Sains Malaysiana 54(10)(2025): 2445-2454
http://doi.org/10.17576/jsm-2025-5410-09

Fabrication of a Laccase-Immobilised Biosensor Based on Carboxylated Multi-Walled Carbon Nanotubes for Sensitive Tyramine Detection

(Pembuatan Biosensor Terpegun Lakase Berdasarkan Tiub Nano Karbon Berbilang Dinding Berkarboksilasi untuk Pengesanan Tyramine Sensitif)

NURUL SYAZWANI KIZZON[1] SYAZA AZHARI[1,*] NURUL HANA MAS'OD & NADRAHTUL HUDA MISRAL[2]

[1]*Faculty of Science and Technology, Universiti Sains Islam Malaysia, Bandar Baru Nilai, 71800 Nilai, Negeri Sembilan, Malaysia*
[2]*Pusat Tamhidi, Universiti Sains Islam Malaysia, Bandar Baru Nilai, 71800 Nilai, Negeri Sembilan, Malaysia*

Received: 2 May 2025/Accepted: 5 August 2025

ABSTRACT

Tyramine is a molecular substance found in foods that can adversely affect consumers due to its toxicity, posing risks to both human health and food quality. Therefore, it is crucial to monitor the excessive concentration of tyramine in meals. Biosensors have attracted considerable attention from researchers due to their benefits, including portability, ease of use, and high specificity. This study aimed to develop a biosensor by specifically modifying multi-walled carbon nanotubes (MWCNTs) with a carboxyl group (-COOH) to immobilise laccase (Lac) enzyme molecules, thereby enhancing the biosensor's characteristics, including faster response times, a wider detection range, and higher sensitivity. The modified screen-printed carbon electrode (SPCE) was characterised using differential pulse voltammetry (DPV), Field Emission Scanning Electron Microscopy (FESEM), and Fourier Transform Infrared Spectroscopy (FTIR). The optimised parameters of the Lac-MWCNT-COOH SPCE biosensor exhibited excellent performance at pH 7 in phosphate buffer solution, within the tested pH range of 5.0-9.0, with 3 µL of laccase enzyme (range 1-5 µL), at 0.2 V deposition potential (range 0.1-0.5 V), and a deposition time of 5 s (range 3-7 s). The SPCE modified was successfully fabricated for tyramine determination, achieving a limit of detection (LOD) of 0.09 mM.

Keywords: Biogenic amine; biosensor; multi-walled carbon nanotubes; tyramine

ABSTRAK

Tyramine adalah sebatian molekul yang terdapat dalam makanan dan boleh memberi kesan buruk kepada pengguna kerana ketoksikannya, sekali gus memberi kesan negatif kepada kesihatan manusia dan kualiti makanan. Oleh itu, adalah penting untuk memantau kepekatan kandungan tyramine yang berlebihan dalam makanan. Penggunaan biosensor telah menarik minat penyelidik kerana kelebihan seperti kebolehgunaan, kemudahan penggunaan dan keupayaan khusus yang tinggi. Kajian ini bertujuan untuk membangunkan biosensor dengan mengubah suai tiub nano karbon berdinding pelbagai (MWCNT) khusus dengan kumpulan karboksil (-COOH) untuk memegun molekul enzim lakase, sekali gus meningkatkan ciri biosensor seperti masa tindak balas yang lebih cepat, julat pengesanan yang lebih luas dan kepekaan yang lebih tinggi. Ciri dan sifat elektrokimia elektrod karbon cetak skrin (SPCE) yang telah diubah suai telah dikaji menggunakan Mikroskopi Elektron Pengimbasan Pancaran Medan (FESEM), Spektroskopi Inframerah Transformasi Fourier (FTIR), voltametri denyut kebezaan (DPV) dan voltametri kitaran (CV). Parameter optimum untuk biosensor Lac-MWCNT-COOH SPCE menunjukkan prestasi yang baik pada pH 7 dalam larutan penampan fosfat (julat 5.0-9.0), dengan 3 µL enzim lakase (julat 1-5 µL), potensi pemendapan 0.2 V (julat 0.1-0.5 V) dan masa pemendapan 5 saat (julat 3-7 saat). Elektrod SPCE yang diubah suai telah berjaya dibangunkan dan direka untuk pengesanan tyramine, menunjukkan had pengesanan (LOD) serendah 0.09 mM.

Kata kunci: Amina biogen; biosensor; tiub nano karbon berdinding pelbagai; tyramine

INTRODUCTION

Food safety has become increasingly important due to the rise in food poisoning cases, some of which have led to serious health issues and even death. Biogenic amines such as tyramine, cadaverine, putrescine, and histamine are commonly found in foods like cheese, fish, wine, meat, beer, and vegetables. These compounds, when present at high levels, can have toxicological effects on the human body, leading to symptoms like palpitations, headaches, diarrhea, and vomiting (Özogul, Kuley & Kenar 2011). As a result, the BA content in food is considered a crucial indicator of food quality and safety, necessitating

constant monitoring to ensure consumer protection. Tyramine is a notable biogenic amine produced through the decarboxylation of the amino acid tyrosine in animals, plants, and microorganisms. Tyramine is known to trigger the secretion of noradrenaline by the sympathetic nervous system, which may contribute to migraines, headaches, and elevated blood pressure (Hungerford 2010). Studies have shown that tyramine concentrations are particularly high in meat and animal-derived products (Salcedo-Sandoval et al. 2015). Conventional techniques for detecting biogenic amines, including gas chromatography (GC), capillary electrophoresis (CE), high-performance liquid chromatography (HPLC), and thin-layer chromatography (TLC), have been extensively utilised, though each comes with specific limitations. These methods often require expensive equipment, skilled personnel, and extensive sample preparation. In the past few years, biosensors have emerged as promising alternatives for detecting biogenic amines due to their sensitivity, durability, and cost-effectiveness. Biosensors operate by using biological recognition elements, such as enzymes, to detect specific analytes, with the resulting signals processed and converted into digital data for analysis. In this study, a biosensor was fabricated using multi-walled carbon nanotubes (MWCNT) modified with a laccase enzyme to detect tyramine levels. Laccase, an enzyme commonly present in fungi, plants, insects, and bacteria, is renowned for its high efficiency and is extensively utilised in various industrial applications (Datta et al. 2021). The biosensor's design is expected to provide an effective means of detecting tyramine, thereby contributing to better food safety monitoring. This study will investigate the electrochemical behavior, optimisation, and quantification of the pH and laccase enzyme levels in the biosensor, comparing the tyramine levels in chicken samples.

