DETECTION OF QUINOLINE USING AMMONIA PLASMA-FUNCTIONALISED MULTI-WALLED CARBON NANOTUBES AND APPLICATION TO REAL SAMPLES

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ABSTRACT

Study on the growth of multi-walled carbon nanotubes (MWCNT) using plasma-enhanced chemical vapour deposition (PECVD) techniques has been carried out in this work. MWCNT screen-printed electrode (SPCE) based on silicon substrate was fabricated for quinoline detection using photolithography methods. The MWCNT working electrodes were modified under ammonia (NH₃) plasma to enhance the surface sensitivity to detect quinoline. The electrocatalytic activity of modified electrode towards oxidation of quinoline was evaluated using cyclic voltammetry (CV) and linear sweep voltammetry (LSV). Under experimental conditions, peak currents increased with an increase in the concentration of quinoline (dynamic range = 2.0 to 25.0 µM) and displayed good linearity ($R^2 = 0.9958$). The limit of detection (LOD) was calculated to be 0.164 µM. The modified electrode was then applied to real samples sourced from both healthy and Ganoderma boninense *infected oil palm roots crude extracts. A linear plot was obtained with a good linearity ($R^2 = 0.9879$), indicating good sensing results.*

INTRODUCTION

Ganoderma is a group of wood degrading fungi with hard fruiting bodies. Various species of

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Ganoderma have been identified in previous studies (Hushiaran *et al.*, 2013). *Ganoderma* sp. has been reported as the major cause of basal stem rot (BSR) and upper stem rot diseases which eventually lead to the death of oil palm. In 1985, Ho and Nawawi collected several hundred sporophores of *Ganoderma* from 5-40 years old oil palms infected with BSR in five oil palm estates in Peninsular Malaysia to identify the species of *Ganoderma*. They found that all the *Ganoderma* sporophores that they collected belonged to a single species, *i.e. Ganoderma boninense*. Recent findings showed that BSR disease as a single major devastating disease constraint to oil palm plantations in Malaysia and Indonesia (Azahar *et al.*, 2014; Lisnawati *et al.*, 2016). Despite the presence of various *Ganoderma* species, *Ganoderma boninense* has been reported as the main pathogen causing the BSR disease in oil palm (Khairuddin, 1990).

Plants produce a variety of primary and secondary metabolites in response to various environmental factors. Secondary metabolites play important roles in plant protection which might involve deterrent or anti-feedant activity, toxicity or acting as precursors to physical defence systems. Numerous specialist herbivores and pathogens do not merely avoid the deterrent or toxic effects of secondary metabolites but actually utilise these compounds as either host recognition signals or nutrients or both (Bennet and Wallsgrove, 1994; Nusaibah *et al.*, 2016).

Ganoderma boninense infection in oil palms surges the release of metabolites which can be used as indicators for early detection of infection in oil palms (Choon, 2011; Dutse *et al.*, 2012; Fang and Ramasamy, 2015; Idris *et al.*, 2006; Idris and Rafidah, 2008; Madihah *et al.*, 2014; Mohd Su'ud *et al.*, 2007; Nurnadiah *et al.*, 2014; Wong *et al.*, 2012). Nusaibah *et al.* (2016) has studied the accumulation of an alkaloid secondary metabolite such as quinoline in oil palm roots at early stages of *Ganoderma boninense* infection. Quinoline which is belong to alkaloids group of secondary metabolites is derived from tryptophan, an amino acid-based precursor (Akanbi *et al.*, 2017). Development of rapid detection of the quinoline compound at the early stage is crucial to prevent the continuous devastating loss of stands in the oil palm plantations.

Electrochemical sensors have extensively been applied in many fields such as biomedical, environmental and agricultural with diverse industrial applications. High requests of electrochemical sensors in the world market are due to their compatibility, fast result and easy to handle (miniature in size, usually micro to nano range). In electrochemical biosensors, measurement of current produced is taken either from oxidation or reduction process. The current response is either due to the concentration of the electroactive species present or its rate of production or consumption. The resulting electrical signal is related to the recognition process by target analyte, and is proportional to the analyte concentration.

In this study, an electrochemical sensor using voltammetry approach was fabricated based on multi-walled carbon nanotubes (MWCNT) material as sensing part. Even though carbon nanotubes (CNT) display many outstanding properties but their surface inertness has restricted the application of CNT as the sensing area on the sensor. To overcome this limitation, modification on the surface of CNT has been one of the most vital aspects in carbon nanotube studies. Production of MWCNT/silicon (Si) screen-printed electrode (SPE) is necessary to provide plasma growth of the nanotubes and also plasma modification on the surface of the working electrodes. Compared to conventional chemical method, plasma method offer several advantages

such as highly dense and uniform CNT films productions with an outstanding reproducibility and adhesion at relatively high deposition rate (Fu and Yu, 2014).

Our previous work has reported the post-treatment effects of applying different plasma atmospheric conditions such as hydrogen (H_2), oxygen (O_2) and ammonia (NH_3) plasma on the electrochemical performances of the MWCNT (Abdul Rahim *et al.*, 2018). Surface modification is a good way to improve the electrical conductivity of the MWCNT. Based on our findings, the NH_3-treated MWCNT show the highest efficiency as compared to the other pre-treatments and control. This is due to the enhanced surface area of the composite, which improved adsorption and associated interaction with that of the analyte molecules at the electrodes.

In the present work, the electrochemical characterisation of the NH_3/MWCNT electrodes for the detection of quinoline and the application to the real samples sourced from both healthy and *Ganoderma boninense* infected oil palm roots crude extracts were carried out to determine the performance of the modified electrode.

