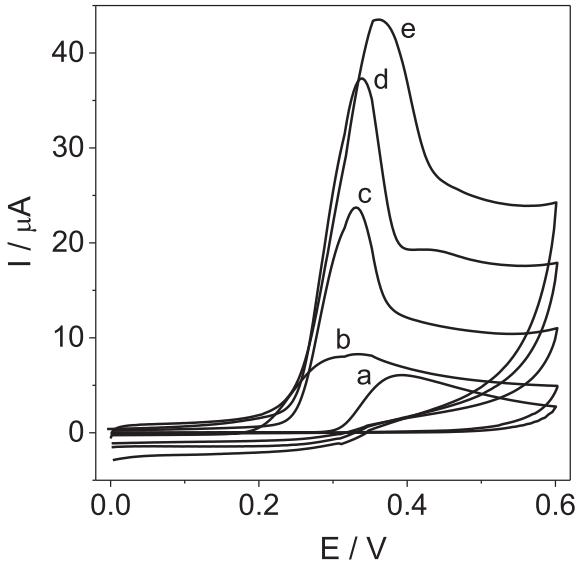

Fig. 1. Cyclic voltammograms of (a) SPCE, (b) SPCE/Lac, (c) SPCE/Lac-Ch, (d) SPCE/$BMIMBF_4$-Lac-Ch and (e) SPCE/MWCNTs/$BMIMBF_4$-Lac-Ch in the presence of 50 μM BPA in 0.04 M BRB pH 5.0. Scan rate 0.01 Vs^{-1}.

With the objective of optimizing the amount of each of the modifying agents, biosensors containing different amounts of each component were prepared and their responses to BPA were tested. Only one component was varied at a time. An increase in the current is observed with increasing 1 $mgmL^{-1}$ MWCNTs volumes on the electrode surface (Fig. 2A) up to 5 μL (26%). The response obtained with a higher amount (6 μL) is not significantly different from the one attained with 5 μL, therefore, this volume was chosen as the optimal MWCNTs loading. In the case of laccase (Fig. 2B), the best signal is obtained for 5 μL and higher loadings cause a decrease in the current since a large amount of enzyme could hamper the electron transfer towards the electrode surface. Regarding the other bioconjugate components, maximum signals are reached at 2 μL for $BMIMBF_4$ and 5 μL for chitosan. These two components are related since for a low chitosan- $BMIMBF_4$ ratio, the biopolymer could not retain the ionic liquid on the electrode surface and for a high ratio, the role of ionic liquid cannot be appreciated. Thus, these amounts were selected for the optimal composition of the developed biosensor.

3.2. Biosensor characterization

SEM was used to study the morphology of the proposed biosensor (Fig. 3 and Fig. S2 of Supplementary Material). Fig. 3 shows the surface modification sequence of the biosensor namely, the bare SPCE (A), the SPCE modified with MWCNTs (B), and the SPCE modified with MWCNTs and the bioconjugate (C). As can be seen, in the developed biosensing platform image (C), the underlying MWCNTs can be observed, covered by $BMIMBF_4$, enzyme and chitosan layer. This can be clearly seen when comparing with the MWCNTs modified electrode (B), where a more defined image of the typical morphology of well-dispersed MWCNTs [53] is obtained since no top layer is covering them. In both cases, a homogeneous and compact surface with a high coverage is observed. The deposited bioconjugate ($BMIMBF_4$-Lac-ch) originated an appropriate milieu for laccase entrapment by merging the solvation capacity and superior ionic conductivity of $BMIMBF_4$ with the structure of the natural biopolymer, supported by the high tensile strength and electric conductivity of the MWCNTs deposited on the SPCE [50,51].

The electrochemical behaviour of the biosensor was characterized by studying its voltammetric response in the presence of a reversible redox probe. In this case, 50 μM 4AMP was chosen since it acts also as a substrate for the enzyme, due to the existence of a phenol in its structure. In the bare SPCE voltammogram (Fig. 4A black dots), a quasi-reversible redox pair (0.05 V) is detected, associated with the formation of a quinone-imine species [54]. This redox pair is shifted to more negative potentials (0.01 V) in the case of SPCE/MWCNTs (Fig. 4A gray dots) and a higher anodic and cathodic peaks current can be observed as well, due to the increase in the surface area and conductivity. When the surface is subsequently modified with MWCNTs and $BMIMBF_4$-Ch compos-

