Laccase immobilized onto graphene oxide nanosheets and electrodeposited gold–cetyltrimethylammonium bromide complex to fabricate a novel catechol biosensor

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Abstract. In this study, a new biosensor is developed with reliable and easy-to-use biodevice properties for catechol determination in real samples. A method is proposed for the fabrication of biosensors to sense catechol based on the adsorption method of laccase immobilization. Hence, a glassy carbon electrode was modified via graphene oxide nanosheets and then it was modified with a gold–cetyltrimethylammonium bromide nanocomposite to adsorb and immobilize laccase on the electrode surface. The results showed laccase immobilization onto the reformed glassy carbon electrode, and a direct electron transfer reaction between laccase and the electrode. The mechanism of electron transferring was EC'. Also, k_s and α were calculated as 0.41 s^{-1} and 0.33, respectively. For this biosensor two linear ranges, 0.1×10^{-6} to 5×10^{-6} M and 16.7×10^{-6} to 166×10^{-6} M, and a detection limit of 1.5×10^{-6} M were obtained.

Keywords. Biosensor; laccase; graphene oxide; gold–cetyltrimethylammonium bromide complex; catechol.

1. Introduction

The health of organisms is affected by phenolic compounds. These compounds release into ground and surface waters is occurred due to their utilization in product fabrication among oil refining, coal conversion, medicines, surfactants, pesticides, colourants, plastics and resin formation [1–3]. European Community announced that the maximum amount of phenols in effluent should be <1 ppm [4]. Phenols can be detected by various analytical techniques, such as spectrophotometry, capillary electrophoresis and liquid and gas chromatographies [5,6]. These techniques do not have high sensitivity and specificity and also they require time, sample pre-treatment, costly equipment and skilled operators [7,8]. Biosensors are sensitive, cost-effective and portable systems compared to the other techniques [9]. Biosensors are used to detect phenol in clinical and biomedical areas, samples of environmental contamination, industrial and pharmaceutical analysis. Electrochemical biosensors are more common [10]. Laccase (Lac), tyrosinase (Tyr) and horseradish peroxidase (HRP) have attracted considerable attention for electrochemical biosensor fabrication in the phenol detection field [11–13]. These three enzymes have different reaction mechanisms. HRP is oxidized by hydrogen peroxide (H_2O_2) and can be acquired again by oxidizing

of phenolic compounds [13,14]. Phenolics without any substituent groups are able to be oxidized by Tyr [10]. Lac can oxidize phenols without substituent groups in meta- and para-positions [15]. Lac biosensors fabrication has been followed for phenol detection as a fascinating subject in a broad range. Lac (EC 1.10.3.2), a copper-containing oxidase [16] exists in insects, fungi, plants and some bacteria [17]. In addition to phenols, it is able to catalyse anilines and benzenethiols oxide-forms with the attendant O_2 reduction and water production [18], via free radical mechanism [19]. Different biosensor fabrications have been reported based on Lac immobilization onto many supports, such as matrix of mesoporous silica sieve/polyvinyl alcohol [20] and modified screen-printed electrodes modified with carbon black [21] through adsorption; cyanuric chloride in chitosan by cross-linking [22]; graphite electrodes [23] and nanohybrid of reduced graphene oxide (rGO)–glycol chitosan [24] by covalent coupling.

The use of nanomaterials and nanoparticles (NPs) affects biosensors' analytic performance. Graphene is an ideal material in the making of electrochemical biosensors because of electrical conductivity, mechanical pliability, high-thermal conductivity, vast specific surface area and good adaptability [25]. It is a good candidate for many applications and also for the construction of sensitive biosensors [26]. Veerapandian

et al [27] proposed the detection of quercetin utilizing a covalent reaction of graphene oxide (GO) with NPs of silver and silica–polyethylene glycol placed on-chip with a good linear range and low detection limit. Modifying the graphene with metal NPs (MNPs) makes it stable and value-effective biosensors. Gold NPs (AuNPs) have special properties including high conductivity, vigorous adsorption ability and promoting electron transfer. Using good stabilizers such as surfactants can accelerate the amount of metal particle formation and also protect the MNPs from agglomeration [28,29]. Amlodipine and enalapril were determined simultaneously using a paste electrode reformed with multi-walled carbon nanotubes (MWCNTs) and cetyltrimethylammonium bromide (CTAB). The outcomes showed that the CTAB could be adsorbed on the oxidized MWCNTs and influenced the electrochemical response of analytes [30]. Surfactants provide better molecule orientation at the electrode interface and affect electron transferring of electroactive species at the surface of bare metal electrode [28]. It was reported that $[\text{AuCl}_4]^-$ interacts with CTAB prior to the reduction process [31] and the reductions of AuCl_4^- to AuCl_2^- and AuCl_2^- to Au^0 are electropositive reactions. AuCl_2^- can bind strongly to the CTAB micelles [32].

