

Acetylcholinesterase Biosensor for the Detection of Methyl Parathion at an Electrochemically Reduced Graphene Oxide-Nafion Modified Glassy Carbon Electrode

Tharini Jeyapragasam¹, R. Saraswathi², Shen-Ming Chen^{3,*}, Tse-Wei Chen³

¹ Rajarajan Institute of Science, Madurai, Tamilnadu, India

² Department of Materials Science, School of Chemistry, Madurai Kamaraj University, Madurai, Tamilnadu, India

³ Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, Taipei, Taiwan

*E-mail: smchen@ntut.edu.tw

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We report the fabrication of a methyl parathion (MP) biosensor based on acetyl cholinesterase (AChE) immobilized on reduced graphene oxide (ERGO)-Nafion (Nf) modified glassy carbon electrode. ERGO-Nf acts as a biocompatible immobilization matrix for AChE for the analysis of MP. Electrochemical oxidation of acetylthiocholine chloride occurs at 0.7 V vs. Ag/AgCl in phosphate buffer solution (pH 7). Various experimental parameters such as effect of scan rate, MP concentration, acetylthiocholine chloride concentration, AChE loading and incubation time have been optimized for the sensor calibration. The calibration curve of MP has been constructed using square wave voltammetry in the concentration range of 2×10^{-9} M to 7×10^{-7} M and the sensor shows good reproducibility and stability. The sensor is found to have an estimated detection limit of 1×10^{-9} M. The biosensor has been applied for the determination of methyl parathion in a vegetable sample.

Keywords: Pesticide biosensor, acetyl cholinesterase, methyl parathion, electrochemically reduced graphene oxide, Nafion.

1. INTRODUCTION

Electrochemical biosensor is an analytical device that combines receptor-transducer functions to provide the information about the biological receptor. They have several advantages such as high sensitivity, selectivity and can be miniaturized for efficient use in on-line monitoring. Various electrochemical biosensors based on amperometry, potentiometry, voltammetry and impedance analysis have been developed for the detection of organophosphorus pesticides. However, they have

certain limitations including low mechanical stability, high diffusion resistance of substrate/bio component assembly and interferences from other compounds present in the real samples [1]. There have been several pioneering contributions made on electrochemical biosensors for organophosphorus and carbamate pesticides and several reviews have appeared in the literature [2-4]. Electrochemical biosensors for pesticides could be constructed using either living microorganisms such as algae, bacteria, fungi and yeast (cell based biosensors) [5], antigen and antibodies (immunosensors) [6], nucleic acids [7] and enzymes [8].

Methyl parathion (*o*, *o*-dimethyl *o*-(4-nitrophenyl) phosphorothioate, MP) is an organophosphorous compound widely used as a pesticide in agriculture [9]. It is used for pest control in a wide variety of crops including cereals, fruits, coffee, potato and sugarcane. It has been reported that MP irreversibly inhibits acetylcholinesterase (AChE) and leads to excessive cholinergic neurotransmission resulting in autonomic dysfunction. AChE and butyrylcholinesterase (BChE) are the two important enzymes widely employed in the detection of organophosphorus pesticides [10]. The AChE (or BChE) is a biorecognition element sensitive to inhibition by organophosphorus as well as carbamate compounds.

Organophosphorus pesticides such as paraoxon and MP and carbamate pesticides such as aldicarb were first determined with an amperometric hydrogen peroxide based AChE-choline oxidase biosensor [11, 12]. AChE enzyme biosensor based on a prussian blue modified electrode was developed for the detection of aldicarb, paraoxon and MP [13]. A cheap and disposable electrochemical biosensor based on AChE immobilized 7, 7, 8, 8-tetracyanoquinodimethane modified screen-printed electrode was reported for the determination of MP in egg, meat, milk and honey [14]. Law and Higson [15] developed a sonochemically fabricated AChE and polyaniline carbon/cobalt phthalocyanine biosensor for the detection of MP by flow injection analysis. Another biosensor based on gold/mercaptobenzothiazole/polyaniline/AChE/polyvinyl acetate was developed for the detection of MP in polar organic solvents [16]. Multi-walled carbon nanotube (MWCNT) was used for electrochemical biosensing of MP and chlorpyrifos pesticides [17]. The electrochemical biosensor was developed using AChE immobilized onto Au nanoparticles–polypyrrole nanowires composite film [18], sol-gel derived silicate network assembling gold nanoparticles [19], silica sol-gel carbon paste electrode [20], silk fibroin - MWCNT matrix [21], immobilized silica sol-gel film modified carbon paste [22] and nano CaCO₃-chitosan [23] for the detection of MP.

A highly sensitive MP sensor was developed based on organophosphate hydrolase loaded CdTe quantum dots onto a carbon nanotube/gold nanocomposite [24]. Recently, highly sensitive AChE enzyme-based biosensors were developed based on Au–Pd bimetallic nanoparticles onto a functionalized graphene [25], ionic liquid functionalized graphene and a Co₃O₄ nanoparticle [26], reduced graphene oxide/silver nanocluster/chitosan nanocomposite [27] and silver nanoparticles [28].

Yang *et al* [29] developed AChE enzymatic biosensor based on SnO₂ nanoparticles–carboxylicgraphene–Nf modified glassy carbon electrode for the detection of MP and carbofuran pesticide. The same group developed biosensors for MP and chlorpyrifos biosensor using platinum nanoparticles–carboxylic graphene–Nf-modified glassy carbon electrode [30], NiO nanoparticles–carboxylic graphene–Nf modified glassy carbon electrode [31] respectively.

In this work, we report the use of a simple chemically modified electrode viz. AChE immobilized electrochemically reduced graphene oxide-Nf for the detection of MP by square wave voltammetry. Various experimental parameters such as effect of scan rate, effect of enzyme concentration and effect of substrate concentration were optimized for the sensing of MP.

