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# Phenolic Compounds Determination Using Laccase-based Electrode Modified with Conducting Polymer Support

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A novel amperometric biosensing electrode for the determination of catechol was investigated by immobilization of laccase in conducting polymer built of dithienotetraphenylsilane. The optimum fabricating conditions of the enzymatic electrode were studied. The resulting laccase-based electrode with the limit of less than 0.4 μM can be used in determination of catechol in the linear range of 5 – 160 μM. the laccase biosensor exhibited good stability, reproducibility, and sensitivity. The effects of potential and pH on the response current of laccase electrode are also presented.

**Keywords:** conducting polymer, laccase, enzymatic electrode, catechol, electrochemical process

## 1. INTRODUCTION

Phenolic compounds broadly used in a diversity of modern industries, including food industry, pesticide industry, are environmentally toxic and pollute the rivers and groundwater [1]. When consumed, phenol-laced water may cause lung, liver, kidney, and genitourinary system damage in both humans and wildlife [2]. Consequently, it is essential to investigate a sensitive and precise sensors able to monitor phenolic compounds concentration in ground water.

Phenols and its derivatives have been detected by chromatography, spectroscopy, votammetry method, capillary electrophoresis as well as colorimetry [3]. However, some of these techniques are expensive, poor sensitivity and usually require time-consuming complicated sample pretreatment. Therefore, it is still necessary to work on developing fast and effective alternative methods for detection phenolic compounds. Due to their simplicity and convenience of miniaturization, electrochemical measuring systems have been widely investigated [4,5]. Electrochemical methods for phenolic compounds determination can be qualified into (1) direct oxidation of phenolic compound

and (2) electroreduction of an electroactive species into which phenolic derivative is enzymatically converted [6,7]. The former demands a highly electrocatalytic electrode, whereas the second often requires an electrode modified with phenol-specific biocatalyst, i.e. laccase.

The laccase (EC 1.10.3.2) is an inviting enzyme as a piece of biosensor [8]. Laccases have been used in various fields including environmental analysis [9,10] and the food industry [11]. They are multi-copper enzymes which oxidize various aromatic and nonaromatic compounds accompanied by four-electron reduction of molecular oxygen to water [12]. Laccase biosensors can directly catalyze the oxidation of catechol to *o*-quinone for quantitative detection [13-15]. This laccase-based monitoring allows electrochemical determination to be performed at low potentials, where redox reactions of the interfering species are not valid.

The efficient immobilization of the enzymatic protein onto the electrode is crucial for the preparation of an effective and stable catalytic system. To utilize such enzymes as efficient sensing parts, the laccase have to be placed on the surface of the electrode. This procedure of immobilization needs to run across convinced qualifications, i.e. protein retention on the electrode, enzyme stability and swift electron transfer.

Utilization in this procedure a semiconducting polymeric material as supporting element may provide relatively homogeneous, stable and adherent carrier. Furthermore, utilizing a modern semiconductors for immobilization permits to engineer a biosensing device for examination the direct electron transfer process of laccase.

Resuming the researches in the field of engineering of protein–conducting material sensitive layers [15], we report in this study the effects of research steered into the characteristic of laccase from *Cerrena unicolor* integrated to electropolymer layer built of the dithienotetraphenylsilane (Fig. 1).

Recently, tetra-arylsilane derivatives have appeared as an interesting group of host material, because of their ultrahigh energy gap and high triplet energy level, and some of them have achieved a high quantum efficiency in host/blue-phosphorescent OLEDs. The common character of the hosts is that they mostly transport holes. They possess good fluorescence quantum yield and ability to oxidative polymerization [16].

Introduction of the bis(thiophene) elements into the tetraphenylsilane main chain may improve the stability and diminish the ionization potential of this type of polymers, on the other hand, the presence of tetraphenylsilane, because of its flexibility, accomplishes the solubility of obtained polymer. Electrochemical features of such polymers hinge on the experimental requirements i.e. solvent polarity or applied potential range.

In this study, dithienotetraphenylsilane-based polymer and laccase were attached to a platinum electrode surface by electrostatic binding. The semiconducting polymer layer was used to enhance the mobility of electrons between surface of electrode and enzymatic active center. This method of immobilization of laccase allow to obtain a bioanalytical tool for monitoring phenolic pollutants, the device with good stability, suitable reproducibility, a proper linear range and low limit of detection.

The morphological characteristic of the analytical system was acquired by atomic force microscopy (AFM). The effect of the laccase immobilization procedure on the analytical efficiency of the developed electrode was evaluated using catechol as an example of phenolic substrates.