In this study, we fabricated a Lac biosensor using GO nanosheets, a cationic surfactant (CTAB) and AuNPs for immobilization of Lac to detect catechol. The modified electrode has high-catalytic activity for catechol determination. GO nanosheets have negative charge due to the presence of many oxygen groups. It was placed onto the bare glassy carbon electrode (GCE); then the second layer, i.e., Au–CTAB complex (Au–CTAB) was electrodeposited, and finally, Lac with negative charge was immobilized.

2. Experimental

2.1 Materials and reagents

Lac (from the source of *Trametes versicolor*, 21.8 U per mg) was provided by Sigma Aldrich. It was used without further purification. Catechol was purchased from Merck. HAuCl_4 and CTAB were obtained from PubChem and Sigma, respectively. All other chemicals were purchased from Merck. Distilled deionized water was used for the preparation of all solutions.

2.2 Apparatus

A Sama 500 electro-analyzer system was used for carrying out voltammetry techniques (differential pulse voltammetry (DPV) and cyclic voltammetry (CV)). Three electrodes (purchased from Azar Electrode) were placed in an electrochemical cell; an Ag/AgCl (saturated KCl) as a reference electrode, GCE as a working electrode and a Pt rod as a counter electrode were used. The electrochemical impedance spectroscopy (EIS) technique was carried out on

an Autolab (Eco Chemie BV, The Netherlands) in the solution comprising 5.0×10^{-3} M $\text{K}_4[\text{Fe}(\text{CN})_6]/\text{K}_3[\text{Fe}(\text{CN})_6]$ and 1.0×10^{-1} M KCl. To characterize the electrode surface morphology, scanning electron microscopy (SEM) and field-emission scanning electron microscopy (FESEM) (MIRA3 TESCAN) were employed.

2.3 Preparation of Lac/nano-Au/CTAB/GO/GCE

GO was obtained by an improved modified Hummers method [33]. A GCE was cleaned with alumina grouts and well emery paper sheet and was washed with distilled deionized water, then dried at room temperature. A suspension of GO was prepared in water (2 mg GO in 10 ml water); after sonicating, 5 μl of its suspension was dropped on the GCE surface and the water was taken out. By the addition of CTAB to the HAuCl_4 solution, a yellow/orange color solution was attained (micelles are formed from a water-insoluble complex). There were electrostatic attractions between the CTAB positive head group and anion complexes to offer the binding interaction [32]. Subsequently, in a yellow/orange colour solution comprising 1.0×10^{-1} M KNO_3 , 1.0×10^{-4} M HAuCl_4 and 5.0×10^{-4} M CTAB, the modified GCE was treated under the fixed potential of -0.4 V (vs. Ag/AgCl) for 300 s to achieve Au–CTAB/GO/GCE. The modified GCE was then picked up and thoroughly washed with distilled deionized water to eliminate the physically absorbed HAuCl_4 and CTAB. Then, it was potential scanned in phosphate buffer to gain a steady state. The enzyme solution was prepared in phosphate buffer (pH 6, 1.0×10^{-1} M) with the concentration of 3.3 mg per ml (Lac has negative charge at pH 6). Lac immobilization on the modified GCE was performed by dropping 5 μl of Lac solution on the electrode surface, and then it was washed with phosphate buffer. While the biosensor was not in use, it was kept in a fridge at 4°C .