2. EXPERIMENTAL

Electrochemical measurements were performed on an electrochemical work station (CH Instruments, USA, Model 680). A one compartment cell with provision for three electrodes comprising glassy carbon electrode (GCE, 0.07 cm²) as the working electrode, silver-silver chloride electrode (Ag/AgCl) as reference and a large platinum foil electrode as counter electrode was used. Surface morphological studies were carried out using scanning electron microscope (SEM) (Hitachi S-3000 H). The surface topography of the samples was obtained using an atomic force microscope (AFM) (Shimadzu 9500). The IR data were obtained using a FT-IR spectrometer (Shimadzu 8400S).

Acetylthiocholine chloride, AChE (from *Electrophorus electricus*, electric eel), bovine serum albumin (BSA), pralidoxime chloride, MP and Nf were purchased from Sigma–Aldrich were used as received. The stock solutions of MP (0.001M) were prepared by dissolving the appropriate quantity of the pesticide in 10 mL of acetonitrile. A measured volume of the stock solution was diluted with the phosphate buffer solutions (PBS, pH 7) to prepare the pesticide solution of desired concentration. A stock solution of AChE was prepared by dissolving 500 U in 1 mL of PBS and to this 1 mg of bovine serum albumin (BSA) was added for stability and stored in a refrigerator. The stock solution was diluted appropriately to obtain various concentrations (0.05, 0.1, 0.5, 1, 1.5 and 2 U) of enzyme. All electrochemical experiments were carried out in 0.1 M PBS (pH 7). All aqueous solutions were prepared using double distilled water. Prior to each experiment, all the solutions were deoxygenated by passing pure N₂ gas for 15 min.

2.1 Preparation of graphene oxide (GO)

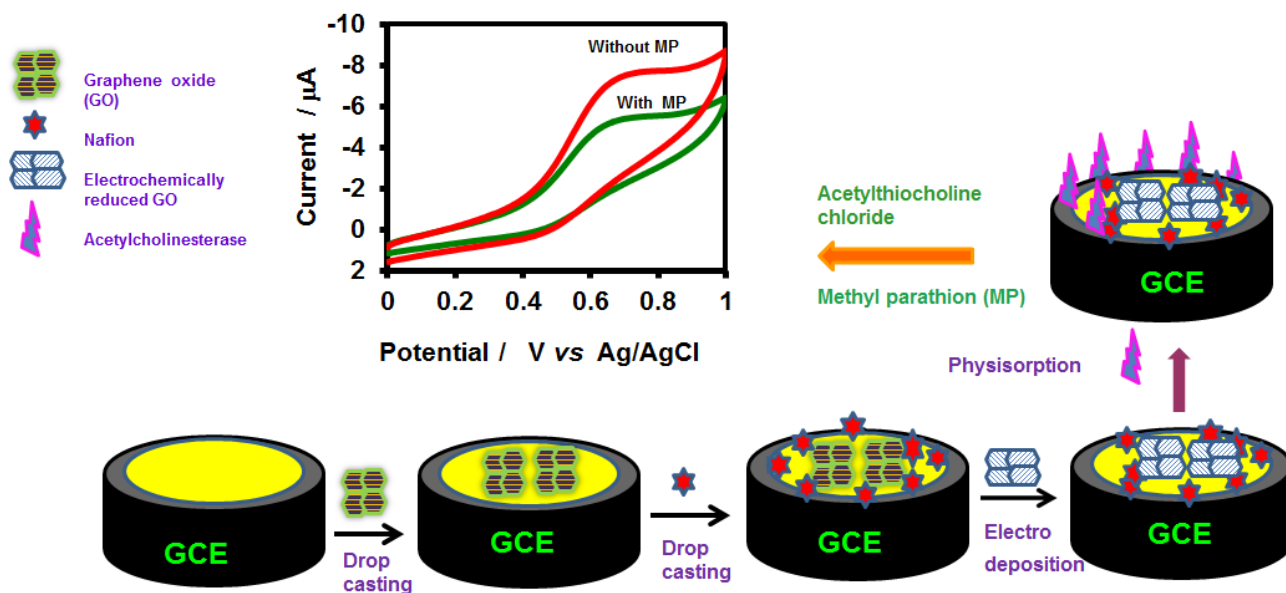
GO was prepared by Staudenmaier method [32-34]. About 5 g graphite was first mixed with sulphuric acid (87.5 mL) and nitric acid (45 mL) and stirred vigorously for 30 min to get dispersion. The mixture was then cooled to about 5 °C by immersion in an ice bath. To the ice-cold mixture potassium chlorate (55 g) was added slowly within 30 min and then added to complete the oxidation of graphite. The mixture was then added into a copious amount of distilled water and then filtered. The obtained graphite oxide solid was rinsed repeatedly and washed with a 5 % HCl three times followed by repeated washing with distilled water until the pH of the filtrate was neutral. The graphite oxide thus obtained was dried and the yield was found to be about 56%. About 2 mg of the graphite oxide was dispersed in 1 mL of water and sonicated for 2 h to get a uniform aqueous dispersion of GO.

2.2 Preparation of Electrochemically Reduced Graphene Oxide-Nafion modified glassy carbon electrode (ERGO-Nf/GCE)

The GO modified GCE (GO/GCE) was prepared by drop coating 5 μL of an aqueous dispersion of GO (2 mg/mL). About 5 μL of 1 wt % Nf was then drop coated on a dry GO/GCE and the surface was allowed to dry for about 20 min. The electrochemically reduced graphene oxide-Nafion (ERGO-Nf) was prepared by cycling the potential in the range between +0.2 to -1.5 V vs Ag/AgCl at 0.05 V s⁻¹ in 0.1 M PBS (pH = 5) for about 15 cycles until the cathodic peak at -1.35 V disappeared completely.