MATERIALS AND METHODS

CHEMICALS

The chemicals employed in this study included laccase enzyme (in powder form), tyramine, 1-ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride (EDC), sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), sodium hydroxide (NaOH), potassium chloride (KCl), hydrochloric acid (HCl) of 37% concentrated HCl and n-hydroxysuccinimide (NHS), which had been purchased from R&M Chemicals. The multi-walled carbon nanotube screen-printed carbon electrode (MWCNT-SPCE) was obtained from Biogenes Technologies Sdn. Bhd. The electrode configuration comprised a carbon auxiliary electrode, a carbon working electrode, and a silver (Ag) reference electrode.

PHYSICAL MEASUREMENTS

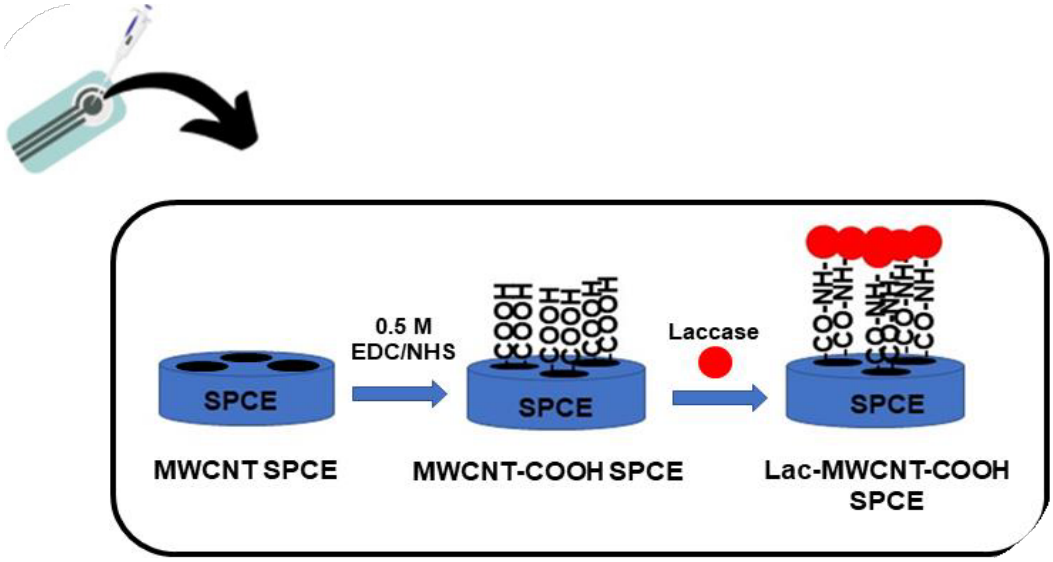
Prior to use, the weight balance and pH meter (Mettler Toledo) were calibrated using buffer solutions at pH values of 5, 6, 7, 8, and 9 to achieve the accurate pH measurements. Electrochemical analysis was performed using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) with a potentiostat (Autolab-PGSTAT) connected to an electrochemical system (NOVA 2.1.4) and linked to a computer for data acquisition.

SOLUTION PREPARATION

A 0.01 M standard solution of tyramine was formulated by dissolving 0.05 g of tyramine powder in a 20 mL volumetric flask and diluting it to the calibration mark with phosphate buffer solution (PBS) at pH 7.0. The flask containing the tyramine solution was wrapped in aluminum foil to protect it from light and stored at 4 °C. For the preparation of PBS, 3.45 g of sodium phosphate monobasic (NaH_2PO_4) and 20.10 g of sodium phosphate dibasic (Na_2HPO_4) were dissolved in a 1000 mL volumetric flask, and the solution was diluted to achieve the target concentration using distilled water. A 0.05 M PBS solution was made by diluting 50 mL of 0.1 M PBS in a 100 mL volumetric flask to achieve the required concentration. Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were utilised for pH adjustment as required. To prepare the 0.1 M NaOH solution, 1.0 g of sodium hydroxide was dissolved in distilled water and diluted to the final volume in a 250 mL volumetric flask. Likewise, 20.5 mL of 37% concentrated hydrochloric acid (HCl) was diluted with distilled water in a separate 250 mL volumetric flask to obtain a 0.1 M HCl solution. PBS 0.05 M at pH 7 was used to dilute 0.05 g of laccase powder to 1 mL of volume. Since the enzyme must be newly generated, a stock solution of 50 mg mL^{-1} (25-unit activity) of the enzyme laccase had been prepared by adding 10 mL of enzyme to a polypropylene microcentrifuge tube and freezing it until the experiment was ready to run.

ELECTRODE MODIFICATION

The laccase enzymes were immobilised onto the modified SPCE using EDC/NHS chemistry. To prepare the modification solution, 0.005 g each of EDC and NHS were dissolved in a 1 mL polypropylene microcentrifuge tube, followed by the addition of 0.05 M PBS (pH 7) up to the final volume. The freshly prepared solution was used on the same day to maintain maximum enzyme activity and was not stored. Any excess solution was removed using tissue before enzyme application. For electrode modification, 10 µL of the 0.5 M EDC/NHS solution was dropped onto the dried MWCNT-SPCE surface. After a 1h reaction at room temperature, 2 µL of the laccase enzyme solution was applied to the MWCNT-COOH SPCE. Subsequently, the electrode was stored at +4 °C for 15 min before being rinsed with PBS to eliminate unbound enzymes. The final biosensor was designated as Lac-MWCNT-COOH SPCE, with the modification steps depicted in Scheme 1.



