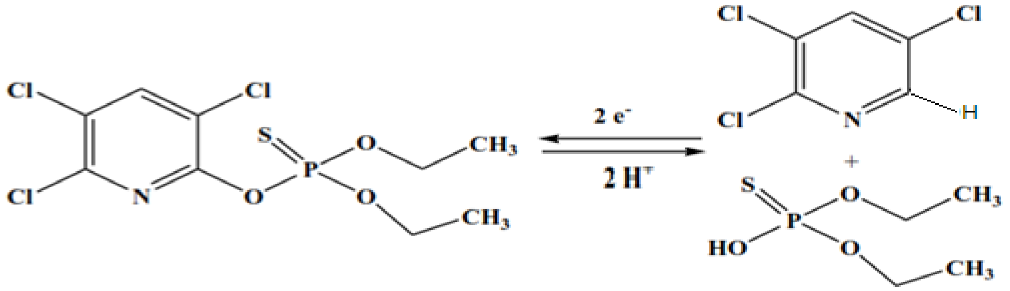
Nano Fe_3O_4–Graphite Paste Modified Electrochemical Sensor for Phosphatic Pesticide -Chlorpyrifos

Tadele Hunde, Mulu Berhe, Abraha Tadese, Mekonnen Tirfu, Amaha Woldu, Berhanu Menasbo and Saini R. C*

Department of Chemistry, College of Natural and Computational Sciences, Mekelle University, P.O. Box 231, Mekelle, Ethiopia (*drrcsaini@gmail.com).

ABSTRACT

A sensitive, cost effective and selective electrochemical sensor has been developed by exploiting iron oxide nano-particles as modifier in the paste of graphite powder. Cyclic voltammetric (CV) and differential pulse voltammetric (DPV) working parameters have been standardized and used practically for the determination of chlorpyrifos (O, O-diethyl O-3, 5, 6-trichloropyridin-2-yl phosphorothioate) pesticide from its aqueous solutions. Both techniques were deployed to investigate the electro-chemical interactions between chlorpyrifos and modified carbon paste sensor along with the redox characteristics at analyte/ sensor interface. The significant enhancement in peak current signals and the improved magnitude of the redox peak potential indicated the awe-inspiring facilitation of the electron transfer process by the modifier at the sensor/ analyte interface. The difference in the redox -potential (ΔEp) and peak current ratio (Ipc/Ipa) have revealed a notable surface enhancement characteristic of the modifier that responds the higher concentration gradient of pesticide at the interface. In lower analyte's concentration range i.e. from 1.0 to 100µM, the peak current varies directly to the pesticide concentration with detection limit of 2.8×10^{-6} mol/L. The relative stability of the modified sensor is fine and the reproducibility of the results is up to 98%, even after a gap of two months. The proposed analytical method is quite successful when applied for the quantification of chlorpyrifos from its aqueous samples. The electrochemical sensing/ detection of the pesticide chlorpyrifos is confirmed by its two electrons redox behavior and the same have been explained by suitable reaction scheme as:



Keywords: Nano-iron oxide, Chlorpyrifos, Modified graphite paste, Voltammetry, Electrochemical method.

1. INTRODUCTION

Synthetic organo-phosphorous compounds continue to be used in immense quantities and in ever-increasing variety with new products being introduced into the agricultural industry in the form of pesticides and insecticides every year without realizing the potential neuro-toxicity of these pesticides and their metabolites (Arvand et al., 2013). These neuro-toxic compounds, which are structurally similar to soman and sarin, can irreversibly inhibit acetyl-cholinesterase and lead to excessive cholinergic neuro-transmission and subsequent signs of toxicity including autonomic dysfunction, involuntary movements, and ultimate death (Arvand et al., 2013; Tan et al., 2010; Li et al., 2004). Therefore, it is important to develop a rapid, selective and sensitive method for the detection of organic-phosphorous compounds for the sake of environmental protection and human health. The conventional techniques for degradation (Pathiraja et al., 2014; Ghalwa and Nasser, 2013) and analysis of organo-phosphorous compounds are chromatography including gas chromatography, liquid chromatography, and thin chromatography coupled with different detectors in different types of spectroscopic techniques (Hua et al., 2010). However, these techniques require technically skilled labor for their operational usage besides being time-consuming, expensive and cannot be used for continuous monitoring. Though, to some extent the biological techniques such as immunoassays and biosensors have also been tried for the determination of organo-phosphorous compounds in agro-crop products (Mauriz et al.,2006; Prabhakar et al., 2008; Prasad et al., 2015; Rotariu et al., 2012; Wei et al., 2014; Zamfir et al., 2011). In addition to the already mentioned limitations encountered in chromatography techniques, the other problem related to the activity of the acetylcholinesterase, which is irreversibly inhibited by organo-phosphorous compounds. Electrochemical analytical techniques, based on chemically modified sensors have proved to be a sensitive and selective process for the determination of organo-phosphorous compounds (Cao et al., 2015; Kumaravel and Chandrasekaran, 2015; Liu et al., 2011; Mulchandani et al., 2001a,b; Mulchandani et al., 1999). Electrochemical detection could be easily and effectively accomplished without tedious separation of the co-components and its application is quite promising in continuous monitoring. Thus, the objective of the present work is to develop a rapid, sensitive, reproducible, and cost-effective simple method for determination of chlorpyrifos in aqueous environmental conditions to maintain quality control effectively.