2.3 Immobilization of AChE onto ERGO-Nf/GCE

About 5 μL of 0.1 U of the enzyme solution was drop coated onto ERGO-Nf/GCE and dried for 1h. Finally, the electrode was rinsed with PBS and stored at 4°C when not in use. The AChE enzyme immobilized electrode is denoted as AChE/ERGO-Nf/GCE. Scheme 1 outlines the various steps involved in the fabrication processes and the methodology of the biosensor.

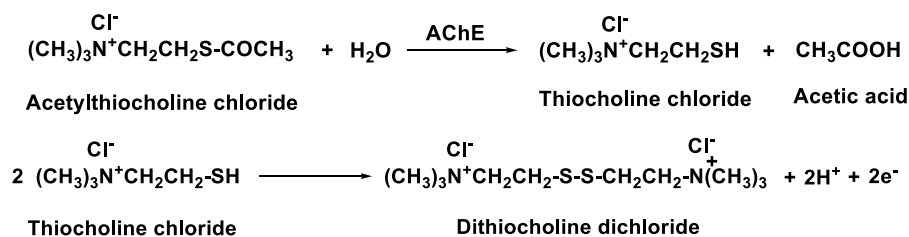


Scheme 1. Preparation of the enzyme immobilized ERGO-Nf/GCE for the detection of methyl parathion

2.4 Measurement procedure

The principle of an electrochemical biosensor based on AChE is shown in Scheme 2. In the absence of the pesticide analyte, the substrate acetylthiocholine chloride is converted by hydrolysis to thiocholine chloride and acetic acid. Thiocholine is electroactive and can be oxidized to dithiocholine

at an appropriate applied voltage. In the presence of the pesticide (inhibitor), the conversion of acetylthiocholine chloride is decreased. The anodic oxidation current of acetylthiocholine chloride is inversely proportional to the concentration of pesticide.



Scheme 2. The principle of an electrochemical AChE biosensor [2]

The inhibition (%) is estimated using the formula shown in Eq. 1 [35].

$$\text{Inhibition}(\%) = \frac{i_{p,control} - i_{p,exp}}{i_{p,control}} \times 100 \qquad (1)$$

where $i_{p,control}$ is the oxidation peak current of acetylthiocholine chloride at the enzyme modified electrode in the absence of pesticide and $i_{p,exp}$ is the peak current of acetylthiocholine chloride at the enzyme modified electrode after inhibition of the enzyme by pesticide.

3. RESULTS AND DISCUSSION

3.1 Morphological characterization of the modified electrodes

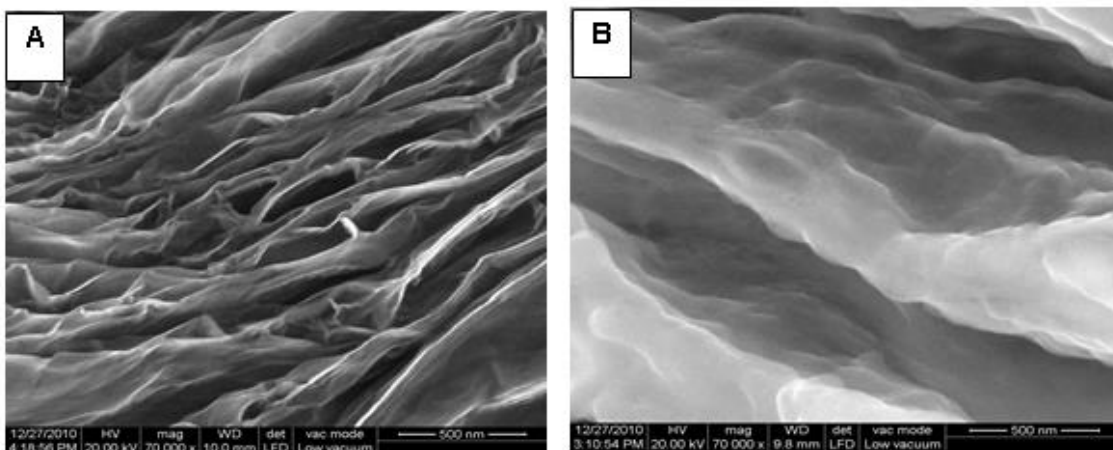


Figure 1. HRSEM images of (A) ERGO-Nf (B) AChE/ERGO-Nf

High Resolution Scanning Electron Microscopy (HRSEM) was used to obtain information on the morphologies of ERGO-Nf/GCE and AChE/ERGO-Nf/GCE. Figs. 1 A & B show the HRSEM images of ERGO-Nf/GCE and AChE/ERGO-Nf/GCE respectively. The HRSEM of ERGO-Nf/GCE is akin to that of ERGO/GCE in that the crumpled graphene sheets can be observed. This means that

the presence of Nafion on the surface has not changed the morphology of graphene. On the other hand, the HRSEM image of the AChE/ERGO-Nf/GCE is different from that of ERGO-Nf/GCE in that the graphene sheets have become somewhat dense and as a result, a rather smooth morphology is observed. The change in the morphology between the two electrodes observed thus confirms the immobilization of AChE.

FT-IR spectroscopy is used to identify the residual functional groups present in ERGO-Nf. Fig. 2A shows the FT-IR spectrum of ERGO-Nf with a broad peak at about 3400 cm^{-1} which can be assigned to the O-H stretching mode of intercalated water molecules. The presence of C=C (1646 cm^{-1}), SO_3^- (1230 cm^{-1}) and OH (3496 cm^{-1}) groups can also be confirmed [31,32].

