Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society

ENZYME-BASED ELECTROCHEMICAL BIOSENSOR ON IMMOBILIZATION OF TYROSINASE ONTO CARBOXYL FUNCTIONALIZED CARBON NANOTUBE FOR DETECTION OF TYRAMINE

(Biosensor Elektrokimia Berasaskan-Enzim Diimobilasi Enzim Tirosinase Ke Karboksil Tiupnano Karbon Untuk Pengesanan Tiramin)

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Received: 12 February 2022; Accepted: 27 March 2022; Published: 27 December 2022

Abstract

Tyramine (TYR) in foods have been regarded as a quality indicator of food freshness for assessing microbial action, which potentially affects human health. Enzyme-based electrochemical biosensor technology represents an excessively massive field that significantly impacts food quality control with incredible potential and rapid tools. Thus, this study aimed to immobilize tyrosinase (tyro) over single-wall carbon nanotubes (SWCNTs) onto the screen-printed carbon electrode (SPCE) in the detection of TYR. The characteristics and electrochemical behaviour of the modified SPCEs were investigated by Fourier transformed infrared spectroscopy (FTIR), cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Under optimum experimental conditions, Tyro-SWCNT-COOH/SPCE biosensor exhibit good performance at scan rate 50 mVs^{-1} (range of 10 to 500 mVs^{-1}), pH 8.0 (range of 7.0 – 10.0), 8 µL enzyme tyrosinase (range of 2 to 10 µL), and 0.5 mg/mL SWCNTs (range of 0.2 - 3.0 mg/ml). The modified SPCEs was successfully applied for tyramine (TYR) determination with a detection limit (LOD) of 0.02 mM.

Keywords: tyrosinase, tyramine, screen-printed electrode, single-walled carbon nanotube

Abstrak

Tiramin (TYR) dalam makanan telah dianggap sebagai penunjuk kualiti kesegaran makanan untuk menilai tindakan mikrob yang berpotensi menjejaskan kesihatan manusia. Teknologi biosensor elektrokimia berasaskan enzim mewakili bidang yang luas dimana mampu memberi kesan ketara terhadap kawalan kualiti makanan dengan potensi dan alat pantas yang luar biasa. Oleh itu, matlamat kajian ini adalah untuk mengalihkan tirosinase (tyro) ke atas tiubnano karbon berdinding tunggal (SWCNT) ke elektrod karbon bercetak skrin (SPCE) dalam pengesanan tiramin (TYR). Ciri-ciri dan tingkah laku elektrokimia SPCE yang diubah suai telah disiasat oleh spektroskopi inframerah (FTIR), voltammetri kitaran (CV) dan voltammetri nadi pembezaan (DPV). Di bawah keadaan percubaan optimum, biosensor Tyro-SWCNT-COOH/SPCE mempamerkan prestasi yang baik pada kadar imbasan 50 mVs^{-1} (julat 10 hingga 500 mVs^{-1}), pH 8.0 (julat 7.0 – 10.0), 8 µl enzim tirosinase (julat daripada 2 hingga 10 µl), dan 0.5 mg/ml

SWCNTs (julat 0.2 – 3.0 mg/ml). SPCE yang diubah suai telah berjaya digunakan untuk penentuan tiramine (TYR) dengan had pengesanan (LOD) sebanyak 0.02 mM.

Kata kunci: tirosinase, tiramin, elektrod bercetak skrin, tiub nano karbon berdinding tunggal

Introduction

Tyramine (TYR) is one of the most abundant compounds in a group of biogenic amines (BAs) that are present in a variety of foods due to their capability to show the activity of certain microorganisms such as *Enterobacteriaceae,* including *E. coli* [1–3]. Today, food safety and quality are the main concerns among consumers and health agencies worldwide [4]. Furthermore, TYR can affect human health by stimulating the release of catecholamines from the sympathetic nervous system, which can increase heart rate and blood pressure, which can lead to migraines, hypertension, cardiac failure, and cause serious intoxication effects when ingested in large quantities [5–8]. Due to their toxicity to human health, the optimization parameter for modification of the electrode in the detection of TYR is important.

The demand for safer food quality has promoted more research into BAs quantification over the past few years. Previous work deals with the development of a sensitive and easily fabricated electrochemical biosensor for TYR detection using tyrosinase (tyro) on a glassy carbon electrode [9], such as electrochemical deposition of poly-(8-anilino-1-naphthalene sulphonic acid) attached with gold nanoparticles (AuNP) on gold electrodes (AuE) by polymerization of the monomer [10,11]. However, amongst all of the modified electrodes, screen-printed electrode (SPCE) is the most suitable to be used based on electrochemical analysis in environmental, clinical or agricultural food areas due to its versatility of fabrication, affordability, nontoxic and customizability with nanomaterials and biological elements such as enzymes resulting in the high surface area of the modified electrode [12].

