

RESEARCH PAPER

## A Novel Laccase Immobilization Approach Using Simultaneously Electrodeposition of 3, 4-Ethylenedioxythiophene, Gold Nanoparticles and Functionalized Multi-Walled Carbon Nanotube to Detect Catechol

Shahad Abdulrasol Albayati<sup>1</sup>, Soheila Kashanian<sup>1,2\*</sup>, Maryam Nazari<sup>1,\*</sup>, Saba Dabirian<sup>1</sup>

<sup>1</sup> Faculty of Chemistry, Razi University, Kermanshah, Iran

<sup>2</sup> Faculty of Chemistry, Sensor and Biosensor Research Center (SBRC) & Nanoscience and Nanotechnology Research Center (NNRC), Razi University, Kermanshah, Iran

### ARTICLE INFO

#### Article History:

Received 28 November 2019

Accepted 23 January 2020

Published 15 February 2020

#### Keywords:

Biosensor

PEDOT

MWCNT

Laccase

Catechol

### ABSTRACT

Laccases are phenoloxidases that can oxidize the phenolic compounds. Catechol (1,2-dihydroxybenzene) is one of the phenolic compounds broadly utilized in industry. Nanomaterials and conductive polymers can be utilized as a support medium for the laccase immobilization and phenolic biosensors. This paper describes a new enzymatic biosensor being developed to determine phenols such as catechol and shows the laccase catalytic reaction in the presence of phenolic substrates. It is based on the glassy carbon electrode fabricated with electrodeposition of poly (3,4-ethylenedioxythiophene) (PEDOT), carboxyl group functionalized multi-walled carbon nanotube (MWCNT<sub>COOH</sub>), and gold nanoparticles in combination, thereafter immobilizing of the laccase on the electropolymerized material on the electrode surface using the covalent binding of laccase to MWCNT<sub>COOH</sub>. The simply fabricated laccase biosensor response was characterized via voltammetry techniques and Fourier transform infrared (FTIR) spectroscopy. Using differential pulse voltammetry (DPV), the biosensor results indicated that the detection limit, sensitivity, and linear range are 0.35  $\mu\text{M}$ , 3.52  $\mu\text{A mM}^{-1}$ , 1 – 4  $\mu\text{M}$ , respectively, as well as the correlation coefficient of 0.95 under optimal conditions. By investigation of scan rate effect on cyclic voltammograms of laccase biosensor, transfer coefficient ( $\alpha$ ) and standard heterogeneous rate constant ( $k_s$ ) were estimated to be 0.68 and 0.083  $\text{s}^{-1}$ , respectively. The proposed biosensor incorporates the favorable electrocatalytic properties of nanomaterials with the PEDOT properties to decrease the oxidation potential and improve the electron transfer rate, detection limit and sensitivity.

#### How to cite this article

Albayati SA., Kashanian S., Nazari M., Dabirian S. A Novel Laccase Immobilization Approach Using Simultaneously Electrodeposition of 3, 4-Ethylenedioxythiophene, Gold Nanoparticles and Functionalized Multi-Walled Carbon Nanotube to Detect Catechol. *Nanochem Res*, 2020; 5(1):94-103. DOI: 10.22036/ncr.2020.01.009

### INTRODUCTION

Biosensors make a bridge between various fields of medicine, agriculture, biotechnology, materials science, optics, electronics, physics, and chemistry [1]. Nowadays, biosensors are

one of the developed domains to introduce novel developments. Biosensors are substitutes of classical analytical systems in the real world that are annoying, costly, and complex and cannot be used for in situ controlling [2]. They include three essential components; transducer, bioreceptor, and electronic circuit [3]. Bioreceptor is a biomolecule

\* Corresponding Author Email: [skashanian@razi.ac.ir](mailto:skashanian@razi.ac.ir)  
[nazari.maryam@razi.ac.ir](mailto:nazari.maryam@razi.ac.ir)

surrounded by the transducer such as DNA, enzyme, protein, antibodies, and whole-cell [4]. Biosensors are an interesting strategy for working at neutral pHs and low temperatures, that they are used as enzymes and catalysts [5]. The enzymes with high stability are very expensive [6]. Enzymes are active biocatalysts and one of the most used methods to enhance the selectivity of the biosensor, which catalyze specific reactions [7]. In addition, the enzymes are usually applied in bioassays, diversified biosensors, and biofuel cells. The limited stability of enzymes is the main obstacle in extensive biological application of enzymes [8].

To make biosensors, using enzymes on solid electrode surfaces, often need immobilization steps. Various strategies involve adsorption, membranes, chemical binding or encapsulation have been used to immobilize enzymes on the surface of solid electrodes successfully [5].

The stabilization of enzymes is of great interest in many applications, and the purpose is to offer prolonged active lifetime at normal environments with common substrates in buffered solutions. To make and store the devices without losing activity, the enzyme should be kept in a dry state, since it is very stable in this state, and is not stable in the long term or in the solution process [9].

There are two ways for electron transfer between the electrode and enzymes; mediated electron transfer (MET) and direct electron transfer (DET) approaches. This capability depends on various factors such as the location of the redox center, the structure of the enzyme, the distance of electron transfer and the orientation of the enzyme on the electrode surface [10]. Modifying the electrode surface and immobilization of enzymes on the electrode surface can easily improve the electron transfer [11].

Laccases are phenoloxidases which are in the category of multi-copper enzymes. Furthermore, laccases have the ability to accelerate the oxidation of phenols and its derivatives including catechol, benzenethiols, and anilines [12, 13]. Laccase biosensors can accelerate the catechol oxidation to *o*-quinone for calculable recognition [14].

