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Laccase immobilization on the electrode surface to design a biosensor for the detection of phenolic compound such as catechol

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ABSTRACT

Biosensors based on the coupling of a biological entity with a suitable transducer offer an effective route to detect phenolic compounds. Phenol and phenolic compounds are among the most toxic environmental pollutants. Laccases are multi-copper oxidases that can oxide phenol and phenolic compounds. A method is described for construction of an electrochemical biosensor to detect phenolic compounds based on covalent immobilization of laccase (Lac) onto polyaniline (PANI) electrodeposited onto a glassy carbon (GC) electrode via glutaraldehyde coupling. The modified electrode was characterized by voltammetry, Fourier transform infrared (FTIR) spectroscopy and atomic force microscopy (AFM) techniques. The results indicated that laccase was immobilized onto modified GC electrode by the covalent interaction between laccase and terminal functional groups of the glutaraldehyde. The laccase immobilized modified electrode showed a direct electron transfer reaction between laccase and the electrode. Linear range, sensitivity, and detection limit for this biosensor were 3.2×10^{-6} to 19.6×10^{-6} M, 706.7 mA L mol^{-1}, 2.07×10^{-6} M respectively.

Key words: Laccase immobilization, biosensor, phenolic compound

1. Introduction

Phenolic compounds are broadly used in the manufacture of products, including coal conversion, petroleum refining, pharmaceuticals, production of dyes, pesticides, surfactants, resins, and plastics and thus readily release into the ground and surface water [1-3]. Many of them are showing harmful effects on plants, animals, and human health and then they are very toxic [4]. The maximum amount of phenols in wastewater allowed by the European Community is lower than 1 ppm [5]. Many technologies have been used to determine phenolic compounds such as spectrophotometry, chromatography, and capillary electrophoresis. However, these methods are time-consuming and the instrumentations are expensive [6]. Biosensors can provide ideal sensing systems to monitor the effects of phenolic compounds on the environment, due to their fast response, high selectivity, cost-effectiveness, simplicity of operation, and manufacturing. Electrochemical biosensors are the most commonly used class of biosensors [7]. The use of oxidative enzymes such as laccase [8], tyrosinase [9] and horseradish peroxidase (HRP) [10] to design electrochemical biosensors for detection of phenolic compounds has received great attention. However, reaction mechanisms of the biosensors based on tyrosinase, laccase and HRP are different for various types of phenolic compounds. Phenolic compounds can be oxidized by HRP and the reduction form of HRP can be oxidized by hydrogen peroxide [11, 12]. Tyrosinase can oxidase phenolic compounds with ortho-position of the phenol ring free of substituent group [13], but laccase can oxidase phenolic compounds with para- and meta-position free of substituent group [14].

Laccase (Lac, EC 1.10.3.2, p-benzenediol: oxygen oxidoreductase) belongs to the group of blue oxidases and represents the largest subgroup of multicopper oxidases [15, 16]. It is able

to catalyze an oxidation of various aromatic substrates with concomitant reduction of O_2 to water. Laccase has wide potential applications due to free radical mechanism [17].

Conducting polymers such as polyaniline (PANI) is suitable for immobilization of various enzymes [18]. Using conducting polymers to design electrochemical biosensors offer many advantages and new possibilities to detect biologically significant compounds [19]. The electrochemical polymerization usually yields a thin polymeric film at the electrode surface. PANI is compatible to most enzymes and can be easily synthesized from aniline monomer in an aqueous solution [20]. PANI exists in two forms, as conducting emeraldine salts (ES) and nonconducting emeraldine base (EB). It is one of the most stable polymers and has high stability to extreme temperature and pH and is also resistant to microbial attack [21]. Many reports have been published on the immobilization of enzymes into the PANI film [22].

In this study, we describe the construction of a biosensor to detect catechol. Polyaniline studied in this work was electropolymerized on glassy carbon electrode and laccase was immobilized on the surface of this electrode via glutaraldehyde crosslinking (coupling). The study was aimed to investigate the application of immobilized enzymes in electrochemical biosensor, specifically employing laccase immobilized via glutaraldehyde coupling to detect catechol (as a model phenolic substrate).

2. Experimental

2.1 Materials and Instrumentations

The monomers aniline (Merck) was distilled twice before use. The thin film of polyaniline was synthesized electrochemically on glassy carbon electrode under cyclic voltammetric conditions in a single compartment glass cell. A three-electrode geometry was

employed during the electrochemical polymerization in which a glassy carbon electrode functioned as working electrode (1.5 cm^2), a Pt rod as counter electrode and Ag/AgCl as reference electrode. The film was electropolymerized in an aqueous solution containing 0.1M monomer(s) and 0.5M H_2SO_4 (Merck) as electrolyte. Laccase (EC=1.10.3.2, from *Trametes versicolor*, 13.6 U/mg) was purchased from Sigma Aldrich and used with further purification by ultrafiltration membrane. The enzyme was immobilized by cross-linking via glutaraldehyde (2.5 %) (Merck) on films, thus restricted the leaching of the enzyme film. These films were left for 30 min and washed with phosphate buffer. Buffers including the NaH_2PO_4 + Na_2HPO_4 were always employed as supporting electrolyte. Then the enzyme electrode was washed thoroughly with 0.1 M phosphate buffer solution and stored at 4°C. All solutions were made up with twice distilled water. All other chemicals were purchased from Merck.

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with a Sama 500 electro analyzer system in a conventional three-electrode cell. Glassy carbon electrode was used as working electrode. An Ag/AgCl electrode (saturated KCl) and a Pt rod were used as reference and counter electrodes, respectively. Fourier transform infrared (FTIR) spectroscopy was performed with an FTIR spectrometer (Bruker) and AFM studies were performed with an atomic force microscope (Nanosurf mobile s).

2.2 Activity assays of free laccase

Laccase activity can be tested by addition of certain amounts of substrate and enzyme to a buffered aqueous solution. Laccase activity in solution was measured by the oxidation rate of 1 mM catechol in 0.1 M phosphate buffer, pH 3 at 25 °C. The increase in absorbance, corresponding to the production rate of catechol oxidized product, was followed by

spectrophotometry at 392 nm (ε = 1460 mol^{-1} L cm^{-1}) for a short period of time. Then the absorbance is graphed as a function of time.