Nanomaterials such as single-wall carbon nanotubes (SWCNT) play an important part in the recent development of enzyme-based biosensors providing advantages such as high catalytic efficiency, rapid response times [13] and enzymes processed with nanoparticles employed to overcome the inherent drawbacks of enzymes [14]. SWCNT is also very good at adsorption, electrical, mechanical, and thermal properties, and it has a lot of surface area, which makes it a good material for the fabrication of electrochemical biosensors and allows them to stay in harsh chemical environments [15]. In addition, SWCNTs that functionalize with carboxylic acid groups (-COOH) help to mitigate this disadvantage by affording the possibility of further chemical derivatization [16].

Enzyme tyro is commonly used to determine analytes containing phenolic compounds [17–19]. Carbon nanotubes act as conductive material and have been commonly used as common approaches for the immobilization of tyro onto various substrates [20]. In previous work by Apetrei, an amperometric TYR biosensor has been developed by immobilizing enzyme tyro on SWCNT-COOH thin film of SPCE electrodes for detection of TYR level in fish products by using amperometric techniques [21]. Other than that, studied by da Silva, the same enzyme tyro was used using a modification of the glassy carbon electrode (GCE) [22].

However, there are only a few reports on tyro immobilized on SPCE with a synthesis of SWCNT-COOH on TYR determination. As a result, more detailed research on the immobilization of tyro over the activated surface of SWCNT-COOH modified SPCE in the detection of TYR is required to determine the best parameter for developing the novel electrode. Thus, in this study, the effect of various compositions on the modification of the electrode Tyro-SWCNT-COOH/SPCE in the detection of TYR was investigated. The optimization focused on the effect of scan rate, pH of the buffer, amount of enzyme and concentration of SWCNT-COOH that contributed to giving the optimum condition of the biosensor in the experiment.

Materials and Methods

Apparatus

Tyrosinase from mushroom (EC 1.14.18.1) ≥1000 unit/mg, carbon nanotube, and single-walled, ≥98%

carbon basis, n-hydroxysuccinimide (NHS), potassium hexacyanoferrate (III) reagentplus®, tyramine, sodium phosphate dibasic (Na_2HPO_4), sodium phosphate monobasic (NaH_2PO_4) were purchased from Sigma Aldrich (St. Louis, MO, USA). Nitric acid (HNO_3) (65%) and sulphuric acid (H_2SO_4) (95-98%) were purchased from R&M Chemicals. The 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) is from G-Bioscience, USA and potassium chloride (KCl) is from Friendemann Schmidt. The stock solution of enzyme tyrosinase and EDC/NHS coupling agent has been aliquoted and stored at -20°C. All reagents used in this research are analytical grade and used without further purification. Water purified with a Milli-Q system (Millipore Milli-Q, Badford, MA, USA) was used for the preparation of all aqueous solutions.

Chemicals and instrumentations

The weight balance and pH measurements (Mettler Toledo) using a pH meter were employed for all pH measurements and calibrated with buffer pH 4, 7 and 10. Elmasonic EASY 120 H was used for ultrasonication of solution, and Beckman counter centrifuges Allegra 64R was used in ultracentrifuge precipitate separation. The electrochemical measurements were obtained using the Autolab (Ecochemie, Utrecht, The Netherlands) potentiostat incorporated with a general-purpose electrochemical system (NOVA 2.1.4), connected to a computer for data processing. The characterization of SWCNT-COOH was carried out using Fourier transform infrared (FTIR) (Perkin Elmer).

Electrodes and electrochemical cell

The electrochemical experiments were carried out using a screen-printed carbon electrode (SPCE) purchased from Rapid Lab Sdn Bhd (Bangi, Malaysia), which was made up of carbon as the working electrode (diameter: 5 mm), silver chloride (AgCl) as the reference electrode and carbon as counter electrodes. Measurement and electrochemical characterization were carried out using cyclic voltammetry (CV), and differential pulse voltammetry (DPV). CV was registered from -0.6 to 1.0 V at a scan rate of 0.05 Vs^{-1} (except otherwise indicated). DPV was registered from 0.0 to 1.0 V with a scan rate of 0.05 Vs^{-1} and a potential of 0.5 V (except otherwise indicated).

Synthesis of SWCNT-COOH

The amount of 200 mg of SWCNTs was dissolved in 200 mL of concentrated sulphuric acid (H_2SO_4) and nitric acid (HNO_3) with a ratio of 3:1. The mixture then undergoes sonication for 6 hours at 60 °C. The solution was allowed to cool, then distilled water was added, and left overnight. The solution was then ultracentrifuged (35000 rpm), the sedimental black solution was collected, and distilled water was used to control the pH until it reached neutral pH 7. After that, the black sedimental solution was collected using a vacuum filtration assembly with (0.45 µm pore size) filter paper. The filtered SWCNT-COOH bedding was dried in an oven overnight at 50ºC, and the obtained SWCNT-COOH powder was characterized using FTIR.

Biosensor fabrication

The 12 µL of 0.5 mg/mL SWCNT-COOH solution was drop cast onto the pre-treated SPCE surface and dried at room temperature for 30 minutes. The 10 µL of 0.5 M EDC/NHS solution was dropped onto the dried SWCNT-COOH/SPCE. After 30 minutes, 10 µL of enzyme tyrosinase solution (2 mg/ml, 200 units activity) was dropped gently onto the SWCNT-COOH/SPCE at 4ºC for 15 minutes, and the electrode was rinsed with PBS to remove the unbonded enzyme. Each analysis was repeated three times.

