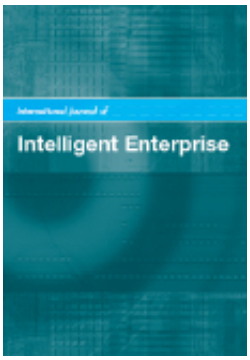

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An intelligent pesticide screening strategy using screen-printing technology

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An intelligent pesticide screening strategy using screen-printing technology

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Abstract: In the present era, agriculture and industrialisation are very necessary to support an exponentially growing population. However, along with this it has also adverse effects on the environment. It is necessary to monitor environmental containments so that risk to human life can be minimised. For this, sensors are fabricated using intelligent strategies to monitor toxic compounds for environmental management. So, in present work, compounds for environmental management. So, in this present work, organophosphorus compounds. Nanoparticles were synthesised and analytical tools were used for characterisation. Intelligent screen-printed gold electrode was modified by layering paste mixture of nanoparticles and c-SWCNTs to form ZnO NPs/c-SWCNTs/SPAuE. Acetylcholinesterase was immobilised onto electrode. Cellulose acetate was applied to prevent enzyme from leaching and electrode fouling. It was tested to detect presence of organophosphorus in samples. This intelligent strategy can also be used for detection of other containments in food, dye removal, heavy metals and medical applications.

Keywords: intelligent; sensor; screening; environment; analytical tool.

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1 Introduction

In last two decades, pesticide use exponentially increased to cope with the requirement of fast growing population. Their toxic nature caused serious health problems in animals and humans. Due to such a wide scale use, these harmful pesticides start accumulating in fruits, vegetables, grains, etc. with time (Aktar et al., 2009a). Pesticides not only affect health but also responsible for environmental pollution as they percolate in ground water through soil (Nicolopoulou-Stamati et al., 2016). They cause irritation to eyes; skin diseases, birth defects, cancer and other neurological disorders (Tran-Minh et al., 1990; Aktar et al., 2009; Gómez-Cortés et al., 2015; Muñoz-Quezada et al., 2016; Jamal, 1997; Ray, 1998). OP compounds inhibit enzyme acetylcholinesterase (AChE, EC 3.1.1.7) which is responsible for the proper functioning of nervous system in human beings. When the OP compounds inhibit enzyme activity this leads to accumulation of Ach which is a neuro transmitter leading to delay in muscle response (Donarski et al., 1989; Chapalamadugu and Chaudhry, 1992). International rules and regulation are also framed and implemented by various agencies for limiting the use of the toxic compounds (Costa, 2012).

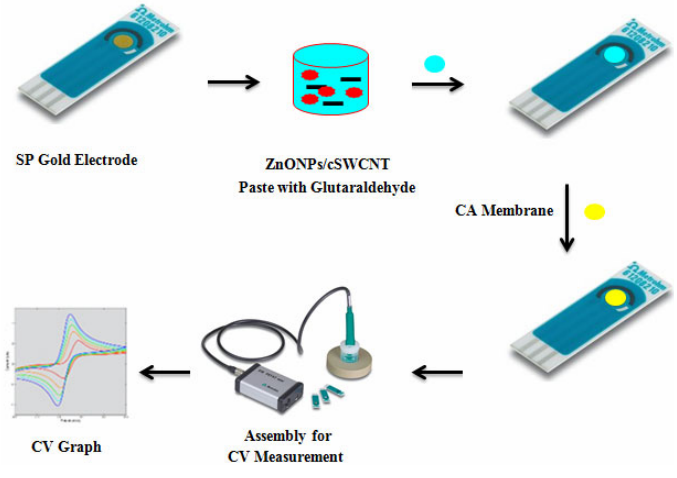
Therefore, in the above concern it is necessary to determine OP compounds presence in various samples of daily use. Many conventional methods are available to detect presence of OP compounds such as HPLC (Mitobe et al., 2001), capillary electrophoresis (Chen et al., 2004), gas column chromatography (Van der Hoff and Van Zoonen, 1999), colorimetry method (Ellman et al., 1961), TLC (Sherma, 2005) and mass spectroscopy (Hernández et al., 2001). All above reported methods are not efficient, accurate, they are labour intensive, and no on site, detection is possible.