2.3 Synthesis of polyaniline on the surface of the electrode

The monomers of aniline (Merck) were distilled twice before use. Aniline can be polymerized electrochemically in either organic or acidic aqueous media. Aniline electropolymerization in aqueous H_2SO_4 was first reported in 1862 [23]. Polyaniline films was synthesized from an aqueous solution of distilled 0.1 M aniline (Merck) and 0.5 M of sulfuric acid (Merck) using electrochemical deposition method. It was carried out by cyclic voltammogram technique at 25°C in one compartment, containing a three-electrode glass cell. The glassy carbon electrode was used as working electrode (1.5 cm^2) and Pt rod and Ag/AgCl were used as counter electrode and reference electrode, respectively. The bare GCE was polished successively, with 0.5 μm Al_2O_3 slurry to a mirror, and ultrasonically cleaned in ethanol and water for 5 min, respectively, then washed with deionized water and dried in air before use. The electrolyte solution was prepared in deionized water. To control the thickness of the films, electropolymerization of this solution was carried out using 25 cycles from -0.5 to 1.3V at a scan rate of 100 mV s^{-1}. The first cycle was applied to induce the polymerization process and the following cycles to achieve the overall coating of the electrode [24]. After synthesis, polymer coated electrode were rinsed thoroughly in deionized water and dried in cold air and then used for subsequent characterization.

The PANI films showed a typical redox response with three redox couples (Fig.1). The first responses occur without loss or gain of protons (leucoemeraldine/ emeraldine) and third redox electrochemical response involves protonation and deprotonation

(emeraldine/pernigraniline) couples are due to the interconversion reactions of PANI upon varying the potential. The middle peaks can be related to the formation of quinones (mostly benzoquinone) as a consequence of a hydrolysis reaction in water [25]. The onset of the polymerization potential of aniline shifts from 0.8 V on the first cycle to 0.4 V on the second cycle, indicating that the PANI formed during first cycle catalyzes the formation of PANI on its surface [26].

2.4 Immobilization of laccase onto polyaniline

Laccase was purified as mentioned in section 2.1. The enzyme solutions were prepared in 0.1M phosphate buffer (pH 7.4) with the working concentration of 2 mg/mL for laccase. The enzyme was immobilized by cross-linking via (2.5 %) glutaraldehyde on polyaniline films. To achieve this kind of immobilization, GC electrode which polyaniline films were constructed on it, was prepared by dipping the modified electrode into carbonate buffer (pH 9.2) solution containing glutaraldehyde (2.5%) for 3 hours and then was thoroughly rinsed in deionized water and dried in air. Laccase immobilization on modified glassy carbon electrode was performed by dipping in 2mg/2mL of laccase for 18 h at 4°C, and then was rinsed with phosphate buffer (pH 7.4). The resulting enzyme electrode was stored in a refrigerator at 4°C when not in use.

2.5 Characterization of immobilized laccase onto polyaniline using various techniques

We used Fourier transform infrared (FTIR) spectroscopy to demonstrate that laccase was immobilized on the electrode. For this, we prepared KBr pills by polishing the surface of the modified electrode on the KBr powder to make pills.

The morphology of the deposited multilayered structures was characterized at nanometer level by AFM (atomic force microscope) that is a tool with different possibilities and limits. Multilayered structures are possible to form because the technique gives the possibility to form multilayered architectures controlled at molecular level. AFM can image biological samples under aqueous conditions with high resolution in three dimensions without the use of any probes.

We used voltammetry technics to study biosensing in the absence and presence of catechol. All the electrochemical experiments were performed with a computer controlled electroanalyzer SAMA 500 conventional cell was used with three-electrodes, using the GC electrode as a working electrode, Ag/AgCl (saturated) (3 M KCl) (Azar electrode) reference electrode and platinum auxiliary electrode. The cyclic voltammograms (CVs) were recorded from -0.5 to 0.8 V and -0.2 to 0.8 V at a scan rate of 100 mV s^{-1} for this biosensor in the absence and presence of catechol, respectively.

3. Results and discussion

3.1 Activity assays of free laccase and comparing kinetic parameters of some Laccases

The laccase activity assay was carried out by monitoring the oxidation of catechol in a reaction mixture for standard conditions at different concentrations of catechol.

If we plot V_o vs. substrate concentration [S], we will see a Michaelis-Menten curve (Fig. 2A). The Michaelis-Menten curve describes the relationship between an enzyme (at constant concentration) and the concentration of substrate, the enzyme's substrate. V_o is the initial rate of production of enzyme product. As [S] increases, V_o eventually becomes independent of [S]**.** The velocity at which this occurs is called V_{max}, and it is the fastest that the given amount of enzyme can operate. The [S] that yields 1/2 V_{max} is another important kinetic parameter called the

Michaelis-Menten constant, designated as K_m. K_m is important since it indicates the [S] at which the enzyme is most effective at altering the rate of the reaction. K_m and V_{max} are characteristics of a reaction that help to characterize the studied enzyme.

To determine K_m and V_{max}, we could determine a set of V_o values at various concentrations of S.

$$\frac{1}{V_0} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}} \quad (1)$$

From the Lineweaver-Burke plot V_{max} and K_m were calculated to be 755 µM/min and 95.9 µM, respectively (Fig. 2B).

The kinetic constants K_m and V_{max} of the kinetic parameters of laccase from *Trametes versicolor* were compared with some laccases (data are shown in Table 1). The K_m values of other laccases [27-29] are higher than that of titled enzyme, which indicates that this enzyme has higher substrate affinity.

3.2 Characterization of immobilized laccase onto polyaniline by variety of techniques

PANI can be activated using glutaraldehyde, to introduce carbonyl group and facilitate covalent immobilization of enzymes via amino groups [21].