2.4 Biosensor characterization using various techniques

For characterization of the modified electrode, the following techniques were employed: Energy Dispersive Spectroscopy (EDS), FESEM, SEM, Fourier-transform infrared (FTIR) spectroscopy and electrochemical methods. Using SEM and FESEM, the morphology of deposited multilayered structures at the nanometre level was demonstrated. EIS was performed in solution containing an electroactive probe ($\text{K}_4[\text{Fe}(\text{CN})_6]/\text{K}_3[\text{Fe}(\text{CN})_6]$) to measure the impedance changes of the prepared electrodes. Voltammetry techniques were used to examine biosensing characteristics in the presence of catechol. Experimentations were carried out with SAMA 500 electro-analyzer to obtain CV and DPV data. An electrochemical cell was utilized with three electrodes using the GCE, Pt rod and Ag/AgCl electrode. Cyclic voltammograms were registered at the scan rate of 1.0×10^{-1} V s^{-1} from -0.5 to 0.8 V in the presence of catechol and $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$.

Figure 1. SEM images of modified electrodes: (a) GO/GCE, (b) Au-CTAB/GO/GCE and (c) GO FTIR spectrum.

Figure 2. (a) FESEM image and (b) EDS spectrum of Lac/Au-CTAB/GO/GCE.

Figure 3. (a) EIS of the bare GCE, Au-CTAB/GCE, Au-CTAB/GO/GCE, Lac/Au-CTAB/GCE and Lac/Au-CTAB/GO/GCE. Inset: the EIS circuit of GCE, Au-CTAB/GCE, Au-CTAB/GO/GCE and Lac/Au-CTAB/GO/GCE. (b) CVs of Au-CTAB/GO/GCE (1) GO/GCE (2), Au-CTAB/GCE (3), GCE (4) Lac/Au-CTAB/GCE (5) and Lac/Au-CTAB/GO/GCE (6) in the presence of $K_3Fe(CN)_6$ ($5.0 \times 10^{-3} M$) and KCl (0.1 M). (c) CVs of GCE (1) GO/GCE (3), Lac/Au-CTAB/GCE (5) and Lac/Au-CTAB/GO/GCE (7) in phosphate buffer (pH 5) and CVs of GCE (2) GO/GCE (4), Lac/Au-CTAB/GCE (6) and Lac/Au-CTAB/GO/GCE (8) in the presence of catechol ($1.0 \times 10^{-3} M$).

3. Results and discussion

3.1 Morphology of electrodes

The SEM images show the surface morphologies of electrodes modified with GO nanosheets and Au-CTAB (figure 1a and b). The bare GCE has a flat surface, so the layer structure of GO at GO/GCE can be detected (figure 1a). For the surface related to Au-CTAB/GO/GCE, there are NPs on GO

sheets and many NPs with the dimensions of <100 nm were distributed uniformly on the electrode surface (figure 1b). It is a good evidence to confirm electrochemical stabilization of Au-CTAB on the surface of GCE merely using a one-step route. The GO sheets increase the electrodeposition of Au-CTAB because of its vast surface area (figure 1b). Additionally, functional groups were detected using FTIR studies. As shown in figure 1c, the spectrum of GO exhibits main peaks at 1059, 1230, 1624 and 1741 cm^{-1} that are related to

Figure 4. (a) The influence of pH on the peak current of catechol redox reaction for Lac biosensor. Inset: the effect of cathodic and anodic currents vs. pH. (b) Dependency of $E^{\circ'}$ against pH.

the bands of C–O–C in alkoxy or epoxy groups, C=C, C–OH and C=O in carboxylic acid and carbonyl groups [34].

Figure 2a shows the FESEM image of Lac/Au–CTAB/GO/GCE morphology. Also, EDS analysis confirmed the presence of Lac. It showed the existence of N, O, C, Cu, Au, Na, K and P components (figure 2b); Cu is related to Lac.

3.2 EIS

EIS demonstrates the impedance variations of the modified electrodes. At the electrode interface, electron-transfer resistance (R_{et}) controls the redox probe electron transfer kinetics and it was obtained from a semicircle diameter. figure 3a illustrates the typical Nyquist plots of GCE, Au–CTAB/GCE, Au–CTAB/GO/GCE, Lac/Au–CTAB/GCE and Lac/Au–CTAB/GO/GCE. R_{et} can be obtained from the circuits that are shown in figure 3a inset. The applied potentials for the modified electrodes were 0.283, 0.268, 0.249, 0.212 and 0.229 V for GCE, Au–CTAB/GCE, Lac/Au–CTAB/GCE, Au–CTAB/GO/GCE and Lac/Au–CTAB/GO/GCE, respectively. The Nyquist plots of GCE, Au–CTAB/GCE, Lac/Au–CTAB/GCE, Au–CTAB/GO/GCE and Lac/Au–CTAB/GO/GCE displayed semicircles with the values of about 30.6, 20.5, 43.9, 1.88 and 3.56 kΩ of R_{et} , respectively. The R_{et} value which is the smallest for Au–CTAB/GO/GCE indicates that the interaction between the Au–CTAB composite and GO stimulates electron transferring of electrochemical probes. For enzymatic modified electrodes, it can be seen that the R_{et} values are obtained larger than for the same modified electrodes without an enzyme.