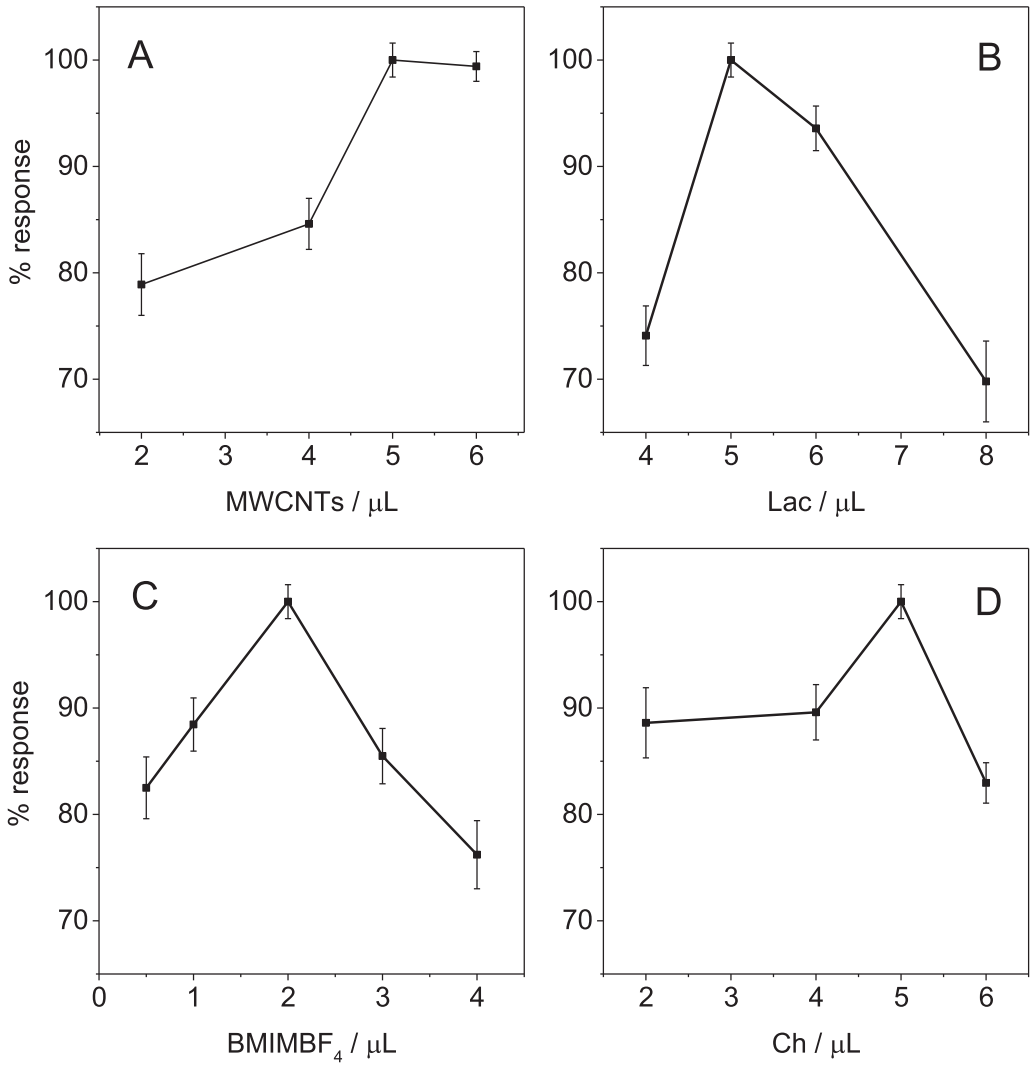

Fig. 2. Relative response of the biosensor constructed with different loadings of (A) 1 mgmL^{-1} MWCNTs (5 μL laccase, 5 μL Ch and 2 μL $BMIMBF_4$), (B) 6.8 UmL^{-1} laccase (5 μL MWCNT, 5 μL Ch and 2 μL $BMIMBF_4$), (C) $BMIMBF_4$ (5 μL MWCNT, 5 μL laccase, 5 μL Ch) and (D) 5 mgmL^{-1} chitosan (5 μL MWCNT, 5 μL laccase, 2 μL $BMIMBF_4$) in the presence of 50 μM BPA in 0.04 M BRB pH 5.0.

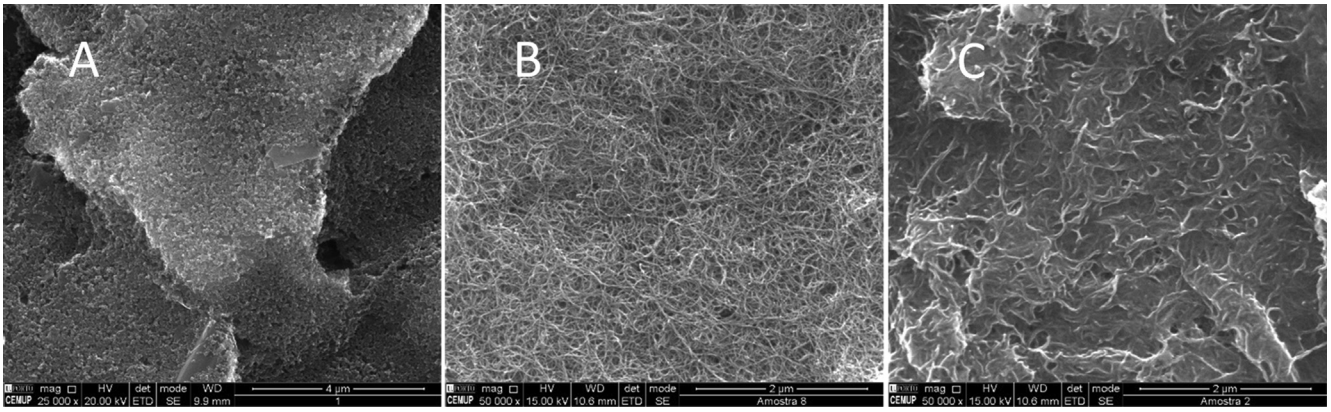

Fig. 3. SEM images of (A) bare SPCE at 25000x magnification, (B) SPCE/MWCNTs and (C) SPCE/MWCNTs/$BMIMBF_4$-Lac-Ch at a 50000x magnification.

ite in the absence of the enzyme (SPCE/MWCNTs/$BMIMBF_4$-Ch) (Fig. 4A gray line), the peak current decreases (40%) mainly due to the presence of chitosan. In the case of SPCE/MWCNTs/$BMIMBF_4$-Lac-Ch (Fig. 4A black line), an increase in the quinone-imine reduction peak current is observed since it is the product of the enzymatic oxidation of 4AMP by laccase [55].

EIS is a very useful technique for characterizing the interfacial features of modified electrodes allowing to complement the CV