To investigate the forming of covalent bonds between PANI, glutaraldehyde and laccase several techniques were used as followed:

3.2.1 Fourier transform infrared (FTIR) spectroscopy

Infrared spectroscopic analysis was performed to study the formation of covalent binding between glutaraldehyde and polyaniline and also covalent binding between laccase and glutaraldehyde.

In the case of PANI, as shown in Fig. 3A there are several characteristic bands. The C-N, N-H stretching and N-H bending vibrations of polyaniline are observed at 1145, 3419, 1615 cm^{-1} respectively [30-32]. A strong peak at 1637 cm^{-1} is related to the C=C stretching vibration [30] mode of the quinoid ring (Fig. 3A). When glutaraldehyde is incorporated into the polyaniline, several new characteristic bands are noticed (Fig. 3B), the C=N, C=O, =C-H and C-H stretching vibrations are observed at 1685, 1720, 2876, 2962 cm^{-1} respectively [30], which indicate that glutaraldehyde interacted with the nitrogen atoms of the polyaniline matrix.

In the FTIR spectrum of immobilized laccase onto PANI, enzyme (Lac) binding is indicated by the appearance of additional absorption bands at 1637 and 1578 cm^{-1} assigned to the secondary amide linkage (C=N bond) [33] of laccase with PANI through glutaraldehyde (Fig. 3C). In addition, C-H stretching vibration is seen in the FTIR spectrum of immobilized laccase that related to CH_2 groups of cross linker (glutaraldehyde). A strong peak at 532 cm^{-1} in Fig. 3C is related to the Cu=N stretching vibration in lac [34].

3.2.2 AFM studies

Atomic force microscopy was used for the topographic characterization of the laccase immobilized onto polyaniline by glutaraldehyde. Fig. 4 shows AFM images of polyaniline electropolymerized onto GCE (Fig. 4 a,b) and laccase immobilized onto polyaniline by glutaraldehyde coupling (Fig. 4 c,d). The GC electrode had no impurity on its surface; the roughness of the surfaces increased significantly as compared with that of the bare GC electrode,

indicating immobilized laccase covered the whole surface. Cross–linked laccases were observed as characteristic mountains. The topographies of laccase films indicate that laccase were fairly well deposited onto PANI surface. Nevertheless they were adsorbed onto the PANI surface as an aggregated pattern in solid-like state with keeping its mountain-like structure.

The surface roughness for PANI layer and PANI layer after immobilization of laccase by glutaraldehyde coupling obtained 77.411 and 89.431 nm, respectively. This result indicates that laccases were successfully immobilized onto polyaniline electrode.

3.2.3 Voltammetric response

The electrochemical behaviors of laccase biosensor were studied using cyclic voltammetry (CV). The cyclic voltammograms of laccase biosensor was investigated in phosphate buffer at pH 5. During the cyclic sweep from -0.5 to -0.8 V, no redox peaks can be seen at the bare electrode. However, a pair of well-defined peaks was observed at the biosensor (data are not shown).

3.2.3.1 Effect of scan rate on the Lac/GA/PANI/GCE

The Cyclic voltammograms of lac-immobilized PANI/GCE via glutaraldehyde coupling at different scan rates show that the redox peak currents of lac increase linearly with scan rates (Fig. 5A). This indicates that the redox reaction is a surface-controlled process, confirming that the immobilized state of lac is stable. Fig. 5B shows the oxidation and reduction peaks, separately, that they have increased with increasing scan rates.

3.2.3.2 Electrochemical behavior of catechol

The detection of phenolic compounds is another important application fields for the Lac modified electrodes. Catechol is used herein as the model phenolic substrate to be detected. The solution pH of 5.0 was selected for catechol determination in subsequent experiments.

The electrochemical behavior of catechol was examined using cyclic voltammetry at modified electrodes (Fig. 6). The redox properties of a compound are readily characterized by cyclic voltammetry. Catechol gave two redox peak potentials for each material that we coated on the surface of the GC electrode, one anodic peak potential (E_p^a) related to oxidation of catechol to o-quinone and one cathodic peak potential (E_p^c) related to reduction of o-quinone to catechol. Fig. 6 shows the current–potential curves for GC, PANI/GC, GA/PANI/GC and Lac/GA/PANI/GC electrodes. In the Table 2 the data about E_p^a and E_p^c and also i_p^a and i_p^c are shown for GC, PANI/GC, GA/PANI/GC and Lac/GA/PANI/GC electrodes. Comparing the anodic currents of the electrodes indicated in Table 2, the Lac/GA/PANI/GCE has the highest anodic current. The difference between anodic and cathodic potentials (E_p^a - E_p^c) for Lac/GA/PANI/GC and other GC electrodes are almost the same. As the laccase biosensor (Lac/GA/PANI/GCE) has the highest current, therefore it has the highest catalytic ability for catechol oxidation.

3.2.3.3 Effect of scan rate

Fig. 7A shows the CVs of 1.0 mM catechol in 0.1 M phosphate buffer at different scan rates. The peak currents were observed to increase with increasing scan rate (Fig. 7B). The oxidation peak increased with increasing scan rates and a linear relationship is found between the peak current and square root of scan rate, with a correlation coefficient of 0.98, Fig. 7B (inset). This behavior indicates a diffusion-controlled process at the electrode surface [35]. From Fig. 7A

it was possible to gather the information shown in Table 3 regarding the influence of the scan rate (v) on the anodic and cathodic peak potentials of this system. The ΔE_p values were 145 mV for n = 4 electrons. In this laccase biosensor i_p^a/i_p^c is greater than one, which indicates that the oxidation of catechol is a quasi-reversible behavior.