2. MATERIALS AND METHODS

2.1. Apparatus

BAS CV-50W electrochemical analyzer (Bioanalytical system Inc., USA) was employed for all voltammetric measurements. A conventional three electrode system was used: unmodified carbon paste electrode (UCPE) or iron oxide modified carbon paste electrode (IOMCPE) as working electrode, silver-silver chloride as reference electrode and a platinum wire as a counter electrode. All potentials were measured with respect to Ag/AgCl as a reference electrode. All the pH values were measured with bench microprocessor pH meter (HANNA Instruments, Italy) which was calibrated with a standard buffer solution (pH 4.01, pH 7.01 and pH 9.02) at 20°C before measurements.

2.2. Chemicals

Chlorpyrifos (O, O-Diethyl O-3, 5, 6-trichloropyridin-2-yl phosphorothioate) and iron oxide were obtained from Adami Tulu Pesticide Factory Adami Tulu, Ethiopia. Graphite powder (BDH, England), NaOH (Scharlau, Spain), paraffin oil light, HCl and $NaH_2PO_4.2H_2O$ (Nice, India), were used for the experiment. All chemicals were analytical grade and used without further purification. Stock solution of chlorpyrifos (2.5 mM) was prepared in 0.1M $NaH_2PO_4.2H_2O$ solution of 7.5pH. For voltammetric characterization and determination of pesticide in samples, phosphate buffer solutions were prepared from a stock solution containing 0.1 M phosphate (Merck, Germany). Working standard solutions of lower concentration were prepared by dilution method. All experiments were carried out in sodium dihydrogen phosphate hydrated buffer solution (0.1M $NaH_2PO_4.2H_2O$; pH 7.5). NaOH and HCl both of concentration 0.1M were used for adjusting the pH of the buffer solution. Double distilled water was used for preparation of all solutions and all dilutions were carried with supporting electrolyte's solution.

2.3. Preparation of Unmodified Graphite Paste Electrode (UGPE)

The unmodified graphite paste electrode was prepared as follows; 70% (w/w) **g**raphite powder and 30% (w/w) light paraffin oil ($d = 0.88\ g.cm^{-3}$) were homogenously mixed by hand for 30 min, using pestle and mortar. The homogenized paste was allowed to rest for 24 hours, and then the paste was packed into the tip of the plastic syringe of 1.0 ml size. The surface of the electrode was smoothed on weighing paper and a copper wire was introduced from the back side of the syringe to provide electrical contact.

2.4. Preparation of Modified Graphite Paste Electrode

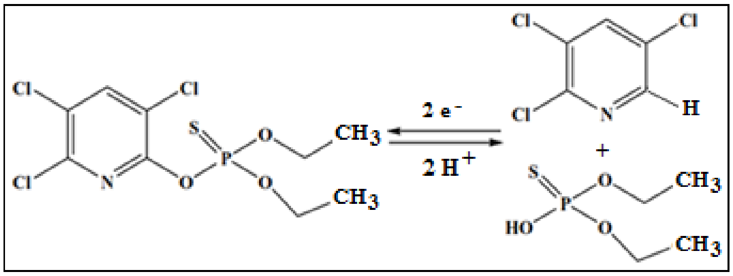
Iron oxide modified graphite paste electrode (IOMGPE) was prepared by mixing 1.35 g of graphite powder and 0.1 g of iron oxide in a small agate mortar for about 5 min. the 0.55 g paraffin oil was added, and then milled again for 30 min to obtain homogenous 5 % (w/w) iron oxide modified carbon paste. The homogenized paste was then allowed to rest for 24 hours.