Electrochemical impedance (EIS) can be used as a tool to confirm the electrode surface modification by the immobilization of AChE. Fig. 2B shows typical Nyquist plots obtained in the frequency range from 1 Hz to 1 MHz at GCE, ERGO/GCE, ERGO-Nf/GCE and AChE/ERGO-Nf/GCE in 0.1 M KCl containing 1 mM $\text{K}_4[\text{Fe}(\text{CN})_6]$. The electron transfer resistance value at bare GCE is $496\ \Omega$ while that of ERGO/GCE is reduced to $128\ \Omega$ which can be attributed to the high conductivity of the electrochemically formed graphene. The electron transfer resistance value increases to $1020\ \Omega$ when the ERGO is covered by Nf. When the AChE is immobilized on the surface of ERGO-Nf/GCE electrode, the value increases substantially to $1802\ \Omega$ due to the increase of the thickness of the interface by the enzyme layer. This result clearly demonstrates that the AChE has been successfully immobilized onto the ERGO-Nf surface.

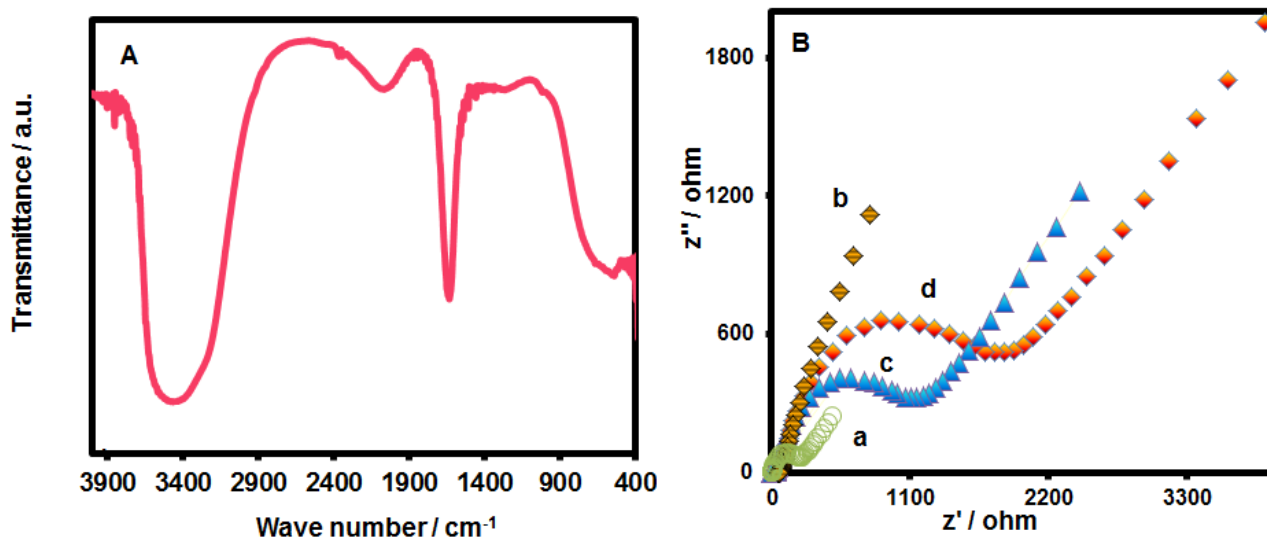


Figure 2. (A) FT-IR spectrum of ERGO-Nf (B) Nyquist plots of (a) GCE (b) ERGO/GCE (c) ERGO-Nf/GCE (d) AChE/ERGO-Nf/GCE in 0.1 M KCl containing 1×10^{-3} M $\text{K}_4\text{Fe}(\text{CN})_6$.

3.2 Electrochemical behavior of AChE/ERGO-Nf/GCE

The cyclic voltammogram of AChE/ERGO-Nf/GCE at 0.1 V s^{-1} in the presence of 1×10^{-3} M acetylthiocholine chloride in PBS (pH 7) shows an irreversible oxidation peak at -0.66 V (Fig. 3A). A single irreversible oxidation peak at -0.66 V is observed which can be ascribed to the oxidation of

acetylthiocholine chloride to thiocholine, catalyzed by the immobilized AChE. The effect of scan rate is shown in Fig.3B. The acetylthiocholine chloride oxidation peak current can be linearly correlated with the square root of scan rate which indicates that the reaction is diffusion-controlled (Inset in Fig 3B). Fig. 3C shows the cyclic voltammogram of AChE/ERGO-Nf/GCE after incubation in 1×10^{-5} M and 2×10^{-5} M MP for 20 min followed by rinsing in 0.1 M PBS (pH 7).