Typical curves for this case, at small values of (reorganization energy for electron transfer) λ (large of v) essentially reversible behavior are found [36, 37]. For large values of λ (small of v), week current is observed on scan reversal (Fig. 7A, for lower scan rates e.g. 20, 40, and 60). When the rate of the charge-transfer reaction is sufficiently slow, the observed behavior depends on standard heterogeneous rate constant (k°) and transfer coefficient (α) as well as the kinetic parameter λ for the following reaction. The irreversible following reactions cause the voltammetric wave to shift toward positive values, and this shift away from $E^{0'}$ causes a decrease in the rate of the charge-transfer reaction [36, 37]. All of these electrochemical results suggest a typical E_qC_i mechanism, which includes a quasireversible electrochemical process (E_q) followed by an irreversible chemical reaction (C_i) [37]. In EC mechanism the peak current ratio (i_p^c/i_p^a) is smaller than one and the variation of the peak current ratio (i_p^c/i_p^a) increases by increasing the scan rate [38]. The anodic currents function ($i_p^a/v^{1/2}$) is shown in Fig. 8A. In this case, the anodic current function ($i_p^a/v^{1/2}$) decreases as the scan rate increases for EC mechanism [39]. Fig. 8B displays the relationship between the anodic and cathodic peak potential (E_P^a) and the logarithm of scan rate (log v) for biosensor in 0.1 M phosphate buffer (pH 5) containing 1mM of catechol. The peak potential (E_P^a) changed linearly versus log v with a linear regression equation of $E_p^a = 0.0581 \text{ Log } v + 0.5326$; $R^2 = 0.979$ (v V/s) in the range from 10 to 250 mV s^{-1}. For a redox monolayer modified electrode, the peak potentials can be represented by Laviron [39] (2) and (3):

(3)

$$E_{pa} = E^{0'} - \frac{2.3RT}{(1-\alpha)nF}\log\frac{(1-\alpha)Fnv}{RTk} \quad (2)$$

$$\log k_s = \alpha\log(1-\alpha) + (1+\alpha)\log\alpha - \log\frac{RT}{nfv} - \frac{\alpha(1-\alpha)Fn\Delta E_p}{2.3RT}$$

Where α is the electron transfer coefficient, n the number of electrons, R, T, F and K are gas, temperature, Faraday constant, heterogeneous electron transfer rate constant, respectively. According to the slope of anodic process, it was calculated that (1-α) n = 1.017. Given 0.3<α<0.7 in general [40, 41], it could be concluded that n = 4 and α = 0.75. So, the redox reaction between laccase and catechol is a four electron transfer process. Also, the heterogeneous electron transfer rate constant could be estimated to be 0.1049 s^{-1}.

The steps of the reactions on the surface of the biosensor in the presence of catechol are shown in Scheme 1. Polymeric films of catechol can be obtained by laccase [42-45].

3.2.3.4 Calibration curve and detection limit

To initially establish linear range, sensitivity and detection limits, we commonly rely on the external standard method. A common misconception is that limit of detection is the smallest concentration that can be measured. Instead, it is the concentration at which we can decide whether an element is present or not, that is, the point where we can just distinguish a signal from the background. Differential pulse voltammetry was used for the direct determination of catechol. Catechol showed a well-defined anodic peak using the following conditions: electrode as a working electrode, differential pulse voltammetry amplitude in 0.1 M phosphate buffer (pH = 5). Catechol showed a linear range from 3.2×10^{-6} to 1.96×10^{-5} M (Fig. 9) with a correlation coefficient of 0.9898. The detection limit was calculated according to $3S_b/b$ formula, where S_b was the standard deviation of the blank measurements (n = 6) and b was the slope of calibration

curve. The detection limit for catechol was 2.07×10^{-6} M and the sensitivity was 706.7 mA L mol^{-1}.

3.2.3.5 Comparing linear range, sensitivity and detection limit to other works

Taking catechol as an example, the performance of phenolic compound biosensor was compared with biosensors based on horse radish peroxidase, tyrosinase and laccase immobilized in different matrices. From Table 4, it is clear that sensitivity of our proposed biosensor for catechol, 706.7 mA L mol^{-1}, appeared higher than other offered biosensors [46-52]. Also detection limit is better (lower) in compare to some cases [48, 51, 52].

4. Conclusions

The covalent immobilization of functional, biological molecules onto a defined conductive surface provides the basis for sophisticated biomolecular architectures with numerous applications for *in vitro* studies on the behavior of biological structures such as proteins and cells, for implant or for biosensor devices. Our data suggest that laccase can be immobilized onto polyaniline (PANI) electrodeposited onto a glassy carbon electrode (GCE) via glutaraldehyde coupling. The modified electrode was characterized by fourier transform infrared (FTIR) spectroscopy, atomic force microscopy (AFM) and cyclic voltammetry (CV) techniques. FTIR was applied to indicate that laccase was immobilized onto the surface of the electrode by glutaraldehyde coupling. Atomic force microscopy was used to study the morphology of the coating formed on GCE. Atomic force microscopy showed the three dimensional morphology of the polyaniline. The roughness values were also obtained from AFM. The roughness after immobilization of laccase onto polyaniline by glutaraldehyde coupling was increased and this is

an evidence for immobilizing of enzyme by coupling method. We propose an EC mechanism for Lac/GA/PANI/GC electrode reaction in the presence of catechol. Linear range, sensitivity, and detection limit for this biosensor were obtained 3.2×10^{-6} to 19.6×10^{-6} M, 706.7 mA L mol^{-1}, 2.07×10^{-6} M respectively.

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Legends

Scheme Legend

Scheme 1: The steps of the reactions on the surface of the biosensor in the presence of catechol

Figure Legends

Fig. 1. Cyclic voltammograms recorded during the synthesis of polyaniline film in aqueous solution of H_2SO_4 as electrolyte. The currents of voltammetry cycles are increasing from (a) to (s).

Fig. 2. (A) The Michaelis-Menten curve. (B) Lineweaver-Burke plot.

Fig. 3. (A) The FTIR of electropolymerized polyaniline onto GCE, (B) The FTIR of electropolymerized polyaniline onto GC electrode coupled by glutaraldehyde, (C) The FTIR of electropolymerized polyaniline onto GC electrode and laccase was immobilized onto it by glutaraldehyde crosslinking

Fig. 4. (a,b) AFM images of electropolymerized polyaniline onto GCE. (c,d) AFM images of laccase immobilized onto polyaniline via GA coupling.