Novel nanomaterials, such as carbon nanotube (CNT) [15] and gold nanoparticles (AuNPs) [16] are the most common promoters used to improve the electron transfer rate and can be utilized as a support medium for the laccase immobilization [17]. They cause to an appropriate moieties orientation onto the electrode surface and diminish

the distance between the transducer surface and electro-active species improving the rate of electron transfer [18]. Amongst all nanomaterials for laccase immobilization, CNT received great attention due to its excellent conductivity, robustness, well-ordered pore structure, higher sensitivity, better selectivity, and high surface area [19]. Nano-sized CNTs can get closer to the active centers of biomolecules and perform better electron transfer [20]. By immobilizing enzymes on functionalized CNTs, the enzyme molecules seem to approach the electrode surface. In addition, enzyme molecules on the electrode surface may be directed by functionalized CNTs, causing the electron transfer process becomes easier and the oxidation potential is reduced [5].

Catechol (1,2-dihydroxybenzene) is one of the phenolic compounds broadly utilized in industry [21]. The European Union and the US Environmental Protection Agency considered catechol as “an environmental pollutant” owing to its toxicity and partial degradation in the ecological system [22, 23]. Of the major conducting polymers, poly (3, 4-ethylenedioxythiophene) (PEDOT) heteroaromatic thiophene has a poor solubility, and therefore its substituted derivatives were synthesized [24]. PEDOT demonstrates some motivating properties, such as optical transparency in its conducting state, high conductivity and stability, and low redox potential and band gap. Polymerization of PEDOT can be conducted by aqueous solutions, and it can be utilized in applications of a biosensor as well [25, 26]. Silva et al. [27] stabilized AuNPs in poly (allylamine hydrochloride) and applied them as a support for the laccase immobilization and were successfully applied in the expansion of a new biosensor for the determination of dopamine.

The purpose of this research was to fabricate a sensitive laccase-based biosensor for catechol recognition. Here, PEDOT, AuNPs, and MWCNT<sub>COOH</sub> were mixed, and then the mixture was electrodeposited on a glassy carbon electrode since their combination effect ensures the quality of biosensor working.

## EXPERIMENTS

### Materials and reagents

Laccase (EC=1.10.3.2, from *Trametes Versicolor*, 21.8 U/mg), N-hydroxysuccinimide (NHS), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were acquired from Sigma-Aldrich. Laccase

was used without further purification. Catechol was purchased from Merck, and its stock solutions were provided in twice distilled water.  $\text{HAuCl}_4$  and 3, 4-ethylenedioxythiophene were purchased from PubChem and Tokyo chemical industry companies, respectively. The other compounds were purchased from Merck.

A Sama 500 electro analyzer system and a three-electrode cell were applied for recording the cyclic voltammetry (CV) and DPV graphs. Glassy carbon electrode, Ag/AgCl electrode (saturated KCl) and a Pt rod were utilized as working, reference and counter electrodes, respectively. To investigate the suitable working electrode or laccase-based biosensor, electrochemical experiments of catechol on the working electrode were studied by CV. FTIR was used for characterization of the electrode surface and enzyme immobilization at the wave-number range of 4000-400  $\text{cm}^{-1}$ .

#### *Preparation of a modified electrode*

Alumina slurries and fine emery paper were used for cleaning the glassy carbon electrode, thereafter was rinsed carefully with distilled water, and permitted to dry at room temperature. Next, a solution containing 0.01 M 3, 4-ethylenedioxythiophene,  $5.0 \times 10^{-4}$  M SDBS, and  $1.0 \times 10^{-4}$  M  $\text{HAuCl}_4$  was confected in 0.1 M  $\text{KNO}_3$  solution.  $\text{MWCNT}_{\text{COOH}}$  powder was dispersed in twice distilled water and sonicated for 4 hours to form a dark and homogeneous suspension.  $\text{MWCNT}_{\text{COOH}}$  suspension was added to the  $\text{KNO}_3$  solution and stirred and sonicated for 30 min, then electrochemically treated onto cleaned electrode under the CV from -0.6 to 1.2 V (vs. Ag/AgCl) for 10 cycles (at a scan rate of 0.05 V/s) to obtain the  $\text{MWCNT}_{\text{COOH}}$ , AuNPs, SDBS, and PEDOT/GCE. Thereafter, the modified electrode was separated from the cell and eluted with distilled water because some molecules may adsorb physically. Then 8  $\mu\text{L}$  of EDC (0.5% w/v), NHS (0.5% w/v), and Laccase (3 mg/mL, pH 3) were dropped on the electrode surface respectively, thereafter kept to dry in the fridge at 4 °C overnight. After drying, the modified electrode was characterized by FTIR and CV techniques to examine the electrochemical behaviors of catechol at biosensor.

#### *Characterization of laccase biosensor*

Voltammetry techniques were applied to study the biosensing systems for catechol detection. The electrochemical experiments were achieved with

a computer-controlled electro-analyzer SAMA 500, conventional cell, and three electrodes; glassy carbon electrode, Ag/AgCl (saturated) (Azar electrode), and platinum auxiliary electrode. The potential range of -0.5 to 0.8 V was selected for recording the cyclic voltammograms in the presence of  $\text{K}_3\text{Fe}(\text{CN})_6$  and catechol separately.

To investigate the proposed biosensor for catechol detection, its different concentrations were applied in phosphate buffer (0.1 M, pH 3), and DPV technique was used to monitor the effects of catechol concentration onto cathodic currents.

For the better investigation, the proposed biosensor was applied in probable interferences with the concentration 100 times higher than catechol. Hydroquinone is an isomer molecule for catechol; also, catechol was investigated in the presence of hydroquinone.