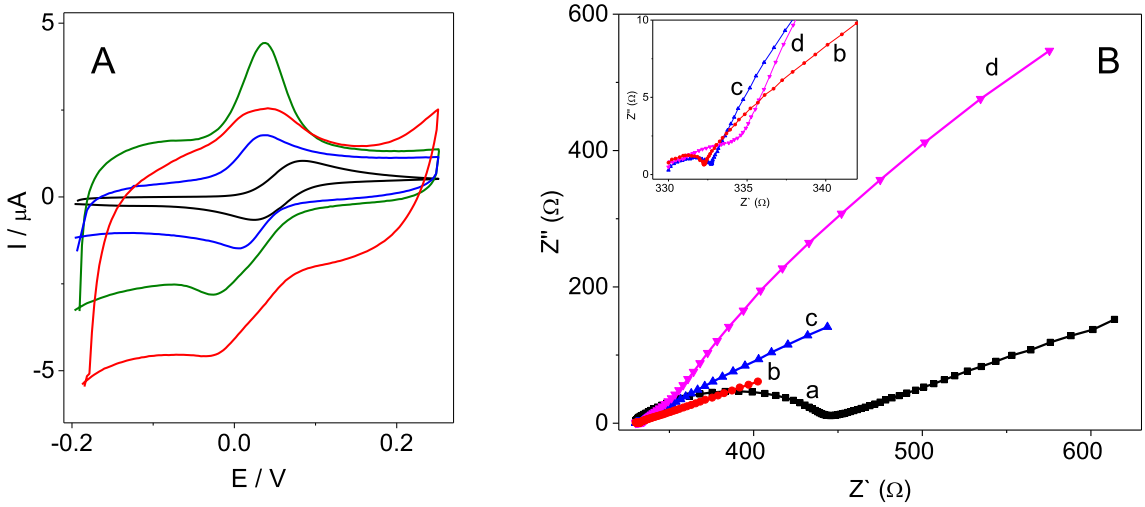

Fig. 4. (A) Cyclic voltammograms of SPCE (black line), SPCE/MWCNTs (green line), SPCE/MWCNTs/ $BMIMBF_4$-Ch (blue line) and SPCE/MWCNTs/$BMIMBF_4$-Lac-Ch (red line) in the presence of 50 µM 4AMP in 0.04 M BRB pH 5.0. Scan rate 0.01 Vs^{-1}. (B) Nyquist diagram of SPCE (a), SPCE/MWCNTs (b), SPCE/MWCNTs/$BMIMBF_4$-Ch (c) and SPCE/MWCNTs/$BMIMBF_4$-Lac-Ch (d) in 10 mM $K_3[Fe(CN)_6]$ / $K_4[Fe(CN)_6]$ in 0.1 M KCl.

data [56]. Therefore, it was employed to assess the effect of modification in the electrode surface properties. The electrochemical impedance spectra of SPCE, SPCE/MWCNTs, SPCE/MWCNTs/ $BMIMBF_4$-Ch and SPCE/MWCNTs/$BMIMBF_4$-Lac-Ch, registered in the presence of the electrochemical indicator$K_3[Fe(CN)_6]$ / $K_4[Fe(CN)_6]$, are shown in Fig. 4B. In the SPCE spectrum (Fig. 4Ba), a semicircle in the high frequency region is perceived, indicating a charge-transfer limited process, with a charge transfer resistance (R_{CT}) of 115.9 Ω, as well as a straight line in the low frequency region, related to a diffusion-limited process [54,57]. In the SPCE/MWCNTs spectrum (Fig. 4Bb), a sharp reduction (97.4%) in R_{CT} (3.0 Ω) is observed, suggesting that the interfacial electron transfer was enhanced due to the great conductive properties of MWCNTs. The presence of the selected ionic liquid and chitosan in SPCE/MWCNTs/$BMIMBF_4$-Ch (Fig. 4Bc) caused a slight increase in the R_{CT} (4.1 Ω), confirming the role of the applied ionic liquid in preserving the conductivity of the modified electrode compensating the insulator properties of chitosan. Finally, in the case of SPCE/MWCNTs/$BMIMBF_4$-Lac-Ch (Fig. 4Bd), the charge transfer resistance is increased to 36.1 Ω, proving the successful immobilization of the enzyme in the bioconjugate. This pattern of variation is in agreement with previous studies [58].

pH is a key parameter in enzymatic biosensor development since the catalytic activity of enzymes, and in particular laccase, is greatly affected by this parameter [19]. Furthermore, the electrochemical reaction taking place on the biosensor surface can also be affected by protonation. Therefore, the effect of pH in the biosensor response was studied in the presence of BPA at pH values between 4 and 6.5 (Fig. 5). As can be seen (Fig. 5A), the highest current is obtained for pH 5.0, which has been described as the optimum pH [39,55] for laccase from *T. versicolor* proving that the enzymatic activity is not affected by entrapment in the bioconjugate. As far as we know, this is the first study that combine the selected three elements ($BMIMBF_4$-Lac-Ch) in a composite to immobilize and maintain the stable and active catalytic capacity of the enzyme.

Moreover, the anodic peak potential is shifted linearly with a slope of 33 mV/pH to lower values as the pH increases (Fig. 5B). This value is almost half the theoretical value envisaged by the Nernst equation of 59 mV/pH [59], suggesting that in the redox process 2 electrons per proton may be exchanged.

With the aim of analysing the electrochemical process, the impact of the scan rate on the BPA oxidation was studied

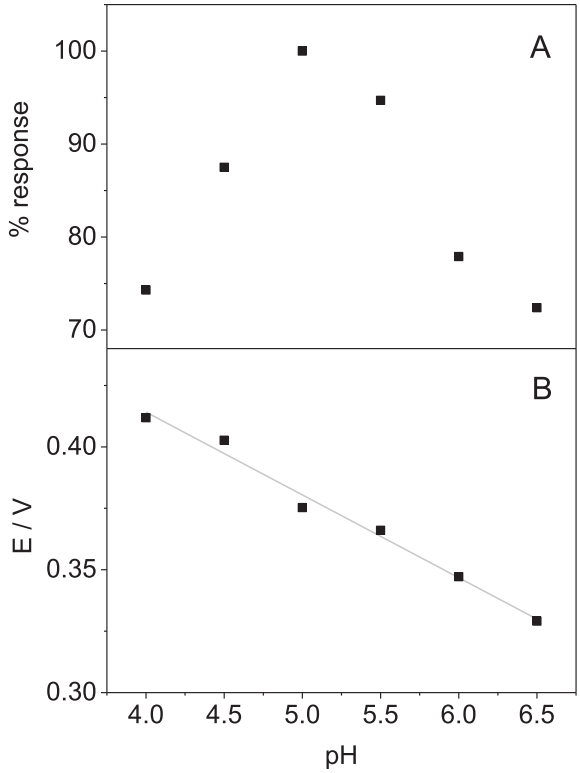

Fig. 5. Effect of pH (A) in the biosensor relative current and (B) in the peak potential in the presence of 50 µM BPA in 0.04 M BRB.

(Fig. S3, Supplementary Material). In the cyclic voltammograms recorded in the presence of 50 µM BPA at different scan rates (Fig. S3A), a displacement in the anodic peak potential on increasing scan rate is noticed, suggesting kinetic limitations in the electrochemical oxidation on the biosensor electrode surface. The anodic peak current follows a linear relationship with the square root of the scan rate (Fig. S3B), indicating that the redox process is controlled by the diffusion of BPA to the electrode surface [60].