3.3 Voltammetric response

Figure 3b demonstrates CVs of electrodes in the presence of $K_3Fe(CN)_6$ and KCl. As it is clear that Au–CTAB/GO

Figure 5. Cyclic voltammograms of the Lac biosensor in the solution comprising of 1.0×10^{-1} M phosphate buffer (pH 5.0) and 1.0×10^{-3} M catechol at diverse scan rates. Scan rates from (i) to (s) are 0.02, 0.04, 0.06, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 V s^{-1} , respectively.

has the largest redox currents and the immobilization of Lac reduces the amounts of redox currents. figure 3c shows the CVs of GCE and modified GCEs in the absence and presence of catechol. As can be seen, there is no any redox peak for the GCE in phosphate buffer. While a clear pair of redox peaks is seen for Lac/Au–CTAB/GO/GCE which can be related to the electroactive surface of the Lac biosensor. The CVs of GCE and Lac/Au–CTAB/GO/GCE in the presence of catechol are shown as well. According to figure 3c, Lac/Au–CTAB/GO/GCE has improved the oxidation and reduction peaks of catechol proportion to GCE due to smaller ΔE_p and significant oxidation and reduction currents.

The pH influences on the current peaks of catechol oxidation are revealed in figure 4a. The changing of catechol redox reaction currents (oxidation and reduction) vs. pH is shown in figure 4a inset. Overall, the signal of current increased with the rising pH (up to pH 5), and then the current signal decreases at pH > 5. The peak currents of catechol gently diminished with the enhancing pH levels from 3.0 to 8.0, ensuing from the lack of protons at upper pH values. It is obvious that the catechol is a protic aromatic molecule, which certainly undergoes deprotonation and turns to anion at higher pHs. As a result, as the pH enhances to upper values, catechol

Table 1. The peak currents, potentials and the ratio of anodic and cathodic currents at different scan rates.

Scan rate, V s ⁻¹	<i>i</i> _p ^a , μA	<i>i</i> _p ^c , μA	<i>E</i> _p ^a , V	<i>E</i> _p ^c , V	<i>i</i> _p ^a / <i>i</i> _p ^c
0.02	6.438	−6.979	0.329	0.24	0.922
0.04	9.011	−9.73	0.336	0.233	0.926
0.06	10.99	−11.94	0.34	0.232	0.920
0.1	14.04	−15.09	0.347	0.227	0.930
0.14	16.57	−17.81	0.354	0.221	0.930
0.16	17.99	−19.33	0.361	0.212	0.931
0.18	19.43	−20.85	0.364	0.211	0.932
0.2	20.11	−21.41	0.366	0.21	0.939
0.25	22.32	−23.6	0.369	0.209	0.946
0.3	24.52	−25.91	0.371	0.204	0.946
0.35	26.38	−27.86	0.375	0.203	0.947
0.4	28.05	−29.44	0.379	0.201	0.953
0.45	29.62	−31.09	0.384	0.198	0.953
0.5	30.79	−32.21	0.387	0.194	0.956
0.6	33	−34.46	0.398	0.188	0.956
0.7	35.21	−36.76	0.406	0.186	0.956
0.8	37.33	−38.96	0.412	0.181	0.958
0.9	38.67	−40.2	0.41	0.178	0.962
1	40.31	−40.89	0.419	0.177	0.986

simply turns to anionic form, causing the electrostatic repulsion between the anionic form of catechol and the surface of reformed electrodes. Furthermore, the solution experienced a deficiency of protons, causing a trouble in electrochemical responses and decline in the current signal [35–39]. Therefore, pH 5 was applied for more experiments of catechol detection at the modified electrode, and the electrochemical performances of Lac-modified electrode were examined using CV in phosphate buffer at pH 5. According to figure 4b, the formal potential (*E*^{o'}) has changed vs. pH and the equation of *E*^{o'} = −0.055V/pH + 0.56 (*R*² = 0.97) was obtained. The amount of slope (0.055) is near 0.059 (the slope value of the Nernstian equation), which means that the number of electrons and protons is equal.