Immobilization of tyrosinase on SWCNT-COOH/SPCE

Immobilized enzymes onto an SPCE using EDC/NHS coupling were prepared by dissolving 0.048 g of EDC (0.25 M) and 0.029 g of NHS (0.25 M) into a 1 mL polypropylene microcentrifuge tube and 0.05 M phosphate buffer solution (PBS) at pH 7.0 up until the mark. The prepared solution was then stored in the freezer.

Optimization procedure

The parameters that were investigated for the optimization of the modified electrode are the effect of pH of the electrolyte (pH 7.0, 8.0, 9.0 and 10.0), effects of SWCNT-COOH concentration (0.2, 0.5, 1.0. 1.5, 2.0 and 3.0 mg/mL) and the amount of enzyme (2, 4, 6, 8 and 10 µL) by keeping other parameters fixed and changing one factor at a time. All the measurements

were run three times, and the error bars show the standard deviation of the three measurements. The electrochemical response of the biosensor was carried out at 0.50 V in phosphate buffer solution (PBS) at 50 mM in the presence of tyramine (TYR) (0.05 M) using differential pulse voltammetry (DPV) by observing the peak current (ΔI_p) response. For the immobilization of the enzyme, tyro was prepared in a solution of 2 mg/ml as a stock solution (activity 2000 units) before being diluted to 0.2 mg/mL (200 units activity) as a working solution. Six different concentrations of enzyme tyro (≥1000 U/mg) ranging between 2, 4, 6, 8 and 10 µL were used in the fabrication of the biosensor and were directly immobilized onto SWCNT-COOH/SPCE after drop cast of EDC/NHS that act as an immobilization holder.

Result and Discussion

Biosensor fabrication

The biocatalytic performance of an immobilized enzyme system depends mostly on the intrinsic properties of both the biomolecule and its support. Single-walled carbon nanotubes (SWCNTs) possess unique features for tyrosinase (tyro) immobilization by adsorption after drop-casting as the first layer on a screen-printed carbon electrode (SPCE). Thus, the optimum concentration condition of SWCNT-COOH was studied on the DPV peak current (ΔI_p) response of modified electrode tyro-SWCNT-COOH/SPCE at 0.2, 0.5, 1.0. 1.5, 2.0 and 3.0 mg/mL SWCNT-COOH towards TYR determination. The resulting biosensor was denoted as Tyro-SWCNTCOOH/SPCE, as shown in Figure 1. The first layer of modification involves SWCNT-COOH to form a thin solid drop solution onto the flat surface of SPCEs, followed by evaporation of the solution. After that, the enzyme tyro was immobilized over SWCNT-COOH by an adsorption process.

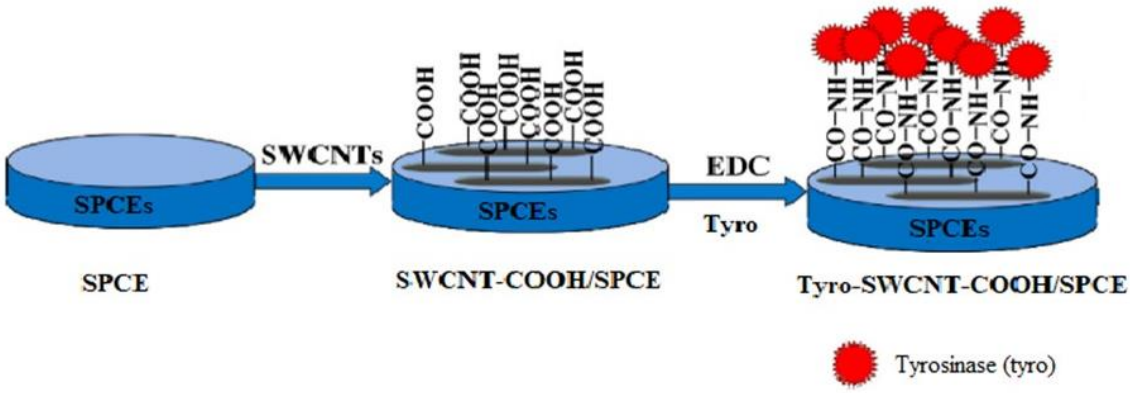

Figure 1. Proposed schematic reaction of the tyrosinase study on the single-walled carbon nanotube carboxyl functionalized (SWCNT-COOH) modified electrode for detection of tyramine (TYR)

Figure 2(a) shows carboxyl functionalized single-walled carbon nanotube (SWCNTCOOH) using the acid treatment method. Chemical manipulation becomes difficult when pristine CNTs are inherently insoluble in organic solvents. Thus, functionalizing the pristine CNTs with carboxylic acid groups will help to mitigate this disadvantage by affording the possibility of further chemical derivatization.