Moreover, the shape obtained in the peak current normalized with the square root of the scan rate *versus* the scan rate plot (Fig. S3C) suggests that the electrochemical reaction for the BPA

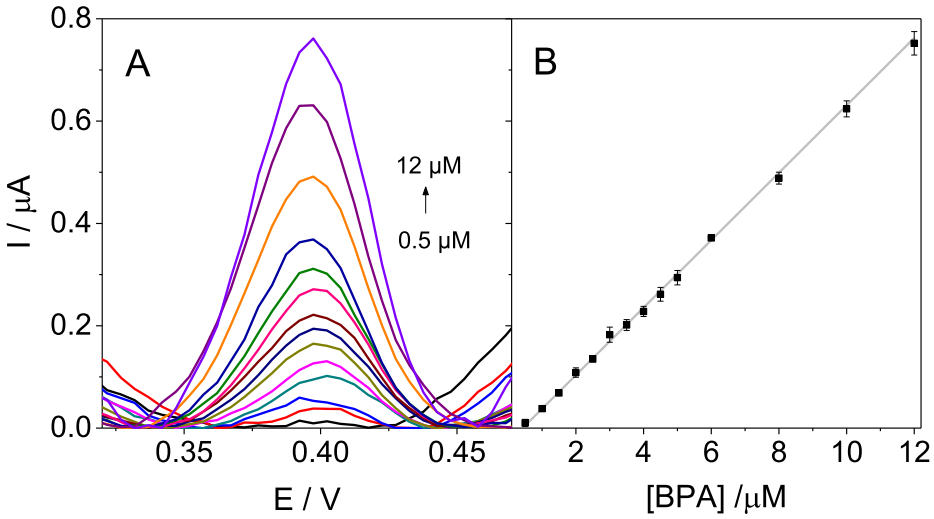

Fig. 6. SWV voltammograms (A) and biosensor current response (B) in the presence of increasing BPA concentrations between 0.5 and 12 μM in 0.04 M BRB pH 5.0. Square wave voltammetric conditions: frequency 1 Hz, pulse amplitude 40 mV and scan increment 5 mV. Scan rate 5 mVs^{-1}.

Table 1
Comparison of laccase-based BPA biosensors.

Biosensor	LOD (nM)	Linear range (μM)	Sensitivity ($\mu A \mu M^{-1}$)	Sample	Reference
SPGE/Lac/nf	35,000	120–500	$(3.3 \pm 0.3) \times 10^{-3}$	–	[14]
SPGE/CB/thionine/Lac/nf	200	0.50–50	$(5.0 \pm 0.1) \times 10^{-3}$	tomato juice	[15]
SPCE/MWCNTs/Ag-ZnO/Lac	6	0.50–2.99	–	water bottles	[13]
SPCE/Gr-Au-Ch/Lac	23	0.05–12	2.71×10^{-1}	plastic products	[16]
SPCE/MWCNTs/$BMIMBF_4$-Lac-Ch	8.4	0.50–12	$(6.59 \pm 0.04) \times 10^{-2}$	river water	this work

SPGE: screen printed graphite electrode; CB: carbon black; nf: nafion; Ag-ZnO: silver doped zinc oxide nanoparticles; SPCE: screen printed carbon electrode; MWCNTs: multi walled carbon nanotubes; Gr-Au-ch: graphene-decorated gold nanoparticles and chitosan composite.

oxidation is a complex mechanism in which a chemical reaction is present in addition to the electrochemical one [61,62].

3.3. Analytical performance

BPA determination was carried out using SWV to reach a higher sensitivity. As can be seen in Fig. 6, the peak current grows linearly (R^2 = 0.998) with increasing BPA concentrations up to 12 μM, with a high sensitivity estimated from the plot slope of $(6.59 \pm 0.04) \times 10^{-2}$ $\mu A \mu M^{-1}$ and a detection limit of 8.4 ± 0.3 nM. The LOD value compares well with the scarce laccase-based BPA biosensors found in the literature (Table 1) [13–16], exhibiting a broader linearity range than the one that has the lowest (and very similar) LOD [13]. Reproducibility was assessed by comparing the current response of three equally prepared biosensors. A relative standard deviation (RSD) value of 5.3% was obtained attesting the excellent reproducibility of the device. In addition, the stability of the developed biosensor was also assessed, and, after one month kept at 4 °C, the biosensor showed 87% of its original response (when exposed to a 10 μM concentration of BPA) corroborating the adopted strategy for laccase immobilization.

The biosensor selectivity is of great importance for its potential applicability in real samples. Therefore, the influence of potential interfering substances existing in river water samples was studied. For that purpose, the response of the biosensor to 10 μM BPA was recorded in the absence and in the presence of two different concentrations of some ions, namely, Cl^-, HCO_3^-, NO_3^-, SO_4^{2-}, K^+, Ca^{2+}, Mg^{2+} and Cu^{2+}. The obtained results (Table 2) showed that, even for the 50-fold concentrations, the studied ions do not significantly affect the biosensor response to BPA. Also, other phenolic compounds that are originated from the chemical industry activities, may be present in environmental waters and thus may interfere in BPA analysis since they have a similar chemical structure. Specifically, 2-aminophenol, catechol, 4-nitrophenol and phenol were also assessed as potential interferents (Table 2). As seen, only phenol significantly interferes in BPA analysis, decreasing the response to about 58.8 ± 3.8%. Overall, the achieved biosensing characteristics, such as low LOD, good reproducibility, stability and selectivity, confirm that the proposed biosensor can be a good strategy for BPA quantification.

Table 2
Interference tests on different ions for BPA determination.

	Current ratio (%)*		
	1:1	1:5	1:50
Cl^-	–	90.2 ± 0.3	88.9 ± 0.3
HCO_3^-	–	91.9 ± 0.3	98.4 ± 0.5
NO_3^-	–	95.2 ± 0.6	92.0 ± 0.4
SO_4^{2-}	–	96.1 ± 0.4	91.7 ± 0.6
K^+	–	93.0 ± 0.6	90.1 ± 0.6
Ca^{2+}	–	92.2 ± 0.5	93.1 ± 0.4
Mg^{2+}	–	93.2 ± 0.7	92.0 ± 0.5
Cu^{2+}	–	93.9 ± 0.5	94.8 ± 0.2
2-aminophenol	91.1 ± 2.0	–	–
4-nitrophenol	100.2 ± 9.0	–	–
phenol	58.8 ± 3.8	–	–
catechol	95.3 ± 7.7	–	–

*Current ratio (%) = $I_{BPA+I}/I_{BPA} \times 100$.
I_{BPA+I}: response of 10 μM BPA in the presence of the interfering compound; I_{BPA}: response of the biosensor to 10 μM BPA.

