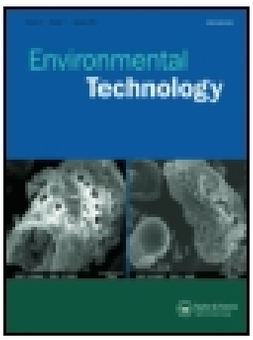


Environmental Technology

ISSN: 0959-3330 (Print) 1479-487X (Online) Journal homepage: http://www.tandfonline.com/loi/tent20

Nafion/AChE-cSWCNT/MWCNT/Au based amperometric biosensor for determination of organophosphorous compounds

Vikas Dhull

To cite this article: Vikas Dhull (2018): Nafion/AChE-cSWCNT/MWCNT/Au based amperometric biosensor for determination of organophosphorous compounds, Environmental Technology, DOI: 10.1080/09593330.2018.1505964

To link to this article: https://doi.org/10.1080/09593330.2018.1505964

Accepted author version posted online: 27 Jul 2018.

Publisher: Taylor & Francis & Informa UK Limited, trading as Taylor & Francis Group

Journal: *Environmental Technology*

DOI: 10.1080/09593330.2018.1505964

Nafion/AChE-cSWCNT/MWCNT/Au based amperometric biosensor for determination of organophosphorous compounds

Vikas Dhull*

*Department of Biotechnology Engineering, University Institute of Engineering & Technology, Maharshi Dayanand University, Rohtak-124001, India

*Corresponding Author

Email: v.nano87@gmail.com, Institutional email: vikasbtdhull.gf.uiet@mdurohtak.ac.in Contact No. 09813488148, Dr. Vikas Dhull, Assistant Professor (Guest Faculty) Department of Biotechnology Engineering, University Institute of Engineering & Technology, Maharshi Dayanand University, Rohtak-124001, India

Abstract

In present study, a biosensor was developed for detection of organophosphorous compounds. Core electrode of working electrode was obtained by depositing paste of Gold nanoparticles and Multi Walled Carbon Nanotubes on gold wire. The acetylcholinesterase enzyme was immobilized on carboxylated Single Walled Carbon Nanotubes and pasted onto core of electrode followed by coating with nafion layer to prevent enzyme leaching from electrode. This electrode was further used as working electrode in the sensor. This sensor worked on AChE inhibition mechanism where signal is inversely proportional to the amount of organophosphorous compounds. The electrocatalytic activity of this sensor was observed at potential of +0.360 mV. The standardized conditions for this sensor were observed as; pH at 7.0, temperature at 30°C, and response time less than 10s. The linear working range of this biosensor was 0.1µM-130µM with lowest detection limit (LOD) of 1.9 nM, 2.3 nM, 2.2 nM and 2.5 nM for Methyl Parathion, Monocrotophos, Chlorpyrifos and Endosulfan respectively. The biosensor showed excellent reusability (upto 55 times) and can be stored stably for 2 months.

Keywords: Acetylcholinesterase; Gold Nanoparticles; Carboxylated Single Walled Carbon Nanotubes; Multi Walled Carbon Nanotubes; Organophosphorus compounds

Introduction

In current scenario, pesticides play an important role for enhancement of agricultural yield. Organophosphate (OP) compounds are one of the important constituents of pesticides due to their high efficiency to eradicate pests and insects. But, organophosphate toxicity causes serious health concerns to human health and environment as they persist there as organic pollutant [1, 2]. They can accumulate in grains, vegetables, fruits etc. and are also responsible for water contamination [3,4]. These compounds inhibit enzyme acetylcholinestrase (AChE, EC 3.1.1.7) which plays a key role in proper working of central nervous system (CNS) in human beings and other animals. Due to this inhibition of AChE, acetylcholine (ACh) neurotransmitter starts accumulating in body which interfere with the muscular responses and causing respiratory problems, myocardial malfunctioning and finally leading to death [5,6]. So, it is very necessary to monitor concentration of OP compounds. Conventional methods are there for determining the presence of these toxic compounds which includes gas chromatography [7], colorimetric method [8], electrophoresis method using capillaries and chips [9], gas column chromatography (GC) [10], mass spectrometry (MS) [11], thin layer

chromatography (TLC) [12, 13] and high-performance liquid chromatography (HPLC) [14]. The above methods suffer from limitations of complex sample preparation which is time consuming; requires trained personals, relies on expensive equipments, missing real time screening, limited number of samples can be tested.

To overcome above limitations biosensors proved boon in analytical sensing being reliable, accurate, real time analysis, fast and sensitive with wide range of detection limit. Amperometric AChE biosensor works on the principle of enzyme [15]. A variety of fabrication strategies have been used by many researchers to develop inhibition-based OP biosensors using variety of materials [16]. Present situation demands, the OP biosensor which can screen large number of samples while preserving sensitivity and it must be reusable. All these three characteristics were not reported jointly in any single OP biosensor. So, the purpose behind this research work was to fabricate a working electode which is stable and is reusability for a longer time and thus truly reflects the desirable characteristics features for effective pesticide monitoring.

The present research describes the fabrication of Nafion/AChE-cSWCNT/MWCNT/Au working electrode for monitoring OP compounds in different samples. The electrode had a linear working range and minimum detection limit of 0.1μM-130μM and 0.01μM respectively for the pesticides. It showed better reusability (55 times) and can be stored stably upto 2 months which in turns proves the economy of developed method.

Materials and Methods

Reagents and equipments

Multi Walled Carbon Nanotubes (MWCNTs) & Carboxylated Single Walled Carbon Nanotubes (cSWCNTs) and Tetrachloroauric acid ($HAuCl_{4)}$ were purchased from SRL, Gold Nanoparticles (Au NPs) were synthesized at Department of Bio & Nanotechnology, Guru Jambheshwar University, Hisar. Gold (Au) wire and Parafin oil were purchased from local market. Nafion was obtained from Sisco Research Laboratories (Mumbai). Purified Acetylcholinesterase (AChE) was obtained from Biosensors and Diagnostics Laboratory, Centre for Biotechnology, Maharshi Dayanand University, Rohtak, Haryana. All the chemicals used throughout the experimental studies were of analytical grade. Electrochemical studies including cyclic voltammetry (CV) were performed on Autolab electrochemical analyser Model PGSTAT 12/30/302. Electrochemical cell consisting of Pt wire, Ag/AgCl electrode and Au wire decorated with Nafion/AChE-cSWCNT/MWCNT. Transmission

Electron Microscopy (TEM) performed at Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, New Delhi with microscope Model JEM-2100F. Fourier transform infrared (FTIR) spectroscopy was carried out at Department of Biotechnology Engineering, University Institute of Engineering & Technology, Maharshi Dayanand University, Rohtak, Haryana with Bruker FT-IR spectrometer. X-ray diffraction (XRD) and Scanning Electron Microscopic (SEM) studies were performed at Department of Physics & Department of Chemistry, Maharshi Dayanand University, Rohtak. Spectrometric measurements were carried out on Shimadzu cooporation UV 2450 spectrophotometer.

Fabrication of Nafion/AChE-SWCNT/MWCNT-Au working electrode

Synthesis of Gold Nanoparticles (AuNPs) and Characterization

AuNPs were synthesized in laboratory by Gole and Murphy (2004) method [17] with modifications. 100ml of $HAuCl_4$ (0.01%) was heated at 100 ºC & stirred continuously. Then, sodium citrate (1%) was added. Within 2-3 minutes the solution color turns to wine red. The solution was heated for 4-5 minutes and allowed to cool at room temperature. Finally, solution was centrifuged, pellets were collected and dried. AuNPs were characterized by TEM at AIRF-JNU, New Delhi, India on commercial basis. UV visible spectroscopy was done at Centre for Biotechnology (CBT), Maharshi Dayanand University (MDU), Rohtak, Haryana, India. XRD characterization of AuNPs was performed at Department of Physics, MDU, Rohtak, Haryana, India.