Figure 5 demonstrates the CVs of 1.0 × 10^{−3} M catechol in phosphate buffer at diverse scan rates (*ν*). Moreover, the peak currents and potentials are shown in table 1. The peaks of current signals are increased with enhancing *ν* (figure 6a) and the square root of *ν* (figure 6b). The correlation coefficient for the plot of currents vs. *ν*^{1/2} is higher than that of it for currents vs. *ν*, demonstrating that a diffusion-controlled process overcomes the surface adsorption-controlled process [40,41]. *ν* influenced the cathodic and anodic peak potentials of the system, and as can be seen, the redox potentials were increased by increasing the logarithm of *ν* (log *ν*) (figure 7a).

Figure 7a demonstrates the relationship between the cathodic and anodic peak potentials (*E*_p^c and *E*_p^a) and a log *ν* for the biosensor in the solution comprising of 1.0 × 10^{−1} M phosphate buffer (pH 5) and 1.0 × 10^{−3} M of catechol. For this biosensor, the peak potentials can be exhibited by Laviron [42] equations (1) and (2):

$$E_p^a = E^{o'} - \frac{2.3RT}{(1-\alpha)nF} \log \frac{(1-\alpha)Fn\nu}{RTk}, \quad (1)$$

Figure 6. (a) The influence of scan rate on cathodic and anodic currents of Lac biosensor. (b) The cathodic and anodic currents of Lac biosensor vs. square of the scan rate.

Figure 7. (a) Dependency of cathodic and anodic potentials scan rates in a logarithmic scale for the Lac biosensor. (b) The plot of $i_p^a \cdot v^{-1/2}$ vs. $\log v$.

Scheme 1. (a) The schematic diagram and (b) the steps of reaction mechanism of Lac/Au-CTAB/GO/GCE in the presence of catechol.

Table 2. The interfering molecules for catechol determination (100 μ M).

Species	$C_{\text{species}}/C_{\text{catechol}}$
Na^+ , K^+ , Br^- , Cl^- , NO_3^- , $H_2PO_4^-$, HPO_4^{2-} , Ca^{2+}	1000
Alanine, glycine, phenylalanine, glucose, citric acid	1000
Phenol, ascorbic acid	10
Uric acid	5
Pyrogallol, dopamine	1

$$\log k_s = \alpha \log (1 - \alpha) + (1 - \alpha) \log \alpha - \log \frac{RT}{nFv} - \frac{\alpha (1 - \alpha) F n \Delta E_p}{2.3RT}, \quad (2)$$

where n , T , F and R are the number of electrons, temperature, Faraday constant and gas constant, respectively; α and k_s are the electron transfer coefficient and heterogeneous electron

transfer rate constant, respectively. Assuming $0.3 < \alpha < 0.7$ is common, it could be resolved that when α is equal to 0.33, n will be obtained as 2.0 using the slope of redox

Figure 8. The biosensor responses to catechol (10^{-4} M) in the presence of interfering molecules.

Figure 9. Catechol calibration curves for the Lac biosensor in two linear ranges: (a) 0.1–5 and (b) 16.7–166 μM .

Table 3. Comparable methods for determination of catechol.

Electrode	Optimum pH	Linear range, μM	LOD, μM	Sensitivity, $\mu\text{A mM}^{-1}$	Reference
Lac/N-OMC/PVA/AuE	5	0.39–8.98	0.31	290	[46]
Lac/AP-rGO/chit/GCE	4.5	15–700	7	14.16	[4]
Lac/AgNPs/CMC/cellulose/GCE	5	4.98–3650	1.64	37.8	[47]
PDA-Lac-NiCNFs/MGCE	5.5	1–9100	0.69	18.4	[48]
Lac-FSM7.0-GCE	5	2–100	2	—	[49]
PEDOT-GONs-Lac/GCE	6	0.036–0.35 and 0.35–2.5	0.032	2.981×10^4 , 3.417×10^3	[50]
Lac/Au-CTAB/GO/GCE	5	0.1–5 and 16.7–166	1.5	37, 16	This work

