Indian Journal of Biochemistry & Biophysics
Vol. 62, January 2025, pp. 46-53
DOI: 10.56042/ijbb.v62i1.15015

Fabrication of an efficient hydroquinone biosensor with laccase immobilized on multiwalled carbon nanotubes

Dimpal J Aghera, Keval Bhatt, Jinal B Thakkar & C Ratna Prabha*

Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara-390 002, Gujarat, India

Received 12 June 2024; *revised 24 October 2024*

Hydroquinone is a phenolic compound commonly found in the raw materials and finished products of various chemical industries. Its widespread usage carries significant risks to aquatic and human life and the environment as well. A laccase biosensor was developed here to determine hydroquinone concentration in water resources. In the present study, the screen-printed electrode of the biosensor was coated with a solution of polyvinyl alcohol containing carboxyl functionalised multiwalled carbon nanotubes (MWCNTs). Laccase extracted from the fungus *Trametes versicolor* was immobilised on MWCNTs using glutaraldehyde. This strategy showed excellent stability, sensitivity, and selectivity, yielding a quick response time of only 10 sec. The electrode surface at different stages of the preparation was characterized using scanning electron microscopy (SEM). The optimized linear range for the bioelectrode spans from 50 µM to 1100 µM. The detection limit achieved with the electrode is 5 µM to 1700 µM. The biosensor detected hydroquinone successfully in real samples, including tap water, sewage water, spiked tap water and spiked sewage water. Further, this biosensor while offering a cost-effective, portable device, establishes the efficiency of carboxyl-functionalised MWCNTs in polyvinyl alcohol as matrix for the design of a variety of biosensors.

Keywords: Cyclic voltammetry, Immobilization, Laccase assay, Multiwalled carbon nanotubes, Scanning electron microscopy, Screen printed electrode

Phenolic compounds are commonly found as raw materials and by-products in chemical industry[1]. They are broadly utilized in many industries like pharmaceuticals, pesticides, dyes, cosmetics, plastics, photography, rubber, paint and tanning[2]. Some of them are highly toxic and carcinogenic, non-degradable pollutants that can contaminate water resources[3].

One of the common phenolic compounds, hydroquinone (1,4-benzenediol) is commonly used in industries such as medicines, cosmetics and pesticides[4]. Hydroquinone is generated during the initial phases of phenol oxidation, leading to a significant acceleration in the toxicity of phenol-containing wastewater. Hydroquinone is highly toxic to all forms of life. Its lower degradability when compared with the original phenol contaminants poses a hazard to the environment even at meagre amounts[5]. Brief exposure to higher concentrations of hydroquinone can cause headache, fatigue, tachycardia, kidney damage and cancer[6]. Therefore, it is important to have a simple and highly efficient detection method for hydroquinone.

Traditionally phenols are detected by chromatographic techniques[7], while spectroscopy and flame ionization are used for detection[8]. Even though, these techniques provide accurate results, they are complex and laborious involving skilled technicians and high throughput instrumentation. Biosensors are associated with the benefits of easy usage, quick analysis, the possibility for onsite testing, high sensitivity and reasonable cost[9].

Laccase (Lac) is a polyphenol oxidase enzyme that contains multiple copper ions and utilizes molecular oxygen to efficiently oxidize different phenolic compounds through a four-electron reduction of oxygen (O_2) to water molecules[10,11]. This unique enzymatic capability of the enzyme allows laccase biosensors to directly detect the oxidation of hydroquinone to p-quinone without the need for solvents. Furthermore, laccase-based biosensors are employed to identify phenols in various contexts, including food analysis and environmental monitoring[12-14].

In recent years, biosensors have found number of applications[15,16]. Direct electron transfer (DET) of

*Correspondence:
Phone: +91-265-2795594 (Off); +91-93272 01349 (Mob)
E-mail: cratna.prabha-biochem@msubaroda.ac.in;
chivukula_r@yahoo.com

enzyme electrode chips is often employed in conjunction with optimal sensitivity, conductivity, wide potential range and greater surface area for ligand immobilization associated with carbon materials, particularly muti-walled carbon nanotubes (MWCNTs) [9,17-22].

As screen-printed electrodes (SPEs) are economical, they are frequently used for making analytical tools to monitor environmental pollution[23,24]. The application of nanomaterials over SPEs improves properties such as sensitivity, ligand loading capacity and response time[25,26].

In the present study, a novel screen-printed carbon electrode (SPCE) has been fabricated by depositing oxidized MWCNTs and a polymer mixture. Subsequently, the laccase enzyme from *Trametes versicolor* was bonded covalently to the film using the cross-linker glutaraldehyde. Laccase (Lac) acts on hydroquinone to produce 1,4-benzoquinone. As shown in Eqs. (1) and (2) protons participate in the reduction process of laccase and combine with oxygen to form water.

$$HQ + O_2 \xrightarrow{\text{Laccase}} 1,4\text{-benzoquinone} + 2H_2O \quad \ldots (1)$$

$$1,4\text{-benzoquinone} + 2e^- + 2H^+ \rightarrow HQ \quad \ldots (2)$$

Electrons generated in the reaction are detected by the electrode.