To overcome these drawbacks the most promising analytical tool, i.e., biosensors is easy, fast, reliable and accurate device for monitoring pesticides level in samples. A variety of biosensors are being fabricated by several researchers worldwide but they also suffer from limitations of less sensitivity, enzyme leaching, fouling, etc. (Dhull et al., 2013). Biosensors using nanomaterials for their fabrication are highly electro catalytic, accurate and linear to detect toxic compounds. Nanomaterials including nanoparticles (gold, zinc, tin, copper, etc.), carbon nanotubes; SWCNTs and MWCNTs and quantum DOTs are also used for fabrication of biosensors (Dhull et al., 2015). Among the above

said nanoparticles, ZnO nanoparticles possess a band gap of 3.37 eV. It is biocompatible with high stability, biomimetic, less toxic as compared to other nanoparticles, high surface area that are used in fabrication of biosensors due to high isoelectric point.

The present research is to develop intelligent strategy for detecting OP compounds using nanomaterial based working electrode by modification of screen printed gold (SPAu) electrode. The modification was done by depositing mixed layer of zinc oxide (ZnO) nanoparticles (NPs) and carboxylated single walled carbon nanotubes (cSWCNTs). Then the enzyme was covalently immobilised on the above-deposited layer and finally covered with the layer of cellulose acetate (CA). The developed biosensor works on the AChE inhibition strategy. The substrate acetylthiocholine (ATCI) was splitted to thiocholine due to the hydrolytic action of AChE immobilised on to SPE. A disulphide compound is formed because of electro catalytic oxidation of thiocholine when potential was applied. During the course of reaction, the electrons are generated depending upon the activity of immobilised enzyme. The overview of the developed biosensor has been shown in Figure 1.

Figure 1 Intelligent strategy for developing the modified SPE based biosensor (see online version for colours)



2 Materials used

2.1 Materials

Zinc acetate (76192), hexamethylenetetramine (HMTA 81451), lithium hydroxide (LiOH 52019) and carboxylated single walled carbon nanotubes (cSWCNTs 18989) were obtained from Sisco Research Laboratories, Mumbai, India. Glutaraldehyde was from MERCK Specialities private limited, 8.20603.0521, Mumbai, India. Zinc oxide nanoparticles (ZnO NPs) synthesised at Department of Biotechnology Engineering, Maharshi Dayanand University, Rohtak, India. Gold (Au) Screen Printed Electrode (6.1208.210) was purchased from Metrohm Limited, Herisau, Switzerland. All the chemicals used in the experimentation were analytical grade.

2.1.1 Instrumentation

Potentiostat Model 910 PSTAT mini was used to record electrochemical studies. Screen Printed Electrode Working Electrode Gold 6.1208.210 (Au), Shimadzu cooperation UV 2450 spectrophotometer, PSA, FTIR was performed at Biotechnology Engineering, UIET, MDU, and Rohtak. XRD at Department of Physics, Maharshi Dayanand University, Rohtak.

2.2 Fabrication of CA/AChE/ZnO NPs/c-SWCNTs/SPE Au based modified screen-printed electrode

2.2.1 Synthesis of zinc oxide nanoparticles (ZnO NPs)

For synthesising ZnO NPs stock solutions of Zinc Acetate (0.1 M), HMTA (0.05 M) and LiOH (1.0 M) were prepared. 50 ml of each stock prepared above; zinc acetate and HMTA were mixed under continuous stirring with the help of magnetic stirrer. Subsequently LiOH was added drop by drop in the above mixture until pH raised to 10. The above solution was heated for three hours at 90°C for formation of precipitates. Then these precipitates were filtered followed by washing with DDW and ethyl alcohol and left for drying at room temperature.

2.2.2 Characterisation of lab synthesised ZnO NPs

Uv-Vis was done for the newly synthesised ZnO NPs for the analysis of optical absorbance activity of the NPs. Shimadzu cooperation UV 2450 spectrophotometer was used and the spectra of the sample was monitored from 300 to 450 nm at room temperature. Water was used as reference. For PSA disposable sising cuvette was used. Water was used as dispersant; temperature 25.0°C, count rate 156.0 kilo counts per second (kcps), testing duration 70 seconds and the measurement position was 4.65 mm. XRD characterisation of ZnO NPs was done using Mini Flex. Desk top X-ray diffractometer. The intensity was recorded using Cu Kα radiation and 2 Theta (θ) values were collected in the range from 25° to 70°.

2.2.3 Preparing paste of ZnO NPs/c-SWCNTs and deposition on screen printed gold electrode (SPAuE)

Carboxylated Single-walled carbon nanotubes (c SWCNT) were mixed with ZnO NPs using glutaraldehyde (2.5%) in ratio of 5:3:2 resulting in consistent paste. The paste obtained was layered on working electrode spot of screen printed electrode. Then electrode left undisturbed for 24 hours so that material can be deposited fully. The ZnO NPs/c-SWCNTs/SPAuE was stored at 4°C after washing.