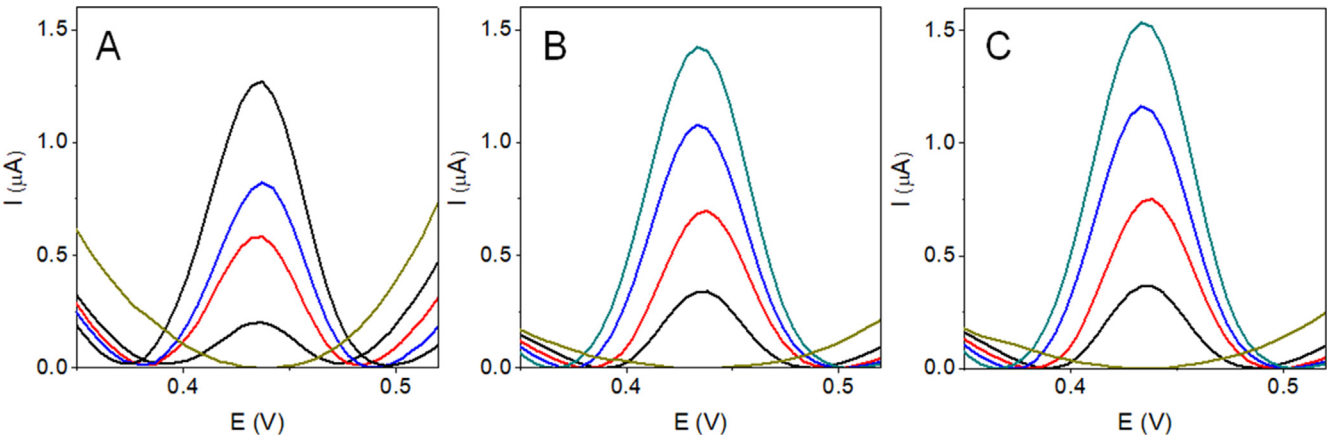

Fig. 7. Typical SWV voltammograms of unspiked (green line) and spiked (10 μM BPA) real samples of (A) Ave river, (B) Douro river at Ribeira site and (C) Douro river at Afurada site (Portugal).

3.4. Real application

With the aim of testing the applicability of the proposed laccase-based biosensor, BPA was determined in water samples from two Portuguese rivers at three different sites (Ave river, and Douro river at Ribeira and Afurada). Since BPA levels in the collected river waters were not detected, recovery assays were carried out at the 10 μM BPA level and the standard additions method was employed to avoid matrix effects (Fig. 7). The found recoveries were 94.6% (Ave river waters), 97.9% (Douro river at Ribeira) and 94.8% (Douro river at Afurada), with RSD ranging from 3.1% (Douro at Afurada) to 6% (Douro at Ribeira) for three different measurements of each same sample. The obtained results indicate that the developed biosensor can be successfully applied for the analysis of BPA in water samples having potential interest for *in situ* environmental monitoring. Still, its application in real samples with endogenous BPA would be valuable. [63].

4. Conclusions

An accurate and sensitive BPA biosensor based on laccase enzymatic activity was developed. Taking advantages of their inherent physical–chemical properties, $BMIMBF_4$ and chitosan were combined to promote a suitable microenvironment for enzymatic electrocatalytic activity while favouring the stability of the bioconjugate. Moreover, performing a prior SPCE modification with MWCNTs improves the biosensor sensitivity and the overall electroanalytical performance. The developed electrochemical biosensor allows sensitive detection within a large linear range with high stability and reproducibility. Its application to the determination of BPA in real samples, such as river water, led to good recoveries and repeatability. These results demonstrate that the proposed biosensor can have a great potential for environmental analysis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioelechem.2021.108033.

References

[1] C. Bolognesi, L. Castle, J.P. Cravedi, K.H. Engel, P. Fowler, R. Franz, K. Grob, R. Gürtler, T. Husøy, W. Mennes, M.R. Milana, A. Penninks, F. Roland, V. Silano, A. Smith, M.F. Tavares Poças, C. Tlustos, F. Toldrá, D. Wölfle, H. Zorn, Scientific opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs, EFSA J. 13 (2015) 3978, https://doi.org/10.2903/j.efsa.2015.3978.

[2] G. Vandermeersch, H.M. Lourenco, D. Alvarez-Munoz, S. Cunha, J. Diogene, G. Cano-Sancho, J.J. Sloth, C. Kwadijk, D. Barcelo, W. Allegaert, K. Bekaert, J.O. Fernandes, A. Marques, J. Robbens, Environmental contaminants of emerging concern in seafood - European database on contaminant levels, Environ. Res. 143 (2015) 29–45, https://doi.org/10.1016/j.envres.2015.06.011.

[3] M. Salimi, A. Esrafili, M. Gholami, A.J. Jafari, R.R. Kalantary, M. Farzadkia, M. Kermani, H.R. Sobhi, Contaminants of emerging concern: a review of new approach in AOP technologies, Environ. Monit. Assess. 189 (2017) 414, https://doi.org/10.1007/s10661-017-6097-x.

[4] S. Almeida, A. Raposo, M. Almeida-Gonzalez, C. Carrascosa, Bisphenol A: Food exposure and impact on human health, Compr. Rev. Food Sci. Food Saf. 17 (2018) 1503–1517, https://doi.org/10.1111/1541-4337.12388.

[5] F. Sun, L. Kang, X. Xiang, H. Li, X. Luo, R. Luo, C. Lu, X. Peng, Recent advances and progress in the detection of bisphenol A, Anal. Bioanal. Chem. 408 (2016) 6913–6927, https://doi.org/10.1007/s00216-016-9791-6.

[6] L.N. Vandenberg, M.V. Maffini, C. Sonnenschein, B.S. Rubin, A.M. Soto, Bisphenol-A and the great divide: A review of controversies in the field of endocrine disruption, Endocr. Rev. 30 (2009) 75–95, https://doi.org/10.1210/er.2008-0021.