Materials and Methods

Reagents and Apparatus

Laccase from *Trametes versicolor*, (38429-1G) was purchased from Sigma Aldrich, India. MWCNTs were purchased from Ad nanotechnologies, India. Glutaraldehyde and polyvinyl alcohol (PVA) were bought from LOBA Chemie Pvt. Ltd., India. Na_2HPO_4 (sodium phosphate dibasic anhydrous), NaH_2PO_4 (sodium phosphate monobasic anhydrous), hydroquinone, BSA, KCl, potassium ferricyanide, sodium acetate and ABTS (2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) were purchased from SRL Pvt. Ltd., India. Screen-printed carbon electrodes (SPCE) were purchased from PalmSens BV, Netherlands. All reagents, except for multi-walled carbon nanotubes (MWCNTs), purchased were of extra pure grade and used without further purification. Autoclaved Milli-Q water was employed throughout the experimental procedures.

Cyclic voltammetry (CV) was carried out from -0.3 to +0.5 V at 0.1 V/s scan rate on screen printed carbon electrode in 50 mM $K_3[Fe(CN_6)]$ with 0.1 M KCl in 50 mM phosphate buffer, pH 5.5. EmStat3$^+$ electrochemical workstation (PalmSens BV, Netherlands) was used to carry out CV measurements. Locally manufactured ultrasonicator bath was used to sonicate MWCNTs in PVA polymer to prepare a suspension. FEG-SEM (Field emission gun scanning electron microscope) (JSM-7600F; Jeol, USA) was used to study the surface of the bare and coated electrodes.

Fabrication of SPCE/PVA/MWCNTs/GA/Laccase electrode

In this study, MWCNT based fabrication chemistry was utilized to prepare the electrode. Screen-printed carbon electrodes used to develop the electrochemical sensor contain working, reference, and counter electrodes, with all three on the same chip. Firstly, 1 mg of MWCNTs was suspended in 0.5 mL of PVA prepared at 1 mg/mL concentration. The suspension was sonicated to homogenize for 3 h in an ultrasonicator bath. Ice packs were kept in the ultrasonicator bath to keep the suspension cool. To prepare the electrode, 4 µL of the MWCNT solution in PVA was dropped on SPCE and air dried at room temperature (RT) for 20 min. Then, 25 µL of laccase (10 mg/mL) dissolved in pH 7.0, 50 mM phosphate buffer was layered on it. The laccase solution was allowed to air dry at RT for around 30 min. Afterwards, 2 µL of 2.5% glutaraldehyde was added on the electrode surface to cross link to PVA. The solution was permitted to air dry at room temperature and the electrode was washed three times with phosphate buffer to remove excessive glutaraldehyde and again allowed it to air dry. The procedure was adopted from our earlier work with a glucose biosensor[26]. This was followed by pre-treating it with 10 µL of 1% BSA for 10 min to block nonspecific binding[27]. The electrode was washed with Milli Q water several times. At a scan rate of 100 mV/s, the electrolyte solution of 50 mM $K_3[Fe(CN)_6]$ in 50 mM phosphate buffer (pH 7.0) was tested for change in the current in a 50 µL system. Then the electrode was washed with phosphate buffer, pH 7.0. The electrode was air-dried at room temperature and stored at 4°C. The electrodes with and without laccase enzyme were compared for performance by running cyclic voltammetry from -0.3 to 0.5 V in 50 µL of 0.1 M KCl and 50 mM $K_3[Fe(CN)_6]$ prepared in 50 mM phosphate buffer, pH 5.5.

Laacsase enzyme activity by ABTS Method

Laccase activity was monitored using the non-phenolic dye 2,2'-azino-bis (3-ethylbenzothiazoline-

6-sulphonic acid) also known as ABTS as the substrate[28]. Laccase oxidizes ABTS to a stable cation radical of blueish green colour. The intensity of the colour monitored at 420 nm, is proportional to the enzyme activity[29]. The reaction mixture used in the assay contained 0.5 mM ABTS, 0.1 M sodium acetate buffer (pH 4.5) and 0.1 mL of laccase in a final volume of 3.0 mL. It was incubated for 5 min. Absorbance of the sample was recorded at 420 nm against a suitable blank.

The temperature stabilities of the free and immobilized enzymes were determined by monitoring their activities between 30°C and 80°C, at 10° increments in temperature, by incubating them for 30 min. at each temperature. Following incubation, the ABTS assay was performed as described above and the following equation was used to calculate the enzyme activity.

$$\text{Enzyme activity (Units/mL)} = \frac{Ab \times V \times 10^6}{T \times \varepsilon \times d}$$

where in, enzyme activity given in Units/mL is the amount of enzyme that oxidizes 1 µmol of ABTS per min. Ab is the absorbance per minute, V is the total volume of enzyme assay in mL, 10^6 is the conversion factor for M to µM, T is the incubation time, ε is the molar absorption coefficient of ABTS (36,000 M^{-1} cm^{-1} at 420 nm), d is the path length of the solution in cm.