2.2.4 Covalent immobilisation of AChE on ZnO NPs/c-SWCNTs/SPAuE

AChE (5 µL) as purified from P. Vulgaris in our earlier reported method (Dhull et al., 2014) was casted on surface of ZnO NPs/c-SWCNTs/SPAuE with micro syringe and dried at room temperature. Finally, AChE/ZnO NPs/c-SWCNTs/SPAuE as working electrode stored at 4°C.

2.2.5 CA membrane coating on AChE/ZnO NPs/c-SWCNTs/SPAuE

The preparation of CA membrane was done using (Hooda et al., 2009) method with slight modifications in the synthesis procedure. CA powder (100 mg) was dissolved in acetone (10 ml). After proper mixing, the resulting solution was poured on AChE/ZnO NPs/ c-SWCNTs/SPAuE using auto pipette and left for drying. After drying modified SPAuE was kept in 0.1 M buffer followed by storing at 4°C.

2.2.6 Characterising CA/AChE/ZnO NPs/c-SWCNTs/SPAuE

The characterisation of above fabricated electrode was done using FTIR. We investigate electrode for the presence of bond and different bond stretches in the electrode at each stage of fabrication.

2.2.7 Study of electrochemical behaviour of working electrode

CA/AChE/ZnO NPs/c-SWCNTs/SPAu was used for electrochemical measurements. To achieve steady state calibration of electrode was done before every use at 0.4 V. The reaction mixture consisted of substrate ATCI and sodium phosphate buffer (0.1 M, pH 7.2). The above mixture was injected into measuring vessel (6.1412.000) provided by Metrohm with 910 PSTAT mini. Then modified SPAuE was connected to the potentiostat and put in the vessel containing the substrate. Enzymatic hydrolysis of ATCI resulted in formation of thiocholine. Further oxidation of thiocholine occurred at silver and silver chloride reference electrode. The current generated due to oxidation of thiocholine was inversely proportional to amount of toxic compound present and exposure time to that compound. Cyclic voltammetric was recorded from –0.2 and +1.0 V at a scan rate 50 mV/s scan rate.

2.3 Optimisation of working parameters for newly fabricated SPE

The SPAuE was investigated for optimum pH, reaction buffers at different pH ranges and interval of 0.5 from 5.0 to 9.0 to 0.1 M final concentration. Three different buffers were used for optimisation of modified electrode; including succinate buffer from pH 5.0 to 6.0), sodium phosphate buffer from pH 6.5 to 7.5) and borate buffer from pH 8.0 to 9.0). Reaction mixture was subjected to temperature range from 10°C to 50°C in order to analyse optimum working temperature SPAuE. The electrode was also subjected to substrate (ATCI) concentration ranging from 0 to 800 µM for the optimum response of biosensor.

2.4 Determining toxic OP compounds in endosulfan using SPE

For determining concentration of OP compound, the CA/AChE/ZnO NPs/c-SWCNTs/ SPAuE was put in the measuring vessel containing 15 ml of phosphate buffer (0.1 M, pH 7), ATCI (0.1 mM). Endosulfan was used for the above determination purpose and left for 10 minutes so that the reaction can occur with the substrate. Then electrochemical analysis was done by cyclic voltammetry (CV) analysis.

2.5 Linearity and minimum detection limit of SPE

These two parameters were investigated by plotting a standard curve with linearity and minimum detection. Minimum detection limit is the amount of analyte being measured, that can give a minimum signal necessary for detection and linearity is the conc. of analyte in which biosensor can provide appropriate signals. The modified SPAuE was investigated for the effect of electroactive species such as sucrose, uric acid and ascorbic acid and heavy metals on the activity of the biosensor. Reactivation of fabricated electrode was done using Sun and Wang (2010). Reusability and storage stability of electrode was also investigated by storing electrode for 60 days at 4°C.

3 Result and discussion

3.1 Characterisation of ZnO nanoparticles

The optical properties of synthesised ZnO Nanoparticles were characterised using UV spectroscopy. The absorption peak was obtained at 370 nm as shown in Figure 2. The PSA of synthesised ZnO nanoparticles showed that the nanoparticles are in average size of 37 nm with Zeta potential of –25.3 as shown in Figures 3 and 4. XRD was done for investigating the crystalline ZnO structure shown in Figure 5. Diffraction peaks appeared at 31.7°, 34.4°, 36.3°, 47.5°, 56.6°, 62.8° and 67.9° which were designated to (100), (002), (101), (102), (110), (103) and (112) respectively. The above peaks specifically correspond to diffraction peaks of zinc crystalline plane and were found in agreement with JCPDS card no. 80-0075.