Effective immobilization of the enzymes is a crucial step in the development of high-performance biosensors. In this study, immobilization of tyro onto SWCNT-COOH/SPCE was employed using EDC/NHS coupling to activate the carboxyl groups of SWCNT-COOH that have been drop cast as the first layer of modification. The EDC/NHS activation method can minimize any adverse effects on the bioactivity of target molecules; thus, it was preferred due to its high conversion efficiency and excellent biocompatibility [23].

The EDC/NHS used in this study was 0.25/0.25 M, where the equal amount of EDC/NHS = 0.25/0.25 M shows the most effective concentration for the carboxyl group [24]. The -COOH functionalized groups on the sidewalk of the SWCNT will be used as the precursor for diimidation for the formation of amide bonds with the amine groups of the enzyme. This will be successfully achieved through the reaction of carboxylic acid groups with EDC/NHS to form an amine-reactive ester group that finally bonds to the -NH group in the enzyme, so that the enzyme is immobilized on the electrode. The conjugation of the enzyme reaction pathway onto SWCNTs is shown in the mechanism given in Figure 2(b).

2(a) 2(b)

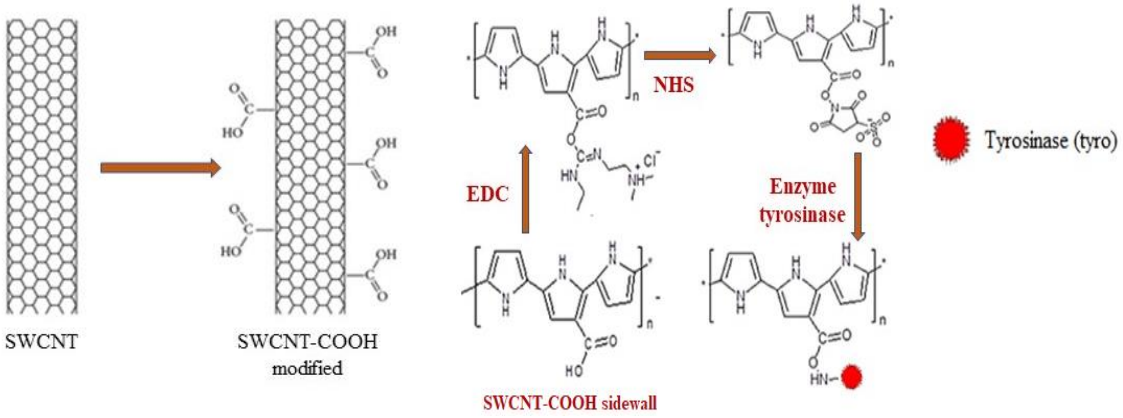

Figure 2. (a) Carboxyl functionalized single-walled carbon nanotube (SWCNT) using an acid treatment method, (b) The EDC/NHS coupling method's mechanism.

Characterizations of the modified electrode

Figure 3 shows the comparison of the total reflection infrared spectra in a range of 4000 - 1000 cm^{-1} for (a) pristine SWCNT and (b) functionalized SWCNT after being treated with H_2SO_4 and HNO_3 acid treatment method, producing SWCNT-COOH. It can be clearly shown that there is a broad peak representing the O-H stretching of hydroxyl groups from carboxyl groups (O=C-OH and C-OH) at about 3296 cm^{-1} [25]. During purification, the partial oxidation reaction occurs, causing the carboxyl group to be present on the surface of pristine SWCNT [26]. The apparent peaks at 1750 cm^{-1} and 1055 cm^{-1} are contributed by the presence of C=O and C-O stretching vibration of the carboxyl group (-COOH), respectively [27]. Meanwhile, the C-H stretch modes of H-C=O in the carbonyl group also indicate the presence of a long-chain alkyl molecule that is attributed to peak 2920 cm^{-1} [28].

FTIR spectra were used to ensure the presence of carboxyl functionalized group -COOH on SWCNT to indicate the successfulness of the synthesis of SWCNT to SWCNT-COOH using the acid treatment method in this study [29]. The functionalization method was used to improve interfacial interactions with nanotubes and to produce a well-dispersed SWCNT composition to drop the SWCNT-COOH dispersion on SPCE during the modification of the electrode. By comparing the FTIR absorption spectra of pristine and SWCNT-COOH, SWCNT oxidized by a mixture of H_2SO_4 and HNO_3 were successfully added with the carboxylic groups (-COOH) to the wall of SWCNT, improving the properties of carbon nanotubes.

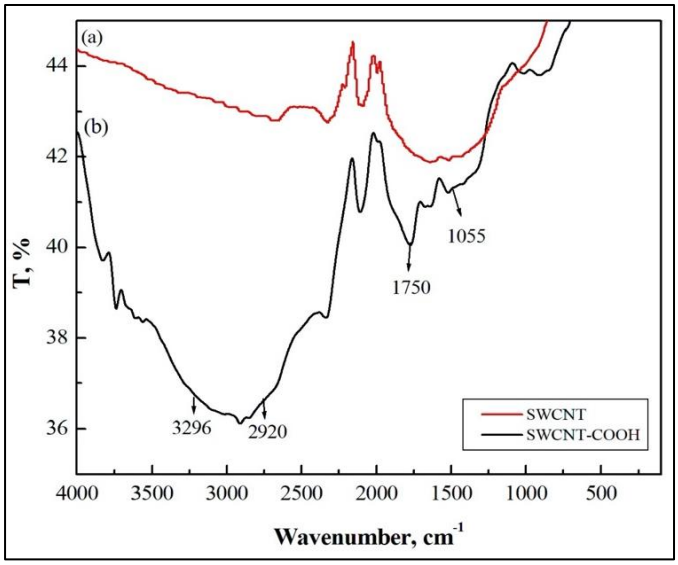

Figure 3. FTIR spectra of (a) pristine single-walled carbon nanotube, (b) functionalized single-walled carbon nanotube