ABTS assay was used to determine whether or not the crosslinking agent glutaraldehyde has any effect on the active site of the enzyme. The activities of the free enzyme and glutaraldehyde treated enzyme were calculated using the equation given above. Surface retention of immobilized enzyme on the electrode was found out by performing ABTS enzyme assay of washout solution of immobilized enzyme from SPCE after washing with milli Q water and comparing its activity with the amount of enzyme used for immobilization.

Results and Discussion

Surface morphology studies

Scanning electron microscopy was used to study the changes in the morphological features of the electrode with step-wise layering method adopted in the preparation. Changes in the electrode morphology at every step are evident from the scanning electron micrographs of (Fig. 1). The electron micrographs given in (Fig. 1A-C) clearly display distinct changes in the surface of the bioelectrode during every stage of its development. In the first step, the bare electrode in (Fig. 1A), was layered with the dispersion of MWCNTs. The MWCNTs displayed a characteristic filamentous appearance as observed in (Fig. 1B). Immobilization of laccase using GA revealed a porous structure formed by the deposition of bulky macromolecules on the electrode surface (Fig. 1C).

Electrochemical behaviour of SPCE/PVA/MWCNTs/ Glutaraldehyde/Laccase electrode

The results of the cyclic voltammetry carried out on bare SPCE and SPCE/PVA/MWCNTs/ Glutaraldehyde/Laccase in 50 mM $K_3[Fe(CN)_6]$, 0.1 M KCl in 50 mM phosphate buffer (pH 7.0) at 100 mV/s scan rate are shown as voltammograms (CVs in) (Fig. 2A). SPCE showed a stronger signal and the current decreased after layering with PVA/ MWCNTs/Glutaraldehyde/Laccase mixture. It is well known that MWCNTs are good for the transportation of electrons but laccase, greatly hindered the electron mobility between electrolyte and electrode. The coated electrode SPCE/PVA/MWCNTs/ Glutaraldehyde/Laccase was then tested by varying scan rates between from 80 to 160 mV/s and the results are shown in (Fig. 2B). The resultant voltammograms showed sharp redox peaks at all the scan rates tested. The values of anodic and cathodic peaks varied linearly with the scan rates. However, as the scan rate increased anodic peaks moved towards