## RESULTS AND DISCUSSION

### *Morphology study of electrodes*

#### *FTIR spectroscopy*

In the FTIR, each type of bond has unlike vibrational frequency, therefore FTIR is considered as a useful tool [28] to check the bonds of the materials. The covalent binding between laccase and  $\text{MWCNT}_{\text{COOH}}$  was confirmed using FTIR spectroscopy. FTIR spectroscopy was performed for two modified electrodes, with and without laccase. Fig. 1 demonstrates the model FTIR spectra of modified electrodes. Lower intensity bands appear at 1165, 1079, and 983  $\text{cm}^{-1}$ , which are responsible for the stretching of ethylenedioxy ring and to the C-S bond. The functional groups of carboxylic acid appeared at 1631  $\text{cm}^{-1}$  (strong band), and 3428  $\text{cm}^{-1}$  (stretch, broad) which are related to the C=O and O-H, respectively. 816  $\text{cm}^{-1}$  is related to N-H bond, and C-N bond is in the range of 1000-1350  $\text{cm}^{-1}$  [29].

#### *Voltammetric response*

The CV of the electrodepositing mixture is shown in Fig. 2.

#### *Effect of pH*

The best pH was determined for the optimization of laccase biosensor, which included the pH from 3.0 to 8.0. It is known that the activity of the enzymes is affected by the change in the medium acidity [30], hence the performance of biosensor is affected as well. There are reasons responsible for selecting the optimal pH. In this case, laccase has

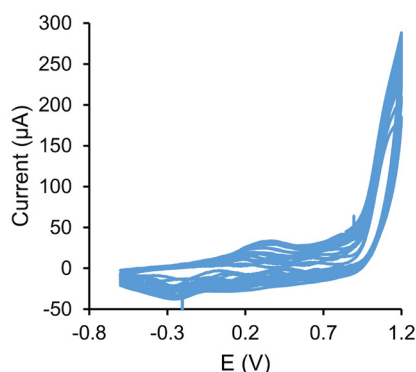


Fig. 2. CVs of electrodepositing of the mixture (MWCNT<sub>COOH</sub>, AuNPs, SDBS, PEDOT).

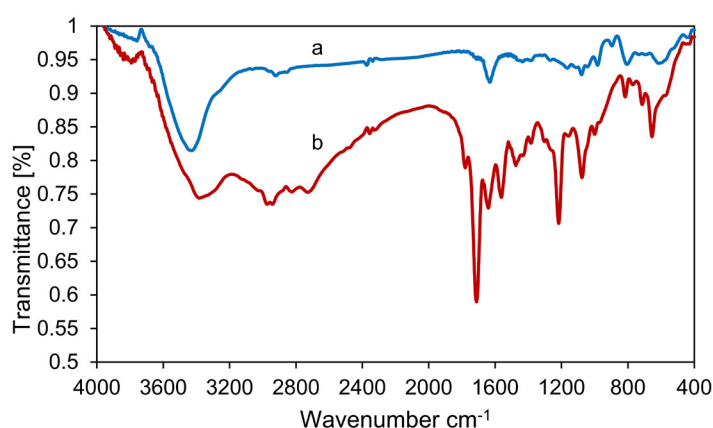


Fig. 1. FTIR spectra of a) MWCNT<sub>COOH</sub>, AuNPs, SDBS, PEDOT/GCE and b) Laccase-MWCNT<sub>COOH</sub>, AuNPs, SDBS, PEDOT/GCE.

the ability to reduce molecular oxygen to water associated with the oxidation of numerous organic substrates such as catechol [31] subsequently, back to the substrate type [32]. Also, the optimal activity of laccase in the pH buffer solution is ranged from 3.0-7.0, when the selected substrate is a phenolic compound [33] and the activity of fungal laccase enhances as the pH is decreased [34]. In addition, the ionic interaction between a charged surface of the support and enzyme has control on shifting the optimum pH for an immobilized enzyme to a lower or higher pH [35].

The biosensor response was considered in phosphate buffer (0.1 M) at the pH range of 3-8 containing  $1 \times 10^{-3}$  M catechol. The results (Table 1) for laccase-based biosensor indicated that the highest sensitivity is obtained at pH 3 due to enhancing the laccase activity in acidic media. At a pH of more than 3, the anodic and cathodic currents were decreased perhaps due to reducing the activity of laccase as shown in Table 1. This is

why, pH 3 buffer solution was selected for further experiments. According to Table 1, anodic and cathodic potentials shifted to negative amounts by increasing pH. The optimum pH for the proposed laccase biosensor was obtained in acidic media which is in accordance with the previous experiments [36, 37].

Fig. 3 shows clearly that the anodic ( $E_p^a$ ) and cathodic ( $E_p^c$ ) peak potentials of a laccase redox couple are highly affected by pH and the formal potential ( $E^0$ ) of laccase has a linear correlation with the pHs 3-8, where  $E^0 = (E_p^a + E_p^c)/2$ . The linear regression equation was found as  $E^0 = -0.0808 \text{ pH} + 0.8979$  with the correlation coefficient of 0.9988.

#### Electrochemical characterization

The various modified electrodes were characterized by CV graphs to examine the changes in their electrochemical behavior.