Electrochemical characterization

Cyclic voltammetry (CV) was conducted at 0.50 V to characterize the electrochemical behavior of bare SPCE and modified SPCE. In this study, CV was performed on three different types of electrodes in 0.05 M ferrocyanide $(Fe(CN)_6)^{3-/4-}$ a solution containing 100 mM KCl as shown in Figure 4 (a) bare SPCE, (b) SWCNT-COOH/SPCE, and (c) Tyro-SWCNT-COOH/SPCE. When the surface of the electrode was modified, the ΔIp_a was increased from 34.86 µA at bare SPCE to 97.40 µA and 109.32 µA at SWCNT-COOH/SPCE and Tyro-SWCNT-COOH/SPCE, respectively. The increase in the peak current proves the electrode was successfully developed, which could be described by the transmission of electrons between the electrode and redox probe. The number of electrons transferred can be determined using the peak separation as a criterion for Nernstian behavior. The Randles-Sevcik equation was used to calculate the active surface area of SPCE.

$$I_{pa} = (2.69 \times 10^5)\, n^{3/2} ACD^{1/2} \nu^{1/2} . \quad (1)$$

Based on Equation (1), the real active surface area of the electrode was calculated by substituting the value of n (number of electrons) = 1 $[Fe(CN)^{3-/4-}$ system], A (electrode area) = (22/7) $(0.25\ cm)^2$ = 0.196 cm^2, C (concentration) = 0.05 mol L^{-1}, D (diffusion coefficient) = 7.6×10^{-6} $cm^2\ s^{-1}$ and ν (scan rate) = 0.05 Vs^{-1}. From the calculation, the value of the geometrical surface area of the electrode is 0.126 $cm^{2,}$ and the electrochemical surface area of the real electrode is much larger, as expected. As tabulated in Table 1, the active surface area of bare SPCE was calculated as 4.2091 cm^2 while it was 11.7604 and 13.1997 cm^2 after modification with SWCNT and enzyme tyro, respectively. Thus, higher electron transfer happened at a larger value of active surface area on the modified electrode [26]. This also proved that the modification was successfully improved at each layer of the electrode.

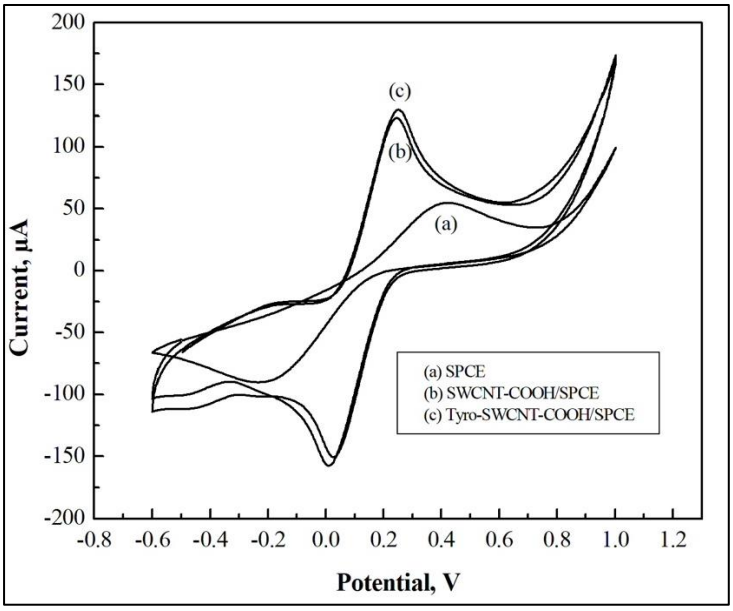

Figure 4. (a) CVs of (a) SPCE, (b) SWCNT-COOH/SPCE, (c) and Tyro-SWCNT-COOH/SPCE (0.05 M $(Fe(CN)_6)^{3-/4-}$ solution containing 100 mM KCl at 50 mVs^{-1})

Table 1: Electrochemical parameters obtained from voltammograms in Figure 4

Electrode	E_{Pc} (V)	E_{Pa} (V)	Ip_c (µA)	Ip_a(µA)	A (cm^2)	Current density (µA/cm^2)
SPCE	0.40	-0.19	-36.67	34.86	4.20	8.28
SWCNT-COOH/SPCE	0.23	0.03	-94.22	97.40	11.76	8.28
Tyro-SWCNT-COOH/SPCE	0.24	0.02	-99.03	109.32	13.19	8.28

Figure 5(a) compares the background current before (solid line) and after modification (dashes-line). The voltammogram of modified SWCNT-COOH/SPCE displayed an increase in peak current as compared to the bare electrode, which can be attributed to the redox behavior of SWCNT-COOH on the electrode surface. Similar voltammogram patterns for carboxyl functionalized on carbon nanotubes (CNT-COOH) were previously reported at different electrode surfaces [30].