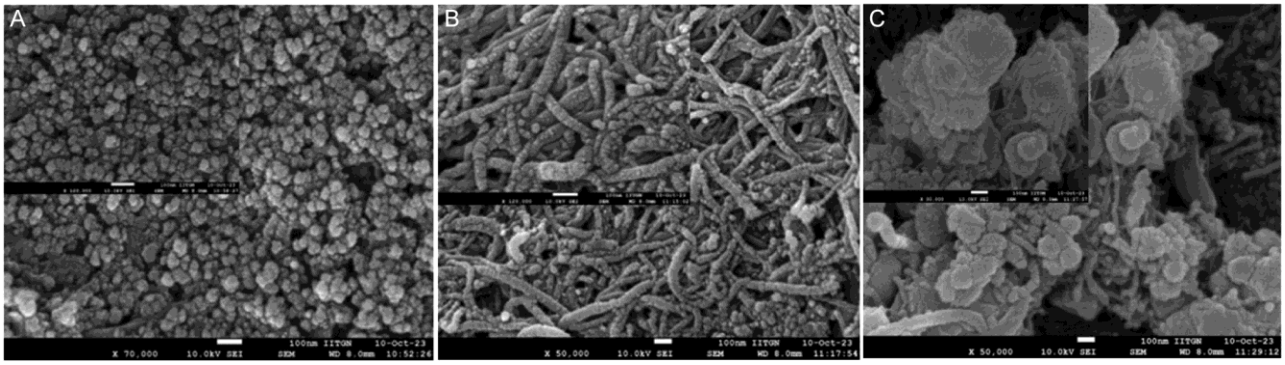

Fig. 1 — SEM images of (A) SPCE, (B) SPCE/MWCNTs, and (C) SPCE/MWCNTs/GA/Laccase

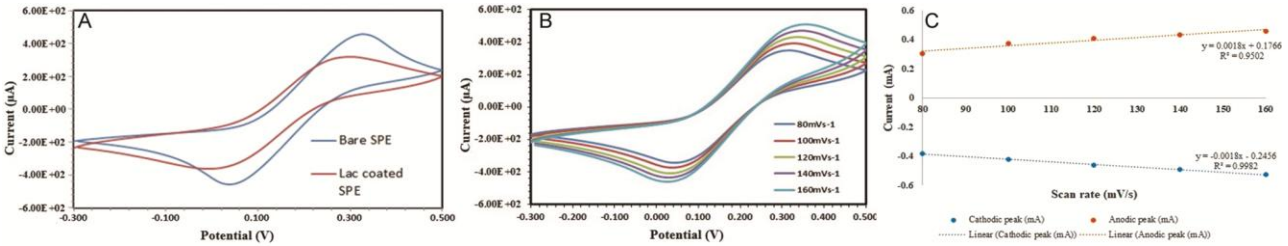

Fig. 2 — Cyclic voltammetry profiles of (A) Bare SPCE and SPCE/MWCNTs/GA/Laccase coated electrode in 50 mM phosphate buffer, pH 7.0 containing 50 mM $K_3[Fe(CN)_6]$ and 0.1 M KCl at 100 mV/s scan rate; (B) SPCE/MWCNTs/GA/Laccase coated electrode at different scan rates from 80 mV/s to 160 mV/s in the same buffer as (A); and (C) Plots of the corresponding anodic and cathodic peak currents *vs* scan rates

higher positive potential and cathodic peaks moved towards higher negative potentials. Figure 2C displays a linear graph of scan rate against the anodic and cathodic peaks. The R^2 values of anodic and cathodic peaks were determined and found to be 0.95 and 0.99, respectively. Thus, cathodic peak showing better correlation was considered for further experimentation to check hydroquinone concentration. Moreover, the ratio of cathodic and anodic peak currents (Ipc/Ipa) approximated to 1. These results are in agreement with the observation that the electron transfer followed a surface-controlled process.

Effect of pH on SPCE/PVA/MWCNTs/Glutaraldehyde/ Laccase

Cyclic voltammetry was carried out at a scan rate of 100 mV/s to examine the effect of pH on the laccase immobilised SPCE in a solution of 50 mM $K_3[Fe(CN)_6]$ and 0.1 M KCl in 50 mM phosphate buffer solution in the pH range of 5.0 to 8.0 (Fig. 3A). The concentration of hydroquinone was 1 mM in the experimental solution. The anodic and cathodic peak currents reduced when the pH rose from 5.0 to 8.0, which can be explained by a decrease in the number of positively charged moieties in the electrolyte.

Laccase immobilized electrode participates in the reduction of O_2 molecule to two water molecules. Also, it is evident from the graph (Fig. 3B) that change in the current with respect to change in the pH follows linearity at cathodic peak with an R^2 value of 0.9759. Hence, cathodic peak was considered as the measure of magnitude of the current at pH 5.5 and was considered as optimal value for all further experiments.

Reproducibility and storage of the electrode

Three screen printed electrodes were coated simultaneously to obtain SPCE/PVA/MWCNT/ GA/Laccase. Current response of all the three electrodes was measured in 50 mM phosphate buffer (0.1 M KCl, 50 mM $K_3[Fe(CN)_6]$; pH 5.5). Margin of

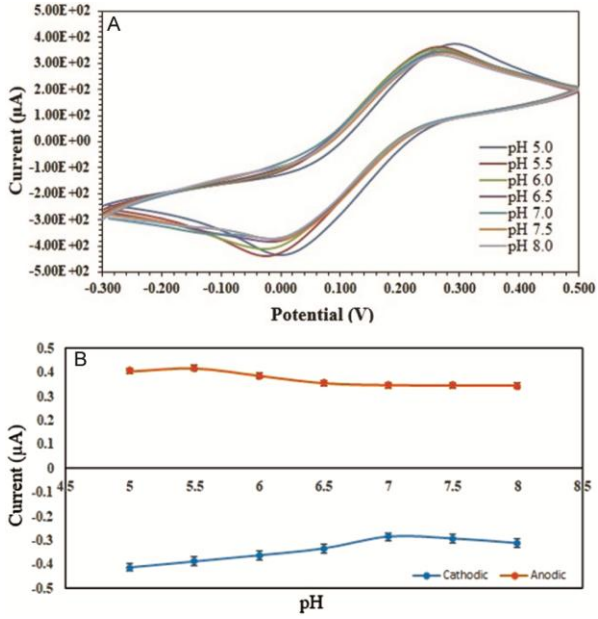

Fig. 3 — (A) Cyclic voltammetry profile of SPCE/MWCNTs/GA/Laccase coated electrode recorded as a function of pH in 50 mM PB (50 mM $K_3[Fe(CN)_6]$) and 0.1 M KCl at 100 mV/s scan rate. (B) Plot showing the effect of pH on the current density of both the peaks of SPCE/ PVA/MWCNT/GA/Laccase electrode in 50 mM PB (50 mM $K_3[Fe(CN)_6]$) and 0.1 M KCl containing 1000 µM hydroquinone at 100 mV/s scan rate

error for the sensor replication procedure was calculated by comparing both anodic and cathodic peak responses. Figure 4 (A) proves that the relative standard deviation (RSD) of cathodic peak is 0.56% and is reproducible. The sensor electrode's enzyme activity was measured every six days while it was kept dry at 4°C. Current response of the sensor was 85.89% compared to its original current response even after the lengthy storage of 30 days (Fig. 4B).