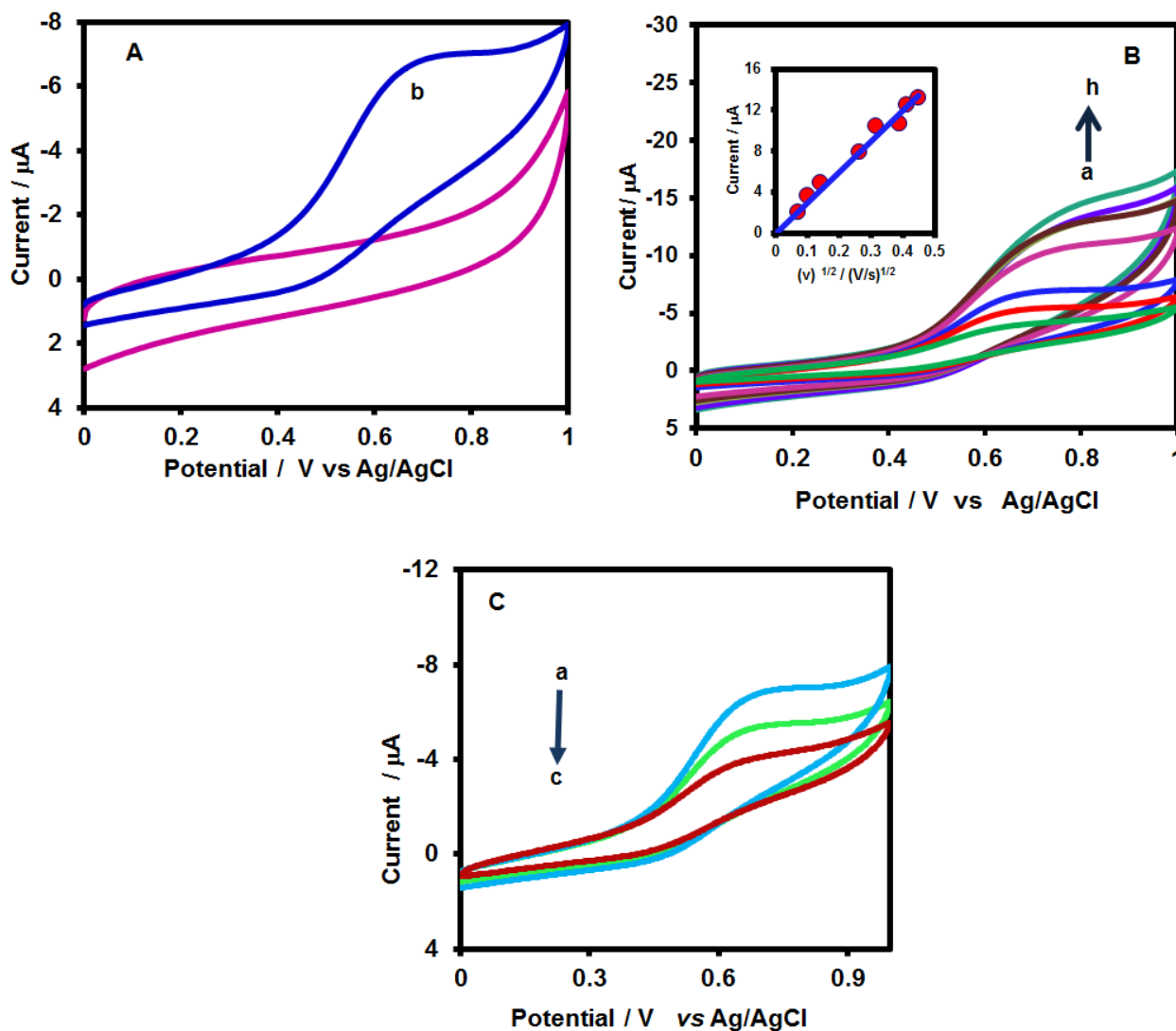


Figure 3. (A) CVs of (a) AChE/ ERGO-Nf/GCE (b) AChE/ ERGO-Nf/GCE in 0.1 M PBS (pH 7.4) containing 1×10^{-3} M ATCl. Concentration of AChE used for immobilization - 0.1 U. (B) Effect of scan rate from { (a) 0.005 (b) 0.010 (c) 0.02 (d) 0.05 (e) 0.07 (f) 0.10 (g) 0.15 (h) 0.2 V s^{-1} }. Inset : Plot of anodic peak current vs square root of scan rate. (C) Cyclic voltammograms of (a) AChE/ ERGO-Nf/GCE (b) AChE/ ERGO-Nf/GCE after incubation in 1×10^{-5} M MP (c) AChE/ERGO-Nf/GCE after incubation in 2×10^{-5} M.

It can be observed that the acetylthiocholine chloride oxidation peak current decreases with increasing concentration of MP. The acetylthiocholine chloride oxidation peak current serves as a quantitative measure of AChE enzyme activity. The decrease in acetylthiocholine chloride oxidation peak current after incubation in MP solutions is due to the irreversible inhibition of the enzyme by the pesticide. The extent of inhibition is observed to depend on the concentration of MP.