Some electrochemical parameters such as  $E_p^c$  and  $E_p^a$ , as well as the corresponding cathodic ( $i_p^c$ )

Table 1. The peak currents, potentials and the difference between the anodic and cathodic potential at various pHs in phosphate buffer (0.1 M) at the scan rate of 0.1 V/s

| pH | $i_p^a$ ( $\mu\text{A}$ ) | $i_p^c$ ( $\mu\text{A}$ ) | $E_p^a$ (V) | $E_p^c$ (V) | $\Delta E$ |
|----|---------------------------|---------------------------|-------------|-------------|------------|
| 3  | 14.68                     | -14.06                    | 0.471       | 0.354       | 0.117      |
| 4  | 14.23                     | -12.68                    | 0.437       | 0.289       | 0.148      |
| 5  | 11.72                     | -11.53                    | 0.389       | 0.213       | 0.176      |
| 6  | 12.86                     | -12                       | 0.334       | 0.168       | 0.166      |
| 7  | 12.61                     | -11.57                    | 0.27        | 0.12        | 0.15       |
| 8  | 13.96                     | -11.79                    | 0.206       | 0.086       | 0.12       |

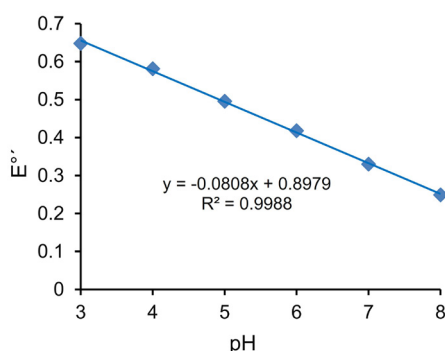
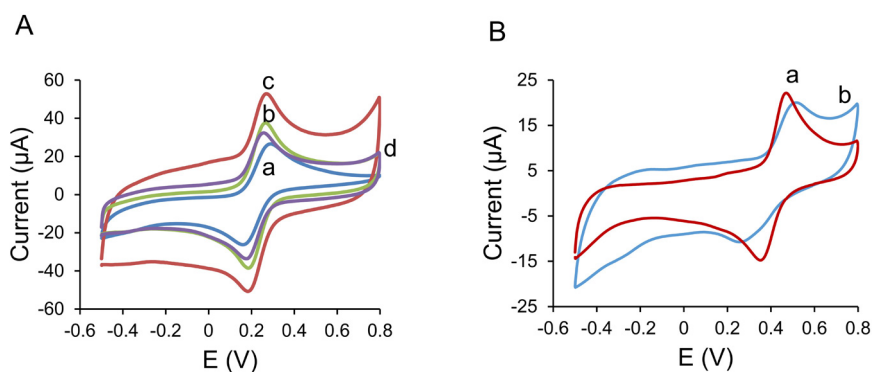
Fig. 3. The formal potential ( $E^0$ ) versus pH.

Fig. 4. (A) CVs of the modified electrodes a) AuNPs, SDBS\GCE b) AuNPs, SDBS, PEDOT\GCE c) MWCNT<sub>COOH</sub>, AuNPs, SDBS, PEDOT\GCE and d) Laccase\MWCNT<sub>COOH</sub>, AuNPs, SDBS, PEDOT\GCE in electrochemical solution containing of 0.1 M KCl and  $K_3Fe(CN)_6$  at constant scan rate (0.1 V/s). (B) CVs of a) MWCNT<sub>COOH</sub>, AuNPs, SDBS, PEDOT\GCE and b) Laccase\MWCNT<sub>COOH</sub>, AuNPs, SDBS, PEDOT\GCE in phosphate buffer (0.1 M, pH 3) containing  $1 \times 10^{-3}$  M catechol at the scan rate of 0.1 V/s.

and anodic ( $i_p^a$ ) peak currents were calculated. Fig. 4 A displays the CVs of the modified electrodes in an electrochemical solution including 0.1 M KCl and  $K_3Fe(CN)_6$  at a constant scan rate (0.1 V/s). The data are shown in Table 2. MWCNT<sub>COOH</sub>, AuNPs, SDBS, and PEDOT\GCE exhibit high activity for the redox reactions of  $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ .

The AuNPs and MWCNT<sub>COOH</sub> have a good effect on the modified electrode due to reducing the difference between redox potentials and increment of the redox currents; they increase the electrode surface area and play a significant role; e.g., a conducting wire that makes the electron transfer easier to occur.

Table 2. The peak currents, potentials and the difference between anodic and cathodic potentials for various modified electrodes in 0.1 M KCl and  $K_3Fe(CN)_6$  at the scan rate of 0.1 V/s

| Modified electrodes                                     | $i_p^a(\mu A)$ | $i_p^c(\mu A)$ | $E_p^a(V)$ | $E_p^c(V)$ | $\Delta E$ |
|---|----------------|----------------|------------|------------|------------|
| (a) GCE   | 8.77           | -10.73         | 0.411      | -0.03      | 0.441      |
| (b) AuNPs,SDBS/GCE                                      | 24.05          | -22.1          | 0.290      | 0.162      | 0.128      |
| (c) AuNPs,SDBS,PEDOT/GCE                                | 28.75          | -31.7          | 0.274      | 0.184      | 0.09       |
| (d) MWCNT <sub>COOH</sub> ,AuNPs,SDBS,PEDOT/GCE         | 30.54          | -32.13         | 0.268      | 0.187      | 0.081      |
| (e) Laccase/MWCNT <sub>COOH</sub> ,AuNPs,SDBS,PEDOT/GCE | 22.77          | -26.14         | 0.257      | 0.182      | 0.075      |

Table 3. The peak currents, potentials and the difference between anodic and cathodic potential for two modified electrodes MWCNT<sub>COOH</sub>, AuNPs, SDBS, PEDOT/GCE and Laccase/MWCNT<sub>COOH</sub>, AuNPs, SDBS, PEDOT/GCE in phosphate buffer (0.1 M, pH 3) at the scan rate of 0.1 V/s.