3.2 FTIR analysis of fabricated electrode

Absorption spectra of ZnO NPs/c-SWCNTs/SPAu showed peaks around 650 cm^{-1}, 1,010 cm^{-1}, 1,541 cm^{-1}, 2,356 cm^{-1}, which are signature peaks of SWCNTs. Minor peaks also appeared between 1,400–1,500 cm^{-1} which appeared because of C = O and O-H bending vibrations. After immobilisation of AChE on ZnO NPs/c-SWCNTs/SPAu electrode, a fresh peak appeared at 1,629 cm^{-1} due to presence of carbonyl stretch. The FTIR analysis confirmed the successful formation of covalent bond between cSWCNTs and AChE enzyme.

3.3 Electrochemical investigation of working electrode

CV was performed for analysis of electrochemical behaviour of newly fabricated screen-printed electrode. As a substrate, ATCI (200 L, 0.05 mM) was combined with phosphate buffer (pH 7.0). For electrochemical measurements, the scan rate was set to 50 mVs^{-1}. Bare electrode showed no peak and a peak at + 360 mV was achieved due to breakdown of thiocholine by enzyme electrode, i.e., CA/AChE/ZnO NPs/ c-SWCNTs/SPAu electrode. The oxidation reaction of thiocholine by immobilised enzyme is shown in Figure 6.

Figure 2 UV visible spectroscopy of synthesised ZnO NPs (see online version for colours)

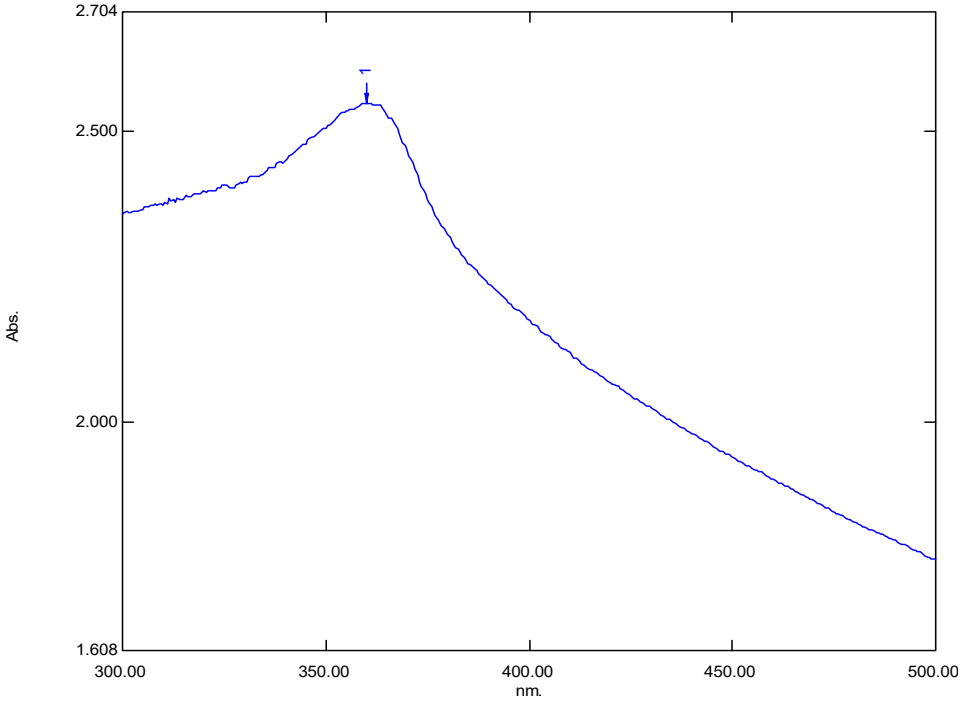


Figure 3 PSA results of synthesised nanoparticles (see online version for colours)