Fig. 5. (A) Cyclic voltammograms of Lac/GA/PANI/GCE in 0.1 mol l^{-1} phosphate buffer pH 5.0 at different scan rates. Scan rates from (a) 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, 1.0 Vs^{-1}, respectively. (B) Effect of scan rate on anodic and cathodic currents.

Fig. 6. Cyclic voltammograms of (a) GC, (b) PANI/GC, (c) GA/PANI/GC, (d) Lac/GA/PANI/GC electrodes in 0.1 M phosphate buffer pH5 containing 1.0×10^{-3}M catechol. Scan rate: 100 mV/s.

Fig. 7. (A) Cyclic voltammograms of Lac/GA/PANI/GCE in 0.1 mol l^{-1} phosphate buffer pH 5.0 containing 10^{-3} M catechol at different scan rates. Scan rates from (a) to (s) are 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 mVs^{-1}, respectively. (B) Effect of scan rate on anodic and cathodic currents. (Insert) Variation of anodic peak current (points) as a function of $\upsilon^{½}$

Fig. 8. (A) anodic peak current function as a function of scan rate. (B) Dependence of peak potentials versus scan rate in logarithmic scale.

Fig. 9. Calibration curve for catechol by phenolic biosensor based on laccase immobilized onto GA/PANI/GCE.

Table Legends

Table 1: Comparing kinetic parameters of some Laccases

Table 2: Compare of some voltammetric data for GC, PANI/GC, GA/PANI/GC, and Lac/GA/PANI/GC electrodes.

Table 3: Voltametric data for laccase biosensor in the presence of catechol

Table 4: A comparison of analytical characteristics towards catechol for biosensors based on different enzymes reported in the literature