The presence of SWCNT-COOH was also verified by CVs measurements in 0.05 M PBS solution containing 100 mM KCl between -0.2 and 1.0 V at a different scan rate of 10, 25, 50, 75, 100, 250, 300, 400 and 500 mVs^{-1}, as shown in Figure 5(b). From the observation, the same pattern is retained from 10 to 100 mVs^{-1} with the appearance of the oxidation peak at 0.1 V, while at a higher scan rate above 250 mVs^{-1}, the redox peak is less visible. According to Venton et al., the rate of diffusion-controlled of the Faradaic currents that occur at the surface of the electrode is proportional to the square root of scan rate resulting in, an increase of background current at a faster rate compared to the Faradaic currents [31]. Thus, the scan rate to be used in this study was at 50 mVs^{-1}.

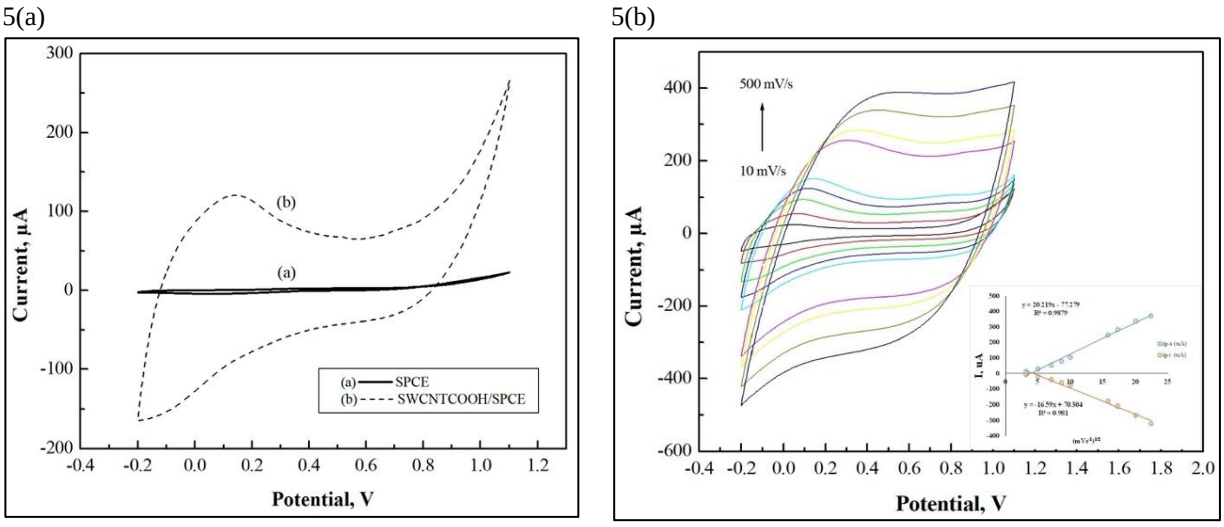

Figure 5: (a) CVs of bare SPCE and SWCNT-COOH/SPCE (50 mM PB buffer solution pH 8.0 containing 100 mM KCl) and (b) CVs of SWCNT-COOH/SPCE (0.05 M PBS solution containing 100 mM KCl) inset: plots of peak current vs square root of scan rate and recorded at 10, 25, 50, 75, 100, 250, 300, 400 and 500 mVs^{-1} inset: plots of peak current vs square root of scan rate.

Optimization of modified electrode composition for determination of tyramine

In this study, the effect of pH was analyzed in a wide range, starting from pH 3.0 to 11.0. Figure 6(a) shows the influence of pH in the detection of tyramine (TYR) at pH 7.0 to 10.0. However, no peak was observed at pH less than 6 and above pH 11 due to the deactivation of tyro under strong acid and alkaline conditions. It can be seen that the activity of tyrosinase (tyro) increased first up to pH 8 and then decreased until pH 10.0. The decreased response of the biosensor might be due to the absence of the biocatalytic activity resulting in enzyme denaturation [32]. Therefore, pH 8.0 was chosen as the optimum pH in all experiments in the electrochemical determination of TYR.

Figure 6(b) shows the effect of the SWCNT-COOH concentration on the modified electrode. This study is important to identify a suitable concentration of SWCNT-COOH on the modified electrode in which increasing performance is correlated with the small size of SWCNT-COOH, which enhanced the large surface area of the electrode. When the enzyme is added in the following stage, the electrode's high specific area allows for the immobilization of higher concentrations of bioreceptor units relative to the biosensor surface [33]. The graph shows an increased pattern starting at 0.2 mg/ml, and a higher reading was observed at 0.5 mg/ml. However, further increasing the concentration of SWCNT-COOH caused a decrease in biosensor response. This can be attributed to the thickness of the nanocomposites that will block the electric conductivity on the surface of the electrode [34]. Thus, 0.5 mg/ml of SWCNT-COOH was selected as the optimum concentration for biosensor fabrication of the SWCNT-COOH layer on SPCE.