AuE, gold electrode; N-OMC, nitrogen-containing ordered mesoporous carbon; PVA, polyvinyl alcohol; rGOs, reduced graphene oxides; 1-AP, 1-aminopyrene; chit, chitosan; CMC, carboxymethyl cellulose; MGCE, magnetic glassy carbon electrode; PDA, polydopamine; NiCNFs, nickel NP-loaded carbon nanofibres; FSM7.0, 7.0 nm pore diameter; PEDOT, poly(3,4-ethylenedioxythiophene); GONs, graphene oxide nanosheets.

potential changes vs. logarithm of scan rate that indicates the electron transfer process is inclusive in the rate-limiting step [43]. Consequently, the redox reaction of catechol is a 2-electron transfer route and also, the number of protons is equal to 2. Also, the k_s could be estimated to be 0.41 s^{-1} which is in agreement with previously reported values of k_s for different Lac biosensors [44].

To postulate the reaction mechanism, it is needed to examine the following: (1) verifying the ratio of i_p^a/i_p^c is < 1 and it increased with enhancing the scan rate. (2) The plot of $i_p^a \cdot \nu^{-1/2}$ vs. $\log \nu$ can be seen in the reducing form as shown in figure 5b. These evidences confirm that the mechanism is EC or EC' [45]. When the oxidized form of Lac affects on catechol, it causes formation of quinone, two electrons and two protons; this is the first step. Then, reduced Lac can be changed to the oxidized form again by reduction of oxygen to water; this is the second step and it is a chemical reaction that produces oxidized Lac that can react again for catechol oxidation; therefore, the mechanism is EC'. Scheme 1 shows the reactions on the biosensor surface. Additionally, the electrochemical mechanism of the proposed biosensor is not reversible and it can be determined using the behaviour of the scan rate effect; the electrochemical

reaction on the biosensor surface is quasi-reversible, due to the following reasons: (1) enhancing of redox peak currents by increasing $\nu^{1/2}$, (2) both the numbers of electrons and protons are equal to 2, and ΔE_p is >0.059 V even at low scan rates (the ΔE_p value was 0.124 V at the scan rate of $1.0 \times 10^{-1} \text{ V s}^{-1}$), (3) ΔE_p is increased by increasing ν , and (4) changing of cathodic potentials to negative values by increasing ν [45].

Also, the four electrodes were fabricated to investigate the reproducibility of the Lac biosensor by the CV technique and relative standard deviation was obtained as 5.59% for catechol ($1.0 \times 10^{-3} \text{ M}$) determination. The biosensor stability was examined by the CV response to $1.0 \times 10^{-3} \text{ M}$ of catechol. After 1 month, the Lac biosensor lost about 37.34% of its first response.

3.4 Interference study

The potential impacts of some overlapping products, including anionic and cationic species, citric acid, some amino acids, glucose, uric acid, ascorbic acid, dopamine, phenol and pyrogallol were used to sense catechol under the same conditions. According to table 2, it perceives that the side effects of organic and inorganic materials are insignificant except for pyrogallol and dopamine. These two interferences, especially dopamine, have the most structural similarity to catechol. For better evaluation of the response of serious interferences including phenol, pyrogallol, ascorbic acid, uric acid and dopamine is shown in figure 8. The biosensor can distinguish catechol in the solution containing potential interferences. There are two distinguishable oxidation peaks for catechol and some interferences including pyrogallol, ascorbic acid and uric acid and their oxidation peaks appear at 0.257, 0.202 and 0.468 V, respectively; while the oxidation peak of catechol appears at 0.35 V.

3.5 Calibration curve and detection limit

The minimum concentration that can be detected is named as limit of detection (LOD), this is the point where we are able to just discriminate signals from the backgrounds. Due to the high sensitivity of the DPV technique, it was used for catechol detection and LOD calculation. A sharp cathodic peak was exhibited using the subsequent circumstances: modified GCE as a working electrode, DPV amplitude in phosphate buffer (pH 5) containing catechol ($1.0 \times 10^{-3} \text{ M}$). Catechol indicated two linear ranges from 0.1×10^{-6} to $5 \times 10^{-6} \text{ M}$ and 16.7×10^{-6} to $166 \times 10^{-6} \text{ M}$ (figure 9a and b) and the LOD was calculated as $1.5 \times 10^{-6} \text{ M}$. This Lac biosensor was compared with some enzymatic-based biosensors, and the resulting data are shown in table 3. From table 3, it is clear that the presented biosensor has a wide linear range and its LOD is lower when compared with some other biosensors.