3.3 Effects of enzyme and substrate concentration, inhibition time on AChE/ERGO-Nf/GCE

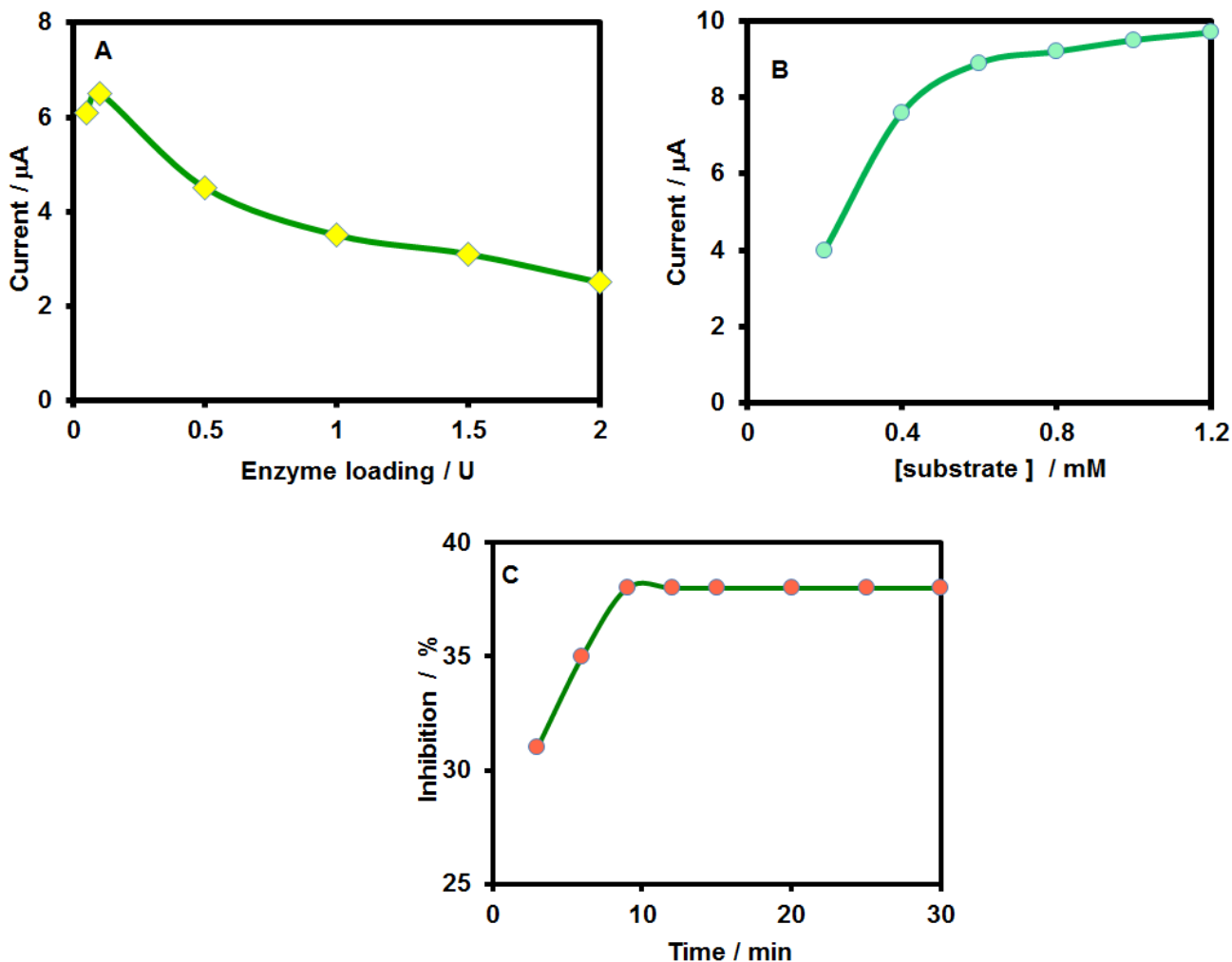


Figure 4. (A) A plot of current magnitude against enzyme loading (B) A plot of current magnitude vs substrate concentration. (C) A plot of inhibition (%) against incubation time.

The performance of the biosensor depend on various factors such as enzyme concentration, substrate concentration and inhibition time [18] and hence it is important to optimize these parameters before attempting on sensor calibration. The effect of AChE enzyme loading is investigated in 0.1 M PBS (pH 7) containing 1×10^{-3} M acetylthiocholine chloride. The enzyme concentration is varied in the range from 0.025 U to 2 U. A plot of acetylthiocholine chloride oxidation peak current vs

enzyme concentration is shown in Fig. 4A. It is observed that the peak current increases with AChE reaching a maximum at 0.1 U and thereafter decreases gradually when the amount of AChE is increased further. This can be attributed to the increase of enzyme layer thickness, which can enhance the electrode resistance and slow down the electron transfer between substrate and electrode. Many authors have reported that a lower enzyme loading is often beneficial to achieve the lowest detection limit [4,10]. The concentration of acetylthiocholine chloride substrate is optimized using square wave voltammetry (SWV). Fig. 4B shows well defined current response of the AChE/ERGO-Nf/GCE for varying concentration of acetylthiocholine chloride ranging from 2×10^{-4} M to 1×10^{-3} M. The SWV peak currents are found to increase with concentration of acetylthiocholine chloride up to 4×10^{-4} M and thereafter tends to reach a limiting value. From the data obtained, the optimum concentration of acetylthiocholine chloride for further experiments is chosen as 1×10^{-3} M. The incubation time plays an important role in lowering the limit of detection of MP. The effect of incubation time on the response of AChE/ERGO-Nf/GCE biosensor is investigated at a concentration of 1×10^{-5} M MP. The incubation period is varied between 3 and 30 min. Then the cyclic voltammograms are recorded in 0.1 M PBS (pH 7) containing 1×10^{-3} M acetylthiocholine chloride. The inhibition (%) is estimated using the formula shown in 1[12]. Fig. 4C shows a plot of Inhibition (%) against incubation time for AChE/ERGO-Nf/GCE in 0.1 M PBS (pH 7) containing 1×10^{-5} M of MP. It is found that a minimum of 12 min. is required to obtain a stable response. However, for calibration experiments, the enzyme electrode is incubated for a constant period of 20 min. to ensure a stable response.

3.4 Calibration plot for MP using SWV

The SWV experiments have been performed in the potential range from 0 to 1 V using the following optimal parameters: pulse amplitude - 0.025 V, scan increment 0.005 V and frequency 15 Hz. Fig. 5 shows typical SWV voltammograms at AChE /ERGO-Nf/GCE in 0.1 M PBS (pH 7) in the MP concentration range between 2.0×10^{-9} M and 7×10^{-8} M. As the concentration of MP is increased, the SWV peak current of 1×10^{-3} of acetylthiocholine chloride is found to decrease due to enzyme inhibition effect. In order to construct the calibration plot, four independent measurements with freshly prepared AChE /ERGO-Nf/GCE were made at each MP concentration for an inhibition period of 20 min. The insert in Fig. 5 shows the calibration plot of the AChE /ERGO-Nf/GCE-based MP biosensor. The Inhibition (%) can be correlated linearly against the MP in the concentration range from 2.0×10^{-9} M – 7×10^{-8} M and can be represented by Eq. 2

$$\text{Inhibition (\%)} = 0.95 [\text{MP}] \quad (2)$$

The detection limit can be calculated using Eq. 3

$$\text{Detection Limit} = \frac{3 \times S}{N} \quad (3)$$

Where S is the standard deviation of mean current measured at the lowest analyte concentration used and N is the slope of the calibration curve. Applying Eq. 3, the detection limit for methyl parathion is estimated to be 1.08×10^{-9} M.