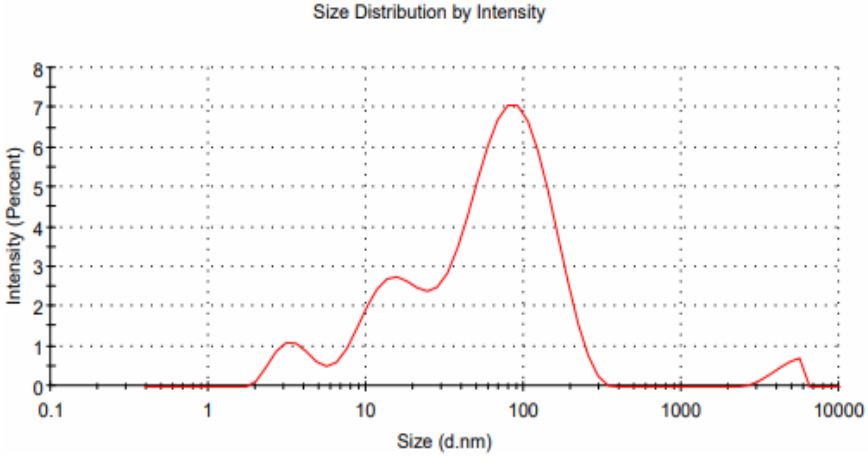


Figure 4 Zeta potential of synthesised nanomaterial (see online version for colours)

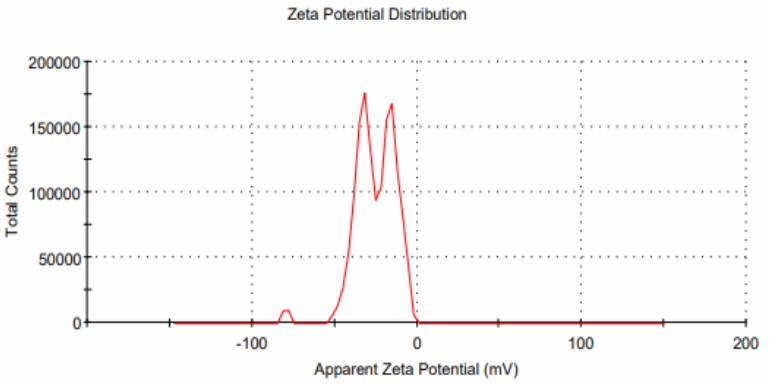


Figure 5 XRD results of synthesised nanoparticles (see online version for colours)

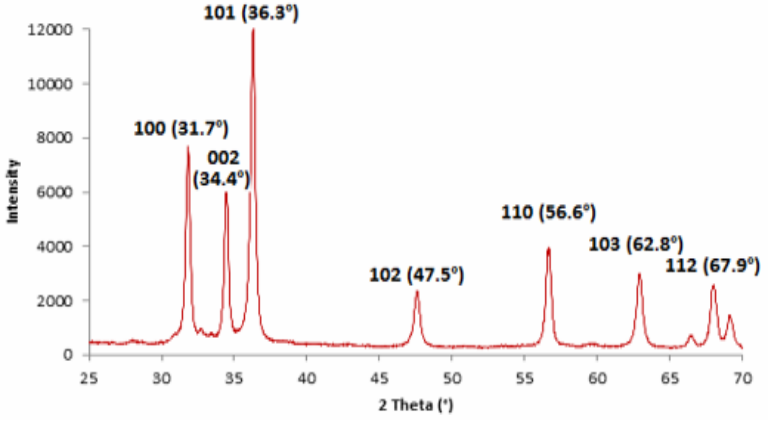
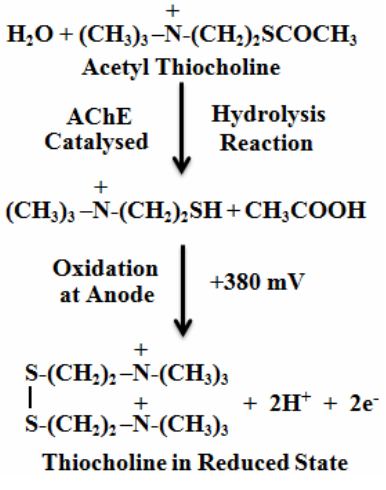


Figure 6 Oxidation reaction of thiocholine



3.4 Optimisation of biosensor

The modified SPAuE optimised at different pH and temperature range. The maximum response was observed 35°C with pH 7.2.