SCHEME 1. Schematic representation of the reaction involving the laccase enzyme on a multi-walled carbon nanotube carboxyl-functionalized (MWCNT-COOH) modified electrode for the detection of tyramine

ANALYTICAL TECHNIQUES FOR ELECTROCHEMICAL STUDIES

A 10 mL aliquot of buffer solution was placed in the voltammetry cell, and the appropriate volume of analyte solution was added using a micropipette. To remove dissolved oxygen, the solution was purged with nitrogen gas while stirring at 300 rpm for a duration of 3 min. The experiment began by scanning the applied potential in the appropriate direction. The cyclic voltammetry (CV) experiment was conducted in a cathodic direction for the preliminary investigation of the newly designed electrodes with the analyte. The potential (E) was set at 0.5 V, the scan rate (V) at 500 mV/s, with the initial potential (E_i) at -200 mV and the final potential (E_f) at -200 mV. For the differential pulse voltammetry (DPV) technique, a 10 mL aliquot of buffer solution was placed in the voltammetry cell, and the required analyte solution was added using a micropipette. The solution was purged with nitrogen gas at 300 rpm for 3 min to remove dissolved oxygen. The experiment commenced with a scan of the applied voltage, using a 5 mV step, a 0.005 V step increment, a 0.06 V amplitude, and a 0.5 V interval duration, covering a voltage range from 0 to 1 V.

OPTIMISATION OF MODIFIED ELECTRODE COMPOSITION

A general differential pulse voltammetry (DPV) analysis was applied to optimise the composition of the modified electrode. The parameters tested for electrode optimisation included the volume of laccase (1, 2, 3, 4, and 5 µL), the electrolyte pH (5.0, 6.0, 7.0, 8.0, and 9.0), deposition times (3, 4, 5, 6, and 7 s), and deposition potential (0.1, 0.2, 0.3, 0.4, and 0.5 V). All measurements were performed in triplicate, and the error bars show the standard deviation of the three measurements (n=3).

MORPHOLOGY STUDIES OF MODIFIED ELECTRODE

FIELD EMISSION SCANNING ELECTRON MICROSCOPY (FESEM)

Field Emission Scanning Electron Microscopy (FESEM) analysis was carried out using a JEOL JSM-IT800 equipped with backscattered electron detection (BED) to obtain topographical information. Solid samples of bare SPCE, MWCNT-COOH SPCE, and Lac-MWCNT-COOH SPCE were prepared in the same manner as for FTIR analysis. Before imaging, the samples were covered with a thin layer of gold to avoid surface charging. Images were acquired at a magnification of 15,000×, ensuring a high-resolution visualisation for detailed examination.

SPECTROSCOPY STUDIES OF MODIFIED ELECTRODE

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

The FTIR analysis was performed using a Thermo Scientific IS-50 FT-IR with the attenuated total reflectance (ATR) mode. The solid samples of MWCNT SPCE and MWCNT-COOH SPCE were prepared by cutting them to a diameter of approximately 25 mm (around 5 mg in weight). The samples were placed in the IR source path, and the resulting spectrum was analysed by the computer, identifying the signals and peaks.

RESULTS AND DISCUSSION

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS

Multi-walled carbon nanotubes (MWCNTs) were functionalised with carboxyl (-COOH) groups to obtain MWCNT-COOH, and the functionalisation was verified using Fourier Transform Infrared Spectroscopy (FTIR). Figure 1 illustrates the FTIR spectra comparison between MWCNT SPCE and MWCNT-COOH SPCE over the wavenumber range of 4000 to 500 cm^{-1}. A broad peak observed at 3250 cm^{-1} corresponds to the O–H stretching vibrations of hydroxyl groups from carboxyl functionalities (O=C–OH and C–OH). The broad absorption band ranging from 3650 to 3250 cm^{-1} indicates the presence of hydrogen bonding. This characteristic band is associated with the presence of hydrate (H_2O), hydroxyl (-OH), ammonium, or amino groups (Nandiyanto, Oktiani & Ragadhita 2019). The stretching vibrations of C=O and C–O from carboxyl groups (-COOH) are evident around 1640 cm^{-1} and approximately 1350 cm^{-1}, reflecting the presence of highly polar C=O bonds. In contrast, the absence of a significant absorption band at 1147 cm^{-1} suggests the lack of a C–O bond. Additionally, the C–H stretching associated with carbonyl groups (H–C=O) appearing at 2926 cm^{-1} indicates the presence of a long-chain alkyl group.

FIELD EMISSION SCANNING ELECTRON MICROSCOPY (FESEM) ANALYSIS

The surface morphology of the bare screen-printed carbon electrode (SPCE) modified with MWCNT-COOH was examined using Field Emission Scanning Electron Microscopy (FESEM) to confirm the presence of carbon nanotubes and enzymes on the modified electrode surface. FESEM images were obtained at a magnification of 15,000×. Figure 2 demonstrates the successful modification process at each stage: (a) bare SPCE, (b) MWCNT-COOH SPCE, and (c) Lac-MWCNT-COOH SPCE. The wavy and crumpled structures visible in image (b) are characteristic of the MWCNT-COOH layer applied to the bare SPCE. In image (c), the enzyme immobilisation led to a distinct porous morphology on the electrode surface. Enzyme immobilisation via EDC/NHS resulted in a porous morphology on the electrode surface, more pronounced than on bare or CNT-modified electrodes. This structure stems from nano-gel networks or 3D scaffolds formed by the enzymes, which entrap water or buffer and collapse into pores upon drying, which aligns with previously reported by Erden, Erdoğan and Kılıç (2019). The photomicrograph also confirms the presence of MWCNTs across the cell membrane, supporting the existence of nanotubes within the cells (Fraczek-Szczypta et al. 2012). This confirms the successful integration of enzymes and carboxyl groups (-COOH) onto the MWCNT walls.