Cyclic voltammetric detection of hydroquinone

Cyclic voltammetric response at cathodic sweep segment of the SPCE/PVA/MWCNTs/GA/Laccase biosensor as a function of hydroquinone (HQ)

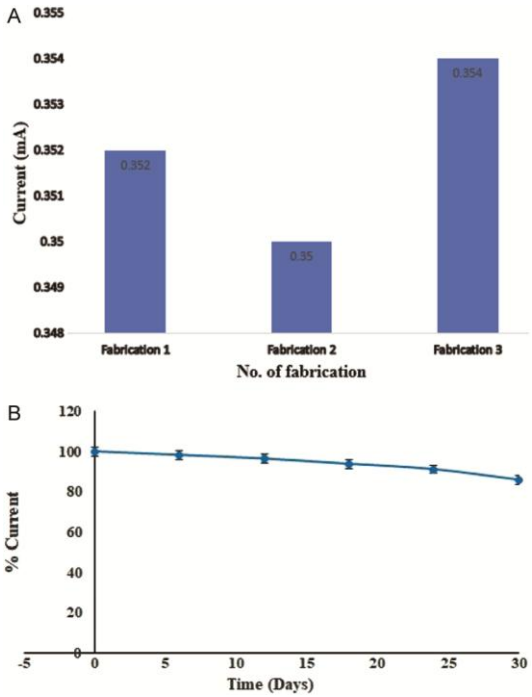

Fig. 4 — (A) Cathodic peak currents of SPCE/PVA/MWCNT/GA/Laccase electrode in triplicates in 50 mM PB (50 mM $K_3[Fe(CN)_6]$, 0.1 M KCl, pH 5.5) at 100 mV/s scan rate (n=3); and (B) Storage stability of electrode at 6 days of interval

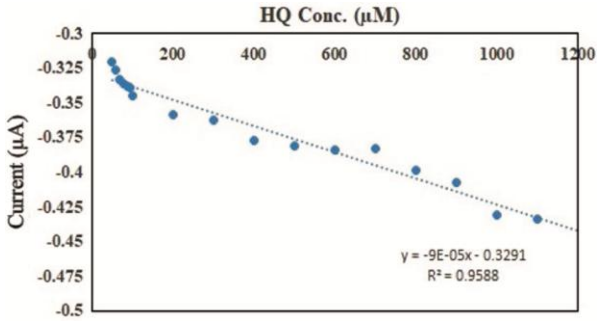

Fig. 5 — Calibration curve of the current Vs the concentration of hydroquinone in 50 mM PB (50 mM $K_3[Fe(CN)_6]$) and 0.1 M KCl containing at 100 mV/s scan rate

concentration was recorded in 50 mM $K_3[Fe(CN)_6]$, 0.1 M KCl in 50 mM phosphate buffer (pH 5.5) at the scan rate of 100 mV/s. It is evident from (Fig. 5) that as the concentration of HQ increases, cathodic peak current increases. This increase in magnitude of the current can be explained by the pH sensitive behaviour of the biosensor. Protons were released during the enzymatic reaction by laccase, which in turn altered the pH of the reaction system. This change in pH ultimately led to change in magnitude of the current. As seen in Figure 5, change in the cathodic peak current magnitude with respect to the HQ concentration followed linear regression model

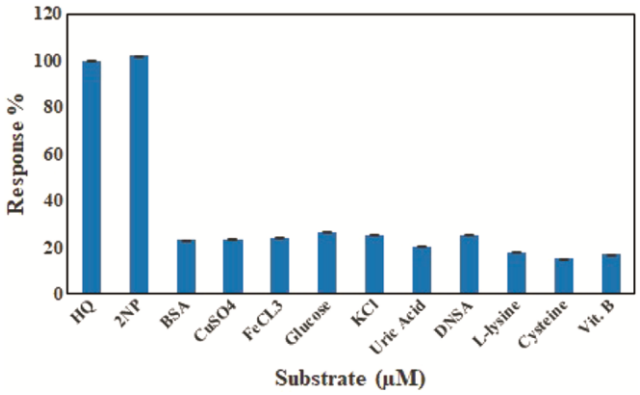

Fig. 6 — Comparison of SPCE/MWCNTs/GA /Laccase in 50 mM PB (50 mM $K_3[Fe(CN)_6]$) and 0.1 M KCl containing different interferent at 100 mV/s scan rate (n=3)

with the R^2 value of 0.974. The lower and higher detection limits of HQ concentration were found to be 50 and 1100 µM, respectively.

Interference study

Selectivity of a sensor is critical when it comes to practical application. To estimate the anti-interference of the biosensor, the impact of some possible interfering substances was examined under ideal conditions. Herein, the substances checked for interference were phenolic compounds (2-Nitro phenol), protein (BSA-bovine serum albumin), small molecules (glucose, cysteine,and L- lysine), $CuSO_4$ (copper sulphate), KCl (potassium chloride),DNSA (3,5- Dinitrosalicyclic acid), Uric Acid. Current response examined by the biosensor in solution with the substrate and the interferents in the ratio of 1:1. (Fig. 6). The current either became low or negligible with the addition of every interferent used in the study.