| Modified electrode                                  | $i_p^a(\mu A)$ | $i_p^c(\mu A)$ | $E_p^a(V)$ | $E_p^c(V)$ | $\Delta E$ |
|---|----------------|----------------|------------|------------|------------|
| MWCNT <sub>COOH</sub> ,AuNPs,SDBS,PEDOT/GCE         | 8.108          | -6.26          | 0.51       | 0.274      | 0.236      |
| Laccase/MWCNT <sub>COOH</sub> ,AuNPs,SDBS,PEDOT/GCE | 14.68          | -14.06         | 0.471      | 0.354      | 0.117      |

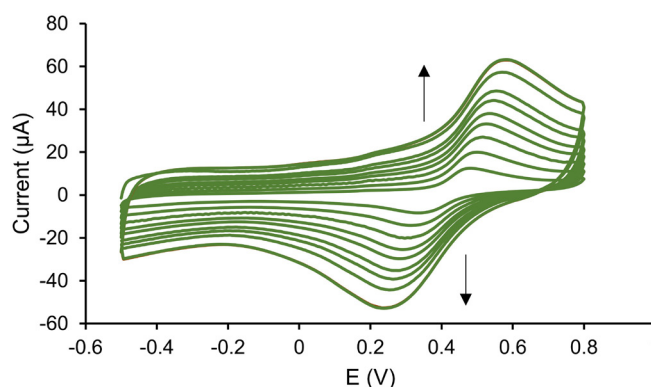


Fig. 5. CVs of Laccase/MWCNT<sub>COOH</sub>, AuNPs, SDBS, PEDOT/GCE at different scan rates: 0.02, 0.04, 0.06, 0.08, 0.1, 0.12, 0.14, 0.16, 0.18, and 0.2 V/s in phosphate buffer (0.1 M, pH 3) containing  $1 \times 10^{-3}$  M catechol.

Fig. 4 B shows CVs of two modified electrodes with and without laccase in the presence of catechol (the data are shown in Table 3). Laccase\MWCNT<sub>COOH</sub>,AuNPs,SDBS,PEDOT\GCE has higher anodic and cathodic currents and also lower different potential peak ( $\Delta E$ ) as comparable with MWCNT<sub>COOH</sub>,AuNPs,SDBS,PEDOT\GCE, so the laccase biosensor is more suitable.

*Investigation of scan rate effect*

The effect of scan rate was considered for Laccase/MWCNT<sub>COOH</sub>, AuNPs, SDBS, and PEDOT/GCE at diverse scan rates in phosphate buffer (0.1 M, pH 3) containing  $1 \times 10^{-3}$  M catechol (Fig. 5). Fig. 6 A demonstrates the relation between anodic and cathodic peak currents with the scan rate, correlation coefficients of 0.96 and 0.98 for anodic and cathodic currents, respectively; this refers to

the process controlled by laccase. Furthermore, it determines the mechanism of adsorption. Fig. 6 B displays the changes of anodic and cathodic potentials versus the logarithm of scan rates. The Potentials of an anodic peak proceed in the positive potential direction, while the potentials of a cathodic peak slightly shifts to the negative potential direction in the case of increasing scan rates. The practical behavior depends on  $\alpha$  and  $k_s$  when the charge-transfer rate is adequately slow [38, 39]. The cathodic peak potential ( $E_p^c$ ) altered linearly versus  $\log v$  with an equation of  $E_p^c = -0.0909 \log v + 0.2134$ ;  $R^2 = 0.92$ , in the range from 0.02 to 0.2 V/s scan rate. The peak potentials can be denoted by Laviron [40] equations number (1) and (2):

$$E_{pa} = E^{0'} - \frac{2.3RT}{(1-\alpha)nF} \log \frac{(1-\alpha)Fv}{RTk} \tag{1}$$





$$E_{pa} \log k_s = \alpha \log(1 - \alpha) + (1 + \alpha) \log \alpha - \log \frac{RT}{nf\nu} - \frac{\alpha(1-\alpha)Fn\Delta E_p}{2.3RT} \quad (2)$$

where  $k$  is heterogeneous electron transfer rate constant,  $n$  is the number of electrons,  $\alpha$  is the electron transfer coefficient,  $R$ ,  $F$ , and  $T$  are gas constant, Faraday constant, and temperature, respectively. Correspondent to the slope of a cathodic process, it was considered that  $(1-\alpha)n = 0.64$ . Given  $0.3 < \alpha < 0.7$  [41], it could be concluded that  $n = 2$ ,  $\alpha = 0.68$ . Also,  $k_s$  was estimated to be  $0.083 \text{ s}^{-1}$ . The redox reaction of catechol with the proposed biosensor was pH dependent to 2 electron numbers. Therefore, two electrons have exchanged in the transfer process and  $k_s$  could be estimated to

be  $0.083 \text{ s}^{-1}$  that is near to  $k_s$  of the other literature [42]; this indicates that the biosensor has been engaged in electron transfer step.

*Calibration curve and detection limit*

Fig. 7A demonstrates DPVs that is obtained in different concentrations for the detection of catechol in phosphate buffer (0.1 M, pH 3) with -0.5 to 0.8 V. Fig. 7B demonstrates the relationship between the cathodic current of the biosensor and the catechol concentrations (calibration curve) obtained by DPV techniques. It was found a linear correlation with a detection limit of  $0.35 \mu\text{M}$  in the concentration range of 1 to  $4 \mu\text{M}$ . Table 5 illustrates the performance of the proposed biosensor which has a lower limit of detection (LOD), and better sensitivity than some other biosensors [42-45].