MATERIALS AND METHODS

Preparation of Working Solution

Quinoline stock solution (1 mol litre^{-1}) was prepared by diluting 8.9 mol litre^{-1} standard solution of quinoline with deionised water. Working solution of quinoline was prepared just before use by diluting the stock solution in 0.1 mol litre^{-1} phosphate buffer solution (PBS) supporting electrolyte.

Preparation of MWCNT Electrodes

Preparation of MWCNT electrodes followed our previously reported studies (Abdul Rahim *et al.*, 2018). Titanium (Ti) conducting layer and cobalt (Co) catalyst film were deposited onto Si substrates with crystallographic orientations (100) [(Si (100)] by using magnetron sputtering at a base chamber pressure of ~10^{-3} mbar and temperature of 42°C in 20 s. MWCNT were then synthesised by using plasma-enhanced chemical vapour deposition (PECVD) in an Oxford Instruments Nanofab-700 system at ambient pressure. The catalyst films underwent annealing process to promote the formation of catalyst nanoparticles for CNT growth in vacuum at 700°C for 10 min in NH_3; 99.99% pure at flow rate of 100 standard cubic centimeter per minute (sccm), with an applied plasma power of 200 W. The MWCNT growth was performed in the same furnace chamber with 50 sccm of acetylene, C_2H_2 (99.99% pure) at a pressure of 1000 mTorr at 700°C for 10 min. Both the pristine and

plasma-modified MWCNT were characterised by using electrochemical technique, *i.e.* potentiostatic system of cyclic voltammetry (CV).

Electrochemical Measurements

Electrochemical measurement was carried out using Dropsens Potentiostat with the screen-printed NH_3-MWCNT/Si electrode. The working and counter electrode were made up of CNT whereas the reference electrode was made up of silver. During electrochemical detection, 0.1 M PBS (pH 7.0) was used as the supporting electrolyte for the detection of quinoline. After 60 s deposition time at -0.6 V, the linear sweep anodic stripping voltammograms (LSASV) were recorded from -0.6 to 0.6 V, and the oxidation peak current at 0.15 V was measured. The scan rate used was 100 mVs⁻¹.

Electrochemical Response of Quinoline on NH_3/MWCNT Electrode

Different modified Si electrodes were used to investigate the oxidation response of 20 μM quinoline using LSASV. *Figure 2* shows the voltammograms of bare and modified electrodes towards detection of quinoline in 0.1 M PBS solution (pH 7.0) under optimal conditions (scan rate = 100 mV s⁻¹; potential range = -0.6 to 0.6 V).

Optimisation of the Working Conditions for Modified Electrode in Quinoline

Various working conditions for the detection of quinoline were optimised. These conditions included influence of pH on the current response, effect of accumulation potential and time, effect of varying scan rates and quinoline concentration. Each experiment was performed in triplicate.

Influence of pH on the Current Response

Different pH values from 4 to 12 of PBS solution (0.1 mol litre⁻¹) were analysed using scan rate of 100 mVs⁻¹. Potential scanning was performed in -0.6 to +0.6 V at scan rate 100 mVs⁻¹; accumulation potential -0.6 V and accumulation time for 50 s. The oxidation peak current was monitored with various pH values used in the experiment.

Effect of Accumulation Potential

Accumulation potential was varied towards positive direction from -1.0 to +0.6 V and applied for electrochemical analysis of NH_3/MWCNT using 0.1 M PBS as supporting electrolyte with accumulation time of 50 s. The oxidation peak current was monitored with various accumulation potential used in the experiment.

Effect of Accumulation Time

Accumulation potential was varied from 30-120 s and used for the electrochemical analysis of NH_3/MWCNT employing 0.1 M PBS (pH=7) as supporting electrolyte with accumulation potential of -0.6 V. The oxidation peak current was monitored with various accumulation time used in the experiment.

Effect of Varying Scan Rates

A series of scan rate from 10 to 100 mVs⁻¹ were used for the electrochemical analysis of NH_3/MWCNT in 0.1 M PBS (pH 7) with accumulation potential of -0.6 V used at 50 s accumulation time. The oxidation peak current was monitored with various scan rate used in the experiment.

Effect of Varying Quinoline Concentration

The NH_3/MWCNT was used to analyse different concentration of quinoline using LSASV technique. Potential scanning was performed in a positive direction at scan rate of 100 mVs⁻¹. The concentration of quinoline applied were 25 μM down to 2 μM diluted in PBS (pH=7) solution. Accumulation potential of -0.6 V was used at 50 s accumulation time.

Electrochemical Characterisation of Developed Electrode for Quinoline Detection

The reproducibility study of the modified NH_3/MWCNT was carried out in the concentration of 10 μmol litre⁻¹ quinoline standard solution. Reproducibility of the method was determined by analysing five different batches of similarly constructed modified SPE placed in quinoline solution and the average and standard deviation (SD) of the peak current were calculated. Stability of the NH_3/MWCNT was studied by recording the peak current every day with the single NH_3/MWCNT.

Analysis of Real Samples

Healthy and artificially inoculated *Ganoderma boninense* infected oil palm roots were used as real samples in this study to investigate the potential of developed electrode.

Inoculation of *Ganoderma boninense* Using Rubber Wood Block and Plant Cultivation

The rubber wood block was prepared according to the method previously described (Idris *et al.*, 2006). Cultures of *Ganoderma boninense* PER 71 were obtained from the stock collection of

the Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, Selangor, Malaysia. Rubber wood blocks of 6 cm x 6 cm x 6 cm were purchased from Wah Heng Rubber Wood Factory Sdn Bhd, Semenyih, Selangor. Cut pieces of *Ganoderma boninense* measuring 1 cm x 1 cm were inoculated into the rubber wood blocks and incubated in the dark for 30 days until the blocks were fully colonised.