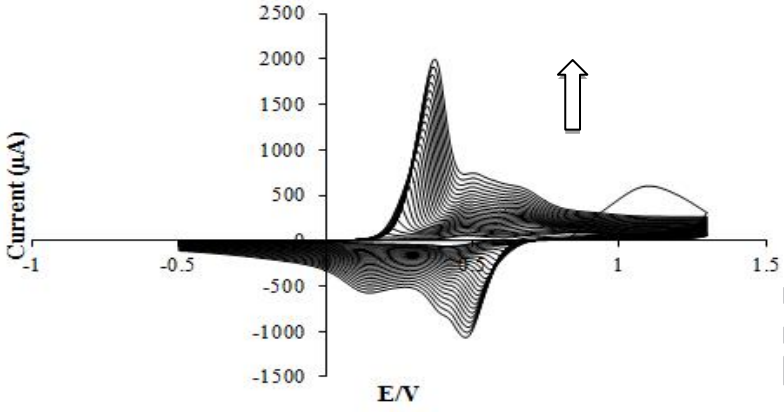


Fig. 1

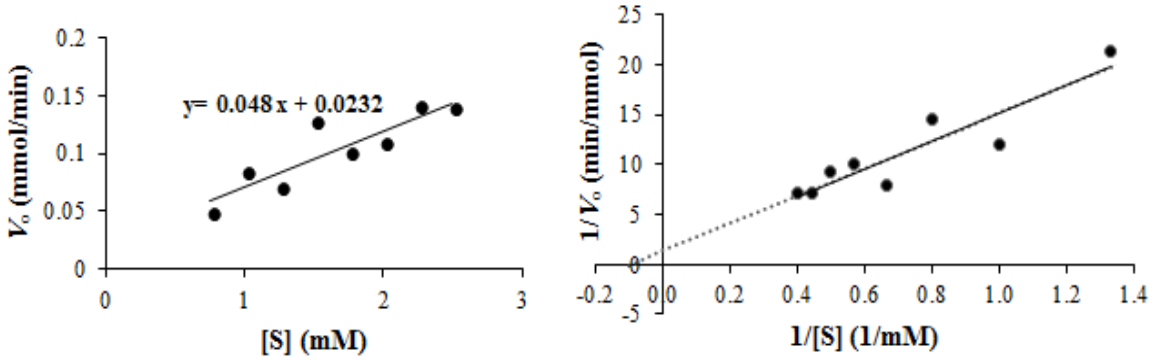


Fig. 2

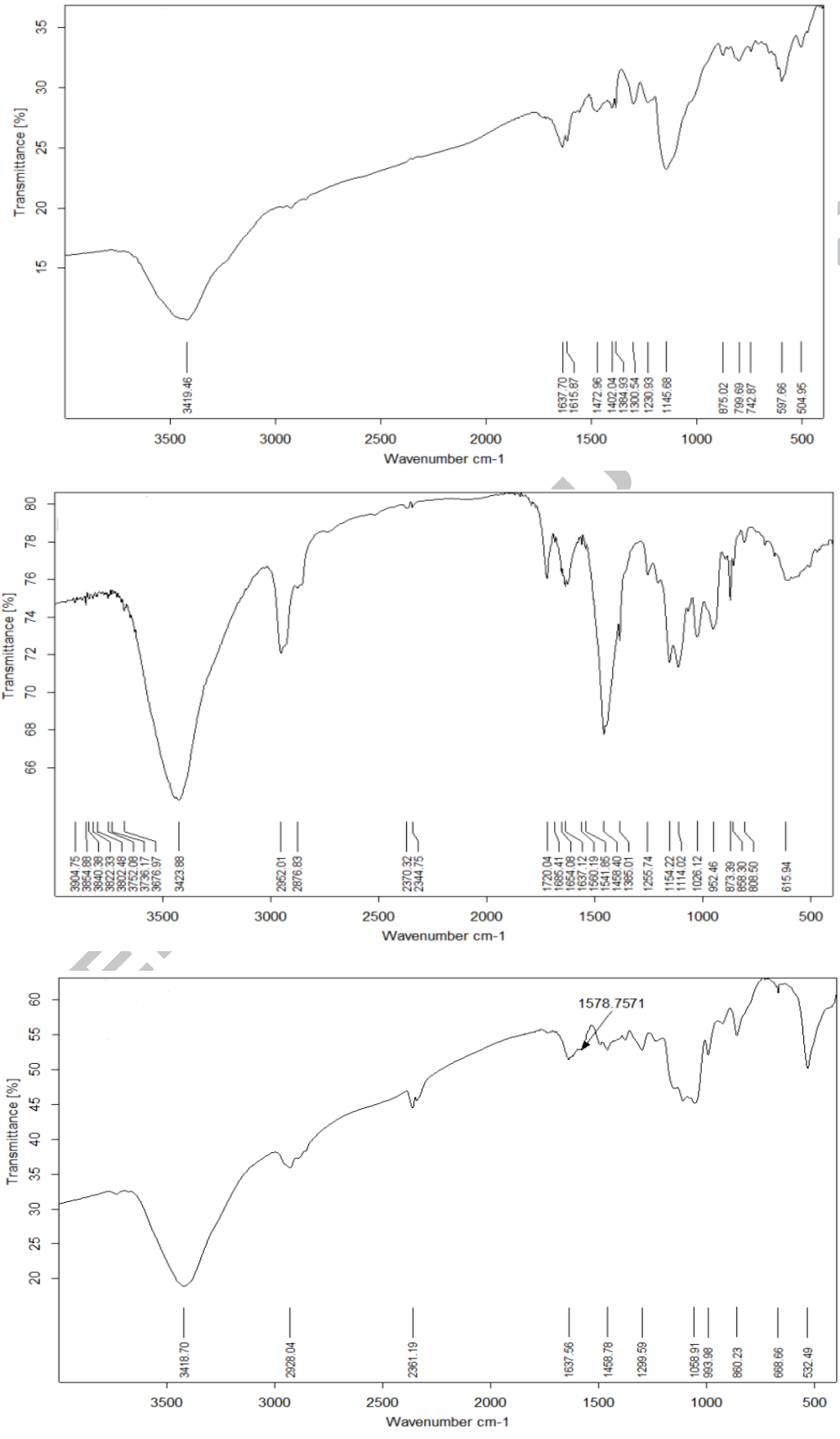


Fig. 3

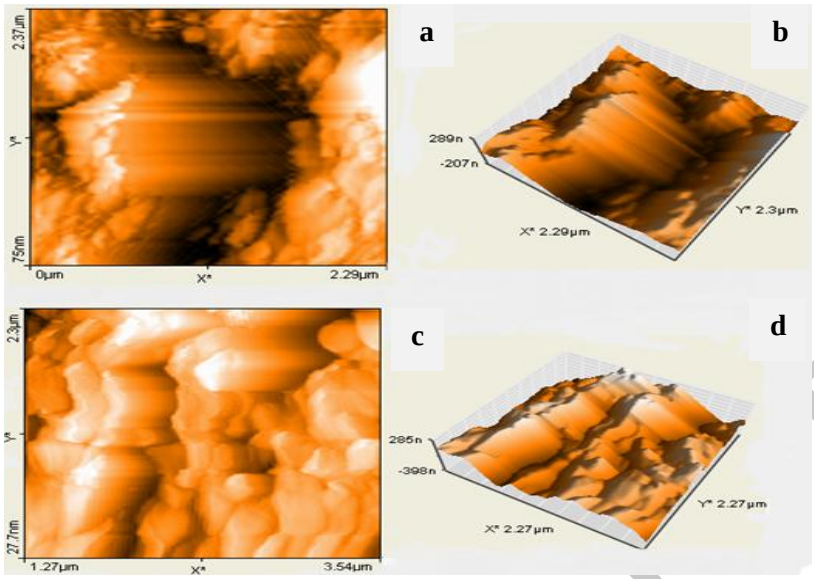


Fig. 4

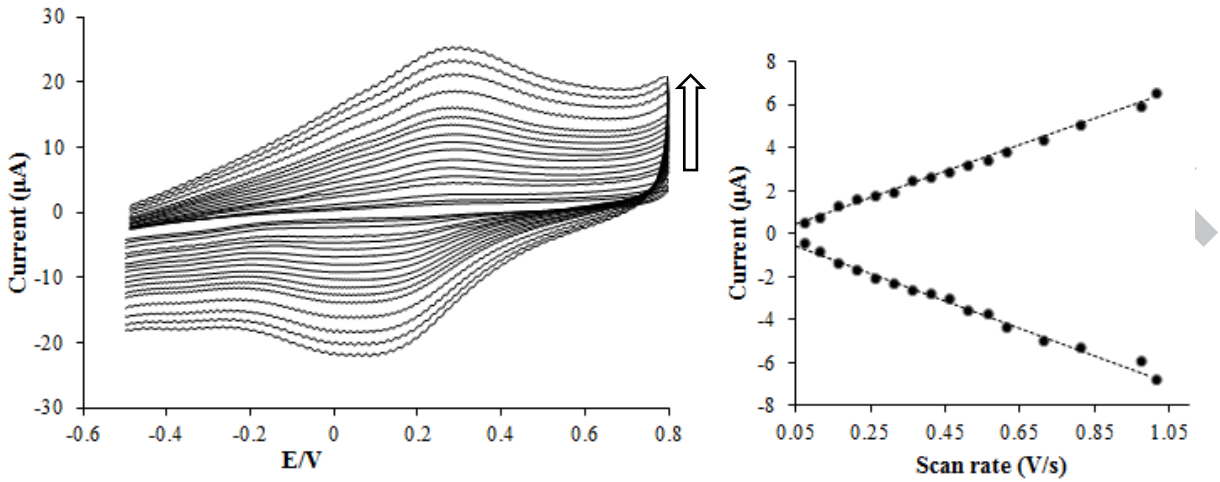


Fig. 5

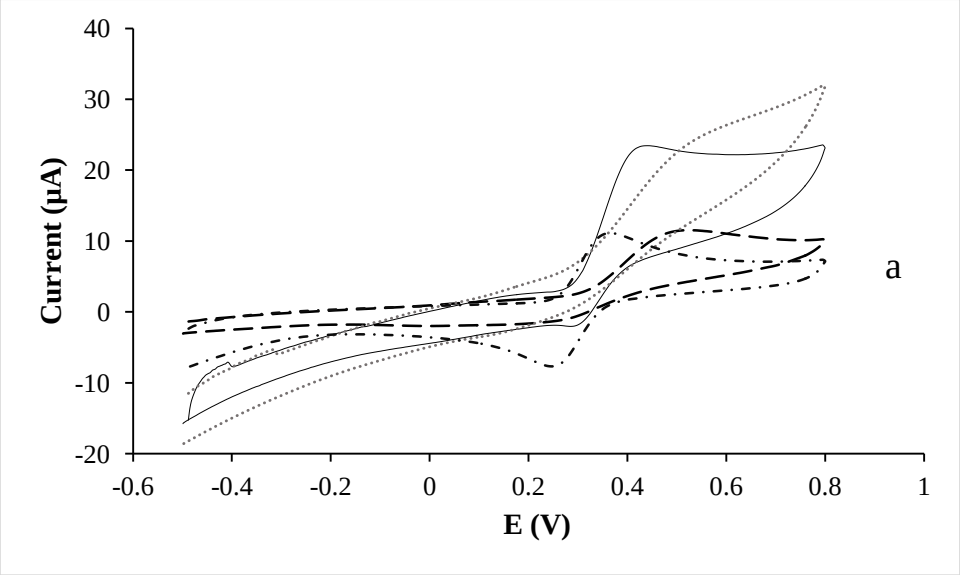