Comparison of temperature stability of free and immobilized enzyme

An important consideration pertaining to the activity of enzymes in a variety of industrial applications is their stability at high temperatures. Enzymes can be grouped according to their thermal stability based on their origin and structural features including post-translational modifications and the processes like immobilization that provide resistance to heat-induced denaturation. Here, 40°C is the temperature at which both free and immobilized enzymes show their highest levels of activity. Additionally, as seen in (Fig. 7), enzyme activity falls marginally up to 70°C but sharply beyond that point. However, when it comes to temperature stability, immobilized enzyme performs better than free enzyme.

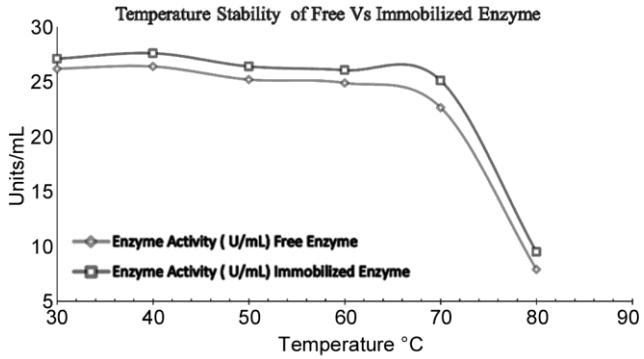

Fig. 7 — Temperature Stability of free *vs* Immobilized Enzyme

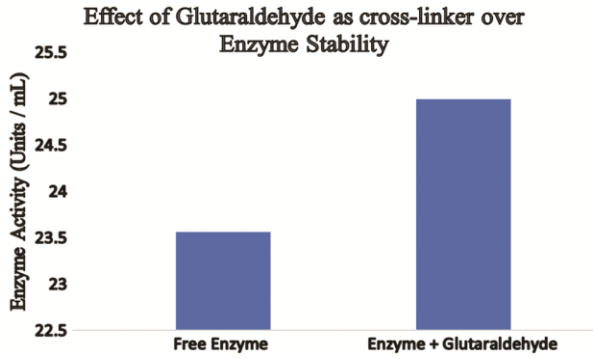

Fig. 8 — Effect of Glutaraldehyde on Enzyme Stability

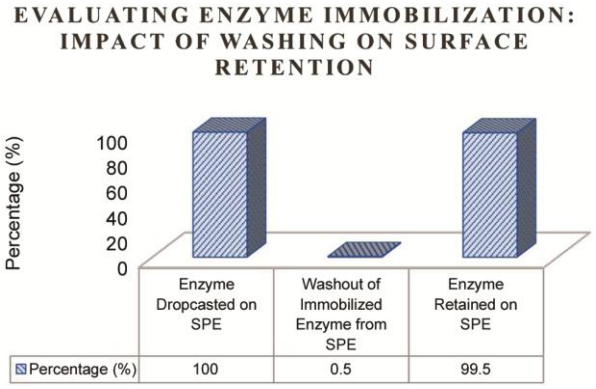

Fig. 9 — Surface Retention of Enzyme for electrode after washed with water

Effect of Glutaraldehyde on enzyme stability

In the fabrication of biosensors, glutaraldehyde is frequently used, especially for forming cross-linked enzyme aggregates (CLEAs). Under operating conditions, the three-dimensional structure of enzymes is stabilized by the crosslinking action of glutaraldehyde, which protects them from undergoing denaturation. To prevent leaching out and to sustain enzyme activity over long stretches of time and several reaction cycles, immobilization by glutaraldehyde is essential. Due to possible structural distortions, glutaraldehyde often reduces enzyme activity at high concentrations; but, at optimally low concentrations, glutaraldehyde can boost stability and sustain activity. Since the concentration of glutaraldehyde used is low, the overall stability of the enzyme is increased and enzyme activity of laccase is not hampered, as seen in the (Fig. 8).

The amount of enzyme retained on the surface of the electrode after immobilization

Economically speaking, immobilized enzymes are preferable since they can be used repeatedly without experiencing a major loss of activity. Because a single batch of enzyme can catalyse the reaction many times, electrode's reusability not only reduces operating costs but also increases total productivity. Therefore, it is essential to assess the stability of the enzyme by determining how strongly it is immobilized on the electrode, and retained even after repeated washings. According to (Fig. 9), it has been determined that the enzyme is retained on the electrode surface after being washed with milli Q water several times.

Real sample detection

To check the performance of the biosensor SPCE/MWCNTs/Laccase/GA with real samples, the electrode was checked against sewage water, sewage water spiked with HQ, tap water, tap water spiked with 1 mM HQ dissolved in a solution of 50 mM PB ($K_3[Fe(CN)_6]$, 0.1 M KCl, pH 5.5). The scan rate for the samples was 100 mV/s. The results showed reliable current change at cathodic sweep segment of cyclic voltammogram. Recovery of the real sample was 0.989 mM, 0.020 mM and 1.015 mM for the sewage water, sewage water spiked with HQ, tap water and tap water spiked with HQ, respectively.