Commercial DxP GH500 germinated seedlings (3 months old) from Sime Darby Seeds & Agricultural Services Sdn Bhd, Banting, Selangor were used in this work. Five germinated seedlings were treated with *G. boninense* inoculated rubber wood blocks and another five germinated seedlings with non-inoculated rubber wood blocks were used as control. Treatment was performed for up to 30 days. Leaf samples were harvested at 30 days post-infection. Liquid nitrogen was used to quench each sample and ground into powder form before being stored at -80°C. Freeze-dry system (Scanvac Labogene) was employed to remove the water content in the samples.

Extraction of Healthy and *Ganoderma boninense* Infected Oil Palm Roots and Sample Preparation

The root powder of infected oil palm (150 mg) was extracted via sonication in 250 ml, 80% commercial-grade methanol (30 min, 40°C). The supernatant was filtered by using filter paper (Whatman, 125 mm) and then evaporated by using rotary evaporator. Healthy and *Ganoderma boninense* infected oil palm roots crude extract stock solutions (200 mg litre⁻¹) were prepared by dissolving 2 mg of healthy and *Ganoderma boninense* infected oil palm roots crude extract in 0.1 ml methanol, followed by dilution in PBS solution (pH 7.0) in 10 ml volumetric flask. Different concentrations (10-100 mg litre⁻¹) of healthy and *G. boninese* crude extract solutions were prepared by serial dilution of the stock solution (200 mg litre⁻¹) of the respective extract.

Application of Developed Electrode to Real Samples

The characterisation of the developed electrode in real samples (healthy and *Ganoderma boninense* infected roots crude extracts) was conducted under the optimised conditions. The linear sweep voltammetry (LSV) measurement were performed for different concentrations of real samples. Reproducibility study was also performed in this work. Each real sample (healthy and *Ganoderma boninense* infected roots crude extracts) was determined in triplicates of LSV cycles and relative standard deviation (RSD) cum percentage recovery were evaluated.

RESULTS AND DISCUSSION

Figure 1 shows the schematic design of screen printed MWCNT electrode. Every single SPE was made up of reference electrode deposited with silver whereas counter and working electrodes were deposited with MWCNT.

In our previous work, we discussed the surface morphologies of as-grown MWCNT film before and after being exposed to NH₃ plasma, by providing the field emission scanning electron microscope (FESEM) images. From the FESEM analysis, the surface defects of the plasma modified MWCNT were improved after exposing to NH₃ plasma. The MWCNT surface changed from smooth to rough and the outer diameters of the modified MWCNT decreased.

Raman spectroscopy has been employed in our previous work to study the quality of MWCNT after treatment with NH₃ plasma (Abdul Rahim *et al.*, 2018). Three different peaks, *i.e.* 1360 cm⁻¹, 1580 cm⁻¹ and 2700 cm⁻¹ which referred to the D, G and 2D bands, respectively were observed. This observation confirmed the MWCNT formation. The existence of both D and 2D bands was due to the presence of defects or amorphous-phase carbon by the NH₃ plasma treatment. The presence of G band indicates the C-C stretching of the sp2 graphite or ordered-phase carbon. The ratio between D and G band intensity ($R = ID/IG$) which provides the information on the relative amounts of structural defects (degree of perfection) of the MWCNT was also carried out in our previous work. The value of R ratio increased after MWCNT was treated with NH₃ plasma. This can be indicated by the formation of electron deficient moieties at the MWCNT surface due to the NH₃ plasma treatment.
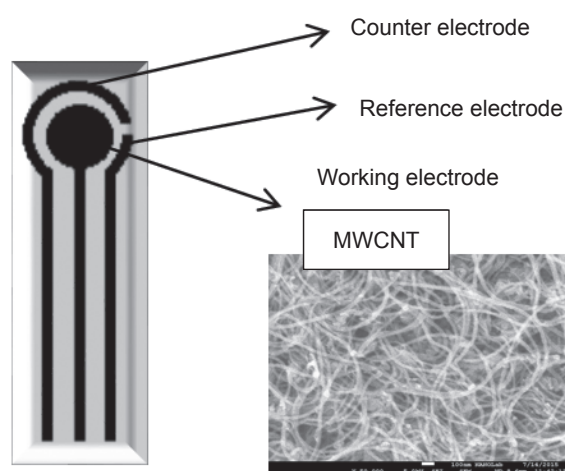

Figure 1. Schematic design of multi-walled carbon nanotubes (MWCNT)/silicon electrode.

Electrochemical Response of Quinoline on NH₃/ MWCNT Electrode

Differently modified Si electrodes were used to investigate the oxidation response of 20 μM quinoline LSASV. *Figure 2* shows the voltammograms of bare and modified electrodes towards detection of quinoline in 0.1 M PBS solution (pH 7.0) under optimal conditions (scan rate = 100 mV s⁻¹; potential range = -0.6 to 0.6 V). There is no significant oxidation peak observed after 60 s accumulation time after single solution of 0.1 M PBS solution (pH 7.0) was introduced to NH₃/MWCNT electrode (*Figure 2a*). An oxidation peak was observed in the curve of bare MWCNT electrode at the peak current of 87 μA with the potential of +0.12 V after addition of 20 μM quinoline into the 0.1 M PBS solution (pH 7.0) (*Figure 2b*). This indicates that the oxidation peak at +0.12 V is due to the oxidation of quinoline. The NH₃/MWCNT electrode was then used to observe the oxidation response of quinoline in 0.1 M PBS solution (pH 7.0). As shown in *Figure 2c*, a sharp and well-defined oxidation peak at +0.12 V

with the current response of 252 μA was observed. The NH₃/MWCNT electrode showed an increase in the oxidation response signal of quinoline when compared to bare MWCNT electrode (*Figure 2*).