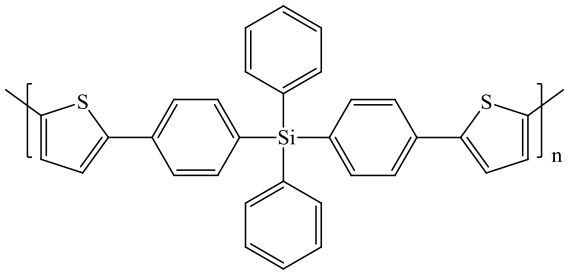


**Figure 1.** Semiconducting polymer based on dithienotetraphenylsilane

## 2. EXPERIMENTAL

### *2.1. Reagents and materials*

Laccase (from *Cerrena unicolor*, EC 1.10.3.2, ≥10 U/mg) as well as other reagents (i.e. catechol) were purchased from Sigma-Aldrich Co. All chemical reagents were analytical grade and were not further purified in usage. The phosphate-citrate buffer solution (McIlvaine, pH 5.2) was prepared by mixing the citric acid and sodium phosphate solutions (in suitable proportions).

The tetraphenylsilane derivative (Fig. 1) was synthesized according to [17].

### *2.2. Apparatus and procedures*

#### *2.2.1. Modification of electrodes*

The platinum electrodes were modified with a thin layer of poly[dithienotetraphenylsilane] and laccase. The electropolymerization of monomers were performed by a potentiostat/galvanostat AUTOLAB PGSTAT128N with GPES software. A three-electrode electrochemical cell (8 ml) equipped with a working platinum electrode, a silver-silver chloride reference electrode (Ag/AgCl), and a coiled platinum wire as the counter electrode, was used for all electrochemical experiments. Before each experiment, the solution was stirred and deoxygenated with nitrogen for 20 min. All the electrochemical measurements were performed at room temperature. Due to synthesize the polymeric layer onto a surface of the clean Pt electrode, dithienotetraphenylsilane (1mM) was dissolved in a acetonitrile solution containing 0.1 M tetrabutylammonium tetrafluoroborate (TBA-TFB). The electrodes were dipped into 8 mL of the monomer solution. The polymer layer deposition was carried out through cyclic voltammetry. The electrode was scanned in a potential range of 0.0 – 1.6 V *vs* Ag/AgCl for 10 cycles, at a scan rate of 50 mV/s. Then, the polymer layer was washed with acetonitrile.

#### *2.2.2. Laccase immobilization*

Electrolytic embedding of laccase on the solids was performed in phosphate-citrate buffer solution (0.1 M, pH 5.2). Prior to the experiment, the buffer solution was stirred and thoroughly purged with nitrogen for 20 min, to create an oxygen-free environment. Due to immobilize the enzyme,

electrode coated with polymer was immersed into 8 ml of buffer containing laccase (0.1 mg/ml). Electrodeposition of laccase was carried out for 15 min under amperometric conditions and applied potential was 0.75 V. Afterwards, electrodes with immobilized enzyme were rinsed three times with buffer solution (pH 5.2) to remove the unbound protein and left in the buffer at 4°C for the further experiments.

To estimate the number of adsorbed molecules of laccase, the semiconducting layer on electrode load was combined with the surface area taken as 8 $mm^2$; the engaged surface area by one laccase molecule was evaluated as $7x10^{-12}$ $mm^2$ from the data of [18] with the presumption of its casual arrangement at the surface. The protein lining was evaluated by Bradford procedure with bovine albumin as the standard [19].

Due to the method, the number of laccase molecules responds to $2.54x10^{11}$.

*2.2.3. Electrochemical measurements*

Cyclic voltammetry experiments were performed with a potentiostat/galvanostat AUTOLAB PGSTAT128N with GPES software. All measurements were carried out in a 8-ml cell with a conventional three-electrode configuration. A working platinum electrode (effective area of 8 $mm^2$) was used together with a coiled platinum wire as the counter electrode and a silver-silver chloride reference electrode (Ag/AgCl).

Before every experiment, the surface of the working electrode was polished with abrasive material (1200), immersed in 1 M $HNO_3$ for 15 min, rinsed with double-distilled water, washed with acetone, dried on air and flame annealed.

*2.2.4. Measurement procedures*

Cyclic voltammetric responses of laccase electrode were recorded in 8 ml of substrate solutions prepared in 0.1 M phosphate-citrate buffer (pH 5.2). As a testing substrate we used:

- catechol, 0.2 mM.