[7] J.R. Rochester, Bisphenol A and human health: A review of the literature, Reprod. Toxicol. 42 (2013) 132–155, https://doi.org/10.1016/j.reprotox.2013.08.008.
[8] E. Carlsen, A. Giwercman, N. Keiding, N.E. Skakkebaek, Declining semen quality and increasing incidence of testicular cancer - is there a common-cause, Environ. Health Perspect. 103 (1995) 137–139, https://doi.org/10.2307/3432523.
[9] R. Steinmetz, N.A. Mitchner, A. Grant, D.L. Allen, R.M. Bigsby, N. Ben-Jonathan, The xenoestrogen bisphenol A induces growth, differentiation, and c-fos gene expression in the female reproductive tract, Endocrinology 139 (1998) 2741–2747, https://doi.org/10.1210/en.139.6.2741.
[10] A.T.E. Vilian, K. Giribabu, S.R. Choe, R. Muruganantham, H. Lee, C. Roh, Y.S. Huh, Y.K. Han, A spick-and-span approach to the immobilization of horseradish peroxidase on Au nanospheres incorporated with a methionine/graphene biomatrix for the determination of endocrine disruptor bisphenol A, Sens. Actuator B-Chem. 251 (2017) 804–812, https://doi.org/10.1016/j.snb.2017.05.122.
[11] N. Ben Messaoud, M.E. Ghica, C. Dridi, M. Ben Ali, C.M.A. Brett, A novel amperometric enzyme inhibition biosensor based on xanthine oxidase immobilised onto glassy carbon electrodes for bisphenol A determination, Talanta 184 (2018) 388–393, https://doi.org/10.1016/j.talanta.2018.03.031.
[12] X. Wang, X. Lu, L. Wu, J. Chen, 3D metal-organic framework as highly efficient biosensing platform for ultrasensitive and rapid detection of bisphenol A, Biosens. Bioelectron. 65 (2015) 295–301, https://doi.org/10.1016/j.bios.2014.10.010.
[13] K. Kunene, M. Sabela, S. Kanchi, K. Bisetty, High performance electrochemical biosensor for Bisphenol A using screen printed electrodes modified with multiwalled carbon nanotubes functionalized with silver-doped zinc oxide, Waste Biomass Valori. 11 (2020) 1085–1096, https://doi.org/10.1007/s12649-018-0505-5.
[14] V. Scognamiglio, I. Pezzotti, G. Pezzotti, J. Cano, I. Manfredonia, K. Buonasera, F. Arduini, D. Moscone, G. Palleschi, M.T. Giardi, Towards an integrated biosensor array for simultaneous and rapid multi-analysis of endocrine disrupting chemicals, Anal. Chim. Acta 751 (2012) 161–170, https://doi.org/10.1016/j.aca.2012.09.010.
[15] M. Portaccio, D. Di Tuoro, F. Arduini, D. Moscone, M. Cammarota, D.G. Mita, M. Lepore, Laccase biosensor based on screen-printed electrode modified with thionine-carbon black nanocomposite, for Bisphenol A detection, Electrochim. Acta 109 (2013) 340–347, https://doi.org/10.1016/j.electacta.2013.07.129.
[16] F.M. Fartas, J. Abdullah, N.A. Yusof, Y. Sulaiman, M.I. Saiman, M.H.M. Zaid, Laccase electrochemical biosensor based on graphene-gold/chitosan nanocomposite film for Bisphenol A detection, Curr. Anal. Chem. 15 (2019) 1–9.
[17] L. Kong, S. Huang, Z. Yue, B. Peng, M. Li, J. Zhang, Sensitive mediator-free tyrosinase biosensor for the determination of 2,4-dichlorophenol, Microchim. Acta 165 (2009) 203–209, https://doi.org/10.1007/s00604-008-0121-3.
[18] M. Fernandez-Fernandez, M.A. Sanroman, D. Moldes, Recent developments and applications of immobilized laccase, Biotechnol. Adv. 31 (2013) 1808–1825, https://doi.org/10.1016/j.biotechadv.2012.02.013.
[19] M.M. Rodriguez-Delgado, G.S. Aleman-Nava, J.M. Rodriguez-Delgado, G. Dieck-Assad, S.O. Martinez-Chapa, D. Barcelo, R. Parra, Laccase-based biosensors for detection of phenolic compounds, Trac-Trends, Anal. Chem. 74 (2015) 21–45, https://doi.org/10.1016/j.trac.2015.05.008.
[20] A.M. Othman, U. Wollenberger, Amperometric biosensor based on coupling aminated laccase to functionalized carbon nanotubes for phenolics detection, Int. J. Biol. Macromol. 153 (2020) 855–864, https://doi.org/10.1016/j.ijbiomac.2020.03.049.
[21] I. Vasilescu, S.A.V. Eremia, M. Kusko, A. Radoi, E. Vasile, G.L. Radu, Molybdenum disulphide and graphene quantum dots as electrode modifiers for laccase biosensor, Biosens. Bioelectron. 75 (2016) 232–237, https://doi.org/10.1016/j.bios.2015.08.051.
[22] C. Salvo-Comino, A. González-Gil, J. Rodriguez-Valentin, C. Garcia-Hernandez, F. Martin-Pedrosa, C. Garcia-Cabezon, M.L. Rodriguez-Mendez, Biosensors platform based on chitosan/AuNPs/phthalocyanine composite films for the electrochemical detection of catechol, Role Surface Struct. Sensors 20 (2020) 2152, https://doi.org/10.3390/s20072152.
[23] A. Kaushika, R. Khan, P.R. Solanki, P. Pandey, J. Alam, S. Ahmad, B.D. Malhotra, Iron oxide nanoparticles-chitosan composite based glucose biosensor, Biosens. Bioelectron. 24 (2008) 676–683, https://doi.org/10.1016/j.bios.2008.06.032.
[24] K. Zhou, Y. Zhu, X. Yang, J. Luo, C. Li, S. Luan, A novel hydrogen peroxide biosensor based on Au-graphene-HRP-chitosan biocomposites, Electrochim. Acta 55 (2010) 3055–3060, https://doi.org/10.1016/j.electacta.2010.01.035.
[25] C. Zhai, X. Sun, W. Zhao, Z. Gong, X. Wang, Acetylcholinesterase biosensor based on chitosan/prussian blue/multiwall carbon nanotubes/hollow gold nanospheres nanocomposite film by one-step electrodeposition, Biosens. Bioelectron. 42 (2013) 124–130, https://doi.org/10.1016/j.bios.2012.10.058.
[26] E. Pashai, G.N. Darzi, M. Jahanshahi, F. Yazdian, M. Rahimnejad, An electrochemical nitric oxide biosensor based on immobilized cytochrome c on a chitosan-gold nanocomposite modified gold electrode, Int. J. Biol. Macromol. 108 (2018) 250–258, https://doi.org/10.1016/j.ijbiomac.2017.11.157.
[27] H. Xu, H. Dai, G. Chen, Direct electrochemistry and electrocatalysis of hemoglobin protein entrapped in graphene and chitosan composite film, Talanta 81 (2010) 334–338, https://doi.org/10.1016/j.talanta.2009.12.006.