Comparison of NH₃/MWCNT electrode with other plasma treatment electrodes such as H₂/MWCNT and O₂/MWCNT has been made in our previous work (Abdul-Rahim *et al.*, 2018). In our previous finding, NH₃/MWCNT electrode showed the highest redox peak currents followed by H₂/MWCNT and O₂/MWCNT. Functionalisation of NH₃ on MWCNT surface had greatly enhanced the surface performance by enlarging the surface area and this led to high accumulation efficiency of quinoline oxidation. Furthermore, improvement of the current signals is correlated to the increment of the diffusion rate of $K_3Fe(CN)_6$ and larger effective surface area that improved the sensitivity of the nanotubes' surfaces (Taurino *et al.*, 2012). Hence, the sensitivity of the electrode surface has been improved for detection of quinoline. The proposed chemical reaction for the oxidation of quinoline is shown in *Figure 3*.
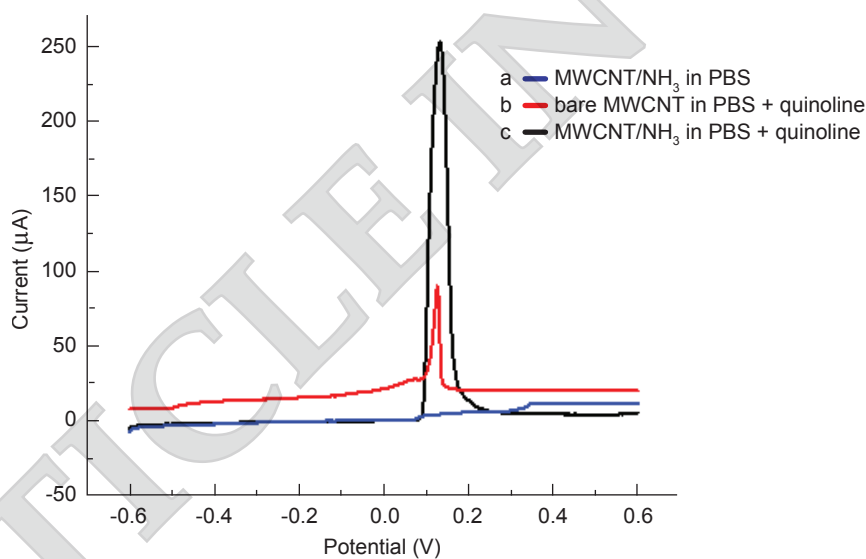

Figure 2. Linear sweep anodic stripping voltammograms (LSASV) curves of (a) ammonia/multi-walled carbon nanotubes (NH₃/MWCNT) electrode in 0.1 M phosphate buffer saline (PBS) solution (pH 7.0), (b) bare MWCNT electrode in 0.1 M PBS solution (pH 7.0) after addition of 20 μM quinoline, (c) NH₃/ MWCNT electrode in 0.1 M PBS solution (pH 7.0) after addition of 20 μM quinoline (accumulation potential of -0.6 V for 50 s).
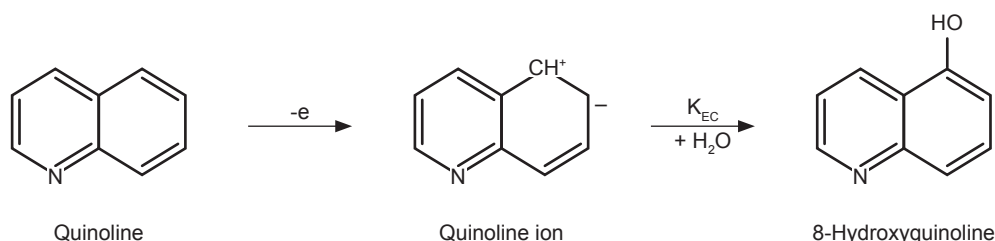

Figure 3. Chemical reaction for the oxidation of quinoline.

Optimisation of the Working Conditions for Modified Electrode in Quinoline

Various working conditions such as influence of pH on the current response, effect of accumulation potential and time, effect of varying scan rates and quinoline concentration for the detection of quinoline has been optimised in this work.

Influence of pH on the Current Response

In order to investigate the effect of pH on oxidation response of quinoline, different pH values of PBS solution were used. *Figure 4* shows the relationship between pH values with the oxidation peak currents of quinoline. When the pH values were increased from 4.0 to 7.0, the oxidation signal of quinoline increased gradually with maximum peak current at pH 7.0. When the pH was increased further, the oxidation peaks decreased drastically from pH 8.0 to 12.0.

At different pH values, the dissociation constant of quinoline also varies. At neutral or alkaline medium, quinoline mainly appears in solution as ion, but at acidic medium, quinoline exists in the form of molecule. The electrophilic reaction occurs more easily for ionic substance than for molecular substance (Wang *et al.,* 2015). Therefore, pH 7.0 of 0.1 M PBS solution was selected to be used for detection of quinoline to achieve high sensitivity.
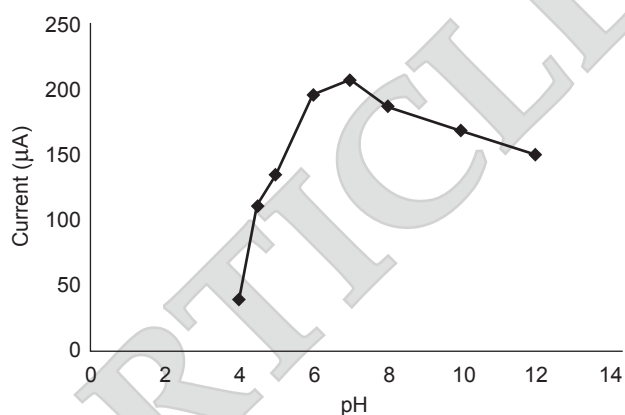

Figure 4. Graph of peak current of 20 μM quinoline in different pH of 0.1 M phosphate buffer solution (PBS) solution on ammonia/multi-walled carbon nanotubes (NH₃/MWCNT) electrode (accumulation potential of -0.6 V for 50 s).