Potential was scanned from -0.1 to 0.7 V *vs* Ag/AgCl for catechol. After holding the electrochemical system at the initial potential for 20 s, voltammograms were recorded by scanning the potential in the cathodic direction, where the substrate underwent a redox process, at a scan rate of 100 mV/s.

The direct electron transfer between laccase and the electrode material in the absence of a mediator was examined by using 8 ml of 0.1 M phosphate-citrate buffer (pH 5.2).

The substrate solution was discharged after each one experiment, so that it had to be subsequently replaced by 8 ml of a fresh substrate solution. All experiments were performed at room temperature, the cell was opened to the air. Every electrode modified with enzyme was scanned 30 times, and the relative deviation of the response current was evaluated to be no more than 2.5%.

*2.2.5. Characterization techniques*

AFM images of enzyme layer covering the electrode were collected in the tapping mode using a AFM Dimension V Veeco. The AFM data was processed by WSxM software [20]. AFM was also used for determination of film thicknesses. The measurements were performed at air-ambient (25°C and 35% relative humidity).We utilized 3 N/m silicon AFM tips with the resonance frequency of 71–88 kHz, and scanning rate of 0.996 Hz (lines per second).

*2.3. Measurement of response current*

Laccase is an enzyme with a copper active sites. In the presence of oxygen, the catalytic reaction of catechol is observed as follows [13], Fig. 2:

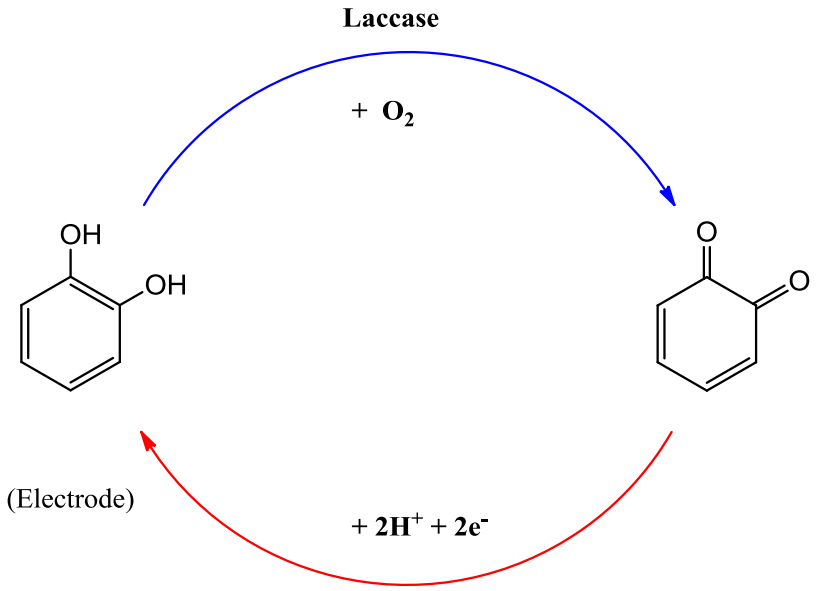


**Figure 2.** Laccase-catalyzed reaction of catechol

The generated in catalytic reaction *o*-quinone is consequently reduced at the electrode. In this study, the response current of the enzymatic electrode was detected by amperometric method during the reduction of *o*-quinone.

## 3. RESULTS AND DISCUSSION

*3.1. Polymer synthesis*

The suitable immobilization of the biocatalyst on solid support is essential for the engineering of every biosensing tool. The architecture of the film used for laccase immobilization should cooperate with the keeping of enzyme efficiency.

In this report polymer built of dithienotetraphenylsilane has been electrochemically synthesized in the presence of 0.1 M TBA-TFB. The polymer occurs in the conducting oxidized state. The overall

charge of the polymer was neutral due to the doping anions which are incorporated into the polymer over the polymerization [15]. Doping is the process of oxidizing or reducing a neutral polymer and providing a counter anion or cation (dopant). Dopants may catalyze the polymerization process through the synthesis and modify the physical features and biological affinity of the conducting polymer [21].

Conducting polymers that possess inherent ion/electron transport properties can supply a platform between the biological event to be studied and the metal electrode for the measuring electronics.

The thiophene derivatives/polythiphenes present a group of compounds characterized by simplicity of preparation, stability under the ambient atmosphere and comparatively high conductivity [17,21]. Moreover, the increase of the area available for enzyme immobilization strengthens the biocatalyst attachment to the solid substrate by increasing the number of weak interactions. Regarding to the inherence of aromatic parts in the polymer backbone, the immobilization is fulfilled with the π–π stacking interactions of the polymer and enzyme. These effective interactions stabilize tertiary structure of proteins [22].