Figure 6(c) depicts the effect of increasing the enzyme amount from 2, 4, 6 and 8 L. However, at 10 µL of tyro, a slight decrease in the biosensor response was observed, which can be attributed to the protein loading on the electrode surface rising due to mass transfer diffusion limitation through the electrode surface. Thus, the amount of enzyme at 8 µL was chosen as the optimum parameter in the modification of the biosensor.

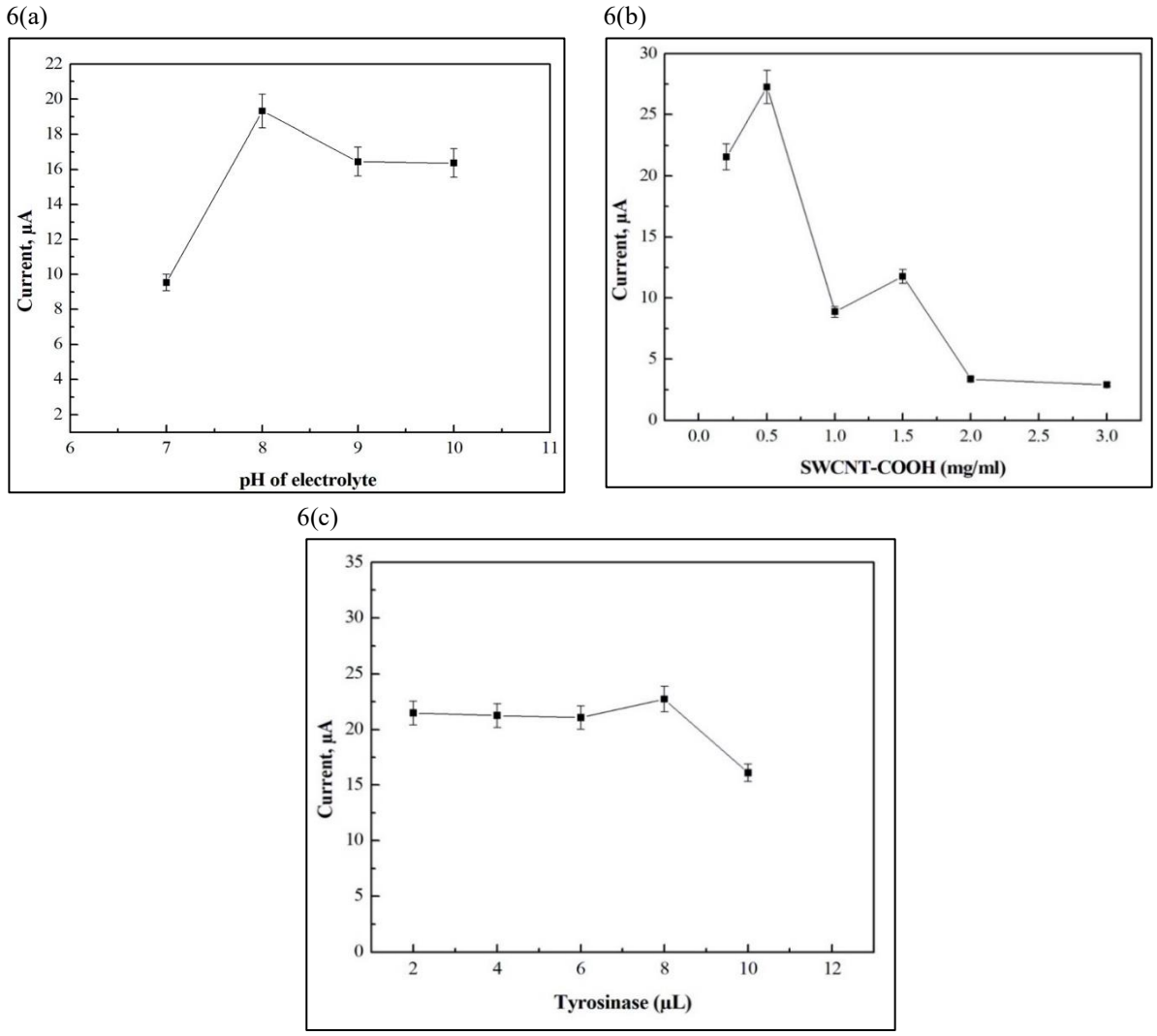

Figure 6. (a) The influence of pH value, (b) concentration of SWCNT-COOH, and (c) amount of tyrosinase on the response of Tyro-SWCNT-COOH/SPCE in 50 mM PB solution containing 0.05 M tyramine at +0.05 V

Figure 7(a) shows the CVs of three-electrode modifications in the presence of 0.05 M tyramine (TYR): bare SPCE (curve a), Tyro-SWCNT-COOH/SPCE (curve b), and SWCNT-COOH/SPCE (curve c) in 0.05 M PB solution pH 8.0, analyzed by CVs from -0.6 to 1.0 V at a scan rate of 100 mVs^{-1}. The peak potential of TYR at modified SWCNT-COOH/SPCE was observed at 0.67 V. While for bare SPCE, no oxidation peak was detected, showing that the modification of electrode SPCE using SWCNT-COOH and enzyme tyro was successfully fabricated. A similar peak potential for TYR oxidation was also reported previously [35].