Influence of Potential and Accumulation Time

Different potentials were measured individually to study the influence of accumulation potential towards the oxidation peak current of quinoline. As shown in *Figure 5a*, the oxidation peak current increased gradually with increasing accumulation potential from -1.0 to -0.6 V and then remained constant in range of -0.6 to 0.6 V. Therefore, the accumulation potential of -0.6 V was used in further measurements.

Another important aspect in stripping technique is the accumulation time. Anodic stripping voltammetry involves the pre-concentration of analytes on the surface of the working electrode. Thus, adequate period is required for analytes to be deposited on the electrode. In the period of pre-concentration step, the working solution is stirred constantly to permit escalation in mass transfer which allows more accumulation of the analyte on the surface of electrode.

Figure 5b represents the effect of accumulation time on the oxidation peak current of 20 μM quinoline. Extending the accumulation time from 30-50 s, the oxidation peak current of quinoline was at the highest amplitude at 50 s. It indicated that the longer accumulation period obviously improved the detection sensitivity. Further increase of the duration time up to 120 s, the oxidation peak current of quinoline remained constant, suggesting that the amount of quinoline saturation occurred at the surface of working electrode. Therefore, 50 s was chosen as the optimum accumulation time for the detection study.
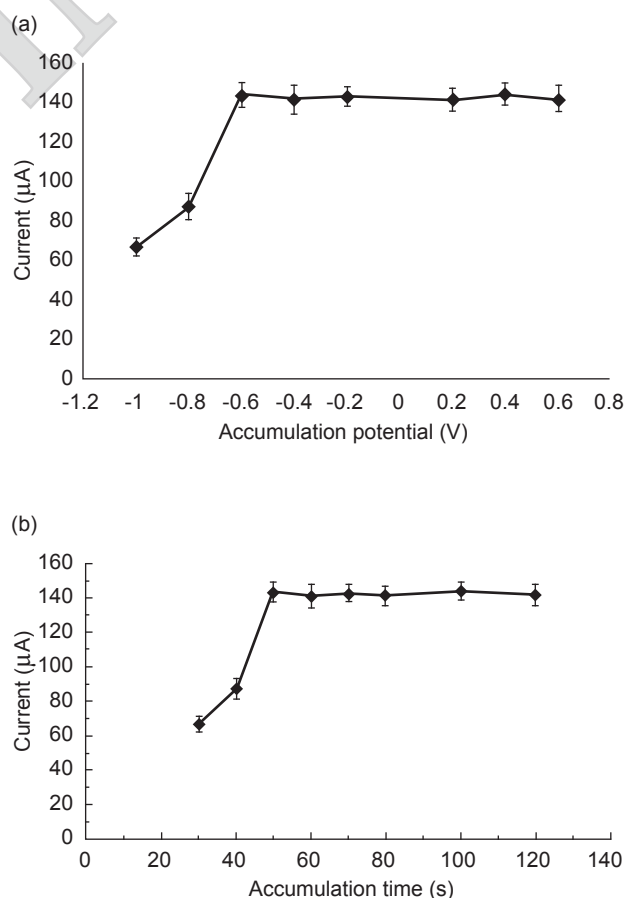
(a)

(b)

Figure 5. (a) Accumulation potential (V) and (b) accumulation time of the oxidation peak current of 20 μM quinoline in 0.1 M phosphate buffer saline (PBS) solution (pH 7.0).

Effect of Varying on Scan Rates

The oxidation responses of quinoline at different scan rates were determined as illustrated in *Figure 6a*. It was found that the oxidation peak current of quinoline at modified NH_3/MWCNT electrode increased linearly with the scan rate (v) in the series from 10 to 100 mV s^{-1}. The responded peak currents (ip) against square root of scan rates ($v^{1/2}$) is illustrated in *Figure 6b*. The plot shows linear relationship of ip towards mVs$^{1/2}$ in the range of 10 to 100 mV s^{-1} with correlation coefficient (R^2) of 0.9875 suggesting a diffusion controlled process of reactants on the electrode surface.

Effect of Varying Quinoline Concentration and Limit of Detection

The NH_3/MWCNT electrode was used to determine different concentrations of quinoline in the range of 2 - 25 µmol litre^{-1} using LSASV under the optimised conditions. *Figure 7a* shows the oxidation peak current of different concentrations of quinoline. It can be observed that a slight shift in the peak potentials of the quinoline with increasing concentration towards positive potential. The calibration graph (*Figure 7b*) was linear over the range of 2.0-2.5 µM quinoline with a detection limit of 0.164 µM. The limit of detection (LOD) was calculated using the 3σ method where the 3σ LOD was derived from 3SD/S (SD - standard deviation, S - the slope of the calibration graph). The linear regression equation was Ip (A) = 9.7669C (µM) +

32.866, and the correlation coefficient (R) was 0.9958, revealing excellent linearity.

As shown in *Table 1*, the LOD of the modified electrode was compared with other reported previously (Cinti *et al.*, 2014; Hosu *et al.*, 2016; Piovesan and Spinelli, 2014; Rouhani and Haji-Ghasemi, 2009; Stevic *et al.*, 2012). It can be suggested that the modified electrode in this work has better sensitivity.

Electrochemical Characterisation of Developed Electrode for Quinoline Detection

The reproducibility study was conducted to determine the precision of the electrodes. Reproducibility result was attained with six similar fabricated NH_3/MWCNT electrodes in 20 µmol litre^{-1} quinoline solution and 0.1 M PBS solution (pH 7.0). An excellent RSD value of 2.2% with average current at 201.83 mA and SD at 4.28 was obtained.