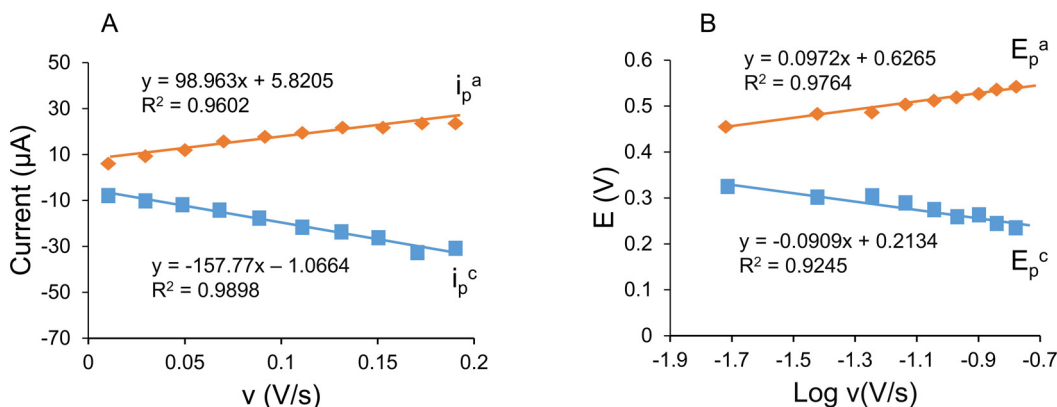


Fig. 6. (A) The relationship between anodic and cathodic peak currents with the scan rate, (B) the peak potentials versus scan rate in logarithmic scale for Laccase/MWCNT<sub>COOH</sub> AuNPs, SDBS, PEDOT/GCE in phosphate buffer (0.1 M, pH 3) containing  $1 \times 10^{-3} \text{ M}$  catechol.

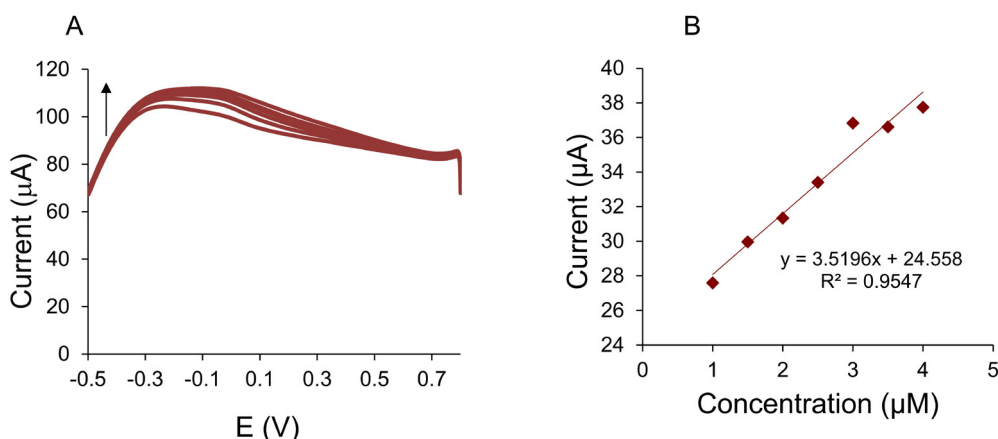


Fig. 7. (A) The relationship between the cathodic current of the biosensor and the catechol concentrations by DPV techniques in phosphate buffer (0.1 M, pH 3) at the scan rate of 0.1 V/s, (B) calibration curve by Laccase/MWCNT<sub>COOH</sub> AuNPs, SDBS, PEDOT/GCE for the detection of catechol in different concentrations.



Table 4. Interferences study for the determination of catechol

| Species   | C <sub>species</sub> /C <sub>catechol</sub> |
|---|---|
| Na <sup>+</sup> , K <sup>+</sup> , Br <sup>-</sup> , Cl <sup>-</sup> , Alanine, Cysteine, Glycine, Glucose, Citric acid | 100   |
| Hydroquinone  | 2   |

Table 5. Comparable biosensors for the determination of catechol

| Biosensor   | Linear range (μM) | Sensitivity (μA mM <sup>-1</sup> ) | LOD (μM) | Ref.      |
|---|-------------------|------------------------------------|----------|-----------|
| Laccase/MWCNT <sub>COOH</sub> ,AuNPs,SDBS,PEDOT/GCE | 1 - 4             | 3.52                               | 0.35     | This work |
| Laccase/GA/PANI/GCE                                 | 3.2-19.6          | 0.706                              | 2.07     | [42]      |
| Laccase/RGO-PdCu NCs/GCE                            | Up to 1155.0      | 12.65 and 5.51                     | 1.5      | [43]      |
| Laccase/AgNPs-CMC/cellulose/GCE                     | 4.98-3650         | -                                  | 1.64     | [44]      |
| Laccase/GA/MWCNT <sub>COOH</sub>                    | 0.5-150           | 2.81 × 10 <sup>3</sup>             | 0.5      | [45]      |

Polyaniline; PANI, Glutaraldehyde; GA, Palladium-copper alloyed nanocages supported on reduced graphene oxide; RGO-PdCu NCs), Carboxymethyl cellulose; CMC

### Interferences study

According to Table 4, the presence of probable interferences was studied by CV, using the concentrations of 100 times higher than that of catechol, which indicated no interferences with catechol response. Hydroquinone is the structural isomer of catechol. When the concentration of hydroquinone was 2 or less than 2 times of catechol, two isolated redox peaks were observed, related to catechol and hydroquinone.