Fabrication of Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au electrode

MWCNTs (50%) along with AuNPs (30%) were mixed with paraffin oil (20%) to obtain the consistency of paste. The paste (MWCNT/AuNPs) was filled in plastic tube (1 cm long and 4 mm wide) and fine Au wire was inserted to obtain an electrical contact. After solidification of the paste, plastic tube was carefully removed. This formed the core of working electrode. Then MWCNT/AuNPs-Au core electrode was washed with double distilled water for removing unbound material and stored at 4ºC. Further, cSWCNTs suspension (2 µL) was mixed with 2 µL of AChE. The mixture was casted onto the surface of electrode core MWCNT/AuNPs-Au using micro syringe and kept to dry at room temperature. In this strategy for fabricating working electrode the SWCNTs and MWCNTs were used in combination. SWCNTs surface was used for immobilizing AChE and on the other hand MWCNTs were used for enhancing electrocatalytic activity of electrode. Finally, 1 µL Nafion (5%) was casted on body surface which acts as binder to hold the AChE-SWCNT. On the core of working electrode, remaining solvent was allowed to evaporate. The resulting

electrode was taken as Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au electrode. The newly fabricated working electrode was stored at 4 °C in a refrigerator.

Characterization of Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au electrode

The working electrode was characterized by using SEM and FTIR. The electrode was cut into small pieces and then put on specimen stub and analysed by SEM. FTIR Spectra of working electrode was also recorded at different stages of its fabrication. The deposited nanomaterial was scraped off from working electrode. The dry potassium bromide (KBr) was mixed with scraped material and grinded properly. After grinding the mixture was pressed with mechanical press. Then, resulting powder was kept in sample holder known as socket of Bruker FT-IR spectrophotometer and spectrum was recorded.

Study of electrochemical behaviour of working electrode

Electrochemical measurements were made using Potentiostat with Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au as working electrode, reference electrode (Ag/AgCl) and counter current electrode (Pt wire). Equilibration of working electrode was done at 0.4 V before each run until steady-state current was obtained at + 0.4V. Acetylthiocholine iodide (ATCI) (150 µL of 0.05 mM) was added into electrochemical cell containing 15 ml of sodium phosphate buffer (0.1 M, pH 7.0). ATCI was hydrolysed enzymatically to thiocholine and Acetic Acid. Further, thiocholine was oxidised Vs Ag/AgCl reference electrode as shown in Fig.1.

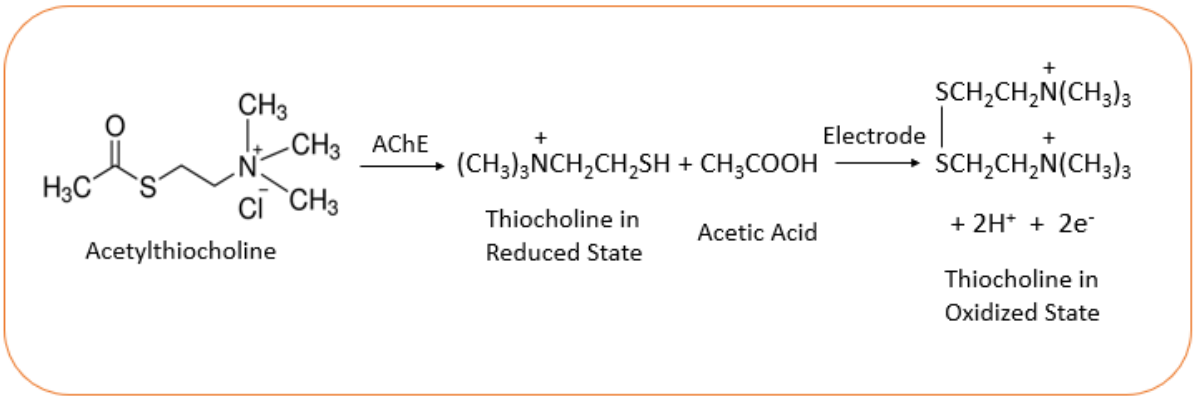


Figure 1: Thiocholine is oxidized at the working electrode under the applied potential.

The oxidation current at anode was inversely proportional to toxic compound present and time of exposure. Voltammetric measurements were monitored from −0.2 and +1.0 V with 50mV/s scan rate.

Optimization of newly developed amperometric method

For determining optimum pH, reaction buffer was varied in pH range with an interval of 0.5 from 5.0 to 9.0 to 0.1 M final concentration. Reaction mixture was subjected to temperature ranging from 10°C to 50°C for the determination of optimum working temperature of this newly developed method. The effect of substrate (ATCI) concentration on biosensor response was also determined in concentration range from 0 to 700 µM.

OP compound determination in Methyl Parathion by newly fabricated electrode

For determining concentration of pesticide, Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au electrode was first kept in phosphate buffer solution (0.1 M, pH 7.0) with two concentrations of methyl parathion (5µM and 10µM) as standard for 10 min and finally injecting it to electrochemical cell containing 15.0 mL, sodium phosphate buffer (0.1 M, pH 7.0) along with ATCl (0.1 mM) to perform electrochemical analysis by CV. Methyl parathion inhibition was determined as follows (Formula 1). The peak current i_P (control) is the representation of ATCl on Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au electrode (without pesticides); the peak current i_P (exp) is of ATCl on Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au electrode with pesticide.

$$\textbf{Percent Inhibition} = \frac{i_{\text{p (control)}} - i_{\text{p (exp)}}}{i_{\text{p (control)}}} \textbf{X100}$$

Formula 1: Expression for calculation of Percent Inhibition

Linearity and Lower Detection Limit

A standard graph was plotted between linearity and minimum detection. LOD is calculated as three times the standard deviation for the smallest concentration of sample detected divided by the sensitivity [18]. The expression for calculating sensitivity and LOD is shown in Formula 2.

$$\text{Sensitivity} = \sum_{i=1}^{n} \frac{\text{Current i}}{\text{Concentration i}}$$

$$\text{LOD} = \frac{3 \text{ X SD of Smallest Sample Detected}}{\text{Sensitivity}}$$

Formula 2: Expression for determining sensitivity & LOD

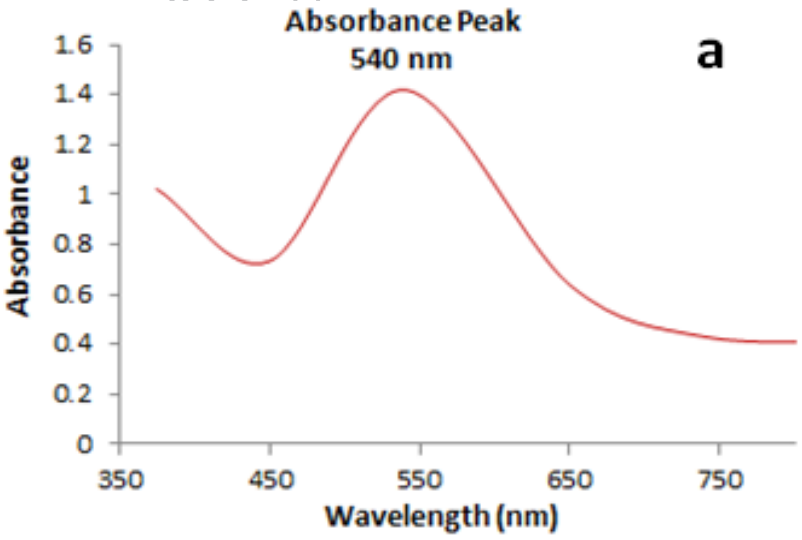
Interference Study, Reusability and Storage stability

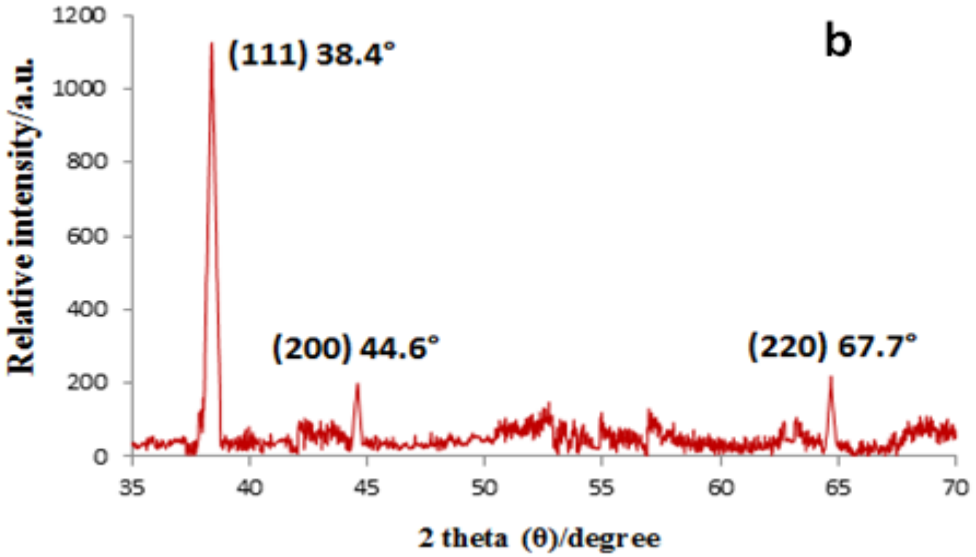
The effect of heavy metals like Cd (II), Pb (II), Cu (II), Ni (II) and Zn (II) was determined. Influence of electroactive species such as glucose, sucrose, uric acid and ascorbic acid were also monitored. The fabricated enzyme electrode was restored at 4°C for 60 days and its response in accordance with activity was monitored. The response was monitored on every alternate day for 2 months in order to evaluate storage stability and reusability. As, the substrate ATCI binds irreversibly to the active sites of AChE, the active sites were reactivated after every uses.