Stability study for NH_3/MWCNT electrode was investigated using single electrode for 12 weeks. *Figure 8* shows that the peak current began to decline from the second week up to week 12. The current response began to decline due to the etching of carbon nanotubes (CNT) lattice on the working electrode. It was observed that the colour of the reference site was degraded when it was placed in open site for more than 10 weeks. The percentage of stability of NH_3/MWCNT electrode was reduced about 50% after 10 weeks and almost 90% in 12 weeks time. This shows that the electrode has quite a high stability.

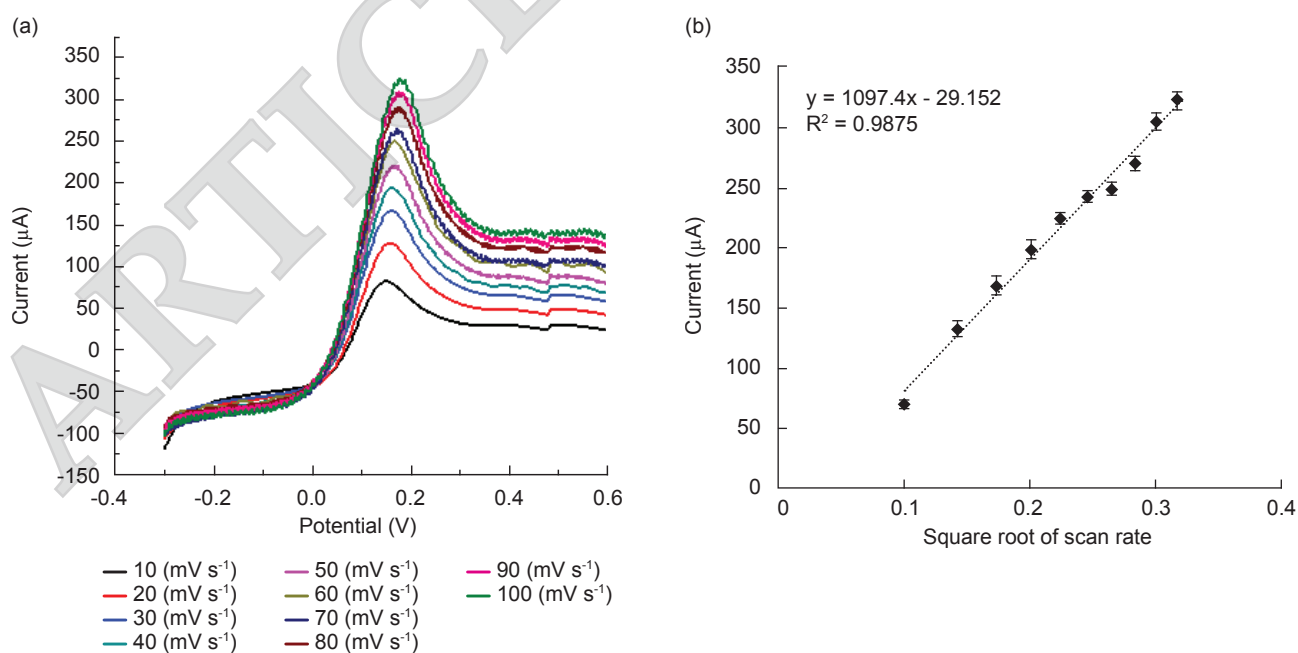*Figure 6. (a) Linear sweeps anodic stripping voltammograms (LSASV) curves of different scan rates of 20 µM quinoline, (b) oxidation peak current versus square root of scan rate of 20 µM quinoline in 0.1 M phosphate buffer saline (PBS) (pH 7.0) on ammonia/multi-walled carbon nanotubes (NH_3/MWCNT) electrode (accumulation potential of -0.6 V for 50 s).*
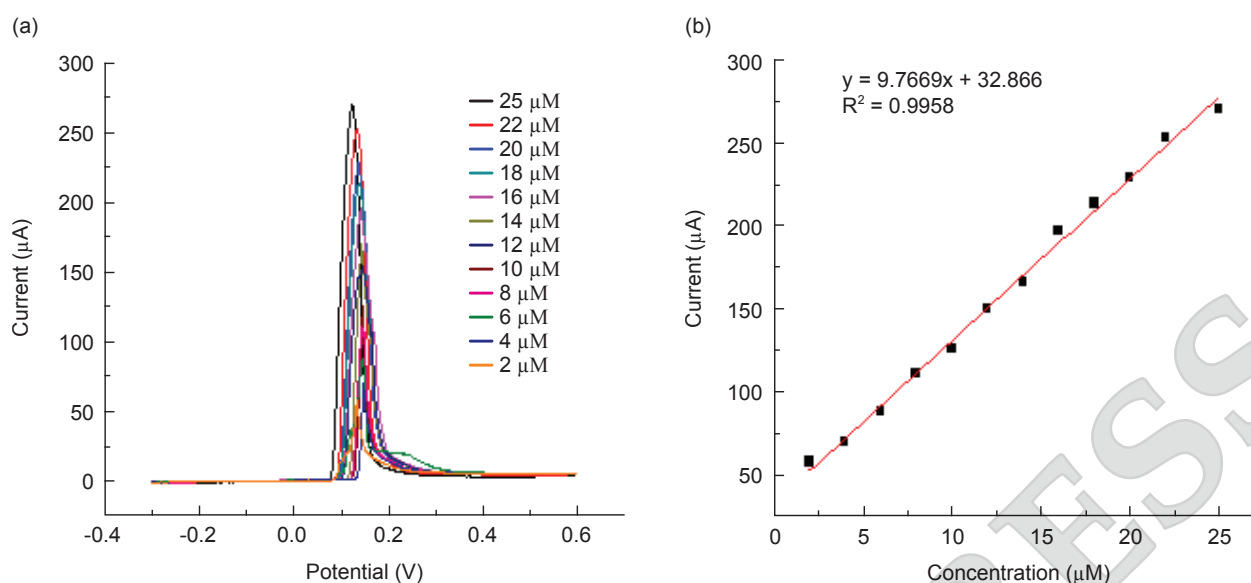
(a)