### CONCLUSION

The proposed biosensor incorporates the favorable electrocatalytic properties of AuNPs, SDBS, and MWCNT<sub>COOH</sub> with the PEDOT properties to decrease the oxidation potential and improve electron transfer rate. FTIR records confirm the presence of amide bonds between laccase and the electrode indicating the immobilization process of laccase. This study shows that the modification of laccase-based biosensor employing PEDOT, SDBS, AuNPs, and MWCNT<sub>COOH</sub> on the glassy carbon electrode improves sensitivity, detection limit, and selectivity for the detection of catechol.

### CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

### REFERENCES

- Aranganathan V, Kavya C, Sheeja MS, Yuvraj C. Construction of Biosensor for Detection of Phenolic Compound Using Thermostabilized *Agaricus bisporus* Tyrosinase. *Arabian Journal for Science and Engineering*. 2016;42(1):11-8.
- Bhardwaj T. A review on immobilization techniques of biosensors. *International Journal of Engineering Research*. 2014;3(5).
- Yang J, Li D, Fu J, Huang F, Wei Q. TiO<sub>2</sub>-CuCNFs based laccase biosensor for enhanced electrocatalysis in hydroquinone detection. *Journal of Electroanalytical Chemistry*. 2016;766:16-23.
- Yang L, Li Y. AFM and impedance spectroscopy characterization of the immobilization of antibodies on indium-tin oxide electrode through self-assembled monolayer of epoxysilane and their capture of *Escherichia coli* O157:H7. *Biosensors and Bioelectronics*. 2005;20(7):1407-16.
- Gouranlou F, Ghourchian H. Ethanol/O<sub>2</sub> biofuel cell using a biocathode consisting of laccase/ HOOC-MWCNTs/ polydiallyldimethylammonium chloride. *Enzyme and Microbial Technology*. 2016;86:127-33.
- Gouranlou F, Ghourchian H. Effect of membrane on power density of ethanol/O<sub>2</sub> biofuel cell. *Nanochemistry Research*. 2018;3(1):24-8.
- Battista E, Lettera V, Villani M, Calestani D, Gentile F, Netti PA, et al. Enzymatic sensing with laccase-functionalized textile organic biosensors. *Organic Electronics*. 2017;40:51-7.
- Burton SG, Cowan DA, Woodley JM. The search for the ideal biocatalyst. *Nature Biotechnology*. 2002;20(1):37-45.
- Moehlenbrock MJ, Minteer SD. Introduction to the Field of Enzyme Immobilization and Stabilization. *Enzyme Stabilization and Immobilization*: Springer New York; 2016. p. 1-7.
- Aghahosseini H, Ramazani A, Azimzadeh Asiabi P, Gouranlou F, Hosseini F, Rezaei A, et al. Glucose-based Biofuel Cells: Nanotechnology as a Vital Science in Biofuel Cells Performance. *Nanochemistry Research*. 2016;1(2):183-204.
- Gouranlou F, Ghourchian H, Kheirmand M, Salimi A. Effect of support on power output of ethanol/O<sub>2</sub> biofuel cell. *Nanochemistry Research*. 2017;2(2):214-22.
- Guo M, Wang H, Huang D, Han Z, Li Q, Wang X, et al. Amperometric catechol biosensor based on laccase immobilized on nitrogen-doped ordered mesoporous carbon (N-OMC)/PVA matrix. *Science and Technology of*