CHARACTERISATION OF MODIFIED ELECTRODE ON CYCLIC VOLTAMMETRY (CV)

To characterise the electrochemical behavior of bare and modified SPCE, the cyclic voltammetry (CV) was conducted at 0.50 V. As shown in Figure 3, different types of electrodes, bare SPCE, MWCNT-COOH SPCE, and Lac-MWCNT-COOH SPCE, were analysed using CV in 0.05 M ferrocyanide $(Fe(CN)_6)^{3-/4-}$ a solution containing 100 mM of KNO_3.

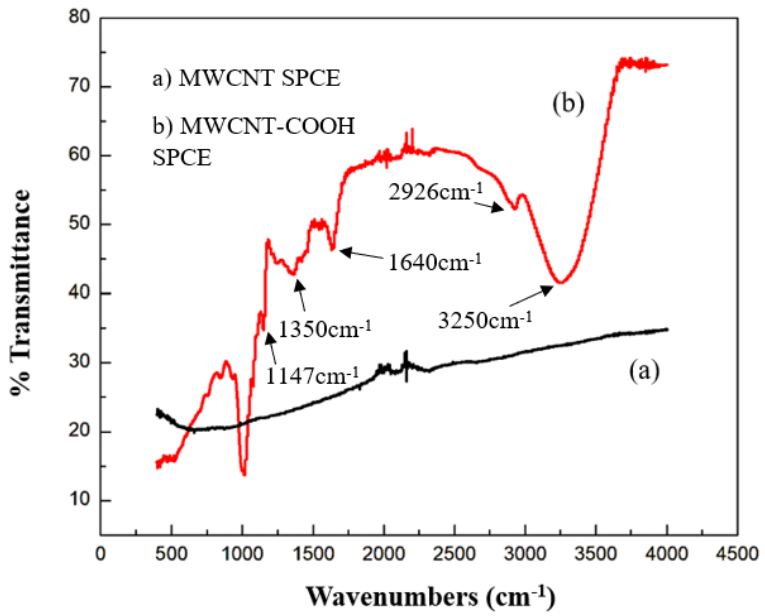


FIGURE 1. Fourier transform infrared (FTIR) spectra of (a) MWCNT SPCE and (b) MWCNT-COOH SPCE

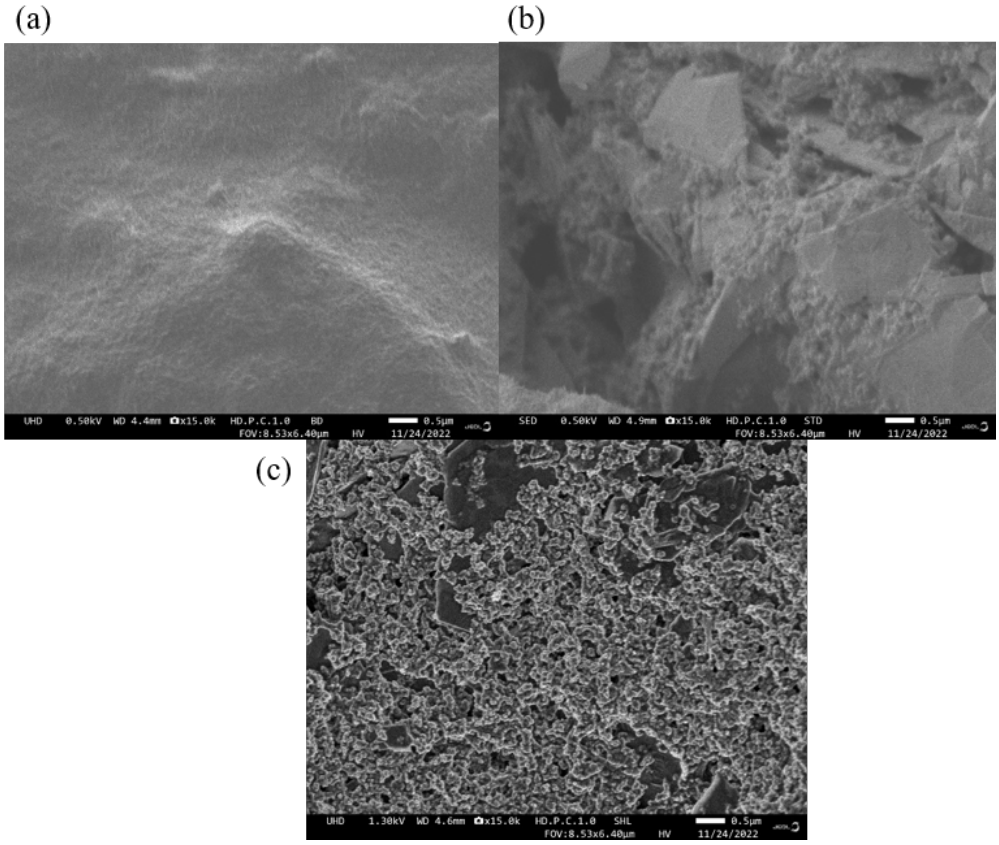


FIGURE 2. Image of FESEM on surface modifications of electrodes (a) SPCE bare, (b) MWCNT-COOH SPCE and (c) Lac-MWCNT-COOH SPCE