In order obtain the conducting polymer on the Pt electrode, the cyclic voltammetry was used. The optimal potential range for polymer deposition was found to be 0.0 – 1.6 V and the polymerization lasted for 10 cycles. The polymer grown under these conditions was of an approximate thickness of 270 nm. The thickness of the layer under experiment conditions was evaluated by means of the tapping mode AFM. Figure 3 exhibits the results of the electrochemical polymerization of the tetraphenylsilane derivative. The voltammograms demonstrate the formation of the electroactive polymer layer on the Pt electrode. The graph exhibits the increase of peak currents in subsequent scans, which suggests the embedding of the conducting polymer.

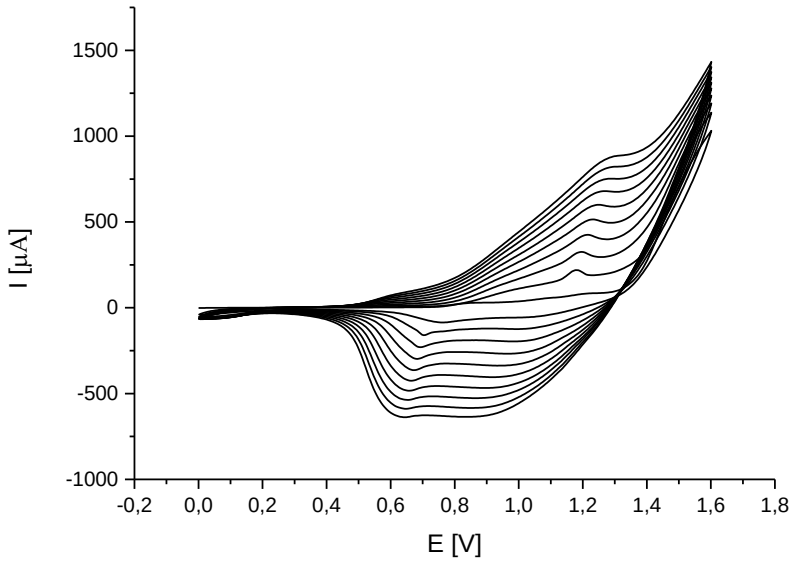


**Figure 3.** Cyclic voltammograms of investigated monomer (dithienotetraphenylsilane - 1mM) in 0.1 M TBA-TFB. Measurement conditions: scan rate 50 mV $s^{-1}$, Ag/AgCl – reference electrode, 10 cycles

*3.2. AFM topography of laccase modified electrode*

Due to illustrate the morphology of surface of laccase electrode the atomic force microscopy (AFM) was adapted. The morphologies of immobilized enzymes were visualized by means of the tapping mode AFM (Fig. 4).

The film equipped with enzymes creates a close-packed matrix with a relatively right structural integrity. The obtained morphological pictures exhibit that the enzyme film (Fig. 4b) is smoother than the one without protein (Fig. 4a). The immobilized laccase was found to create an aggregated/compacted structure in solid-like state, maintaining casual cloud-like shape.

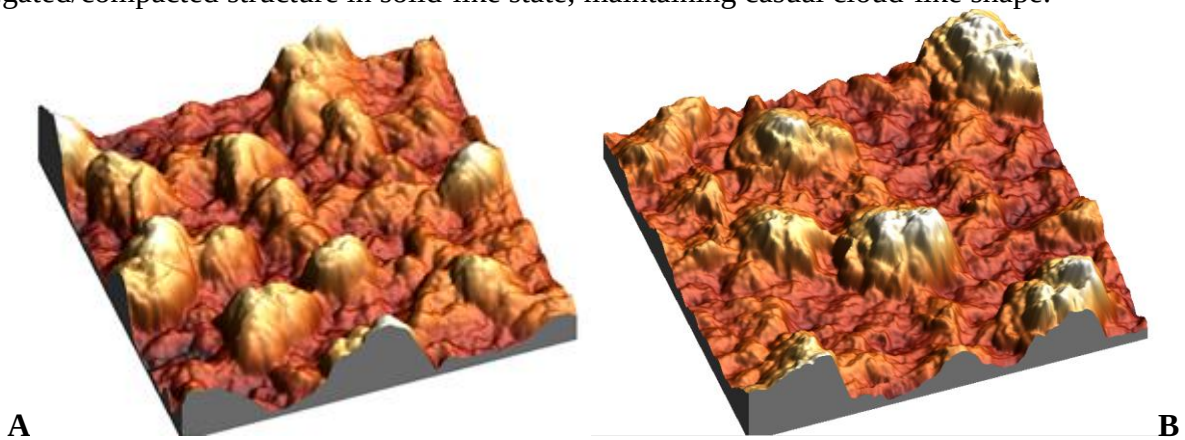


**Figure 4.** AFM images of electrode surfaces modified with laccase (b) and laccase free electrode (a); 5x5 μm

*3.3. Direct electrochemistry of laccase-modified electrode in presence of catechol*

Enzyme-mediated reactions were carried out in substrate solutions at air-opened conditions.