4. Conclusions

A new and simple GCE fabrication was described with the nanosheets of GO and Au-CTAB for the immobilization of Lac and direct electrochemical detection of catechol. Electrodeposition of Au-CTAB onto GO/GCE makes an electroactive surface that showed high current and very low R_{et} compared with GO/GCE and Au-CTAB/GCE. Also, it causes to immobilize biological species especially Lac in this work; usage and immobilization of Lac make a selective and sensitive catechol biosensor. According to the results, Lac was successfully immobilized onto the modified electrode via electrostatic adsorption. Different techniques were used to characterize the biosensor. SEM and FESEM showed the coating morphologies on the GCE. SEM images demonstrate two-dimensional morphology of the GO, the electrodeposited Au-CTAB, and FESEM showed the immobilized Lac. The Lac biosensor displayed the large R_{et} when compared with the other modified GCEs. Biosensor cyclic voltammograms showed a direct electron transfer reaction between the electrode and Lac with two electrons and two protons and a quasi-reversible mechanism. It should be noticed that the EC' mechanism was determined for the electrochemical reaction. Moreover, α and k_s were obtained as 0.33 and 0.41 s^{-1} , respectively. For this biosensor, two linear ranges and low LOD were obtained. Furthermore, the biosensor had acceptable reproducibility and storage stability and satisfying selectivity.

References

- [1] Chen H, Yao J, Wang F, Zhou Y, Chen K, Zhuang R *et al* 2010 *Sci. Total Environ.* **408** 1043
- [2] Han E, Yang Y, He Z, Cai J, Zhang X and Dong X 2015 *Anal. Biochem.* **486** 102
- [3] Wang C, Feng Y, Gao P, Ren N and Li B L 2012 *Sci. Total Environ.* **431** 366
- [4] Zhou X H, Liu L H, Bai X and Shi H C 2013 *Sens. Actuators B Chem.* **181** 661
- [5] Gorla F A, Duarte E H, Sartori E R and Tarley C R T 2016 *Microchem. J.* **124** 65
- [6] Ren J, Kang T F, Xue R, Ge C N and Cheng S Y 2011 *Microchim. Acta* **174** 303
- [7] Feng S, Zhang Y, Zhong Y, Li Y and Li S 2014 *J. Electroanal. Chem.* **733** 1
- [8] Maya F, Estela J M and Cerdà V 2010 *Talanta* **81** 1
- [9] Yang J, Li D, Fu J, Huang F and Wei Q 2016 *J. Electroanal. Chem.* **766** 16
- [10] Nazari M, Kashanian S and Rafipour R 2015 *Spectrochim. Acta, Part A* **145** 130
- [11] Li D W, Luo L, Lv P F, Wang Q Q, Lu K Y, Wei A F *et al* 2016 *Bioinorg. Chem. Appl.* **2016** 5360361 10
- [12] Karim F and Fakhruddin A 2012 *Rev. Environ. Sci. Biotechnol.* **11** 261
- [13] Mohammad R, Ahmad M and Heng L Y 2013 *Sensors* **13** 10014