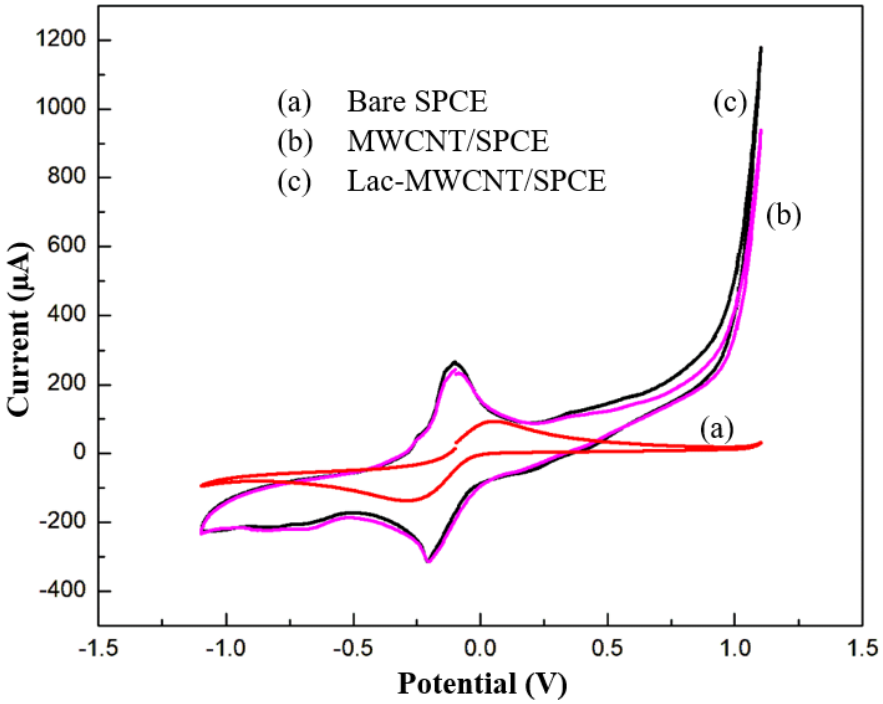


FIGURE 3. Cyclic voltammograms of (a) bare SPCE, (b) MWCNT-COOH SPCE, and (c) Lac-MWCNT-COOH SPCE were recorded in a 0.05 M ferrocyanide $(Fe(CN)_6)^{3-/4-}$ solution containing 100 mM KNO_3 as the supporting electrolyte

The ΔI_{pc} was increased after surface modification, from 122.04 µA at bare SPCE to 207.1 µA and 234.16 µA at MWCNT-COOH SPCE and Lac-MWCNT- COOH SPCE, respectively. It showed that the electrode was successfully modified due to the rise in the peak current, as well as explained the transmission of electrons between the electrode and redox reactions (Forouzandeh, Kumaravel & Pillai 2020). The active surface area comparison was tabulated in Table 1.

The presence of MWCNT-COOH was confirmed through cyclic voltammetry (CV) measurements conducted in a 0.05 M PBS solution at a fixed potential of 0.5 V, using varying scan rates of 10, 25, 30, 50, 75, 100, 150, 200, 250, 300, 400, 500, 700, 750, and 1000 $V \cdot s^{-1}$, as illustrated in Figure 4. The results show that from 10 to 750 $V \cdot s^{-1}$, a consistent pattern is maintained, with an oxidation peak observed at 0.1 V. However, at scan rates above 1000 $V \cdot s^{-1}$, the redox peaks become less distinct. According to Venton and Cao (2020), fast-scan cyclic voltammetry generates substantial background charging currents, as the charging current is directly proportional to the scan rate. Additionally, at higher scan rates, the diffusion rate exceeds the reaction rate, enabling more electrolytic ions to reach the electrode–electrolyte interface, though fewer ions participate in charge transfer reactions. As a result, the current increases at higher scan rates. In this study, a scan rate of 100 $V \cdot s^{-1}$ was selected for further analysis.

Figure 5(a) shows a comparison of cyclic voltammograms before and after electrode modification: bare SPCE (curve a), MWCNT-COOH SPCE (curve b), and Lac-MWCNT-COOH SPCE (curve c). The results show that the MWCNT-COOH modified electrode exhibited an increased peak current relative to the bare SPCE, indicating enhanced redox activity at the electrode surface. Furthermore, the enzyme-functionalised MWCNT-COOH electrode demonstrated an even higher current response compared to the non-enzyme-modified counterpart. This improvement is credited to enhanced electron transfer, which is facilitated by the larger active surface area of the modified electrode (Yang et al. 2015). These findings confirm the successful stepwise modification of the electrode.

OPTIMISATION OF MODIFIED ELECTRODE USING DIFFERENCE PULSE VOLTAMMETRY (DPV)

Figure 6(a) illustrates the effect of pH on tyramine detection across a range of pH 5.0 to 9.0. The data show that laccase activity increases up to pH 7.0, after which it declines as the pH approaches 9.0. This reduction in activity is attributed to enzyme deactivation in strongly alkaline conditions. According to Masód, Azhari and Sathishkumar (2022), no oxidation peak is observed in strongly acidic environments (pH 3 to pH 6) due to enzyme deactivation. The reduced activity of laccase is caused by enzyme denaturation, which leads to a gradual decline in catalytic efficiency over time, ultimately impairing biocatalytic performance. Therefore, pH 7.0 was identified as the optimal condition for tyramine detection, representing the enzyme's most active state.

Figure 6(b) presents the effect of varying enzyme volumes (1 µL to 5 µL) on the electrode's performance, with 3 µL identified as the optimum amount. An increase in biocatalytic activity was observed up to 3 µL, followed by a decline at 4 µL and 5 µL. This reduction is attributed to protein overloading on the electrode surface, which limits molecular diffusion (Debe 2011). When the diffusion rate of reactant molecules becomes lower than the reaction rate, mass transfer is restricted, resulting in decreased biocatalytic efficiency.

Figure 6(c) illustrates the effect of varying deposition potentials from 0.1 V to 0.5 V on sensor performance. Deposition potential is considered a crucial parameter in electrochemical measurements, as it significantly influences both the sensitivity of detection and the duration of the analytical process (Kolliopoulos, Metters & Banks 2013). As the potential increases from 0.1 V, the peak current rises sharply, reaching a maximum at around 0.2 V. Beyond this point, the peak begins to decline. At 0.5 V, the redox probe reaction is already underway, leading to an increase in biocatalytic activity. This behavior suggests that while a certain level of potential enhances the reaction, excessive values may reduce efficiency due to early redox activity or potential over-oxidation effects.

Figure 6(d) analysed the effect of deposition time from 3 s to 7 s. The graph shows an increased pattern starting at 3 s and going up to 5 s. It is obvious that the function reaches the maximum value at deposition time of 5 s. This may be due to the decreased deposition of laccase at times less than 5 s and the removal of some co-deposited laccase molecules by hydrogen attack at times greater than 5 s. Based on this, 5 s gives the optimal value for the deposition time in the next experiments.