Since when the affinity of laccase to $O_2$ is high (binding constant 0.01 - 0.02 nM) [23], and because of the comparatively high oxygen concentration in water at room temperature (ca. 0.26 mM) [24,25], the air-opened water solution of laccase has saturated with $O_2$ all active centers. Consequently, the observed catalytic current hinges precisely on the substrate concentration.

In this manner, as we have reported earlier, the electrode and redox centers of the laccase placed onto the modified electrode are considered to be a donor/acceptor pair [15].

The redox process at high potentials (0.5 V-0.8 V) belongs to the T1 laccase copper center [25]. The cyclic voltammograms of prepared electrodes (modified and unmodified with enzyme) in the air-opened states in 0.1 M phosphate-citrate buffer (pH 5.2) and in presence of catechol as a redox mediator are depicted in Figure 5 at scan rate 100 mV/s. Due to laccase, on the voltammogram it is possible to find a pair of explicit, well-defined and stable redox peaks, which are presumably assigned to the direct electron transfer between the enzyme active center and the electrode surface. From Fig. 5 is known, the redox operation is a reversible electrochemical progress. The explicit anodic and cathodic peak potentials are placed at 0.45 and 0.2 V, respectively (*vs* Ag/AgCl). The formal potential ($E^{0,}$) for laccase from *Cerrena unicolor* (immobilized in designed template) was estimated from the mid worth of the anodic and the cathodic potential values, and the value was found as 0.325 V. This

value is quite close to the $E^0$ values for T1 copper of laccase (originating from *Rhus vernicifera* and *Melanocarpus albomyces*) [26].

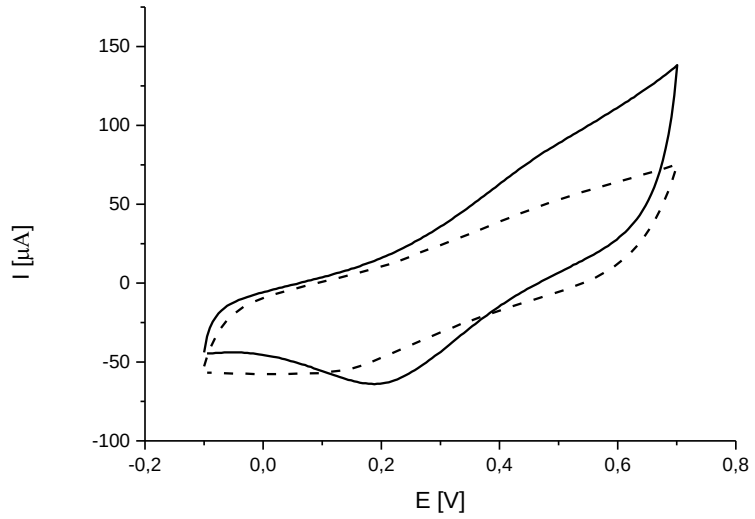


**Figure 5.** Modified electrodes responses recorded in 0.2 mM catechol, pH 5.2; solid line – Pt electrode modified with copolymer and laccase, dashed line – Pt electrode modified with polymer. Measurement conditions: scan rate 100 mV $s^{-1}$, Ag/AgCl – reference electrode

Figure 2 registers a scheme of the reaction concerned the oxidation of catechol catalyzed by laccase on the electrode surface. At the beginning, catechol in presence of enzyme and oxygen, is oxidized to the corresponding *o*-quinone. Then, the quinone is electrochemically reduced on the electrode surface at a potential of 0.2 V vs Ag/AgCl. Subsequently, the arisen current observed in the electrochemical reduction of *o*-quinone is proportional to the catechol concentration.

Figure 5 shows the effect of potential on the response current of the enzymatic electrode in the condition of experiments (0.2 mM catechol, pH 5.2). The current increased notably when the potential was reduced from 0.2 V to -0.1 V due to the growing driving force for the fast reduction of *o*-quinones at low potential [14].