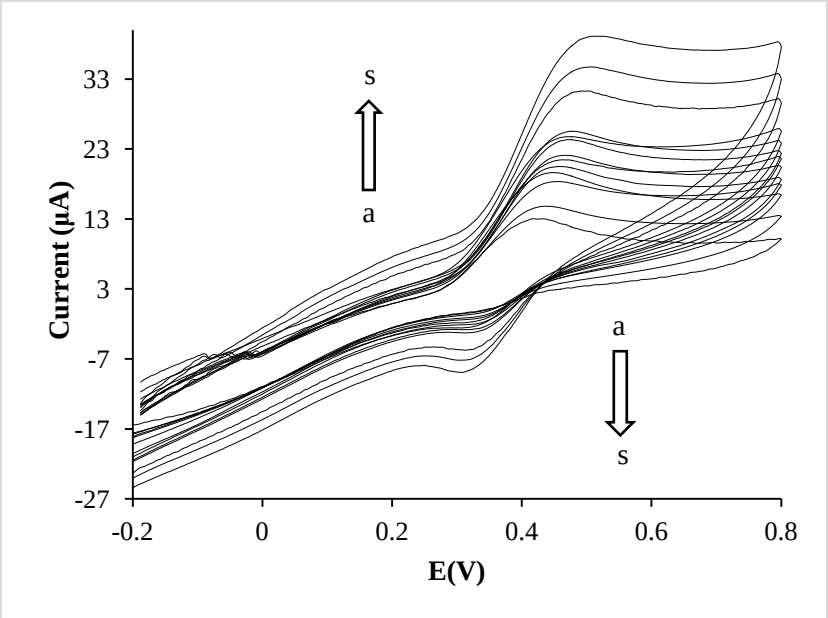


Fig. 6

A



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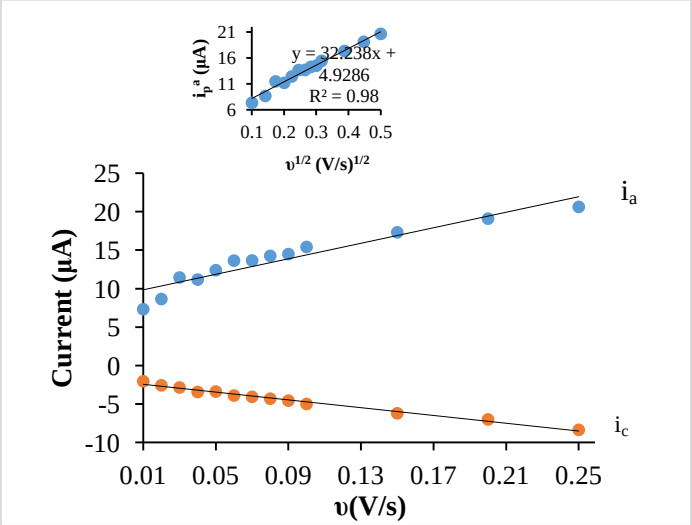
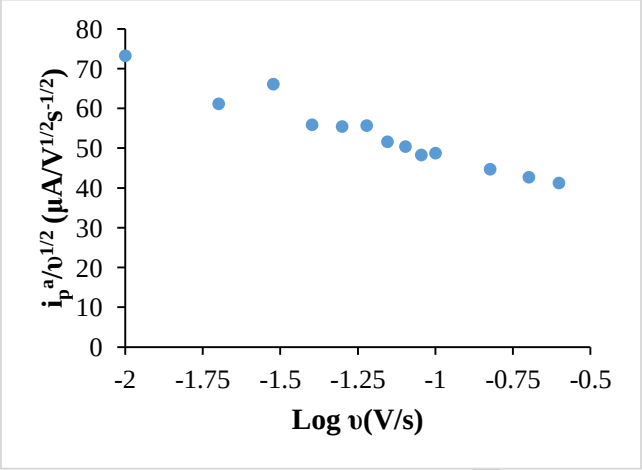


Fig. 7

A



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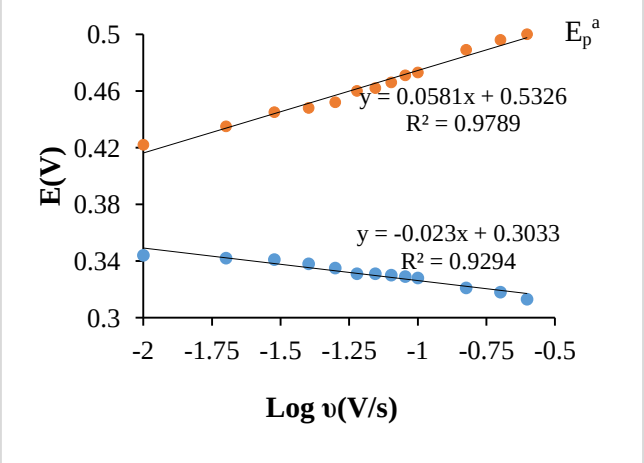