DETERMINATION OF TYRAMINE BY DIFFERENTIAL PULSE VOLTAMMETRY (DPV)

The standard addition method was employed to validate the peak potential of tyramine at the Lac-MWCNT-COOH SPCE using differential pulse voltammetry (DPV). From the calibration plot of the linear graph in Figure 7, the current response (µA) vs. the concentration of tyramine (mM) was increasing linearly with the elevation of the tyramine concentration from 5 mM, 10 mM, 15 mM, 20 mM to 25 mM in 0.05 M PBS at pH 7 at the scan rate of 100 mV^{-1} with the correlation coefficient (R^2) of 0.9996. The lower detection limit (LOD) was calculated as 0.09 mM according to the equation standard error (SD) of intercept/a = 1/3.3, and the limit of quantitation (LOQ) value was 0.267055 according to the equation standard error (SD) of intercept/a = 1/10 (Montes et al. 2015). It can be concluded that the Lac-MWCNT-COOH SPCE is a sensitive biosensor for tyramine detection that offers a satisfactory linear range, lower detection limits, and high sensitivity.

TABLE 1. Electrochemical parameters were obtained from cyclic voltammograms recorded in a 0.05 M ferrocyanide $(Fe(CN)_6)^{3-/4-}$ solution containing 100 mM KNO_3 as the supporting electrolyte

Electrode	E_{pc} (V)	E_{pa} (V)	I_{pc} (µA)	I_{pa} (µA)	A (cm^2)
Bare SPCE	-0.01205	-0.27328	122.04	-111.52	1.47×10^{-5}
MWCNT-COOH SPCE	-0.09018	-0.20248	207.10	-224.65	2.50×10^{-5}
Lac-MWCNT-COOH SPCE	-0.0975	-0.20737	234.16	-238.03	2.83×10^{-5}

Cathodic peak potential (Epc), Anodic peak potential (Epa), Cathodic peak current (Ipc), Anodic peak current (Ipa) and Electroactive surface area (A)

TABLE 2. I_{pa}/I_{pc} Ratios of (a) bare SPCE, (b) MWCNT-COOH SPCE, and (c) Lac-MWCNT-COOH SPCE

Electrode	I_{pc} (µA)	I_{pa} (µA)	I_{pa}/I_{pc} ratio
Bare SPCE	122.04	111.52	0.914
MWCNT-COOH SPCE	207.10	-224.65	1.085
Lac-MWCNT-COOH SPCE	234.16	-238.03	1.017

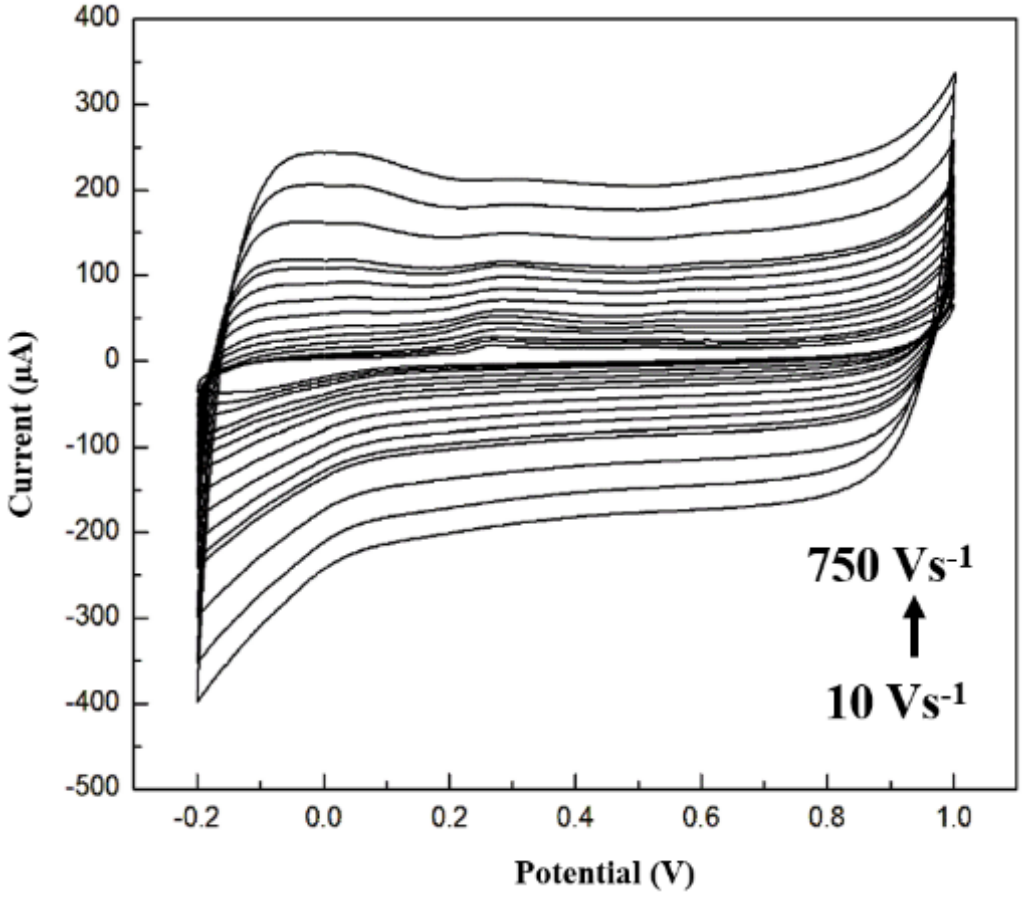


FIGURE 4. Cyclic voltammetry of MWCNT-COOH SPCE in 0.05 M PBS solution at different scan rate