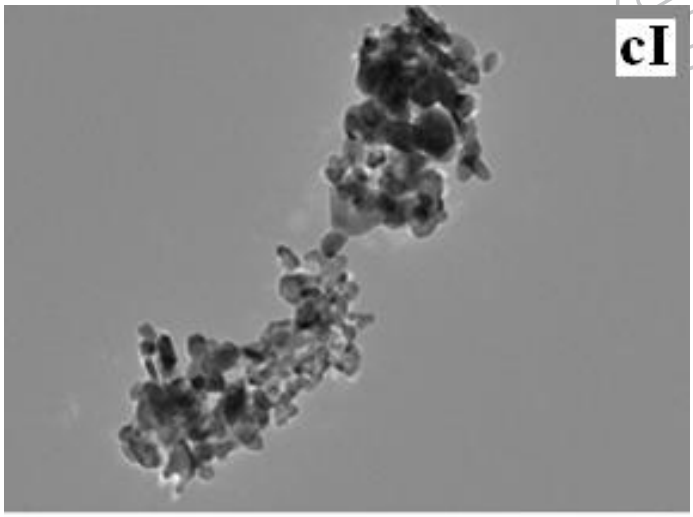
Results & discussion

Characterization of AuNPs

UV visible spectroscopy was done for analysing absorbance of AuNPs (Fig.2a). Absorbance peak was obtained at 540 nm, which confirms the synthesis of AuNPs. XRD (Fig.2b) was performed for AuNPs and the diffraction peaks appeared at 38.4°, 44.6° and 64.7° which were designated to (111), (200) and (220) respectively. The above XRD peaks are specific diffraction peaks of gold crystalline plane. The diffraction peaks appeared was in agreement with JCPDS card no. 089-3697. Spherical shape of AuNPs and their average size was found in range of 20 to 30 nm which were confirmed by TEM (Fig.2cI, 2cII).







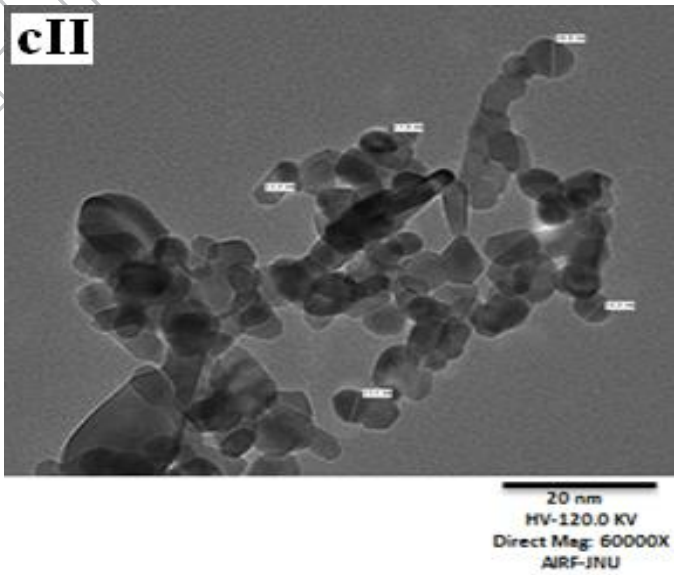
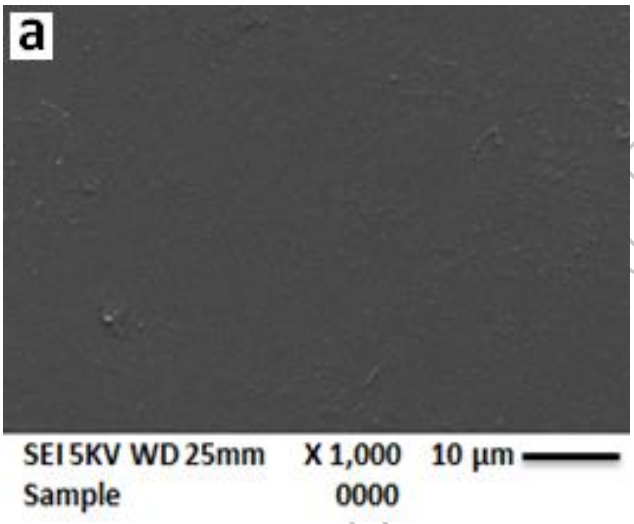


Figure 2: (a) UV spectrum of AuNPs. (b) XRD diffraction of AuNPs. (cI) TEM of AuNPs shows spherical shape of NPs and (cII) TEM showing size of AuNPs.

Morphological studies of working electrode

Morphological characterization was done by SEM. Fig.3a showing the SEM image of bare gold (Au) wire with uniform morphology. Fig.3b showing SEM image of MWCNT/AuNPs-Au. This showed mesh like morphology which confirmed successful deposition of nanomaterial on bare Au electrode. Figure 3c showing morphology of final working electrode (Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au) with immobilized enzyme.



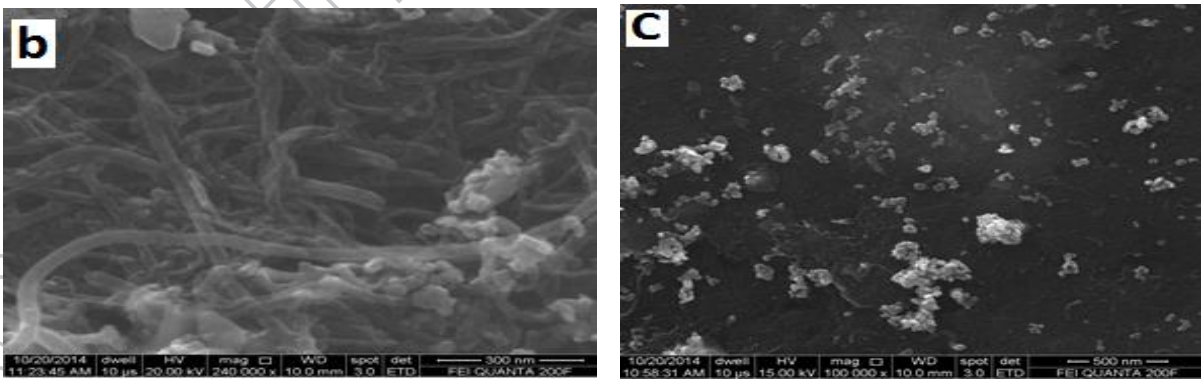
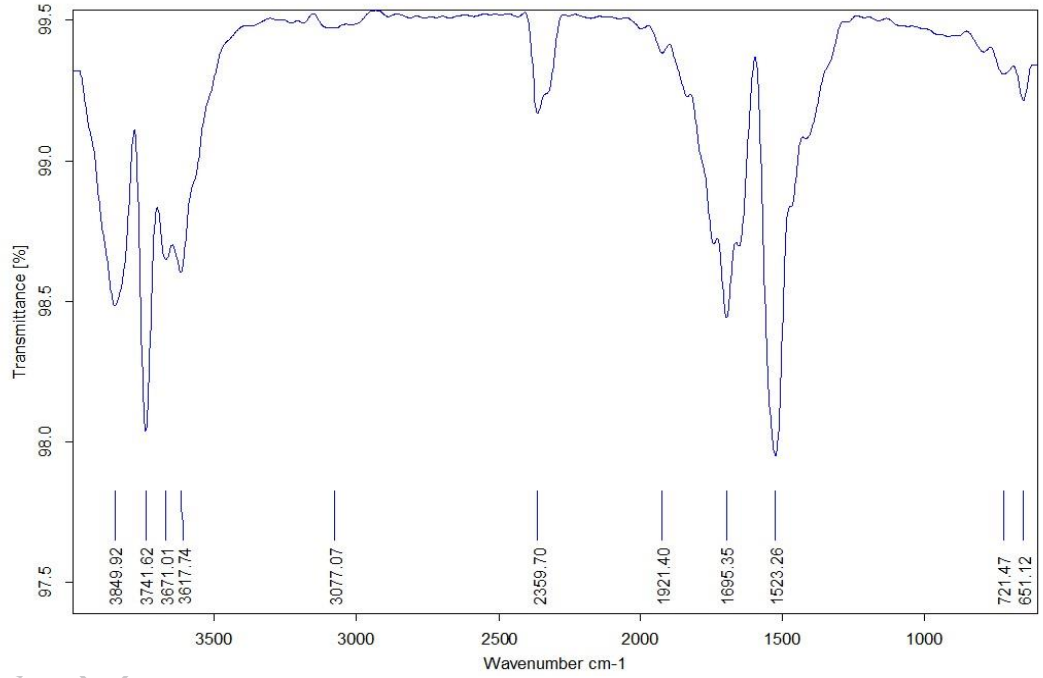