Comparison of the present laccase-based biosensor with other previously reported laccase-based biosensors

Even though, there are several papers published on laccase based sensors they are used for varied purposes[4,18,30-33]. A comparison of the laccase based sensor designed here with the other sensors described in literature shows that the present one has a broader range and a comparable and functional detection limit for the detection of hydroquinone.

The SPCE/MWCNTs/GA/Laccase electrode represents a significant advancement in electro-chemical sensing technology, offering several distinct advantages over existing electrode strategies. With a wide linear range spanning from 50 to 1100 µM and a low detection limit of 5 µM, this electrode demonstrates exceptional sensitivity and precision in detecting analytes across various concentration levels.

The incorporation of multi-walled carbon nanotubes (MWCNTs) and polyvinyl alcohol (PVA) into the electrode structure enhances its stability, ensuring prolonged shelflife and consistent performance over time.

Moreover, the use of laccase and glutaraldehyde (GA) in the electrode composition suggests potential biocompatibility, rendering it suitable for applications involving biological samples or environments. The synergistic effects of MWCNTs, GA, and PVA contribute to improved sensitivity, enabling the detection of even lower concentrations with high accuracy. Additionally, the ease of fabrication and versatility of the electrode make it suitable for a wide range of electrochemical sensing applications, including environmental monitoring, biomedical diagnostics, and industrial process control. Overall, the SPCE/MWCNTs/GA/Laccase electrode represents a promising platform for advancing research in electrochemical sensing and detection.

Conclusion

In summary, SPCE/MWCNTs/GA/Laccase biosensor was successfully fabricated approaching a novel chemistry for fabrication. Enzyme laccase was immobilized on the MWCNTs with glutaraldehyde covalent crosslinking. The electrode showed the linearity for hydroquinone (HQ) from 50 to 1100 µM with the detection limit of 5 to 1700 µM. The electrode was checked for its specificity in the presence of various interfering molecules. The biosensor showed good specificity for hydroquinone. The biosensor was checked against real samples for its practical application. The biosensor can be replicated with the SD of 0.56%. The biosensor is expected to serve as promising tool to detect hydroquinone. It will be interesting to immobilize other enzymes to develop biosensors for a variety of molecules with the same fabrication chemistry.

References

1 Lou C, Jing T, Zhou J, Tian J, Zheng Y, Wang C, Zhao Z, Lin J, Liu H, Zhao C & Guo Z, Laccase immobilized polyaniline/magnetic graphene composite electrode for detecting hydroquinone. *Int J Biol Macromol*, 149 (2020) 1130.

2 Wang HF, Wu YY & Yan XP, Conjugation of glucose oxidase onto Mn-Doped ZnS quantum dots for phosphorescent sensing of glucose in biological Fluids. *Anal Chem*, 85 (2013) 1920.

3 Abosadeh DJ, Kashanian S, Nazari M & Parnianchi F, Fabrication of a Novel Phenolic Compound Biosensor Using Laccase Enzyme and Metal-organic Coordination Polymers. *Anal Bioanal Chem Res*, 8 (2021) 467.

4 Tang W, Zhang M, Li W & Zeng X, An electrochemical sensor based on polyaniline for monitoring hydroquinone and its damage on DNA. *Talanta*, 127 (2014) 262.

5 Enguita FJ & Leitão AL, Hydroquinone: Environmental Pollution, Toxicity, and Microbial Answers. *BioMed Res Int*, Article ID 542168, (2013) 1.

6 Zhao G, Li M, Hu Z, Li H & Cao TJ, Electrocatalytic redox of hydroquinone by two forms of L-proline. *Mol Catal A: Chemical*, 255 (2006) 86.

7 Clement RE, Eiceman GA & Koester CJ, Environmental analysis. *Anal Chem*, 67 (1995) 221R.

8 Robbins RJ, Phenolic acids in foods: An overview of analytical methodology. *J Agric Food Chem*, 51 (2003) 2866.

9 Gupta S, Murthy CN & Prabha CR, Recent advances in carbon nanotube based electrochemical biosensors. *IntJ Biol Macromol*, 108 (2018) 687.

10 Claus H, Laccases: structure, reactions, distribution. *Micron*, 35 (2004) 93.

11 Barton SC, Kim HH, Binyamin G, Zhang YC & Heller A, Electroreduction of O_2 to water on the ''wired'' laccase cathode. *J Phys Chem B*, 105 (2001) 11917.

12 Gamella M, Campuzano S, Reviejo AJ & Pingarrón JM, Electrochemical Estimation of the Polyphenol Index in Wines Using a Laccase Biosensor. *J Agric Food Chem*, 54 (2006) 7960.

13 Torrecilla JS, Mena ML, Yáñez-Sedeño P & García J, Quantification of phenolic compounds in olive oil mill wastewater by artificial neural network/laccase biosensor. *J Agric Food Chem*, 55 (2007) 7418.