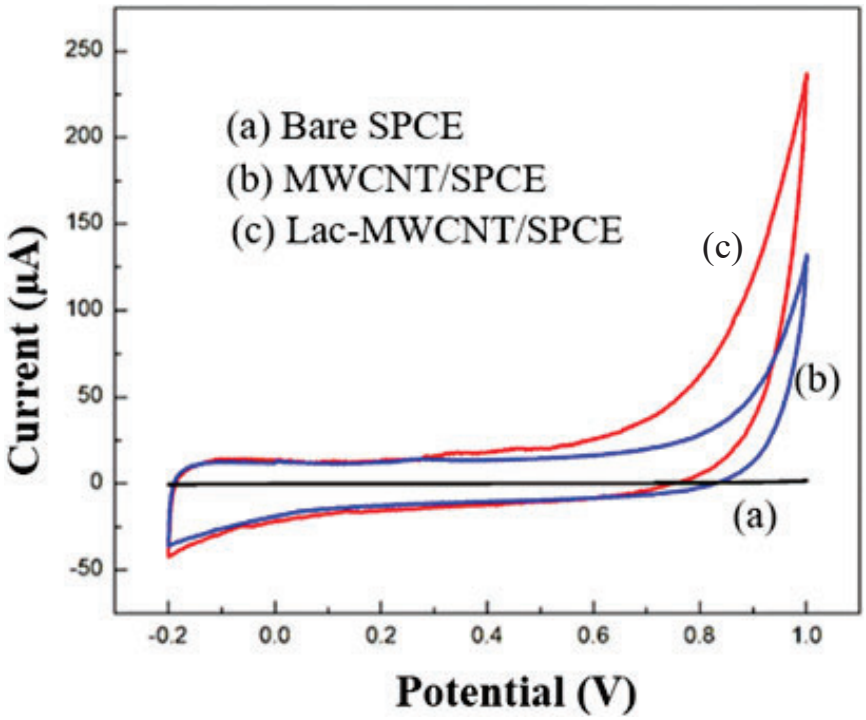


FIGURE 5. (a) Cyclic voltammetry was performed on (a)bare SPCE, (b)MWCNT-COOH SPCE, and (c)Lac-MWCNT-COOH SPCE in 0.05 M phosphate buffer solution (PBS) at pH 7.0, using a scan rate of 100 $mV \cdot s^{-1}$

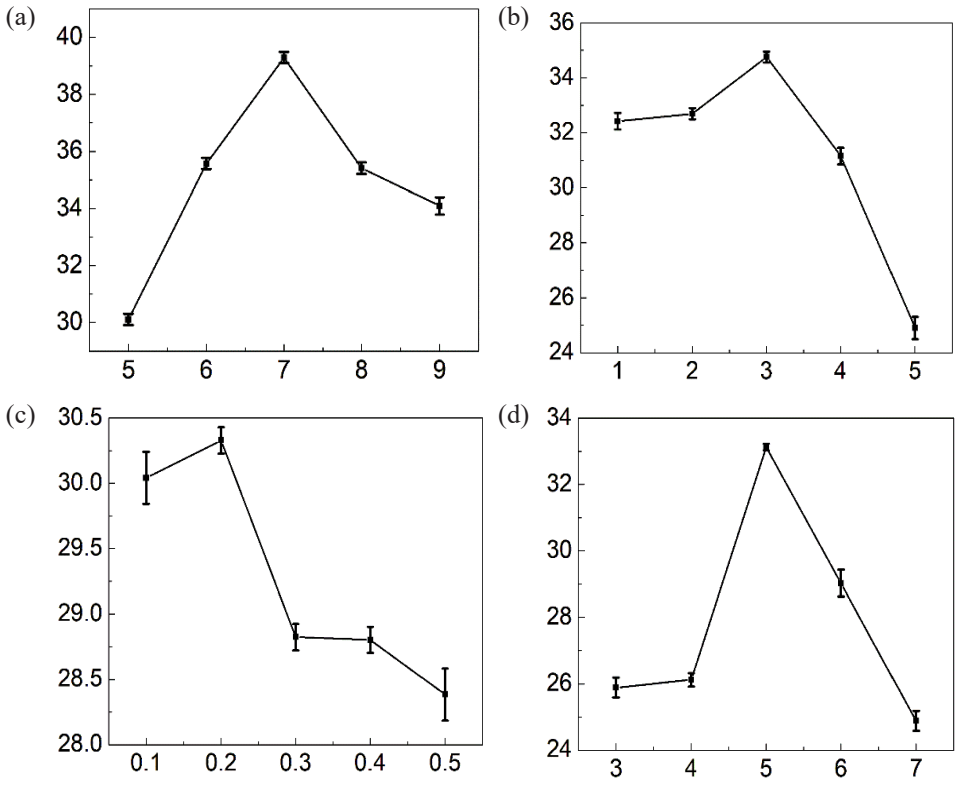


FIGURE 6. (a) The effect of pH analyte, (b) The effect amount of enzyme (µL), (c) The effect of deposition potential (V), (d) The deposition time (s) on the reaction of Lac-MWCNT-COOH SPCE in 0.05 M PBS solution containing 0.002 M tyramine at +0.05 V