However, from Figure 5 (where the cathodic peak imputed to the electrocatalytic reduction of *o*-quinone occurs at 0.2 V) a potential value of 0.1 V was chosen to carry out the chronoamperometric measurements, since when it is suitable to supply an appropriate intensity ratio, reducing interferences suitable to other redox species. Thereby, chronoamperometric surveys for different catechol concentrations proved that a steady state current is acquired after 10 s.

### *3.4. Influence of pH on response current*

The influence of pH on response current of enzymatic electrode in the buffer solutions containing 0.2 mM catechol is presented in Figure 6. It can be observed that response current slowly increases as the change of pH from 2.5 to 5.2, and then quickly decreases due to subsequent pH growth. At pH ranges between 2.5-5.2, the increase of response current was connected with growth of the enzyme activity. But when pH was much higher than 5 reduced response current was attributed to

the implication of protons in the reduction of *o*-quinone. The optimum pH was found as 5.2 and it is in agreement with previous experiments [15] and native laccase activity [27]. It indicates significantly that polymerization process doesn't influence negatively on dimensional structure of protein.

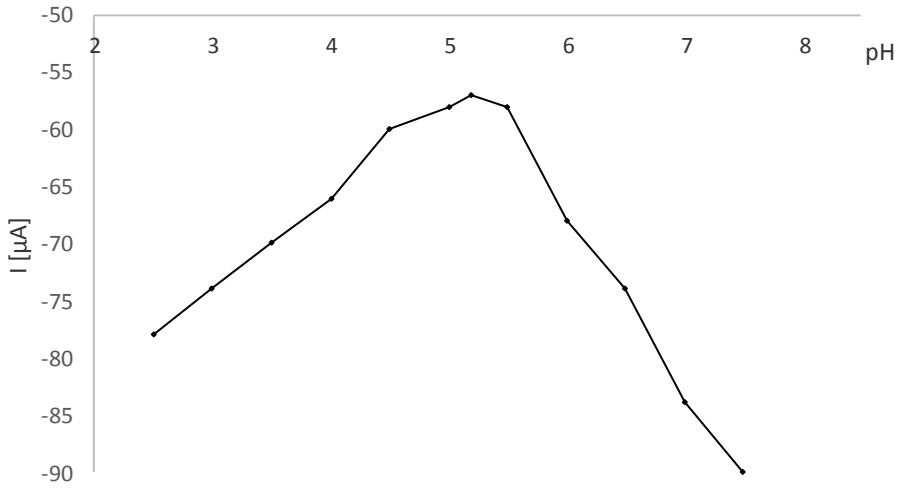


**Figure 6.** The behaviour of response current in different pH, 0.2 mM catechol, 01.V

*3.5. Amperometric response of the laccase electrode*

Catechol was the phenol derivative chosen for this survey as a rather responsive laccase substrate, as well due to its toxicity, difficulty to eliminate from wastewater and possibility of causing nervous system lesions [28].

The analytical character of the engineered biosystem, such as linear concentration range, sensitivity, detection limit and reproducibility were estimated, and are demonstrated in Table 1 and Fig. 7.

The relationship between response current of laccase electrode and the catechol concentration in McIlvaine buffer (pH 5.2) at 0.1 V was investigated. As exhibited in Fig. 7 the response current grows up linearly with increasing of catechol concentration in the range 5–160 μM, what indicates on the 1$^{st}$-order reaction. Subsequently, the response current grows gradually with coincident growth of catechol concentration. The detection limit of this sensor was found as 0.4 μM what is in agreement with data presented by Guo et al. as well as Qu et al. [29,30].

In Table 1 we presented also performance of different laccase biosensors in presence of catechol. It is firstly noticed that detection limit of presented system (0.04 μM) is better (or comparable) than those of shown modified laccase biosensor. We attributed it to the larger specific surface area of presented branched polymers [17]. modified enzymatic electrode, which are better than those listed in Table 1, it means that polymer built of dithienotetraphenylsilane surface can be loaded more laccase molecules to participate in catalytic reaction. The linear range (5-200 μM) is also better when compares with biosensors modified with polyaniline as well as poly(3-methylthiophene) [31,32].

It was reported that polyaniline is biocompatible *in vitro* and in long-term animal *in vivo* studies [33]. Due to the fact, reported modified biosensor could be ascribed to good biocompatibility.