The standard addition method was investigated to confirm the peak potential of TYR at Tyro-SWCNT-COH/SPCE using differential pulse voltammetry (DPV). Figure 7(b) showed the continuous successive additions of TYR in PBS (0.05 M, pH 8.0) ranging from 0.05 mM to 0.15 mM. The oxidation peak appeared at 0.67 V, and the peak current gradually increased with TYR concentration. From the calibration plot, the correlation coefficient (R^2) was found to be 0.9749 with

a low limit of detection (LOD) value of 0.02 mM. It can be concluded that the Tyro-SWCNT-COOH/SPCE is a sensitive biosensor for TYR detection, which offers lower detection limits.

7(a) 7(b)

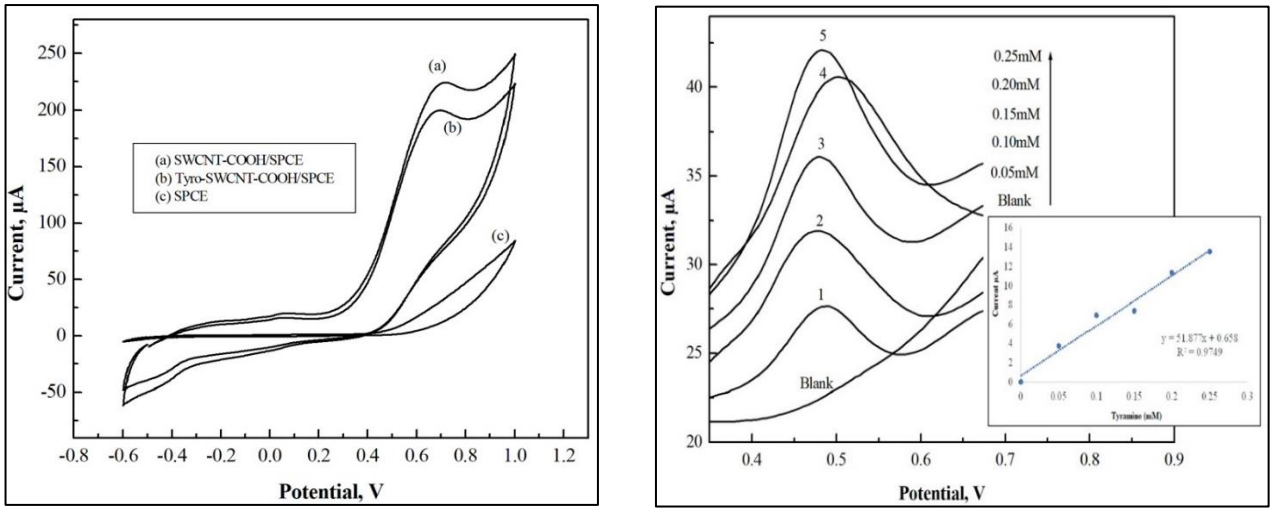

Figure 7. (a) CVs of SPCE, SWCNT-COOH/SPCE, Tyro-SWCNT-COOH/SPCE and in the presence of 0.05 M Tyramine in 0.05 M PB buffer solution pH 8.0. (b) Differential pulse voltammetry (DPV) response of tyro-SWCNT-COOH/SPCE in the presence of tyramine (0.05 to 0.15 mM) in 0.05 M PBS of pH 8.0 at a scan rate of 100 mVs^{-1}. Calibration plot of the current response vs the concentration of tyramine

Conclusion

In this work, a novel biosensor based on Tyro-SWCNT-COOH/SPCE was successfully fabricated, characterized, and optimized for tyramine (TYR) detection. Single-walled carbon nanotubes carboxyl functionalized (SWCNT-COOH) were successfully synthesized and used as a biocompatible matrix for tyrosinase (tyro) immobilization. The SWCNT functionalized -COOH contributed to a suitable microenvironment for immobilization of enzyme, thus retaining the bioactivity of a tyro. The optimum parameters used for this research for the Tyro-SWCNT-COOH/SPCE biosensor exhibit good performance at a scan rate of 50 mVs^{-1}, pH 8.0 of phosphate buffer solution (PBS), 8 µl enzyme tyrosinase and 0.5 mg/ml of SWCNTs. The developed biosensor showed a good lower correlation coefficient (R^2) 0.9749 and detection limits (LOD) of 0.02 mM. All these satisfactory results show that SWCNT-COOH and tyro have good properties as electroactive nanomaterials for biosensor surface modification in the determination of TYR. The biosensor developed could be used to determine TYR in real food sample analysis in the future.

Acknowledgement

This work was supported by the Ministry of Higher Education Malaysia (MOHE) for the financial support through Fundamental Research Grant Scheme for Research Acculturation of Early Career Researchers (FRGS-RACER) [Grant code: USIM/FRGS-RACER/FST/50919].

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