- Advanced Materials. 2014;15(3):035005.
13. Almeida I, Henriques F, Carvalho MD, Viana AS. Carbon disulfide mediated self-assembly of Laccase and iron oxide nanoparticles on gold surfaces for biosensing applications. *Journal of Colloid and Interface Science*. 2017;485:242-50.
  14. Cabaj J, Jędrychowska A, Zając D, Krawiec S, Sołoducho J. Phenolic compounds determination using laccase-based electrode modified with conducting polymer support. *Int J Electrochem Sci*. 2016;11:609-20.
  15. Bourourou M, Elouarzaki K, Lalaoui N, Agnès C, Le Goff A, Holzinger M, et al. Supramolecular Immobilization of Laccase on Carbon Nanotube Electrodes Functionalized with (Methylpyrenylaminomethyl)anthraquinone for Direct Electron Reduction of Oxygen. *Chemistry - A European Journal*. 2013;19(28):9371-5.
  16. Dagys M, Laurynėnas A, Ratautas D, Kulys J, Vidžiūnaitė R, Talaikis M, et al. Oxygen electroreduction catalysed by laccase wired to gold nanoparticles via the trinuclear copper cluster. *Energy & Environmental Science*. 2017;10(2):498-502.
  17. Gholivand MB, Khodadadian M. Amperometric cholesterol biosensor based on the direct electrochemistry of cholesterol oxidase and catalase on a graphene/ionic liquid-modified glassy carbon electrode. *Biosensors and Bioelectronics*. 2014;53:472-8.
  18. Hayat A, Rhouti A, Mishra RK, Alonso GA, Nasir M, Istambouli G, et al. An electrochemical sensor based on TiO<sub>2</sub>/activated carbon nanocomposite modified screen printed electrode and its performance for phenolic compounds detection in water samples. *International Journal of Environmental Analytical Chemistry*. 2016;96(3):237-46.
  19. Wu F, Su L, Yu P, Mao L. Role of Organic Solvents in Immobilizing Fungus Laccase on Single-Walled Carbon Nanotubes for Improved Current Response in Direct Bioelectrocatalysis. *Journal of the American Chemical Society*. 2017;139(4):1565-74.
  20. Gouranlou F, Ghourchian H. Enhancement of ethanol-oxygen biofuel cell output using a CNT based nanocomposite as bioanode. *Biosensors and Bioelectronics*. 2016;78:337-43.
  21. Zhou Y, Tang L, Zeng G, Chen J, Cai Y, Zhang Y, et al. Mesoporous carbon nitride based biosensor for highly sensitive and selective analysis of phenol and catechol in compost bioremediation. *Biosensors and Bioelectronics*. 2014;61:519-25.
  22. Zhang Y, Xiao S, Xie J, Yang Z, Pang P, Gao Y. Simultaneous electrochemical determination of catechol and hydroquinone based on graphene-TiO<sub>2</sub> nanocomposite modified glassy carbon electrode. *Sensors and Actuators B: Chemical*. 2014;204:102-8.
  23. Qu J, Wang Y, Dong Y, Zhu Z, Xing H. Sensitive determination of catechol using a glassy carbon electrode modified with l-cysteine and ZnS:Ni/ZnS quantum dots. *Analytical Methods*. 2015;7(1):260-5.
  24. Otkrokhov GV, Morozova OV, Vasil'eva IS, Shumakovich GP, Zaitseva EA, Khlopova ME, et al. Biocatalytic synthesis of conducting polymers and prospects for its application. *Biochemistry (Moscow)*. 2013;78(13):1539-53.
  25. Park C, Lee C, Kwon O. Conducting Polymer Based Nanobiosensors. *Polymers*. 2016;8(7):249.
  26. Lin K-C, Tsai T-H, Chen S-M. Performing enzyme-free H<sub>2</sub>O<sub>2</sub> biosensor and simultaneous determination for AA, DA, and UA by MWCNT-PEDOT film. *Biosensors and Bioelectronics*. 2010;26(2):608-14.
  27. Silva TR, Vieira IC. A biosensor based on gold nanoparticles stabilized in poly(allylamine hydrochloride) and decorated with laccase for determination of dopamine. *The Analyst*. 2016;141(1):216-24.
  28. Otto JE. Development and characterization of carbon nanotubes for sensor applications. 2005.
  29. Pavia DL, Lampman GM, Kriz GS, Vyvyan JA. Introduction to spectroscopy: Cengage Learning; 2008.
  30. Viswanath B, Rajesh B, Janardhan A, Kumar AP, Narasimha G. Fungal Laccases and Their Applications in Bioremediation. *Enzyme Research*. 2014;2014:1-21.
  31. Singh S, Jain DVS, Singla ML. Sol-gel based composite of gold nanoparticles as matrix for tyrosinase for amperometric catechol biosensor. *Sensors and Actuators B: Chemical*. 2013;182:161-9.
  32. Chandra R, Chowdhary P. Properties of bacterial laccases and their application in bioremediation of industrial wastes. *Environmental Science: Processes & Impacts*. 2015;17(2):326-42.
  33. Bollag J-M, Leonowicz A. Comparative Studies of Extracellular Fungal Laccases. *Applied and Environmental Microbiology*. 1984;48(4):849-54.
  - [34. Chen L, Zou M, Hong FF. Evaluation of Fungal Laccase Immobilized on Natural Nanostructured Bacterial Cellulose. *Frontiers in Microbiology*. 2015;6(1245).
  35. Zhang H, Xun E, Wang J, Chen G, Cheng T, Wang Z, et al. Immobilization of Laccase for Oxidative Coupling of Trans-Resveratrol and Its Derivatives. *International Journal of Molecular Sciences*. 2012;13(5):5998-6008.
  36. Wang F, Guo C, Yang L-r, Liu C-Z. Magnetic mesoporous silica nanoparticles: Fabrication and their laccase immobilization performance. *Bioresource Technology*. 2010;101(23):8931-5.
  37. Ferreira MS, Silva Filho AF, Martins VL, Andrade MM, Giarola DA, Dall'Antonia LH. Effect of mediator added to modified paste carbon electrodes with immobilized laccase from *Aspergillus oryzae*. *Acta Scientiarum Technologia*. 2015;37(2):265.
  38. Bard AJ, Faulkner LR. Fundamentals and applications. *Electrochemical Methods*. 2001;2(482):580-632.
  39. Rafipour R, Kashanian S, Hashemi S, Shahabadi N, Omidfar K. An electrochemical biosensor based on cobalt nanoparticles synthesized in iron storage protein molecules to determine ascorbic acid. *Biotechnology and Applied Biochemistry*. 2015;63(5):740-5.
  40. Laviron E. General expression of the linear potential sweep voltammogram in the case of diffusionless electrochemical systems. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*. 1979;101(1):19-28.
  41. Nazari M, Kashanian S, Moradipour P, Maleki N. A novel fabrication of sensor using ZnO-Al<sub>2</sub>O<sub>3</sub> ceramic nanofibers to simultaneously detect catechol and hydroquinone. *Journal of Electroanalytical Chemistry*. 2018;812:122-31.
  42. Nazari M, Kashanian S, Rafipour R. Laccase immobilization on the electrode surface to design a biosensor for the detection of phenolic compound such as catechol. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2015;145:130-8.
  43. Mei L-P, Feng J-J, Wu L, Zhou J-Y, Chen J-R, Wang A-J.

- Novel phenol biosensor based on laccase immobilized on reduced graphene oxide supported palladium–copper alloyed nanocages. *Biosensors and Bioelectronics*. 2015;74:347-52.
44. Fu J, Li D, Li G, Huang F, Wei Q. Carboxymethyl cellulose assisted immobilization of silver nanoparticles onto cellulose nanofibers for the detection of catechol. *Journal of Electroanalytical Chemistry*. 2015;738:92-9.
45. Romero-Arcos M, Garnica-Romo MG, Martínez-Flores HE. Characterization of Amperometric Laccase Biosensor Based on Carbon Nanotube. *Procedia Technology*. 2017;27:279-81.