Fig. 8

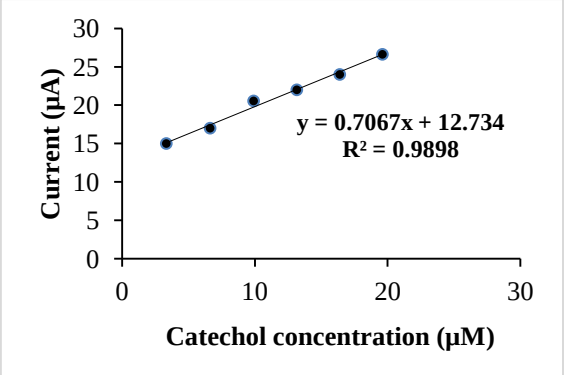
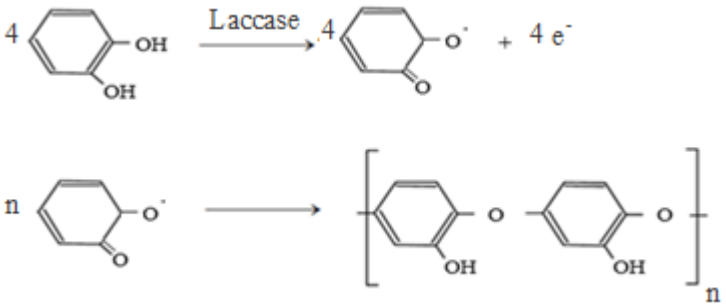


Fig. 9



Scheme 1

Table 1

Enzyme	$pH_{optimum}$	k_m(μM)	V_{max}(μM/min)	Reference
Laccase from *Gandoderma lucidum*	3.5	521	19650	[27]
Laccase from *Botrytis cinerea*	3.5	100	-	[28]
Laccase from *Mycena purpureofusca*	2.2	296	64.5	[29]
Laccase from *Trametes versicolor*	3	95.5	755	This work

Table 2

Electrode	E_p^a	i_p^a	E_p^c	i_p^c	$E_p^a - E_p^c$
GC	0.365	9.581	0.247	-8.984	0.118
PANI/GC	0.522	13.36	-	-	-
GA/PANI/GC	0.525	8.751	-	-	-
Lac/GA/PANI/GC	0.473	15.42	0.328	-4.996	0.145

Table 3

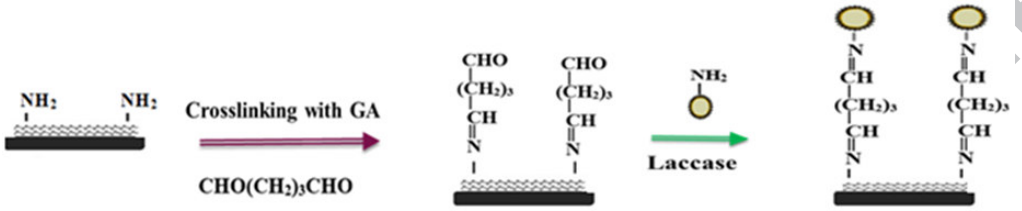
i_p^c/i_p^a	i_p^c (μA)	E_p^c (V)	v (mV/s)
0.279	-2.042	0.344	10
0.297	-2.572	0.342	20
0.249	-2.853	0.341	30
0.307	-3.435	0.338	40
0.273	-3.381	0.335	50
0.286	-3.896	0.331	60
0.298	-4.071	0.331	70
0.302	-4.305	0.330	80
0.315	-4.559	0.329	90
0.324	-4.996	0.328	100
0.358	-6.208	0.321	150
0.367	-7.003	0.318	200
0.405	-8.364	0.313	250

Table 4

Biosensor	Sensitivity [mA L mol^{-1}]	Linear range [mol/L]	Detection limit [mol/L]	Reference
Amperometric biosensor based on recombinant laccases immobilized using bovine serum and glutaraldehyde onto graphite electrode	3.8	1.0×10^{-6}-1.3×10^{-5}	Not reported	[50]
Cross-linked enzyme crystal of laccase embedded in polyvinylpropylidone gel	Not reported	5.0×10^{-5}-1.0×10^{-3}	Not reported	[46]
Layer-by-layer immobilization of tyrosinase on latex particles	150	2.0×10^{-6}-2.0×10^{-5}	Not reported	[47]
Optical biosensor based on immobilization of laccase and MBTH in stacked films	Not reported	5.0×10^{-4}-8.0×10^{-4}	3.3×10^{-4}	[52]
Amperometric biosensor based on multiwalled carbon nanotube poly(pyrrole)-horseradish peroxidase nanobiocomposite film	2.0	1.6×10^{-6}-8.0×10^{-6}	0.93	[51]
Amperometric biosensor based on immobilization of laccase on graphite screen printed electrodes modified with ferrocene	3.021	Not reported	1.0×10^{-5}	[48]
Laccase - nafion based biosensor	68.7	1.2×10^{-6}-1.0×10^{-5}	4.3×10^{-7}	[49]
Immobilization of laccase onto polyaniline by glutaraldehyde coupling	706.7	3.2×10^{-6}-1.96×10^{-5}	2.07×10^{-6}	This work

Graphical Abstract

Construction of an electrochemical biosensor for detection of phenolic compounds based on covalent immobilization of laccase (Lac) onto polyaniline (PANI) electrodeposited onto a glassy carbon electrode (GCE) via glutaraldehyde coupling.



Highlights

Laccase can oxide phenol and phenolic compounds such as catechol.

Biosensor based on laccase may determine the concentration of catechol.

We constructed a biosensor based on immobilization of laccase to detect catechol.

Laccase was immobilized onto polyaniline by glutaraldehyde coupling.