Table 1 shows a comparison of the developed biosensor with those reported in literature. The AChE/ERGO-Nf/GCE biosensor has the primary advantage of simpler fabrication along with a lower limit of detection in a reasonable linear range and can be expected to be practically applicable.

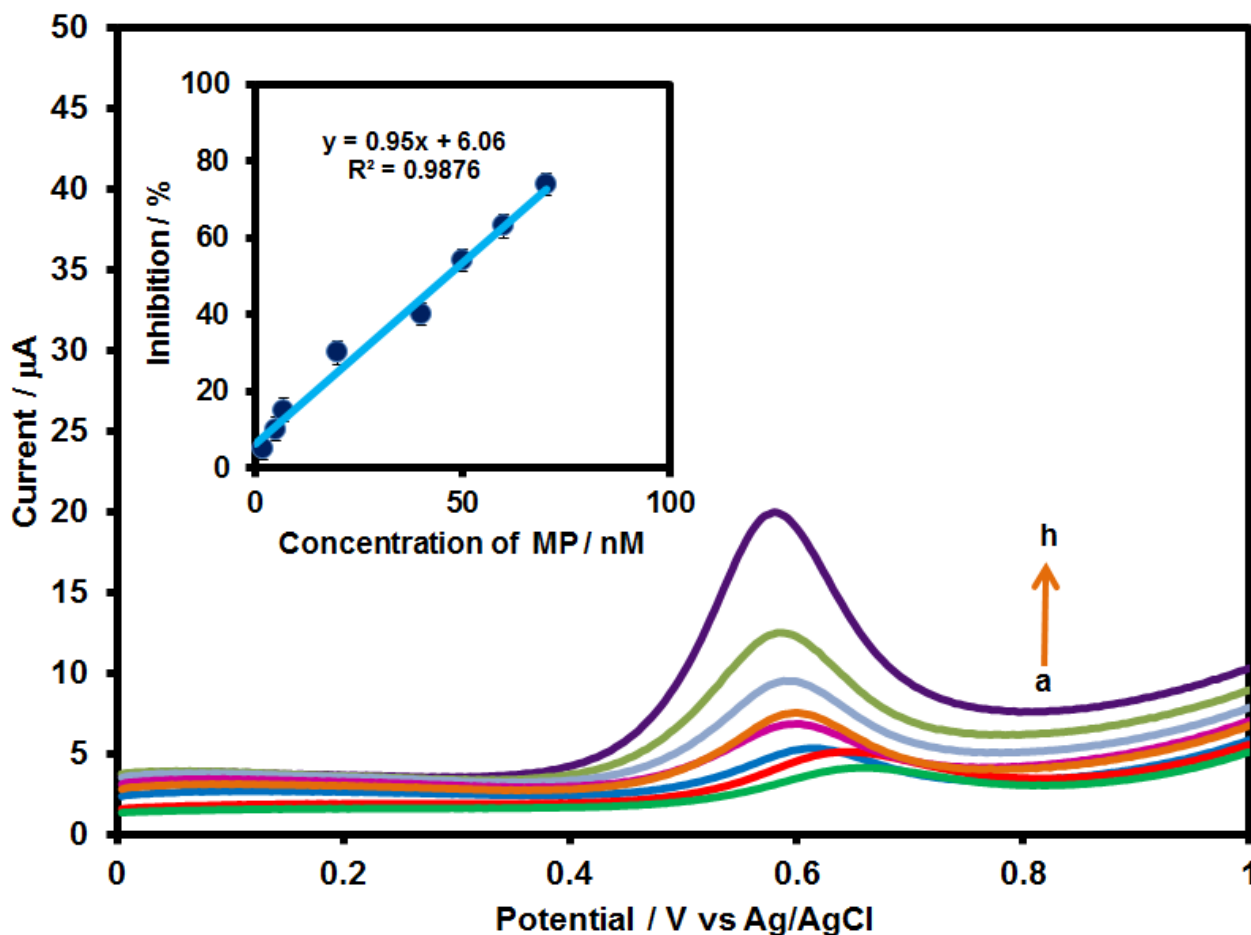


Figure 5. Square wave voltammograms at AChE/ERGO-Nf/GCE in 0.1 M PBS (pH 7.4) containing 1×10^{-3} M ATCl at different concentrations of MP in nM a) 2 b) 5 c) 7 d) 20 e) 40 f) 50 g) 60 and h) 70. Concentration of AChE used for immobilization - 0.1 U. Insert: Calibration plot for methyl parathion biosensor.

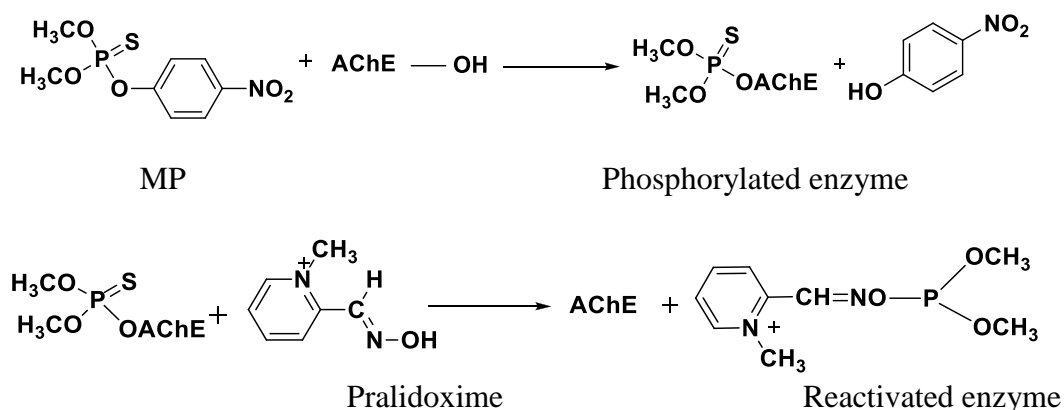
3.5 Reproducibility of AChE/ERGO-Nf/GCE

The reproducibility of the AChE/ERGO-Nf/GCE is determined by performing intra-assay and inter-assay experiments using cyclic voltammetry. The intra-assay is performed by assaying AChE/ERGO-Nf/GCE for five replicate measurements in 1×10^{-3} M acetylthiocholine chloride after incubating in 1×10^{-5} M MP solution for 20 min. Similarly, the inter-assay precision or fabrication reproducibility is ascertained with five freshly prepared enzyme electrodes. The relative standard