3.4.1 Studying the response of applied potential

The response of modified SPAuE was investigated with respect to potential applied for measurements. A potential ranging from 0.0 V to +0.8 V was used. The current response to applying potential increased from 0.0 V to +0.4 V, and following this saturation, the response increased from +0.4 V to +0.8 V. From above it was inferred that optimum applied potential of +0.4 V was suitable for optimum working potential for pesticides determination as shown in Figure 7. This vale is higher than CNT/AChE (0.3 V) (Oliveira and Mascaro, 2011), alumina sol-gel/sonogel-CE (0.21 V) (Zejli et al., 2008), and lower than carbon black (CB)/GCE (0.9V) (Lee et al., 2010), PAN membranes based working electrode (0.8 V) (Ivanov et al., 2002), silica sol-gel film coated on GCE (0.6 V) (Anitha et al., 2004), silica sol-gel/CPE (0.6 V) (Raghu et al., 2012).

Figure 7 Response of applied potential on the current response of modified SPE

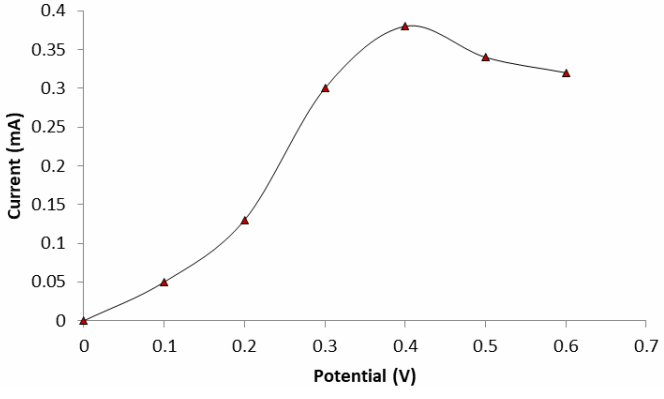
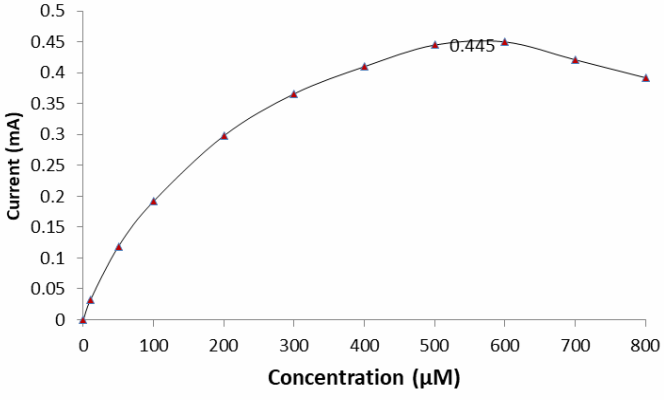


Figure 8 ATCL concentration effect on the response current of modified SPE



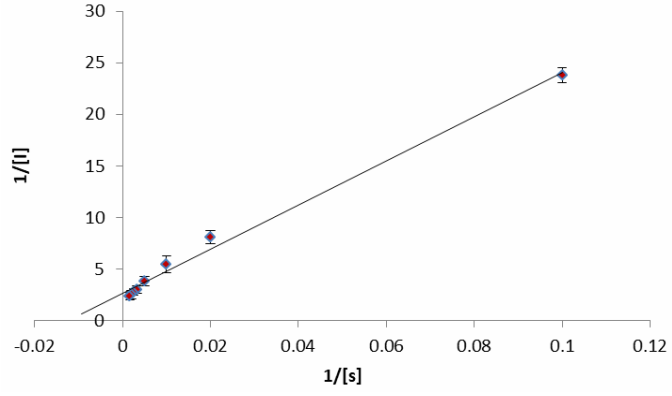
3.4.2 Studying the response of substrate concentration on electrode working

Effect of substrate concentration on modified SPAuE was optimised up to a concentration of 800 µM. A hyperbolic relation was observed between the immobilised enzyme activity and the substrate up to 500 µM, with no further increase (Figure 8). A sensitivity 0.390 mA/µm/L was observed for the fabricated biosensor. This is better than earlier reports (Yang et al., 2005; Shadlaghani et al., 2019). So, 500 µM substrate concentrations were selected as optimum concentration for further experimentation.