(b)

Figure 7. (a) Linear sweeps anodic stripping voltammograms (LSASV) of ammonia/multi-walled carbon nanotubes (NH₃/MWCNT) electrode in 0.1 M phosphate buffer saline (PBS) solution (pH 7.0) with different concentrations of quinoline, (b) current intensity versus concentration of quinoline in 0.1 M PBS solution (pH 7.0) (accumulation potential of -0.6 V for 50 s).

TABLE 1. ELECTROANALYTICAL APPROACHES USED TO DETECT METABOLITES WITH THE ELECTRODE MATERIALS, SENSITIVITY AND LIMIT OF DETECTION (LOD) OF THE METHOD USED

No.	Metabolite	Electroanalytical technique	Sensitivity / linear range (µM)	LOD (µM)	References
1.	6-Aminoquinoline	Cyclic voltammetry	1-10	0.43	Stevic *et al.* (2012)
2.	Quinoline yellow	Potentiometry	50-100 000	40	Rouhani and Haji-Ghasemi (2009)
3.	Quercetin	Cyclic and square-wave voltammetries	0.5-5.5	0.17	Piovesan and Spinelli (2014)
4.	Peroxynitrite	Cyclic voltammetry	2-300	0.2	Hosu *et al.* (2016)
5.	Hydrogen peroxide	Cyclic voltammetry	0-4.5	0.2	Cinti *et al.* (2014)

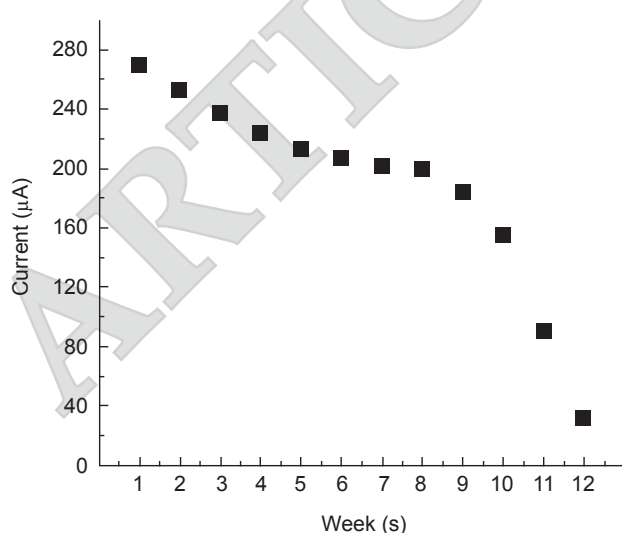*Figure 8. Stability study for 25 µM quinoline in 0.1 M phosphate buffer saline (PBS) solution (pH 7.0) on ammonia/multi-walled carbon nanotubes (NH₃/MWCNT) electrode (accumulation potential of -0.6 V for 50 s).*

Application of Modified Electrode to Real Samples

To validate the methodology, the proposed NH₃/MWCNT electrode was applied using real samples sourced from both healthy and *Ganoderma boninense* infected oil palm roots crude extract solutions.

Healthy and *Ganoderma boninense* infected oil palm roots crude extracts were analysed using LSASV. *Figure 9* shows the comparison of LSASV analysis of NH₃/MWCNT electrode in blank methanol, oil palm roots crude extracts solutions (healthy and *Ganoderma boninense* infected) at concentration of 100 mg litre⁻¹ in methanol and 0.1 M PBS solution (pH 7.0). No current peak was observed after running the LSASV analysis of modified electrode with blank methanol solution. Methanol was used as an extraction solvent in the preparation of roots crude extracts. Therefore, methanol was added into the supporting electrolyte of 0.1 M PBS solution (pH 7.0).

The voltammograms clearly showed that the intensity of *Ganoderma boninense* infected oil palm roots crude extracts was higher compared to the healthy oil palm roots crude extracts. This is due to the role of secondary metabolite such as quinoline as defence mechanism of plants towards any attack by pathogen. The nature of metabolite exhibits anti-microbial properties that help in the early detection of disease in plants (Meyer *et al.*, 2016).

A study on different concentrations of *Ganoderma boninense* infected oil palm roots crude extracts was also carried out. *Figure 10* shows the (a) voltammograms and (b) linear plot of *Ganoderma boninense* infected oil palm roots crude extracts ranging from 100 mg litre^{-1} down to 10 mg litre^{-1} concentrations. It can be observed that as the concentration reduced, the peak current showed lower readings. The linear plot gave a linear equation of y = 0.4753x - 2.2894 with correlation coefficient (R^2) = 0.9879.