[28] Y. Liu, M.K. Wang, F. Zhao, Z.A. Xu, S.J. Dong, The direct electron transfer of glucose oxidase and glucose biosensor based on carbon nanotubes/chitosan matrix, Biosens. Bioelectron. 21 (2005) 984–988, https://doi.org/10.1016/j.bios.2005.03.003.
[29] F. Xi, L. Liu, Q. Wu, X. Lin, One-step construction of biosensor based on chitosan-ionic liquid-horseradish peroxidase biocomposite formed by electrodeposition, Biosens. Bioelectron. 24 (2008) 29–34, https://doi.org/10.1016/j.bios.2008.03.023.
[30] L. Wang, X. Zhang, H. Xiong, S. Wang, A novel nitromethane biosensor based on biocompatible conductive redox graphene-chitosan/hemoglobin/graphene/room temperature ionic liquid matrix, Biosens. Bioelectron. 26 (2010) 991–995, https://doi.org/10.1016/j.bios.2010.08.027.
[31] C. Ruan, T. Li, Q. Niu, M. Lu, J. Lou, W. Gao, W. Sun, Electrochemical myoglobin biosensor based on graphene–ionic liquid–chitosan bionanocomposites: Direct electrochemistry and electrocatalysis, Electrochim. Acta 64 (2012) 183–189, https://doi.org/10.1016/j.electacta.2012.01.005.
[32] J.S. Moulthrop, R.P. Swatloski, G. Moyna, R.D. Rogers, High-resolution C-13 NMR studies of cellulose and cellulose oligomers in ionic liquid solutions, Chem. Commun. (2005) 1557–1559, https://doi.org/10.1039/b417745b.
[33] B.M. Quinn, Z.F. Ding, R. Moulton, A.J. Bard, Novel electrochemical studies of ionic liquids, Langmuir 18 (2002) 1734–1742, https://doi.org/10.1021/la011458x.
[34] X. Wang, J. Hao, Recent advances in ionic liquid-based electrochemical biosensors, Sci. Bull. 61 (2016) 1281–1295, https://doi.org/10.1007/s11434-016-1151-6.
[35] S. Karimi, H. Ghourchian, P. Rahimi, H.A. Rafiee-Pour, A nanocomposite based biosensor for cholesterol determination, Anal. Methods 4 (2012) 3225–3231, https://doi.org/10.1039/C2AY25826A.
[36] K.S. Galhardo, R.M. Torresi, S.I. Córdoba de Torresi, Improving the performance of a glucose biosensor using an ionic liquid for enzyme immobilization. On the chemical interaction between the biomolecule, the ionic liquid and the cross-linking agent, Electrochim. Acta 73 (2012) 123–128, https://doi.org/10.1016/j.electacta.2011.10.088.
[37] X. Liu, H. Feng, J. Zhang, R. Zhao, D.K.Y. Wong, Hydrogen peroxide detection at a horseradish peroxidase biosensor with a Au nanoparticle–dotted titanate nanotube|hydrophobic ionic liquid scaffold, Biosens. Bioelectron. 32 (2012) 188–194, https://doi.org/10.1016/j.bios.2011.12.002.
[38] X. Zeng, X. Li, L. Xing, X. Liu, S. Luo, W. Wei, B. Kong, Y. Li, Electrodeposition of chitosan-ionic liquid-glucose oxidase biocomposite onto nano-gold electrode for amperometric glucose sensing, Biosens. Bioelectron. 24 (2009) 2898–2903, https://doi.org/10.1016/j.bios.2009.02.027.
[39] E. Zapp, D. Brondani, I.C. Vieira, C.W. Scheeren, J. Dupont, A.M.J. Barbosa, V.S. Ferreira, Biomonitoring of methomyl pesticide by laccase inhibition on sensor containing platinum nanoparticles in ionic liquid phase supported in montmorillonite, Sens. Actuator B-Chem. 155 (2011) 331–339, https://doi.org/10.1016/j.snb.2011.04.015.
[40] C. Salvo-Comino, C. Garcia-Hernandez, C. Garcia-Cabezon, M.L. Rodriguez-Mendez, Promoting laccase sensing activity for catechol detection using LBL assemblies of chitosan/ionic liquid/phthalocyanine as immobilization surfaces, Bioelectrochemistry 132 (2020), https://doi.org/10.1016/j.bioelechem.2019.107407 107407.
[41] I.S. Kucherenko, O.O. Soldatkin, D.Y. Kucherenko, O. Soldatkina V., S. Dzyadevych V., Advances in nanomaterial application in enzyme-based electrochemical biosensors: a review, Nanoscale Adv. 1 (2019) 4560-4577. 10.1039/c9na00491b.
[42] Q. Zhao, Z.H. Gan, Q.K. Zhuang, Electrochemical sensors based on carbon nanotubes, Electroanalysis 14 (2002) 1609–1613, https://doi.org/10.1002/elan.200290000.
[43] J.G.S. Moo, A. Veksha, W. Oh, A. Giannis, W.D.C. Udayanga, S. Lin, L. Ge, G. Lisak, Plastic derived carbon nanotubes for electrocatalytic oxygen reduction reaction: Effects of plastic feedstock and synthesis temperature, Electrochem. Commun. 101 (2019) 11–18, https://doi.org/10.1016/j.elecom.2019.02.014.
[44] A. Veksha, A. Ahamed, X.Y. Wu, L. Liang, W.P. Chan, A. Giannis, G. Lisak, Technical and environmental assessment of laboratory scale approach for sustainable management of marine plastic litter, J. Hazard. Mater. 421 (2022), https://doi.org/10.1016/j.jhazmat.2021.126717 126717.
[45] T.C. Egbosiuba, A.A. Abdulkareem, J.O. Tijani, J.I. Ani, V. Krikstolaityte, M. Srinivasan, A. Veksha, G. Lisak, Taguchi optimization design of diameter-controlled synthesis of multi walled carbon nanotubes for the adsorption of Pb (II) and Ni(II) from chemical industry wastewater, Chemosphere 266 (2021), https://doi.org/10.1016/j.chemosphere.2020.128937 128937.
[46] T.C. Egbosiuba, M.C. Egwunyenga, J.O. Tijani, S. Mustapha, A.S. Abdulkareem, A. S. Kovo, V. Krikstolaityte, A. Veksha, M. Wagner, G. Lisak, Activated multi-walled carbon nanotubes decorated with zero valent nickel nanoparticles for arsenic, cadmium and lead adsorption from wastewater in a batch and continuous flow modes, J. Hazard. Mater. 423 (2022), https://doi.org/10.1016/j.jhazmat.2021.126993 126993.
[47] A. Ahamed, L. Ge, K. Zhao, A. Veksha, J. Bobacka, G. Lisak, Environmental footprint of voltammetric sensors based on screen-printed electrodes: An assessment towards "green" sensor manufacturing, Chemosphere 278 (2021), https://doi.org/10.1016/j.chemosphere.2021.130462 130462.
[48] F. Faridbod, M.R. Ganjali, P. Norouzi, S. Riahi, H. Rashedi, Application of room temperature ionic liquids in electrochemical sensors and biosensors, Ionic Liquids: Appl. Perspectives (2011), https://doi.org/10.5772/14702.
[49] W. Wei, H.H. Jin, G.C. Zhao, A reagentless nitrite biosensor based on direct electron transfer of hemoglobin on a room temperature ionic liquid/carbon