Figure 3: SEM images of (a) Au wire without modification, (b) MWCNT/AuNPs assembled on Au wire and (c) Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au (working electrode).

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR absorption spectra for the working electrode without enzyme and having immobilized enzyme has been represented in Fig.4. Spectra of MWCNT/AuNPs-Au showed absorption peaks around 651.12 cm^{-1}, 721.47 cm^{-1}, 1523.26 cm^{-1}, 1695.35 cm^{-1}, 2359.70 cm^{-1}, 3077.07 cm^{-1} and 3617.07 cm^{-1} to 3849.92 cm^{-1}. These peaks correspond to graphitic signature peaks of CNTs as shown in Fig.4a. The peak around 1500 cm^{-1} generated as a result of bending vibrations of C=O and O-H stretches. After AChE immobilization on the working electrode surface, the peaks were obtained at 1525.24 cm^{-1}, 1695.08 cm^{-1}, 2358.14 cm^{-1}, 2891.91 cm^{-1}, 3141.60 cm^{-1} and 3615.27 cm^{-1} to 3842.71 cm^{-1}. The broad peaks around 1695.08 cm^{-1} corresponds to carbonyl stretch and peaks at 3141.60 cm^{-1} represents the amide N-H stretch as shown in Fig.4b. These carbonyl and amino stretches are responsible for formation of covalent bond between AChE and working electrode. This may also result in prevention of enzyme leaching and electrode fouling finally adding to the reusability of enzyme.



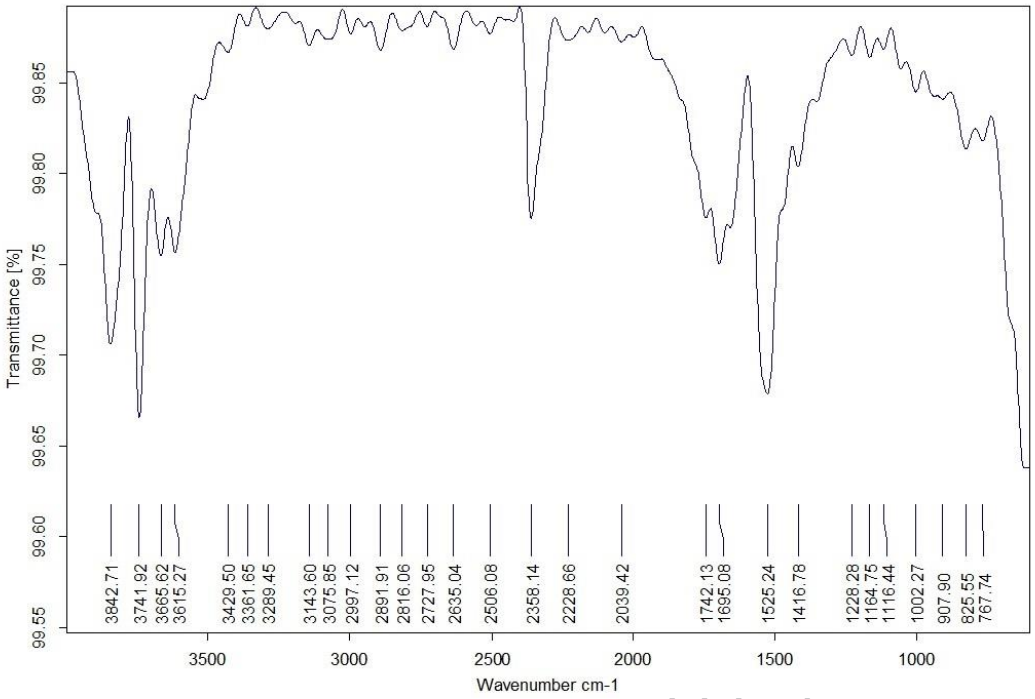


Figure 4: FTIR of (a) MWCNT/AuNPs-Au and (b) AChE-cSWCNT/MWCNT/AuNPs-Au electrode.

Cyclic Voltammetric Study of AChE Biosensor

CV was carried out in presence of ATCI (150 µL, 0.05 mM) in phosphate buffer (pH 7.0) with 20 mVs^{-1} scan rates. Fig.5 showed the CV of bare Au (curve a) wire with no peak. Curve b showed a peak at +265 mV which was of MWCNT/AuNPs-Au electrode and curve c showed an oxidation peak at + 360 mV due to oxidation of thiocholine by enzyme immobilized working electrode i.e., Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au electrode. The oxidation will occur at the interface of the working electrode which is dipped in the solution. The substrate will pass through the nafion layer and will bind to the active site of AChE enzyme which immobilized on cSWCNTs. Further the redox reaction will transfer charge from SWCNTs to the mixture of AuNPs/MWCNTs and further to the Au wire which completes the circuit in three electrode system and the results are obtained.

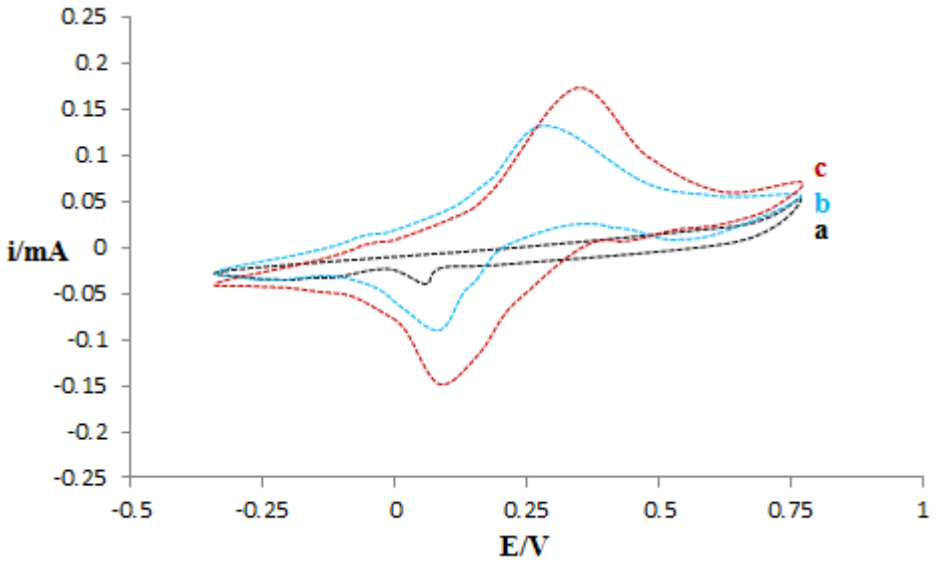


Figure 5: CV of (a) bare Au wire, (b) MWCNT/AuNPs-Au and (c) Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au.