Reproducibility result was attained with six similar fabricated NH$_3$/MWCNT electrodes in 100 mg litre^{-1} *Ganoderma boninense* infected oil palm roots crude extracts solution and 0.1 M PBS solution (pH 7.0). An excellent RSD value of 3.11%, SD at 4.28 with average current 46.87 µA was obtained in this study.
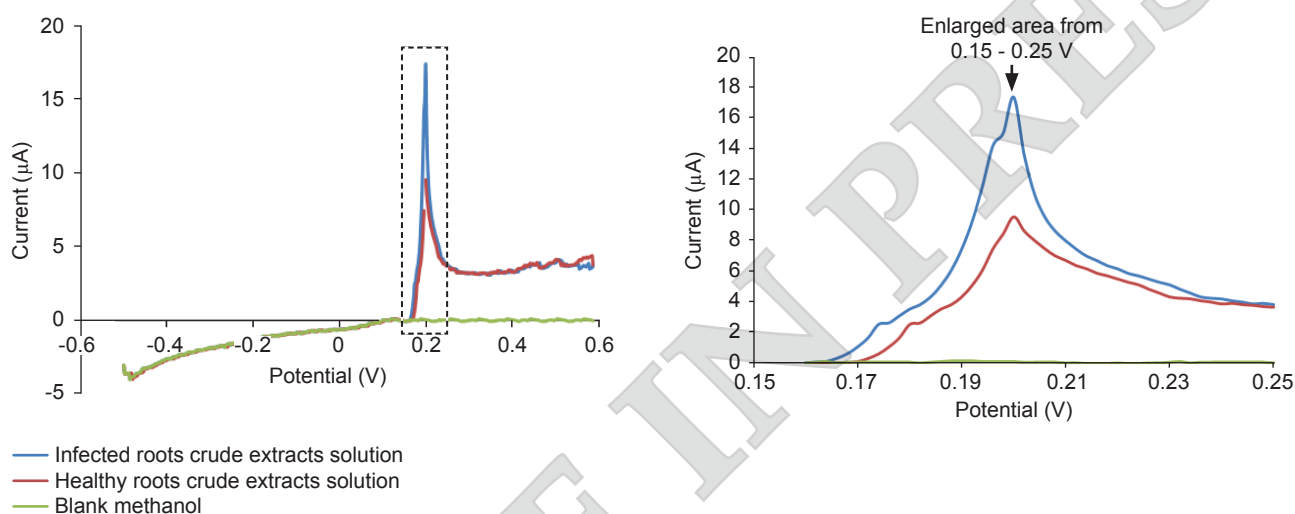

Figure 9. Voltammograms of blank methanol, oil palm roots crude extracts solutions (healthy and Ganoderma boninense *infected) at concentration of 100 mg litre^{-1} in methanol and 0.1 M phosphate buffer saline (PBS) solution (pH 7.0). (a)Potential (V) scale from -0.6 to 0.6 V and (b) enlargement of potential (V) scale from 0.15 to 0.25 V.*
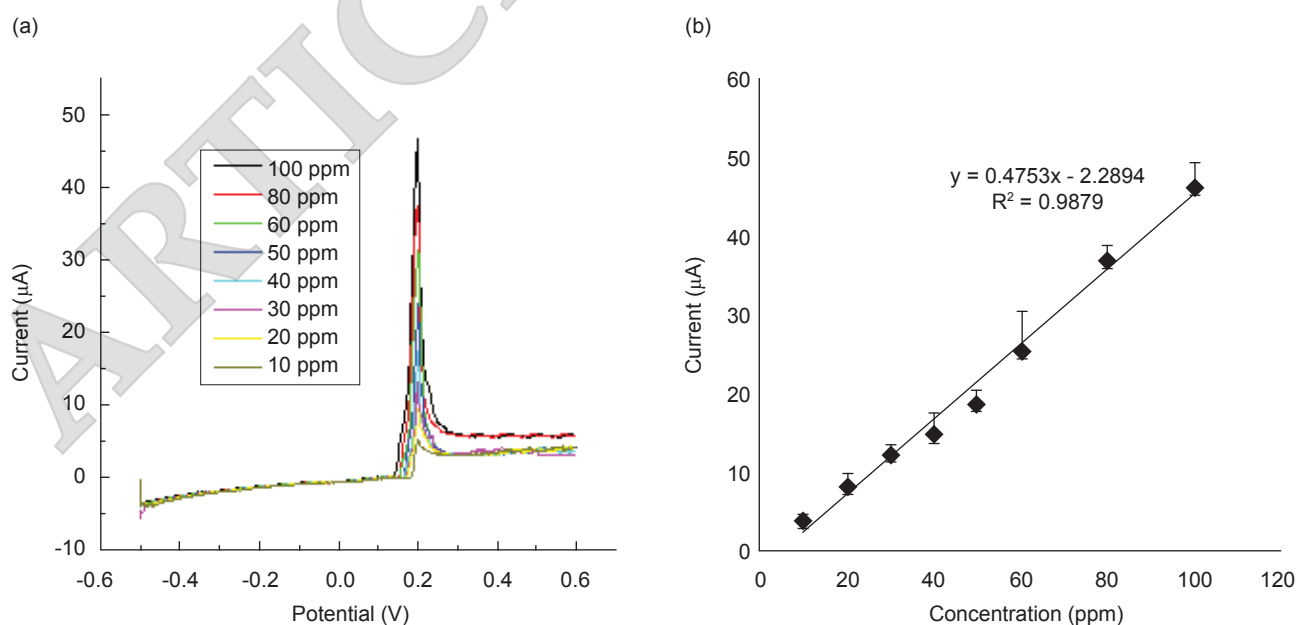

Figure 10. (a) Voltammograms and (b) linear plot of the Ganoderma boninense *infected oil palm roots crude extract solutions at different concentrations (100 mg litre^{-1} down to 10 mg litre^{-1}) (accumulation potential - 0.6 V for 50 s).*

CONCLUSION

A new fabricated NH$_3$/MWCNT electrode-based electrochemical sensor through photolithography process was successfully developed for the detection of quinoline. The linear range for quinoline detection was 2.0-2.5 µM under the optimised experimental condition which was applicable for real samples determination, *i.e.* healthy and *Ganoderma boninense* infected oil palm roots. The electrode also demonstrated good accuracy, stability and reproducibility, which might be due to contributive unique characteristics of NH$_3$-treated MWCNT interaction between them.

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