Assortment of modified graphite paste containing different mass percentage of iron oxide (10, 15, 20, 25, 30 and 35 w/w) was prepared in the same way. A desired amount of the so prepared graphite pastes were packed into the cavity of the syringes holding copper wire at their back in order to get variety of graphite modified electrodes. The surface of each fabricated electrode was then refined on a clean paper before being used.

3. RESULTS AND DISCUSSION

3.1. Electrochemical Behavior of Chlorpyrifos at UGPE and IOMGPE

The electro-catalytic characteristics of chlorpyrifos in its 2.5mM solution using Fe_3O_4 induced graphite paste sensor and the UGPE have been examined with the help of cyclic voltametric technique (CV) between -400mV and 1100mV as working potential and the results of the study are illustrated in Fig-1. It is evident from this figure that there is a peak-less redox curve from the studied pesticide at UGPE vs. Ag/AgCl. Contrary to this, on conducting the experimental work with IOMGPE under similar conditions using said pesticide, there is a strong oxidation signal at potential 99mV i.e. (E_{pA}) and a strong reduction signal at potential 138mV i.e. (E_{pC}). The peak potentials separation gap, $\Delta Ep = E_{pA} - E_{pC} = 39mV$ at scan rate (ν) = $50mVs^{-1}$ has been observed. The observed ΔE_p around 30 – 40 mV justifies the two electron ($2e^-$) reversible redox behavior of chlorpyrifos at the modified sensor/ analyte interface. The reaction scheme for the same has been proposed as: $2H_2O \rightarrow 2OH^{\cdot} + 2H^+ + 2e^-$………… (1)



Scheme 1: Reaction scheme involving $2e^-$ redox behavior of the pesticide.

The surveillance found the magnitude of redox peak current around 2.6×10^{-5}A. This suggests that the iron oxide possess a notable surface enhancement effect to chlorpyrifos at interface and results the high accretion of the pesticide into the GPE. Such fact was also reported in the literature (Mulchandani et al., 2001a). Therefore, the lofty accumulation efficiency of IOMGPE towards chlorpyrifos on its surface can be responsible for improving the concentration gradient interface as well as the fineness of observed redox signals.

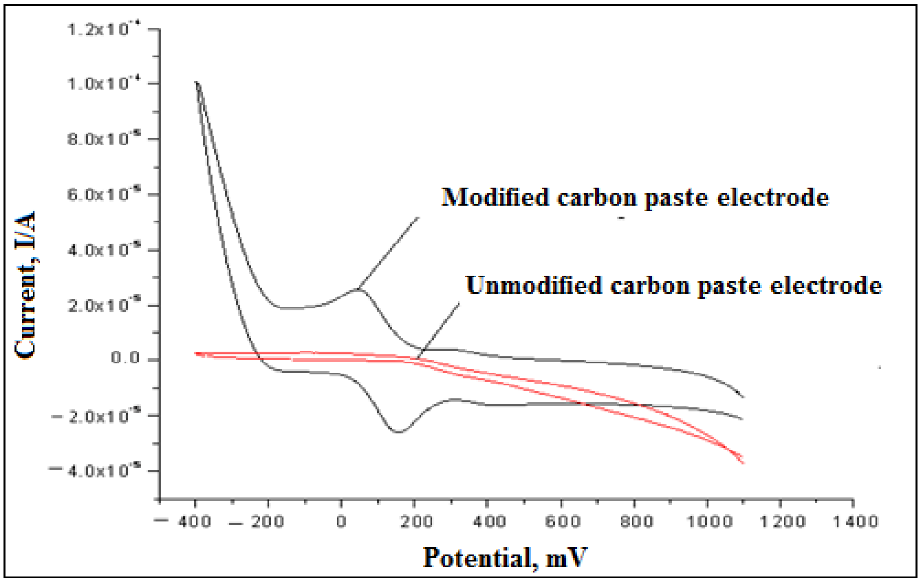


Figure 1. Cyclic voltammograms of 2.5mM chlorpyrifos solution in presence of IOMGPE (black) and UGPE (red) at scan rate 50mV/s.

3.2. Effect of supporting electrolyte

Ground work experiments have been performed to select the best supporting electrolyte for the redox peaks of chlorpyrifos. Various types of 0.1 M acids or buffers such as acetate buffer, perchloric acid, sodium-sulphate, phosphate buffer etc. were tried as possible supporting electrolytes. The results obtained using phosphate buffer was better in signal resolution, crest serration, peak response and peak severance. Thus, the phosphate buffer solution was chosen as a best supporting electrolyte for the present study, because of its excellent signal's rejoinder and reproducibility.