Optimization of Biosensor

The effect of pH, temperature etc. were studied for their effect on working of fabricated biosensor. The working electrode reflects maximum response at pH 7.0 as shown in Fig. 6. It has been noticed that the oxidation current increases from pH 5.0 to 7.0 and then declines from 7.0 to 9.0. Due to the physiological pH of the phosphate buffer (0.1 M, pH 7.0) has been selected for the further experiment [41]. The reported pH is similar to chitosan/gold modified with ferric oxide nanoparticles at pH 7.0 [19], lower than MWCNTs/zirconium oxide nanoparticles onto glassy carbon electrode at pH 7.4 [20], carbon nanosphere modified electrode at pH 7.5 [21], chitosan/tin oxide nanoparticles/graphene nanocomposite at pH 7.4 [22], 3D graphene oxide network and MWCNT composite at pH 7.4 [23]; the incubation temperature was 30°C which is similar to gold electrode modified with chitosan and ferric oxide nanoparticles [19], similar to nanofiber based PVA/BSA membrane electrode [24], this optimal working temperature is higher than modified glassy carbon electrode with MWCNTs and zirconium nanoparticles [20] . For studying response of applied potential, working potential was applied from 0.0 V to +0.6 V. It was observed that there is an increase in current response with applied potential of 0.0 V to +0.36 V, finally reached at saturation from +0.36 V to +0.6 V. Therefore, +0.36 V was taken as working potential for newly fabricated biosensor for pesticides detection. This potential is higher than ferric oxide with chitosan modified gold electrode and glassy carbon electrode modified with nanocomposite of

MWCNTs, zirconium oxide nanoparticles and PVA/BSA membrane-based biosensor [19,20,24],

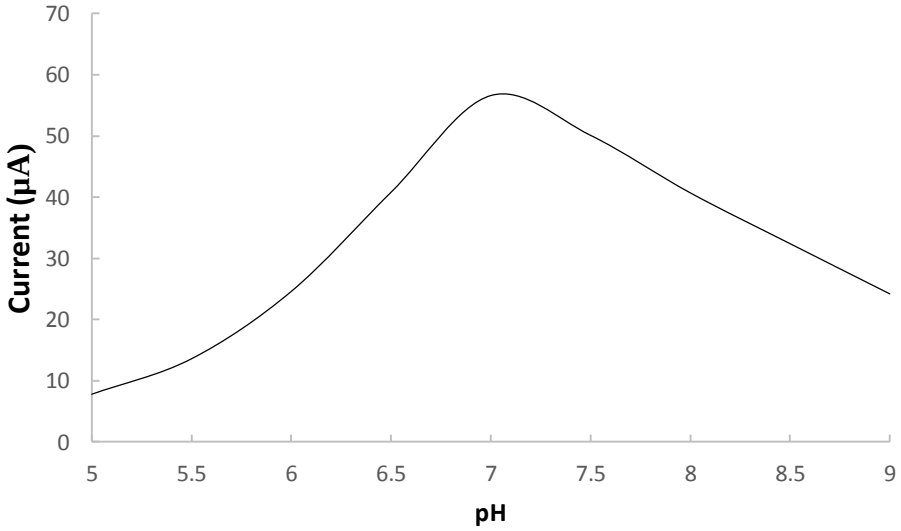


Figure 6: Optimum working pH of the biosensor.

Effect of ATCI Concentration and Lineweaver-Burk plot

The ATCI concentration was optimized for its effect on biosensor response in the concentration range upto 700µM. The biosensor showed a hyperbolic relationship between activity and substrate concentration upto 600µM, after which no increase was observed as shown in Fig. 7. This is higher than PAA/OPH-ZnONP/c-MWCNTs modified silver electrode with substrate concentration of 500µM and modified PVC beaker-based OP sensor with 400µM [25,26], Lineweaver-Burk plot between the reciprocals of ATCl concentration and response of biosensor in mA current for Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au electrode was linear. K_m (app) was determined by calculating the slope of Lineweaver-Burk plot. I_{max} (app) was determined with intercept for reciprocal plot of current vs. ATCl concentrations. K_m (app) and I_{max} (app) was 137.93 µM and 0.49 mA (Lineweaver-Burk plot Fig.8). The K_m (app) is less than glassy carbon electrode (GCE) modified with platinum nanoparticles (Pt NPs), carboxylic graphene (CGR) and nafion (NF) [42], more than nafion (NA) and Ag nanoparticles supported on amine functionalized reduced graphene oxide (rGO-NH_2) working electrode [43] and also better than SnO_2 nanoparticles (SnO_2 NPs), CGR and NF modified GCE [44].

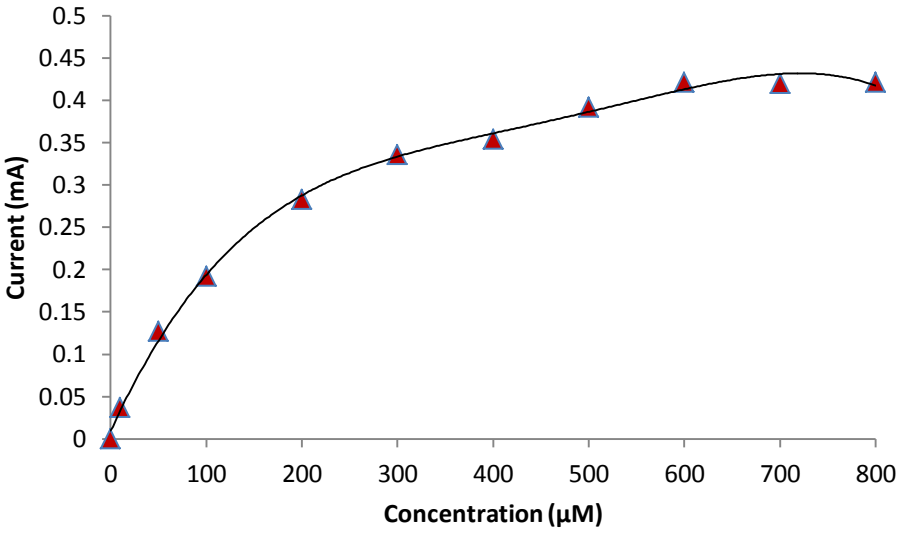


Figure 7: Optimal substrate (ATCI) concentration for the biosensor.

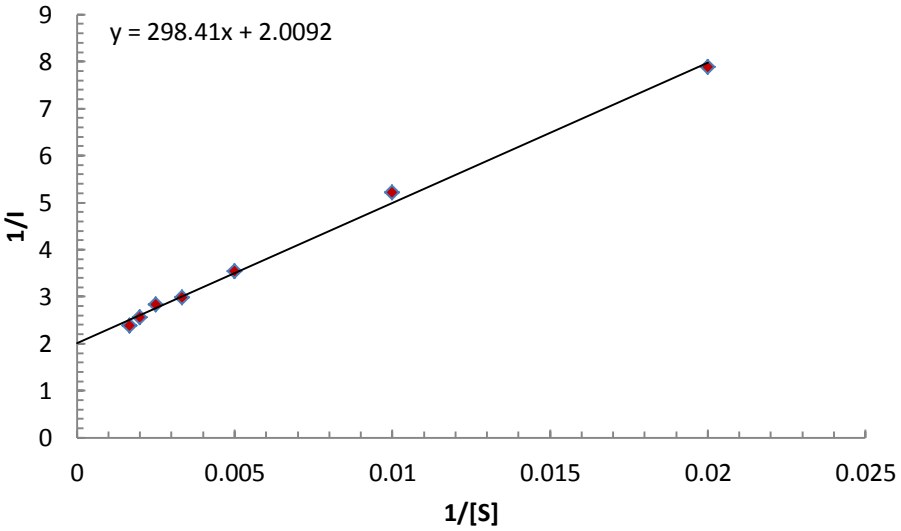


Figure 8: Lineweaver-Burk plot for response of ATCl concentration on the current of newly fabricated working electrode.

Time of Incubation & Response Time of working electrode

Methyl Parathion, Monocrotophos, Chlorpyrifos and Endosulfan were tested for their effect on activity of immobilized enzyme. The incubation time was taken ranging from 2 to 20 min with an interval of 2 min. Fig.9 showed that the inhibition effect of immobilized enzyme is directly proportional to incubation time unless it had reached to saturation level. After 10 min less decrease was observed in activity of immobilized enzyme. So, 10 min was selected as incubation time. This is similar to bioconjugate of gold nanoparticles and calcium carbonate deposited on Au electrode [27], composite of iron nanoparticles and MWCNTs on Au

electrode [28], iron oxide nanoparticles and MWCNTs on ITO electrode [29], lower than zirconium oxide and chitosan on glassy carbon electrode [30], gold-platinum bimetallic nanoparticles on glassy carbon electrode [31], gold nanoparticles and MWCNTs deposited on glassy carbon electrode [32]. The inset in Fig.9 shows the optimum response time. The response time was studied ranging from 0 to 15 seconds and optimum response time was observed at < 10s. The use of nanomaterial in the fabrication of working electrode enhanced the thermal stability and prevents the electrode denaturation even at high temperature.