3.4.3 Line weaver-Burk plot

A liner response was observed when line weaver-Burk relation was plotted between the substrate concentration and current (mA) response for CA/AChE/ZnO NPs/c-SWCNTs/SPAu electrode. The slope of the Line weaver-Burk plot was used to calculate Km (app). The intercept for the reciprocal plot of current vs. ATCl concentrations was utilised to calculate I_{max} (app).K_m (app) and I_{max} (app) was 82.76 µM and 0.38 mA as shown in Figure 9. This is lower than earlier reported methods of biosensor fabrication, e.g., AChE-CHIT assembled on glassy carbon electrode (Km: 0.24 mM) (Wang et al., 2011), AChE-AuNPs/sol-gel film (0.45 mM) (Zejli et al., 2008), CNT in combination with dimethyl formide film (0.66 mM) (Vakurov et al., 2004) and polyethyleneimine modified electrode (1.5 mM) (Joshi et al., 2005) This concludes that the covalently immobilised AChE possesses improved affinity to that of the substrate. This improvement is due to use of SWCNTs in combination with ZnO NPs which increased the electron transfer in efficient manner.

Figure 9 Plot showing response between substrate concentration and current

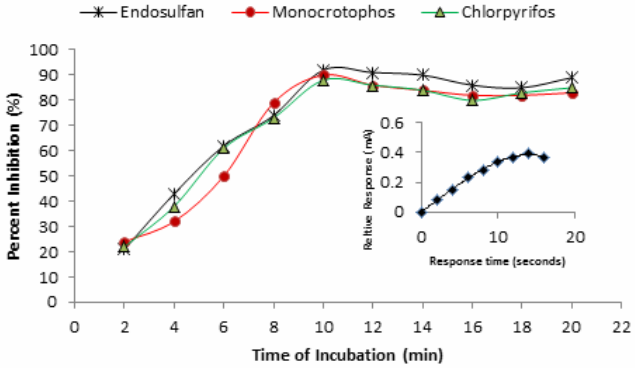


3.4.4 Incubation time and response time

Three pesticides endosulfan, monocrotophos and chlorpyrifos were used for the analysis of their effect on the covalently bounded enzyme activity. The time required for the reaction between the enzyme and substrate to occur was tested for 2 to 20 minutes with two minute intervals. The inhibition of immobilised enzyme by Op compound has direct relation to time of incubation before saturation level approaches. After 12 min a negligible decrease was observed enzyme activity. So, the reaction mixture was

incubated for 12 min before the pesticide determination. The optimum response time of the newly fabricated biosensor is shown in inset of Figure 10. The observed electrode response time was 14 s, which is faster than previously reported methods for detecting OP chemical concentration in diverse materials (Yang et al., 2005; Viswanathan et al., 2009; Ghosh et al., 2006).

Figure 10 Response of incubation time with percentage inhibition the applied potential was + 0.4v vs. the reference electrode (ATCI 500 m, 1.0 mm pesticide) (see online version for colours)



Note: Inset shows the 14-second response time.

3.5 Linearity and detection limit

The modified SPE was analysed for its linearity and minimum detection limit using various samples. The reaction mixture containing sample was incubated for 12 min so that the substrate and enzyme can react properly. The linearity for endosulfan, monocrotophos and chlorpyrifos was observed 2 µM–52 µM, 2 µM–58 µM, and 0.3 µM–140 µM, respectively as shown in Figure 11. Figure 12 represented a minimum detection limit of 2 µM.

Figure 11 Linearity study of pesticides (see online version for colours)

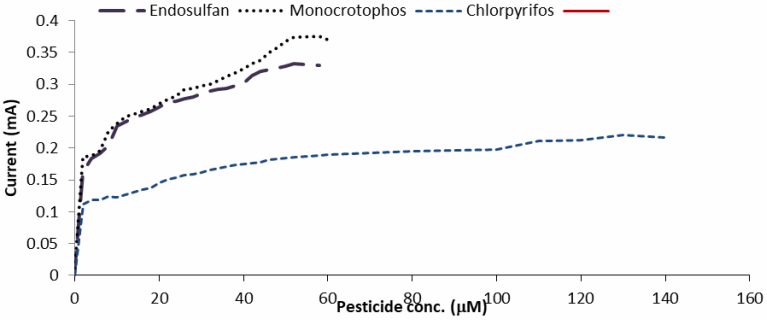
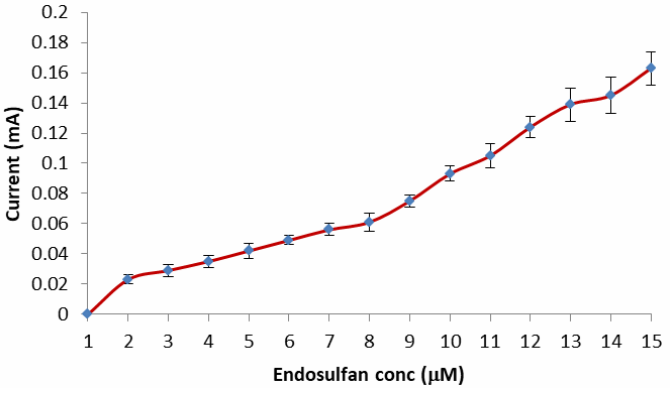