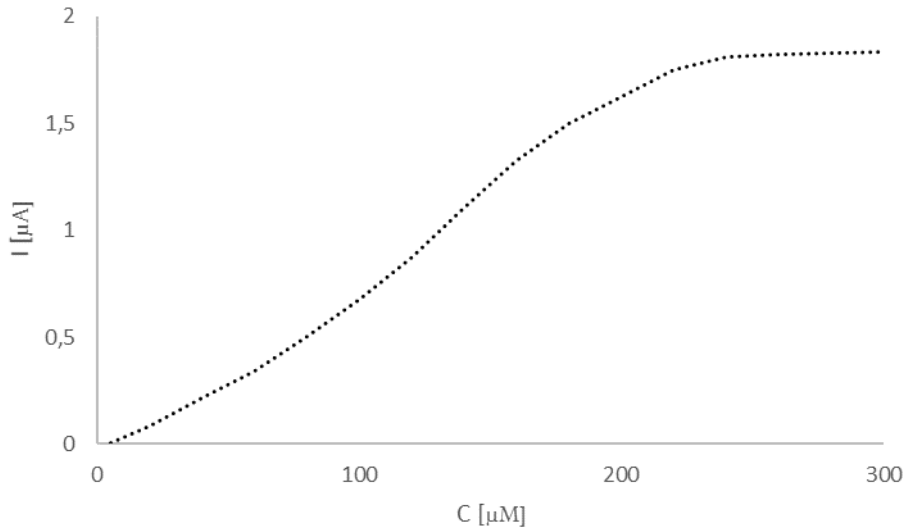


**Figure 7.** The relationship between response current and catechol concentration in 0.1 M phosphate-citrate buffer (pH 5.25). Applied potential 0.1 V

**Table 1.** Properties of laccase-based biosensors

| **Biosensor** | **Substrate** | **Linear range (µM)** | **Sensitivity (µA $mM^{-1}$)** | **Detection limit (µM)** | **Ref.** |
|---|---|---|---|---|---|
| Presented biosensor | catechol | 5.0-200 | 2.86±0.11 | 0.04 | this work |
| Chitosan based biosensor | catechol | 1.0-100 | not reported | 29 | [29] |
| Polyvinyl alcohol based biosensor | catechol | 0.92-16.55 | 59 | 0.31 | [30] |
| Poly(3-methylthiophene) based biosensor | catechol | 0.08-14 | not reported | 0.01 | [31] |
| Polyaniline based biosensor | catechol | 3.2-19.6 | 0.706 | 2,07 | [32] |

The reproducibility of designed biosensor was estimated via the comparison of different electrodes. Ten various laccase electrodes were examined separately for the detection of catechol, providing a considerate standard deviation value of 3%. This fact indicates significantly an effective as well as reproducible immobilization process of laccase in synthesized polymer film. An adequate reproducibility may be attributed to the controllable procedure of the sensor construction.

The life-time and of the enzymatic electrode was evaluated by using square-wave voltammetry survey in 0.2 mM of catechol during 3 months, whereas the electrodes were stored at 4$^{o}$C. The biosensor has maintained 92% of its initial current response over this time what is similar to results of Tan et al. [14]. The operational stability of laccase electrode was measured in the same conditions. A comparative deviation of 2.5% was obtained for 30 consecutive measurements.

The obtained results seem to be acceptable, and signify that laccase was immobilized in a biocompatible environment. Then, the procedure permits to maintain the stability and life-time of the immobilized enzyme.

## 4. CONCLUSIONS

The survey has proved facile and convenient method for catechol determination based on laccase-based oxidation of catechol. The resulting sensor (built of electrode modified with electroconducting polymer and laccase) exhibits good performance, strong affinity between enzyme and catechol, fast response to the substrate, explicit linear range. Moreover, the operational stability as well as life-time are significant advantages of the enzymatic electrode. The detail parameters analysis demonstrated its potential utility in phenolic compounds monitoring.