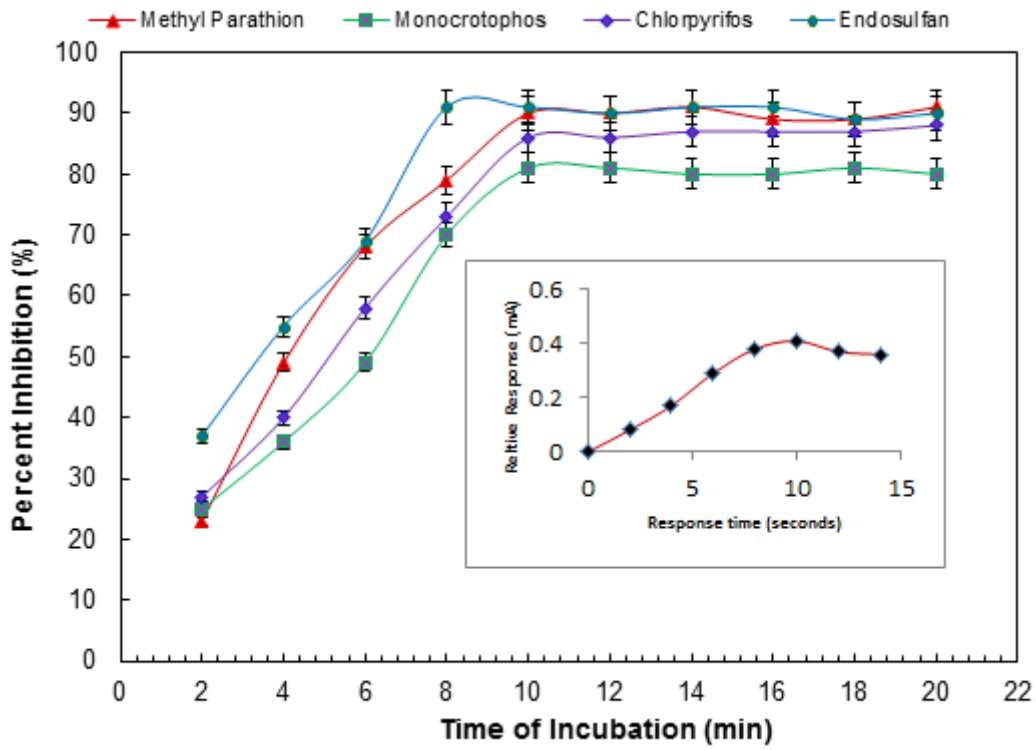


Figure 9: Response of time of incubation with Percentage inhibition. Potential applied was + 0.4V vs. reference electrode (ATCI 600 µM, 1.0 nM pesticide). Inset showing the response time of <10 second.

Evaluation of Newly Fabricated Biosensor

Biosensor was evaluated by studying linearity, detection limit, analytical recovery and finally precision and correlation. The linearity and detection limit of newly fabricated electrode were determined for various pesticides. Incubation was kept 10 min. At enzyme inhibition level (15–25%), the linearity for methyl parathion, monocrotophos, chlorpyrifos and endosulfan was observed 1nM–46 µM, 1nM -52 µM, 1nM-52 µM and 20nM-130 µM, respectively. The linearity is better than earlier reported methods using gold nanoparticles and prussian blue modified GCE [33], MWCNTs and gold composite on GCE [34], gold nanoparticles electrodeposited on GCE [35] The observed sensitivity was 0.0167 mA/nM with LOD of 1.9

nM for Methyl Parathion, 0.0167 mA/nM sensitivity & with LOD of 2.3 nM for Monocrotophos, 0.0147 mA/nM sensitivity with LOD of 2.2 nM for Chlorpyrifos and 0.0058 mA/nM sensitivity with 2.5 nM LOD for Endosulfan. For evaluating reliability of developed method, the percent analytical recovery of known pesticide concentration was measured. The mean recovery of pesticide (5 µM, 10 µM and 20 µM) was 98.6%, 99.2% and 94.4% respectively. Reproducibility and reliability was determined for the developed method, the pesticide level in single use (in batch) and after restoring at -20 °C for 7 days (between batches) were evaluated. The coefficient of variation (CVs) within batch was <2% and between batch was < 1.0%. The results obtained were better than earlier methods. For studying correlation, the values obtained by standard and present method were plotted (standard method X and present method Y) and they agreed with each other with a correlation (0.987) as shown in Fig.10. This observation showed that the developed method is reliable.

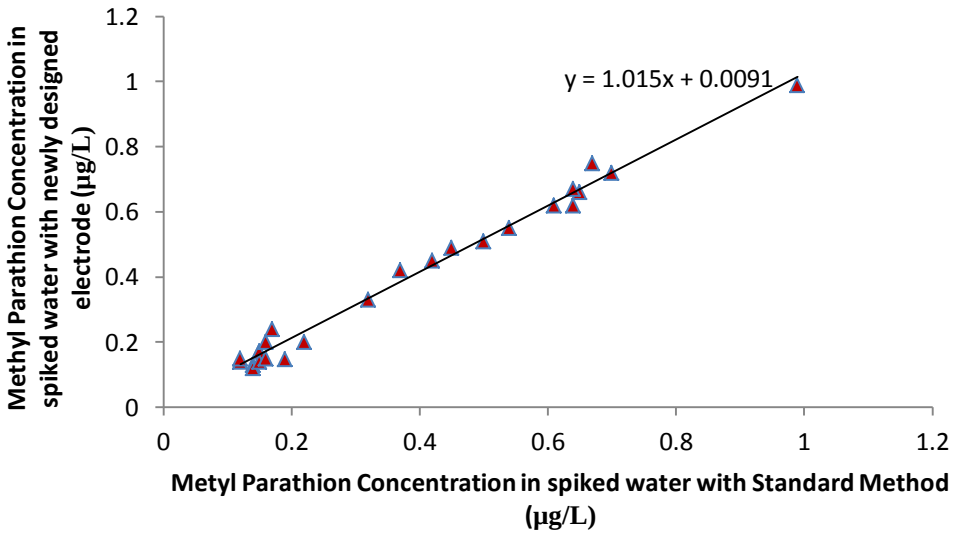


Figure 10: Correlation between pesticide levels in spiked water.

Interference study, Reusability and Storage Stability

The effect of heavy metals ions and other electroactive species on the activity of Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au electrode-based biosensor was studied. Zinc and copper metal ions showed stimulation of the activity of biosensor. All other heavy metal ions showed a minor decrease in the activity at the applied working potential. Interference of 7-9% had been observed with glucose, fructose, sucrose and ascorbic acid which was almost negligible as shown in Fig. 11. False positive results were not observed. The optimum

working potential was very low i.e., +0.360 mV and is a credit to the fabrication design. The newly fabricated electrode lost 30 % activity upto a period of 2 months as shown in Fig.12. This storage stability of biosensor is better than tin oxide decorated graphene onto GCE [36], composite of graphite nanoplatelets and chitosan on GCE [37], CdS decorated graphene nanocomposite [38] and MWCNTs-chitosan composited modified GCE [39]. The working electrode was washed for reactivation of active sites occupied by substrate ATCI after every use with 2 Pralidoxime. As a result of this, conformational changes occur at active site of enzyme and substrate ATCI will be released [40]. The stability observed could be due to covalent immobilization of enzyme on cMWCNTs/AuNPs-Au electrode. Nafion coating could also provide stability by preventing enzyme leaching from working electrode. The biosensor was also tested for the recovery of pesticide in the real vegetable sample and the results have been represented in Table 1. The present work was also compared to the earlier developed methods as shown in Table 2.