Figure 12 Minimum detection study of endosulfan at very low concentrations (see online version for colours)



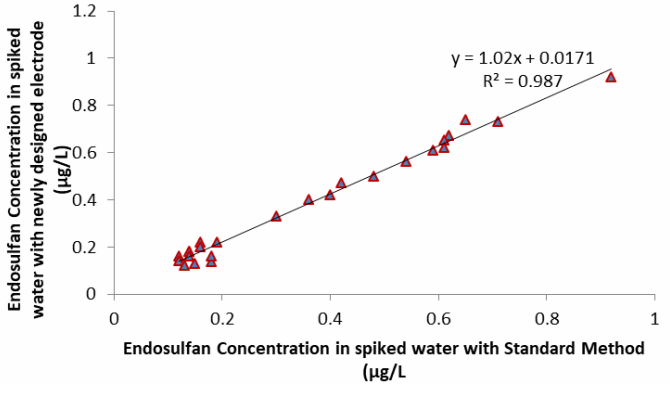
3.6 *Percent analytical recovery*

In order to check the reliability of modified SPE, a known concentration of pesticide was added to the reaction mixture for analysing percent analytical recovery. The recovery rate observed were 96.6%, 95.2% and 97.4% for the three pesticides which is better than recently reported method.

3.7 *Precision*

Pesticide levels in samples were determined using a newly developed biosensor. This was tested in a single use (in batch) and after seven days of storage at –20°C (between batches). The coefficient of variation within batch was 2% and 1.0% between batches. The results obtained showed good reproducibility and reliability which were better than earlier methods.

Figure 13 Graph shows correlated pesticide levels in spiked water (see online version for colours)



3.8 Correlation

A graph has been plotted between samples spiked with endosulfan for analysing the correlation using standard method (X) and with newly fabricated nanomaterial-based biosensor method (Y). A good correlation of 0.987 as presented in Figure 13 has been observed between the two methods.

3.9 Interference study

Heavy metals and electroactive species found in real samples may affect response of biosensor. The performance of biosensor stimulated with presence of zinc and copper ions in the sample. A slight decrease in the activity has been observed when other heavy metal present. With electroactive species such as glucose, fructose, sucrose interference of 9%–10% was detected. This achievement is due to the use of cSWCNTs and ZnO nanoparticles in the fabrication strategy.

3.10 Reactivation of modified SPE

During the OP compound determination process in samples, the active sites of immobilised AChE were occupied by the OP compounds irreversibly. It required the reactivation of the enzyme electrode before every use. Phosphate buffered saline (0.1 mole/l, pH 8) can be used to reactivate the enzyme. Also we can use pyridine-2-aldoxime (2 PAM), Oximes as chemical reactivators and 4-formylpyridinum bromide dioxime (TMB 4). We had used PBS as reported by Wang et al. (2011). The electrode resumed 95% of its activity in 20 minutes after dipping in PBS.

3.11 Reusability and storage stability

A 40% decrease in activity of modified SPE has been noticed over period of 60 days. After using the working electrode, it was restored at 4°C. The enhanced stability resulted from the covalent immobilisation of enzyme and polymer coating which prevented leaching of enzyme AChE in the reaction mixture and finally enhanced the reusability. The reusability was > 45 times.

4 Conclusions

In this study, we effectively developed an intelligent strategy for the detection of harmful OP chemicals in an array of substances. Screen printed gold electrode was used in which the working spot was modified using composite of cSWCNTs and ZnO nanoparticles. The enzyme was covalently immobilised on above deposited material, which was confirmed by FTIR. The newly fabricated electrode was found to be highly electro catalytic due to presence of composite material. This high electrical conductivity of working electrode provides us low working potential. The CA/AChE/ZnO NPs/ c-SWCNTs/SPAuE fabricated biosensor responded in less than 14 s with incubation time of 12 minutes. CA layer on electrode helped in preventing enzyme-leaching leading to

reusability and storage stability. This new improved SPAuE-based biosensor is useful to analyse numerous OP chemicals on-site.

These smart devices can also be exploited for their applications in detection of various compounds in real samples for medical applications, in food and brewage industries, as they are highly electro catalytic and efficient in detection of compounds.

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