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## References

1. K. R. Roger, J. Y. Baker, J. Wang, F. Lu, *Field Anal. Chem. Technol*. 3 (1999) 160-169.
2. S. Gupta, G. Ashrith, D. Chandra, A. K. Gupta, K. W. Finkel, J. S. Guntupalli, *Clin. Toxicol*. 46 (2008) 250-253.
3. J. Meng, C. Shi, B. Wei, W. Yu, C. Deng, X. Zhang, *J. Chromatogr. A*, 1218 (2011) 2841-2847.
4. S. Liu, J. Yu, H. Ju, *Electroanal. Chem*. 540 (2003) 61-67.
5. Rajesh, S. S. Pandey, W. Takashima, K. Kaneto, *Curr. Appl. Phys*. 5 (2005) 184-188.
6. F. Liu, Y. Piao, J.S. Seo, *Biosens. Bioelectron*. 50 (2013) 387-392.
7. J. Njagi, C. Ispas, S. Andresscu, *Anal. Chem*. 80 (2008) 7266-7274.
8. W. Johnston, M. J. Cooney, B. Y. Liaw, R. Sapra, M. W. Adams, *Enz. Microb. Technol*. 36 (2005) 540-549.
9. K. Modaressi, K. E. Taylor, J. K. Bewtra, and N. Biswas, *Water Resear*. 39 (2005) 4309–4316.
10. S. R. Couto, and J. L. T. Herrera, *Biotechnol. Advan*. 24 (2006) 500–513.
11. G. Songulashvili, V. Elisashvili, S. P. Wasser, E. Nevo, and Y. Hadar, *Enzyme Microbial. Technol*. 41 (2007) 57–61.
12. S. C. Barton, H.-H. Kim, G. Binyamin, Y. Zhang, and A. Heller, *J. Phys. Chem. B*, 105 (2001) 11917–11921.
13. L. Deng, F. Wang, H. Chen, L. Shang, L. Wang, T. Wang, and S. Dong, *Biosens. Bioelectron*. 24 (2008) 329–333.
14. Y. Tan, J. Kan, and S. Li, *Sens. Actuat. B*, 152 (2011) 285–291.
15. A. Jędrychowska, J. Cabaj, A. Świst, J. Sołoducho, *J. Electroanal. Chem*. 720-721 (2014) 64-70.

16. H. Liu, G. Cheng, D. Hu, F. Shen, Y. Lv, G. Sun, B. Yang, P. Lu, Y. Ma, *Advan. Funct. Mat*. 22 (2012) 2830-2836.
17. D .Zając, J. Sołoducho, T. Jarosz, M. Lapkowski, S. Roszak, *Electrochim. Acta*, 173 (2015) 105-116.
18. K. Piontek, M. Antorini, T. Choinowski, *J. Biol. Chem*. 277 (2002) 37663-37669.
19. M.M. Bradford, *Anal. Biochem*. 72 (1976) 248–254.
20. I. Horcas, R. Fernandez, J.M. Gomez-Rodriguez, J. Colchero, J. Gomez–Herrero, A.M. Baro, *Rev. Sci. Instrum*. 78 (2007) 013705-1-013705-8.
21. L.B. Groenendaal, F. Jonas, D. Freitag, H. Pielartzik, J.R. Reynolds, *Adv. Mater*. 12(7) (2000) 481–494.
22. D. S. Uzun, A. N. Unlu, M. Sendur, E. F. Kanik, S. Timur, L. Toppare, *Colloid. Surf. B*, 112 (2013) 74-80.
23. B. A. Kuznetsov, G. P. Shumakovich, O. V. Koroleva, A. I. Yaropolov, *Biosens. Bioelectron*. 16 (2001) 73-84.
24. J. Rodakiewicz-Nowak, S. M. Kasture, B. Dudek, J. Haber, *J. Mol. Catal., B Enzym*. 11 (2000) 1-11.
25. S. Shleev, M. Pita, A.I. Yaropolov, T. Ruzgas, Lo Gorton, *Electroanal.* 18 (2006) 1901-1908.
26. D. Brodani, B. de Souza, B. S. Souza, A. Neves, I. C. Vieira, *Biosens. Bioelectron*. 42 (2013) 242-247.
27. A. Rekuć, J. Bryjak, K. Szymańska, A. B. Jarzębski, *Process Biochem*. 44 (2009) 191-198.
28. J. Qu, T. Lou, Y. Wang, Y. Dong, H. Xing, *Analyt. Lett*. 48 (2015) 1842-1853.
29. M. Guo, H. Wang, D. Huang, Z. Han, Q. Li, X. Wang, J. Chen, *Sci. Technol. Advan. Mat*. 15 (2014) 1-9.
30. J. Qu, Y. Wang, J. Guo, T. Lou, Y. Dong, *Analyt. Lett*. 47 (2014) 2537-2547.
31. V. Sethuraman, P. Muthuraja, M. Sethupathy, P. Manisankar, *Electroanal*. 26 (2014) 1958-1965.
32. M. Nazari, S. Kashanian, R. Rafipour, *Spectrochim. Acta A*, 145 (2015) 130-138.
33. H. Chen, S. Li, S. Wang, Y. Tan, J. Kan, *Int. J. Polym Mat. Polym. Biomat*. 62 (2013) 620-626.