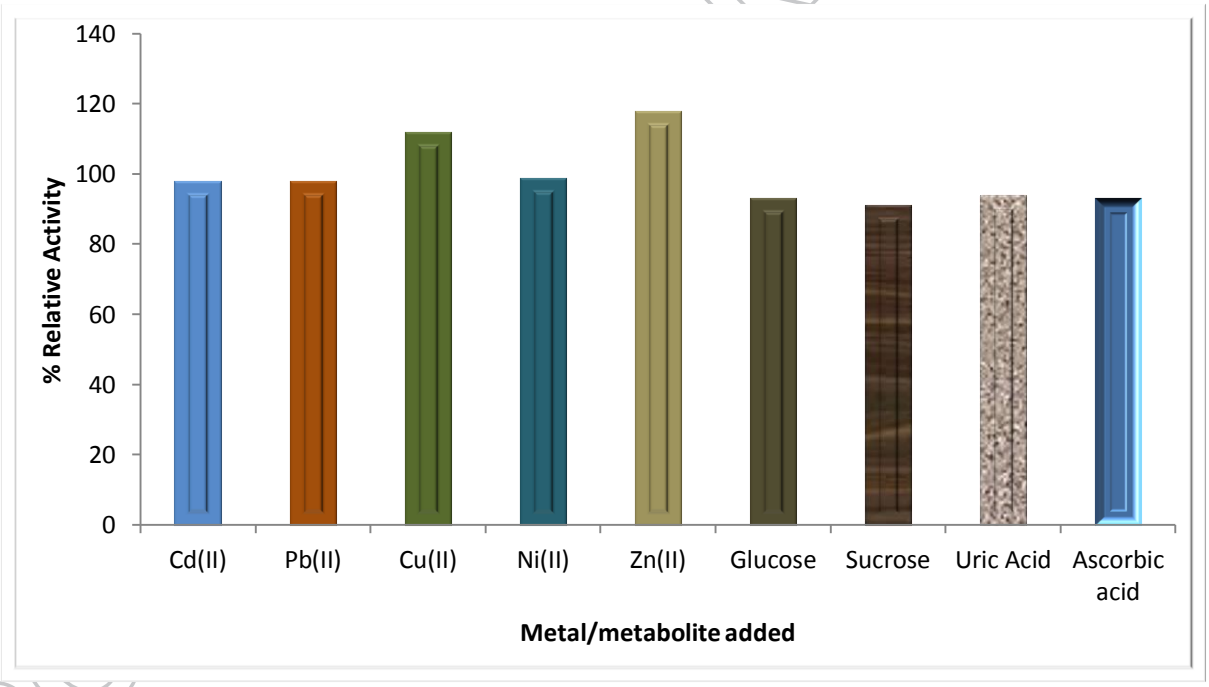


Figure 11: Interference study of the working electrode.

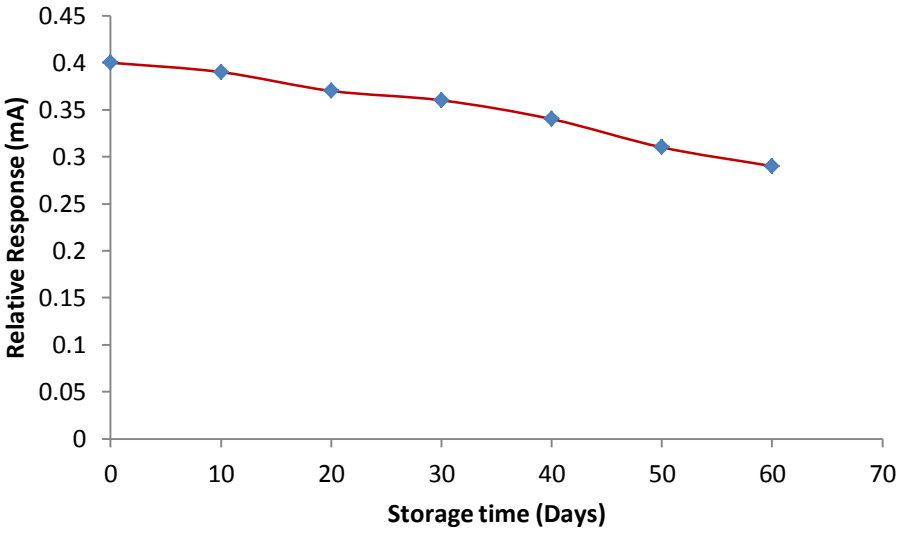


Figure 12: Storage stability showing 30 Percent loss in activity after continuous use of 2 months.

Table 1: Percent recovery of methyl parathion in vegetable samples

Vegetable	Recovery (%) by Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au electrode	RSD (%)
Cabbage	98.5	3.1
Onion	97.1	3.7
Spinach	95.4	2.8

Conclusion

The new fabrication strategy for development of working electrode for on sight monitoring of organophosphorus compounds was tested successfully. The desirable properties of an OP monitoring device were achieved through Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au based biosensor. The use of CNTs in combination with Au nanoparticles has enhanced the electrocatalytic properties of the developed electrode and reduces the working potential up to +0.360mV and hence the possibility of false positivity and additional response due to other

electrocatalytic species in the sample were prevented. The linear working range of biosensor was 0.1µM-130µM with LOD better than that of earlier reported methods. The Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au based biosensor showed a response time of <10 s after incubation period of 10 minutes. Use of Nafion protects the covalently bound enzyme from leaching which leads to high stability (60 days) and reusability (>55 times). The nafion layering also protects the electrode from fouling. This method showed a good correlation (R^2 = 0.987) with the standard method. The developed sensor can be used for determination of various OP compounds in different samples on site.

Table 2: Represents comparison between present method and earlier reported methods.

Detection Mode	Transducer Used	Immobilization Type	Detection Limit (µM)	Linearity (µM)	Enzyme Inhibitor	Incubation Time (min)	Storage Stability (days)	Reference
Amperometric	PAN/AuNPs/Pt	Covalent	0.026×10^{-5}	3.6×10^{-7}-3.6×10^{-4}	Paraoxon	20	30	18
Amperometric	AuNPs/PB/GCE	Adsorption	3.5×10^{-9}	4.48×10^{-3}-4.48×10^{-2}	Monocrotophos	10	30	19
Amperometric	AuNPs/GCE	Adsorption	7.0×10^{-3}	28×10-3-170×10^{-3}	Methamidophos	10	7	20
Amperometric	CHIT-GNPs/Au	Chemisorption/ esorption	0.1×10^{-3}	0.3×10^{-3}-60.5×10^{-3}	Malathion	13	NR	21
Amperometric	AuNPs/Au	Adsorption	33×10^{-3}	10×10^{-3}-135×10^{-3}	Carbofuran	20	7	22
Amperometric	cSWCNT/MWCNT/AuNPs-Au	Covalent	0.1	0.1-130	Parathion, Monocrotophos, Chlorpyrifos and Endosulfan	10	60	Present Method

Acknowledgement

The author thanks the financial support from the Department of Science & Technology, New Delhi, for providing INSPIRE fellowship as SRF-P (IF110655) during PhD. Author is also thankful to AIRF, Jawaharlal Nehru University, New Delhi for providing instrumentation facilities for TEM and Department of Chemistry (SMITA Research Lab), Department of Textile Technology, Indian Institute of Technology, New Delhi for providing SEM facility.

Conflict of Interest

The Author Declares no conflict of interest in publication of this research article.

References

1. Du D, Chen SZ, Song DD, et al. Development of acetylcholinesterase biosensor based on CdTe quantum dots/gold nanoparticles modified chitosan microspheres interface, Biosens Bioelectron. 2008;24:475–479.