nanotube-modified electrode, Microchim. Acta 164 (2009) 167–171, https://doi.org/10.1007/s00604-008-0053-y.
[50] X. Shangguan, J. Zheng, H. Zhang, H. Tang, Direct electrochemistry and electrocatalysis behaviors of glucose oxidase based on hyaluronic acid-carbon nanotubes-ionic liquid composite film, Chin. J. Chem . 28 (2010) 1890–1896, https://doi.org/10.1002/cjoc.201090315.
[51] F.O. Gomes, L.B. Maia, C. Delerue-Matos, I. Moura, J.J.G. Moura, S. Morais, Third-generation electrochemical biosensor based on nitric oxide reductase immobilized in a multiwalled carbon nanotubes/1-n-butyl-3-methylimidazolium tetrafluoroborate nanocomposite for nitric oxide detection, Sensors Actuat. B-Chem. 285 (2019) 445–452, https://doi.org/10.1016/j.snb.2019.01.074.
[52] Oliveira, T M B F, S. Morais, New generation of electrochemical sensors based on multi-walled carbon nanotubes, Appl. Sci. 8 (2018) 1925. 10.3390/app8101925.
[53] P. Fanjul-Bolado, P. Queipo, P.J. Lamas-Ardisana, A. Costa-Garcia, Manufacture and evaluation of carbon nanotube modified screen-printed electrodes as electrochemical tools, Talanta 74 (2007) 427–433, https://doi.org/10.1016/j.talanta.2007.07.035.
[54] T.M.B.F. Oliveira, M.F. Barroso, S. Morais, M. Araujo, C. Freire, P. de Lima-Neto, A.N. Correia, M.B.P. Pinto Oliveira, C. Delerue-Matos, Sensitive bi-enzymatic biosensor based on polyphenoloxidases-gold nanoparticles-chitosan hybrid film-graphene doped carbon paste electrode for carbamates detection, Bioelectrochemistry 98 (2014) 20–29, https://doi.org/10.1016/j.bioelechem.2014.02.003.
[55] F.W.P. Ribeiro, M.F. Barroso, S. Morais, S. Viswanathan, P. de Lima-Neto, A.N. Correia, M.B.P. Pinto Oliveira, C. Delerue-Matos, Simple laccase-based biosensor for formetanate hydrochloride quantification in fruits, Bioelectrochemistry 95 (2014) 7–14, https://doi.org/10.1016/j.bioelechem.2013.09.005.
[56] E. Katz, I. Willner, Probing biomolecular interactions at conductive and semiconductive surfaces by impedance spectroscopy: Routes to impedimetric immunosensors, DNA-Sensors, and enzyme biosensors, Electroanalysis 15 (2003) 913–947, https://doi.org/10.1002/elan.200390114.
[57] M.E. Orazem, B. Tribollet, Electrochemical Impedance Spectroscopy, John Wiley & Sons, New Jersey, 2008.
[58] A.I. Gopalan, K. Lee, D. Ragupathy, Development of a stable cholesterol biosensor based on multi-walled carbon nanotubes-gold nanoparticles composite covered with a layer of chitosan-room-temperature ionic liquid network, Biosens. Bioelectron. 24 (2009) 2211–2217, https://doi.org/10.1016/j.bios.2008.11.034.
[59] A.J. Bard, L.R. Faulkner, Electrochemical methods: fundamentals and applications, 2nd ed., John Wiley & Sons, New York, 2001.
[60] C.P. Andrieux, J.M. Saveant, Heterogeneous (chemically modified electrodes, polymer electrodes) vs homogeneous catalysis of electrochemical reactions, J. Electroanal. Chem. 93 (1978) 163–168, https://doi.org/10.1016/S0022-0728(78)80230-7.
[61] M. Zaib, M.M. Athar, Electrochemical evaluation of phanerocheaete chrysosporium based carbon paste electrode with potassium ferricyanide redox system, Int. J. Electrochem. Sci. 10 (2015) 6690–6702.
[62] M. Zaib, M.M. Athar, Electrochemical characterization of a Porphyridium cruentum-modified carbon paste electrode by cyclic voltammetry, Instrum Sci. Technol. 46 (2018) 408–425, https://doi.org/10.1080/10739149.2017.1394879.
[63] S. Chen, J. Chen, X. Zhu, Solid phase extraction of bisphenol A using magnetic core-shell (Fe3O4@SiO2) nanoparticles coated with an ionic liquid, and its quantitation by HPLC, Microchim. Acta 183 (2016) 1315–1321, https://doi.org/10.1007/s00604-016-1757-z.