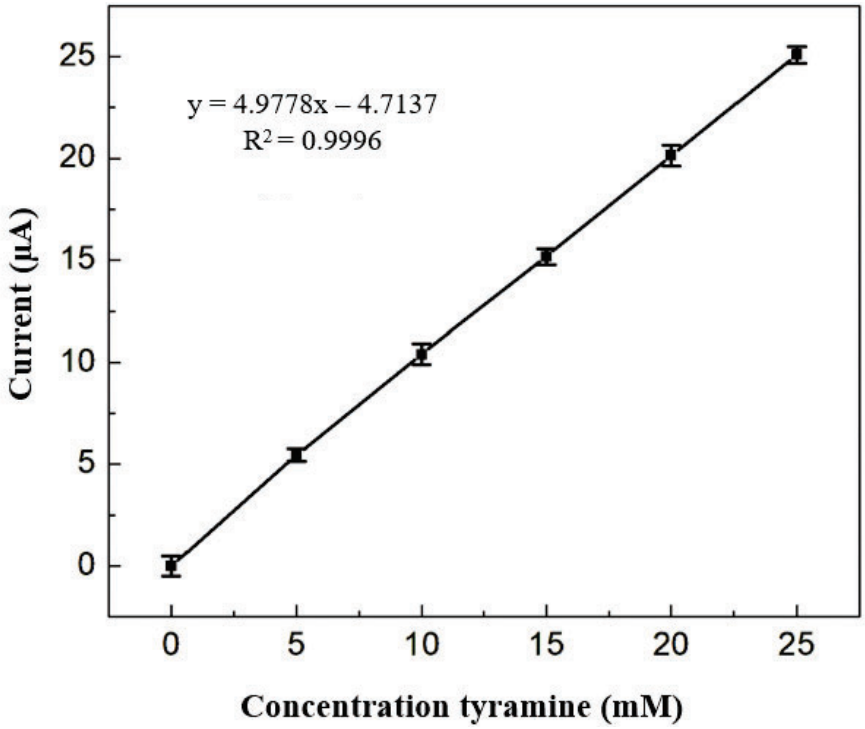


FIGURE 7. Calibration plot of linear graph of the current response (µA) vs the concentration of tyramine (mM)

CONCLUSION

In this study, the biosensor Lac-MWCNT-COOH SPCE was successfully fabricated with the aid of EDC/NHS immobilisation solution, characterised using FTIR and FESEM, optimised using four different parameters (pH, amount of laccase enzyme, deposition time, and potential) for tyramine detection. Multi-walled carbon nanotubes are used as a biocompatible matrix for laccase immobilisation on the electrode surface. It can be summarised that the Lac-MWCNT-COOH SPCE biosensor demonstrated optimal performance under the following conditions: phosphate buffer solution (PBS) at pH7, 3 µL of laccase enzyme, a deposition potential of 0.2 V, and a deposition time of 5 s. This biosensor showed good correlation coefficient (R^2) at 0.9996 with the low detection of 0.09 mM and quantification limit of 0.267 mM. All these results indicated that the laccase enzyme has good properties for biosensor surface modification in the determination of tyramine concentration.

ACKNOWLEDGEMENTS

This research was funded by Universiti Sains Islam Malaysia Research Grant Scheme (PPPI/TRANSDISIPLINARI/FST/USIM/17623). We would like to express our sincere gratitude to Pusat Tamhidi and the Faculty of Science and Technology, Universiti Sains Islam Malaysia, for providing the essential research facilities throughout the course of this study.

REFERENCES

Datta, S., Veena, R., Samuel, M.S. & Selvarajan, E. 2021. Immobilization of laccases and applications for the detection and remediation of pollutants: A review. *Environmental Chemistry Letters* 19(1): 521-538.

Debe, M.K. 2011. Effect of electrode surface area distribution on high current density performance of PEM fuel cells. *Journal of The Electrochemical Society* 159(1): B53-B66. https://doi.org/10.1149/2.032201jes

Erden, P., Erdoğan, Z. & Kılıç, E. 2019. Amperometric biosensors for tyramine determination based on graphene oxide and polyvinylferrocene modified screen-printed electrodes. *Electroanalysis* 31(12): 2368-2378.

Forouzandeh, P., Kumaravel, V. & Pillai, S.C. 2020. Electrode materials for supercapacitors: A review of recent advances. *Catalysts* 10(9): 1-73. https://doi.org/10.3390/catal10090969

Fraczek-Szczypta, A., Menaszek, E., Syeda, T.B., Misra, A., Alavijeh, M., Adu, J. & Blazewicz, S. 2012. Effect of MWCNT surface and chemical modification on *in vitro* cellular response. *Journal of Nanoparticle Research* 14(10): 1-14. https://doi.org/10.1007/s11051-012-1181-1

Hungerford, J.M. 2010. Scombroid poisoning: A review. *Toxicon* 56(2): 231-243. https://doi.org/10.1016/j.toxicon.2010.02.006

Kolliopoulos, A.V., Metters, J.P. & Banks, C.E. 2013. Screen printed graphite electrochemical sensors for the voltammetric determination of antimony(iii). *Analytical Methods* 5(14): 3490-3496. https://doi.org/10.1039/c3ay40683k

Masód, N.H., Azhari, S. & Sathishkumar, P. 2022. Food spoilage: Detection of biogenic amines in food samples by enzyme-based electrochemical biosensors. *Malaysian Journal of Chemistry* 24(3): 74-87. https://pubchem.ncbi.nlm.nih.gov/

Montes, R., Bartrolí, J., Baeza, M. & Céspedes, F. 2015. Improvement of the detection limit for biosensors: Advances on the optimization of biocomposite composition. *Microchemical Journal* 119: 66-74. https://doi.org/10.1016/j.microc.2014.11.004

Nandiyanto, A.B.D., Oktiani, R. & Ragadhita, R. 2019. How to read and interpret FTIR spectroscope of organic material. *Indonesian Journal of Science & Technology* 4(1): 97-118.

Özogul, F., Kuley, E. & Kenar, M. 2011. Effects of rosemary and sage tea extract on biogenic amines formation of sardine (*Sardina pilchardus*) fillets. *International Journal of Food Science and Technology* 46(4): 761-766. https://doi.org/10.1111/j.1365-2621.2011.02560.x

Salcedo-Sandoval, L., Ruiz-Capillas, C., Cofrades, S., Triki, M. & Jiménez-Colmenero, F. 2015. Shelf-life of n-3 PUFA enriched frankfurters formulated with a konjac-based oil bulking agent. *LWT* 62(1): 711-717. https://doi.org/10.1016/j.lwt.2015.01.043

Venton, B.J. & Cao, Q. 2020. Fundamentals of fast-scan cyclic voltammetry for dopamine detection. *Analyst* 145(4): 1158-1168. https://doi.org/10.1039/C9AN01586H

Yang, C., Denno, M.E., Pyakurel, P. & Venton, B.J. 2015. Recent trends in carbon nanomaterial-based electrochemical sensors for biomolecules: A review. *Analytica Chimica Acta* 887: 17-37. https://doi.org/10.1016/j.aca.2015.05.049

*Corresponding author; email: syaza@usim.edu.my