14 Quan D & Shin W, Amperometric Detection of catechol and catecholamines by immobilized laccase from DeniLite. *Electroanalysis*, 16 (2004) 1576.

15 Wulandari A, Sunarti TC, Fahma F, Noor E & Enomae T, Encapsulation of purple sweet potato's anthocyanin in CMC-PVA matrix for development of paper strips as a colorimetric biosensor. *Indian J Biochem Biophys*, 58 (2021) 292.

16 Mehta S, Bakshi S, Choudhury S, Bose S & Nayak R, Hierarchical gold nanostructures based sensor for sensitive and fast detection of cancer biomarker. *Indian J Biochem Biophys*, 58 (2021) 136.

17 Lamas-Ardisana PJ, Queipo P, Fanjul-Bolado P & Costa-García A, Multiwalled carbon nanotube modified screen-printed electrodes for the detection of p-aminophenol: Optimisation and application in alkaline phosphatase-based assays. *Anal Chim Acta*, 615 (2008) 30.

18 Yang J, Li D, Fu J, Huang F & Wei Q, TiO_2-CuCNFs based laccase biosensor for enhanced electrocatalysis in hydroquinone detection. *J Electroanal Chem*, 766 (2016) 16.

19 Das P, Das M, Chinnadayyala SR, Singha IM & Goswami P, Recent advances on developing 3rd generation enzyme electrode for biosensor applications. *Biosens Bioelectron*, 79 (2016) 386.

20 Qu J, Lou T, Kang S & Du X, Simultaneous determination of catechol and hydroquinone using a self-assembled laccase biosensor based on nanofilm. *Sens Lett*, 11 (2013) 1567.

21 Thakkar JB, Gupta S & Prabha CR, Acetyl choline esterase doped multiwalled carbon nanotubes for the detection of organophosphorus pesticide using cyclic voltammetry. *Int J Biol Macromol*, 137 (2019) 895.

22 Thakkar JB, Aghera DJ, Trivedi B & Prabha CR, Design and characterization of a biosensor with lipase immobilized nanoparticles in polymer film for the detection of triglycerides. *Int J Biol Macromol*, 229 (2023) 136.

23 Verrastro M, Cicco N, Crispo F, Morone A, Dinescu M, Dumitru M, Favati F & Centonze D, Amperometric biosensor based on laccase immobilized onto a screen-printed electrode by matrix assisted pulsed laser evaporation. *Talanta*, 154 (2016) 438.

24 Mohtar L, Aranda P, Messina GA, Nazareno MA, Pereira SV, Raba J & Bertolino FA, Amperometric biosensor based on laccase immobilized onto a nanostructured screen-printed electrode for determination of polyphenols in propolis. *Microchem J*, 144 (2018) 13.

25 Taleat Z, Khoshroo A & Mazloum-Ardakani M, Screen-printed electrodes for biosensing: a review (2008–2013). *Microchim Acta*, 181 (2014) 865.

26 Gupta S, Prabha CR & Murthy CN, Functionalized multi-walled carbon nanotubes/polyvinyl alcohol membrane coated glassy carbon electrode for efficient enzyme immobilization and glucose sensing. *J Environ Chem Eng*, 4 (2016) 3734.

27 Ishikawa FN, Chang HK, Curreli M, Liao HI, Olson CA, Chen PC, Zhang R, Roberts RW, Sun R, Cote RJ, Thompson E & Zhou C, Label-Free, Electrical detection of the SARS virus N-Protein with nanowire biosensors utilizing antibody mimics as capture probes. *ACS Nano*, 3 (2009) 1219

28 Bourbonnais R, Leech D & Paice MG, Lignin-derived compounds as efficient laccase mediators for decolorization of different types of recalcitrant dyes. *Biochim Biophys Acta*, 1379 (1998) 381.

29 Majcherczyk A, Johannes C & Huttermann A, Oxidation of polycyclic aromatic hydrocarbons (PAH) by laccase of Trametes versicolor. *Enzyme Microb Technol*, 22 (1998) 335.

30 Cevher SC, Bekmezci SA, Soylemez S, Udum YA, Toppare L & Çırpan A, Indenoquinoxalinone based conjugated polymer substrate for laccase biosensor. *Mater Chem Phys*, 257 (2021) 123788.

31 Wang Y, Zhai F, Hasebe Y, Jia H & Zhang Z, A highly sensitive electrochemical biosensor for phenol derivatives using a graphene oxide-modified tyrosinase electrode. *Bioelectrochem*, 122 (2018) 174.

32 Li D, Yang J, Zhou J, Wei Q & Huang F, Direct electrochemistry of laccase and a hydroquinone biosensing application employing ZnO loaded carbon nanofibers. *RSC Adv*, 4 (2014) 61831.

33 Zhang Y, Lv Z, Zhou J, Fang Y, Wu H, Xin F, Zhang W, Ma J, Xu N, He A, Dong W & Jiang M, Amperometric Biosensors Based on Recombinant Bacterial Laccase CotA for Hydroquinone Determination. *Electroanalysis*, 32 (2020) 142.