deviation values of intra-assay and inter-assay are calculated to be 4.69% and 7.97% respectively, indicating acceptable reproducibility.

3.6 Reactivation of AChE/ERGO-Nf/GCE

AChE inhibited by MP can be reactivated by the use of pralidoxime (2-pyridine aldoxime methyl chloride). AChE reacts with MP to form phosphorylated AChE as shown in Scheme 2. Phosphorylated AChE is characterized by the chemical bond between the phosphorus atom of organophosphate and the oxygen atom of the hydroxyl group of serine in the catalytic center of AChE [36]. The phosphorylated enzyme is extremely unstable and has a half-life of a fraction of a millisecond. Phosphorylated AChE, when bound to MP is attacked by pralidoxime (Scheme 3) to form AChE and phosphorylated aldoxime, enabling the release of pesticides from AChE. AChE/ERGO/Nf/GCE could resume 80% original activity after reactivation.



Scheme 3. Strategy for AChE Enzyme reactivation [36]

3.7 Real sample analysis

The application of AChE/ERGO-Nf/GCE has been evaluated by real sample analysis using potato samples. Vegetable sample (potato) was obtained from the local markets and cleaned using double distilled water. Two samples of 5 g each was kept in distilled water spiked with 2×10^{-8} M and 4×10^{-8} M concentration of MP. After a standing time of 24 h, the two samples were extracted with 30 mL of ether. The supernatants were then filtered through a 0.45 μm membrane and then evaporated to dryness [37]. About 2 mL of ethanol was added to the dry residue and diluted to 100 mL with 0.1M PBS (pH 7). The amount of MP found in the potato samples is estimated by SWV measurement and by substituting SWV peak current or inhibition (%) in the calibration equation (Eq. 2). The % recovery is then calculated from these data (Table 2). It is inferred that nearly 90 % recovery is possible.

Table 1. Comparison of AChE/ERGO/Nf/GCE biosensor performance with those reported in literature for the detection of methyl parathion

S.No	Modified Electrode	Detection technique	Linear range (μM)	Detection limit (nm)	Ref
1	Sol-gel immobilized AChE ^a /CPE ^b	CV	0.38 – 2800	300	22
2	AChE-nanoCaCO ₃ -chi ^c /GCE ^d	CV	0.019 – 14.2	3.8	23
3	AChE-SF ^e /MWNTs ^f /GCE	DPV	3.5 – 2000	0.5	21
4	Au ^g /ssDNA ^h -SWCNT ⁱ /PANI ^j /AChE	SWV	$1 \times 10^{-5} - 1$	0.001	17
5	AChE-AuNPs ^k -PPy ^l	CV	0.019 – 17.1	7.6	18
6	AChE/SiSG ^m /CPE	CV	0.38-3.7	150	20
7	AChE/ ⁿ PtNPs/ ^o CGR-NF/GCE	CV	$1 \times 10^{-7} - 1 \times 10^{-2}$	5×10^{-5}	30
8	AChE/ ^p NiONPs/CGR-NF/GCE	CV	$1 \times 10^{-7} - 1 \times 10^{-2}$	5×10^{-5}	31
9	AChE/ ^r SnO ₂ NPs/CGR-NF/GCE	CV	$1 \times 10^{-7} - 1 \times 10^{-2}$	5×10^{-5}	29
10	AChE/ERGO/Nf/GCE	SWV	$2 \times 10^{-3} - 0.7$	1	Present work

^aAChE-Acetylcholinesterase, ^bCPE-carbon paste electrode, ^cchi- chitosan, ^dGCE-glassy carbon electrode, ^eSF-silk fibroin, ^fMWNTs-multiwall carbon nanotube, ^gAu-Gold electrode, ^h ssDNA-Single-strand oligonucleotide, ⁱSWCNT- single walled carbon nanotubes, ^jPANI- polyaniline matrix, ^kAuNPs-gold nanoparticle, ^lPPy-polypyrrole nanowires, ^mSiSG-silica sol-gel, ⁿPt NPs-Platinum nanoparticle, ^oCGR/NF-pcarboxylic graphene and nafion, ^pNiONPs-nickel oxide nanoparticles, ^rSnO₂NPs-Tin oxide nanoparticle.

Table 2. Recovery study of MP present in two potato samples at AChE/ERGO-Nf/GCE

Sample	Added (nM)	Found (nM)	Recovery %
1	20	18	90.0
2	40	37	92.5

4. CONCLUSION

This work demonstrated a simple method for the immobilization of AChE on ERGO-Nf/GCE. The AChE/ERGO-Nf/GCE greatly facilitated electron transfer leading to a stable biosensor for rapid determination of methyl parathion with a detection limit as low as 1.08×10^{-9} M. The influence of

enzyme, substrate concentration, incubation time, real samples analysis has been investigated. The resulting biosensor shows good precision, stability and reproducibility. The application of the biosensor in real sample analysis has shown promising results expanding the scope of the work to the development of a prototype biosensor device.

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