2. Gavrilescu M. Fate of Pesticides in the Environment and its Bioremediation. Eng Life Sci. 2005;5:497-526.
3. Lozowicka B, Kaczynski P, Paritova AE, et al. Pesticide residues in grain from Kazakhstan and potential health risks associated with exposure to detected pesticides. Food Chem Toxicol. 2014;64:238-248.
4. Ohar Z, Lahav O, Ostfeld A. Optimal sensor placement for detecting organophosphate intrusions into water distribution systems. Water Res. 2015;73:193-203.
5. Ranjan A, Chauhan A, Jindal T. In-silico and in-vitro evaluation of human acetylcholinesterase inhibition by organophosphates. Environ Toxicol Pharmacol. 2018;57:131-140.
6. Pundir CS, Chauhan N. Acetylcholinesterase inhibition-based biosensors for pesticide determination: A review. Anal Biochem. 2012;429:19-31.
7. Vreuls JJ, Swen RJ, Goudriaan VP, et al. Automated on-line gel permeation chromatography–gas chromatography for the determination of organophosphorus pesticides in olive oil. J Chromatogr A. 1996;750:275–286.
8. Ellman GL, Courtney KD, Andres V, et al. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7:88–95.
9. Lu WJ, Chen YL, Zhu JH, et al. The combination of flow injection with electrophoresis using capillaries and chips. Electrophor. 2009;30:83–91.
10. Hoff GR, Zoonen PV. Trace analysis of pesticides by gas chromatography. J Chromatogr A. 1999;843:301–322.
11. Hernandez F, Sancho JV, Pozo O, et al. Rapid direct determination of pesticides and metabolites in environmental water samples at sub-mg/L level by on-line solid-phase extraction–liquid chromatography–electrospray tandem mass spectrometry. J Chromatogr A. 2001;939:1–11.
12. Sherma J. Thin-layer chromatography of pesticides: a review of applications for 2002–2004. Acta Chromatogr. 2005;15:5–30.
13. Sutherland TD, Horne I, Russell RJ, et al. Gene cloning and molecular characterization of a two-enzyme system catalyzing the oxidative detoxification of b endosulfan. App Environ Microbiol. 2002;68:6237– 6245.
14. Mitobe H, Ibaraki T, Tanabe A, et al. High performance liquid chromatographic determination of pesticides in soluble phase and suspended phase in river water. Toxicol Environ Chem. 2001;81:97–110.

15. Dhull V, Gahlaut A, Dilbaghi N, et al. Acetylcholinesterase biosensors for electrochemical detection of organophosphorus compounds: A review. Biochem Res Int, 2013;2013:1-18.
16. Rajangam B, Daniel DK, Krastanov AI. Progress in enzyme inhibition-based detection of pesticides. Eng Life Sci. 2018;18:4–19.
17. Gole CJ, Murphy. Seed-Mediated Synthesis of Gold Nanorods: Role of the Size and Nature of the Seed. Chem Mater. 2004;16:3633-3640.
18. Sahin K, Dooley DM, Cropek AC, et al. A dual enzyme electrochemical assay for the detection of organophosphorus compounds using organophosphorus hydrolase and horseradish peroxidase. Sens Actu B Chem. 2011;158:353-360.
19. Chauhan N, Pundir CS. Amperometric determination of acetylcholine—A neurotransmitter, by chitosan/gold-coated ferric oxide nanoparticles modified gold electrode. Biosens Bioelectron. 2014;61:1-8.
20. Pundir S, Chauhan N, Narang J, et al. Amperometric choline biosensor based on multiwalled carbon nanotubes/zirconium oxide nanoparticles electrodeposited on glassy carbon electrode. Anal Biochem. 2012;427:26-32.
21. Cai JR, Zhou LN, Han E. A sensitive Amperometric Acetylcholine Biosensor Based on Carbon Nanosphere and Acetylcholinesterase Modified Electrode for Detection of Pesticide Residues. Anal Sci. 2014;30:669-673.
22. Cui HF, Wu WW, Li MM, et al. A highly stable acetylcholinesterase biosensor based on chitosan-TiO2graphene nanocomposites for detection of organophosphate pesticides. Biosens Bioelectron. 2018;99:223-229.
23. Li Y, Zhao R, Shi L, et al. Acetylcholinesterase biosensor based on electrochemically inducing 3D graphene oxide network/multi-walled carbon nanotube composites for detection of pesticides. RCS Adv. 2017;7:53570-53577.
24. Moradzadegan A, Ranaei-Siadat SO, Ebrahim-Habibi A, et al. Immobilization of acetylcholinesterase in nanofibrous PVA/BSA membranes by electrospinning. Eng Life Sci. 2010;10:57-64.
25. Dahiya M, Dhull V, Kumar S, et al. Fabrication and Optimization of PAA/OPH-ZnONP/c-MWCNTs Based Silver Electrode for Amperometric Determination of Organophosphorus Compounds. Sens Lett. 2015;13:1-9.

26. Gothwal A, Beniwal P, Dhull V, et al. Preparation of Electrochemical Biosensor for Detection of Organophosphorus Pesticides, International Journal of Analytical Chemistry. 2014;2014:1-8.
27. Chauhan N, Narang J, Pundir CS. Immobilization of rat brain acetylcholinesterase on ZnS and poly(indole-5-carboxylic acid) modified Au electrode for detection of organophosphorus insecticides. Biosens Bioelectron. 2011;29:82–88.
28. Chauhan N, Pundir CS. An amperometric biosensor based on acetylcholinesterase immobilized onto iron oxide nanoparticles/multiwalled carbon nanotubes modified gold electrode for measurement of organophosphorus insecticides. Anal Chim Acta. 2011;701:66–74.
29. Chauhan N, Pundir CS. An amperometric acetylcholinesterase sensor based on Fe_3O_4 nanoparticle/multi-walled carbon nanotube-modified ITO-coated glass plate for the detection of pesticides. Electrochim Acta. 2012;67:79–86.
30. Yang Y, Guo M, Yang M, et al. Determination of pesticides in vegetable samples using an acetylcholinesterase biosensor based on nanoparticles ZrO_2/chitosan composite film. Int J Environ Anal Chem. 2005;85:163–175.
31. Upadhyay S, Rao GR, Sharma MK, et al. Immobilization of acetylcholinesterase–choline oxidase on a gold–platinum bimetallic nanoparticles modified glassy carbon electrode for the sensitive detection of organophosphate pesticides, carbamates, and nerve agents. Biosens Bioelectron. 2009;25:832–838.
32. Jha N, Ramaprabhu S. Development of Au nanoparticles dispersed carbon nanotube-based biosensor for the detection of paraoxon. Nanoscale 2010;2:806–810.
33. Wu S, Lan X, Zhao W, et al. Controlled immobilization of acetylcholinesterase on improved hydrophobic gold nanoparticle/Prussian blue modified surface for ultra-trace organophosphate pesticide detection. Biosens Bioelectron. 2011;27:82–87.
34. Du D, Wang M, Cai J, et al. One-step synthesis of multiwalled carbon nanotubes–gold nanocomposites for fabricating amperometric acetylcholinesterase biosensor. Sens Actu B. 2010;143:524–529.
35. Rong LY, Yong GZ, Fen LY, et al. Immobilization of acetylcholinesterase on one-dimensional gold nanoparticles for detection of organophosphorous insecticides. Sci China Chem. 2010;53:820–825.
36. Wang K, Li HN, Wu J, et al. TiO2-decorated graphene nanohybrids for fabricating an amperometric acetylcholinesterase biosensor. Analyst. 2011;136:3349–3354.

37. Gong J, Liu T, Song D, et al. One-step fabrication of three-dimensional porous calcium carbonate–chitosan composite film as the immobilization matrix of acetylcholinesterase and its biosensing on pesticide. Electrochem Commun. 2009;11:1873–1876.
38. Wang K, Liu Q, Dai L, et al. A highly sensitive and rapid organophosphate biosensor based on enhancement of CdS-decorated graphene nanocomposite. Anal Chim Acta. 2011;695:84–88.
39. Du D, Huang X, Cai J, et al. Comparison of pesticide sensitivity by electrochemical test based on acetylcholinesterase biosensor. Biosens Bioelectron. 2007;23:285–289.
40. Sun X, Wang X. Acetylcholinesterase biosensor based on Prussian blue-modified electrode for detecting organophosphorous pesticides. Biosens Bioelectron. 2010;25: 2611–2614.
41. Guler M, Turkoglu V, Kivrak A. Electrochemical detection of malathion pesticide using acetylcholinesterase biosensor based on glassy carbon electrode modified with conducting polymer film. Environ Sci Pollut Res. 2016;23:12343–12351.
42. Yang L, Wang G, Liu Y. An acetylcholinesterase biosensor based on platinum nanoparticles carboxylic graphene-nafion modified electrode for detection of pesticides. Anal Biochem. 2013;437:144-149.
43. Guler M, Turkoglu V, Basi Z. Determination of malathion, methidathion, and chlorpyrifos ethyl pesticides using acetylcholinesterase biosensor based on Nafion/Ag@rGO-NH_2 nanocomposites. Electrochim. Acta. 2017;240:129-135.
44. Yang L, Zhou O, Wang G, et al. Acetylcholinesterase biosensor based on SnO_2 nanoparticles carboxylic graphene–nafion modified electrode for detection of pesticides. Biosens Bioelectron. 2013;49:25-31.