- [14] Wu C, Liu Z, Sun H, Wang X and Xu P 2016 *Biosens. Bioelectron.* **79** 843
- [15] Rodríguez-Delgado M M, Alemán-Nava G S, Rodríguez-Delgado J M, Dieck-Assad G, Martínez-Chapa S O, Barceló D *et al* 2015 *TrAC, Trends Anal. Chem.* **74** 21
- [16] Cabaj J, Jędrychowska A, Zajac D, Krawiec S and Sołoducho J 2016 *Int. J. Electrochem. Sci.* **11** 609
- [17] Chen B, Xu W Q, Pan X R and Lu L 2015 *Int. J. Biol. Macromol.* **76** 39
- [18] Vasilescu I, Eremia S A V, Kusko M, Radoi A, Vasile E and Radu G L 2016 *Biosens. Bioelectron.* **75** 232
- [19] Morozova O, Shumakovich G, Shleev S and Yaropolov Y I 2007 *Appl. Biochem. Microbiol.* **43** 523
- [20] Dai Z, Guo M, Wang X, Wang H and Chen W 2014 *J. Nanomater.* **2014** 2
- [21] Portaccio M, Di Tuoro D, Arduini F, Moscone D, Cammarota M, Mita D G *et al* 2013 *Electrochim. Acta* **109** 340
- [22] Franzoi A C, Vieira I C, Dupont J, Scheeren C W and de Oliveira L F 2009 *Analyst* **134** 2320
- [23] Gutierrez-Sanchez C, Shleev S, De Lacey A L and Pita M 2015 *Chem. Pap.* **69** 237
- [24] Boujakhrou A, Jimenez-Falcao S, Martinez-Ruiz P, Sanchez A, Diez P, Pingarron J M *et al* 2016 *Analyst* **141** 4162
- [25] Yang L, Zhao H, Li Y and Li C P 2015 *Sens. Actuators B* **207** 1
- [26] Liu J, Lin G, Xiao C, Xue Y, Yang A, Ren H *et al* 2015 *Biosens. Bioelectron.* **71** 82
- [27] Veerapandian M, Lévaray N, Lee M H, Giasson S, Zhu X *et al* 2015 *ACS Appl. Mater. Interfaces* **7** 14552
- [28] Ma H, Yin B, Wang S, Jiao Y, Pan W, Huang S *et al* 2004 *ChemPhysChem* **5** 68
- [29] Bakshi M S, Sharma P and Banipal T S 2007 *Mater. Lett.* **61** 5004
- [30] Valezi C F, Duarte E H, Mansano G R, Dall'Antonia L H, Tarley C R T and Sartori E R 2014 *Sens. Actuators B* **205** 234
- [31] Khan Z, Al-Thabaiti S A and Bashir O 2016 *Dyes Pigm.* **124** 210
- [32] Rodríguez-Fernández J, Pérez-Juste J, Mulvaney P and Liz-Marzán L M 2005 *J. Phys. Chem. B* **109** 14257
- [33] Marciano D C, Kosynkin D V, Berlin J M, Sinitiskii A, Sun Z, Slesarev A *et al* 2010 *ACS Nano* **4** 4806
- [34] Pavia D L, Lampman G M, Kriz G S and Vyvyan J A 2014 *Introduction to spectroscopy*, 5 edn (USA: Cengage Learning)
- [35] Song D, Xia J, Zhang F, Bi S, Xiang W, Wang Z *et al* 2015 *Sens. Actuators B* **206** 111
- [36] Chen R, Wang Q, Li Y, Gu Y, Tang L and Li C 2015 *RSC Adv.* **5** 44165
- [37] Quan D and Shin W 2004 *Electroanalysis* **16** 1576
- [38] Fan L, Li X and Kan X 2016 *Electrochim. Acta* **213** 504
- [39] Qi H and Zhang C 2005 *Electroanalysis* **17** 832
- [40] Qu J, Lou T, Wang Y, Dong Y and Xing H 2015 *Anal. Lett.* **48** 1842
- [41] Sarika C, Shivakumar M, Shivakumara C, Krishnamurthy G, Narasimha Murthy B and Lekshmi I 2017 *Artif. Cells Nanomed. Biotechnol.* **45** 625
- [42] Laviron E 1979 *J. Electroanal. Chem. Interfacial Electrochem.* **101** 19
- [43] Rafipour R, Kashanian S and Tarighat F A 2014 *IET Nanobiotechnol.* **8** 196
- [44] Li G, Sun K, Li D, Lv P, Wang Q, Huang F *et al* 2016 *Colloids Surf. A* **509** 408
- [45] Bard A J, Faulkner L R, Leddy J, Zoski C G 2001 *Electrochemical methods: fundamentals and applications*, 2 edn. (New York: Wiley)
- [46] Guo M, Wang H, Huang D, Han Z, Li Q, Wang X *et al* 2014 *J. Sci. Technol. Adv. Mater.* **15** 035005
- [47] Fu J, Li D, Li G, Huang F and Wei Q 2015 *J. Electroanal. Chem.* **738** 92
- [48] Li D, Luo L, Pang Z, Ding L, Wang Q, Ke H *et al* 2014 *ACS Appl. Mater. Interfaces* **6** 5144
- [49] Shimomura T, Itoh T, Sumiya T, Hanaoka T, Mizukami F and Ono M 2011 *Sens. Actuators B* **153** 361
- [50] Maleki N, Kashanian S, Maleki E and Nazari M 2017 *Biochem. Eng. J.* **128** 1