3.3. Effect of pH

According to the earlier report (Kiran, 2013) magnitude of pH of an electrolytic solution is an important factor that put noteworthy effect on the redox behavior of electro-active species at the interface. So, the variation of pH has been made in the range of 5.0 to 11.0 and its impacts on the electro-chemical characteristics of the pesticide under study were investigated by using phosphate buffer solution at a scan rate 50 mVs^{-1} using cyclic voltammetric method. The plot of redox peak current as a function of buffer pH using IOMCPE in 0.1 M phosphate buffer solution has been illustrated in figure 2. The magnitude of peak current increases with increasing pH from 5.0 to 7.5 and thereafter it presents a continuous decreasing trend at higher pH values. The better sensitivity and shape of the voltammogram was observed at 7.5 pH. Therefore, pH = 7.5 was chosen for the entire voltammetric experiment.

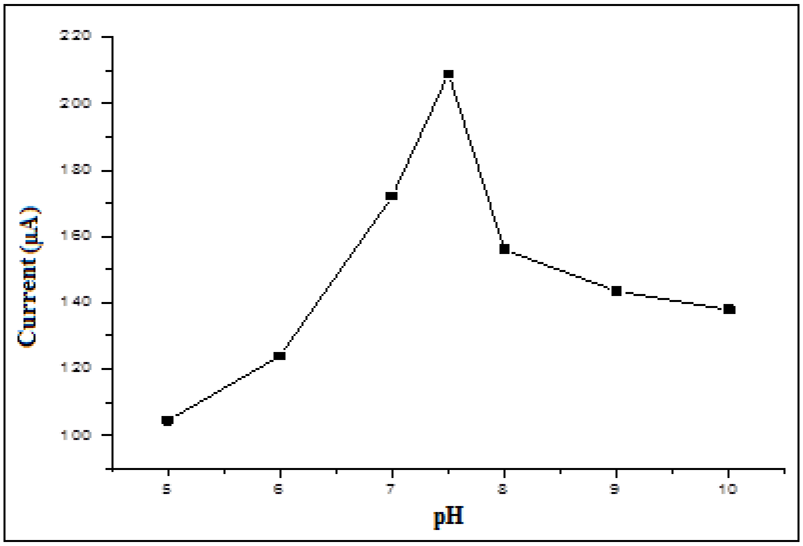


Figure 2. The pH Effect of on the observed peak current in 2.5mM chlorpyrifos solution at the interface of IOMGPE.

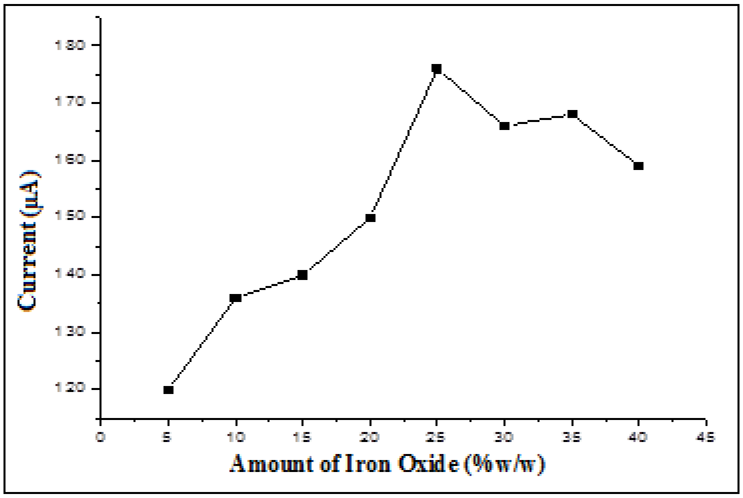


Figure 3. The effect of amount of iron oxide in MGPE (by wt) on the anodic peak current of 2.5 mM Chlorpyrifos in 0.1 M PBS at pH 7.5 and scan rate 50 mVs^{-1}.

3.4. Effect of electrode composition

Cyclic voltammograms of 2.5mM chlorpyrifos in phosphate buffer solution of pH 7.5 at carbon paste electrode modified with different mass percentage (% w/w) of iron oxide were recorded at a scan rate $50 mVs^{-1}$. The magnitude of observed peak current corresponding to each composition as a function of iron oxide composition in graphite paste has been presented in figure 3. It is evident from this figure that as the composition of the modifier in the GPE increase up to 25% (w/w) the intensity of observed redox current increase. On further rise of iron oxide concentration in the graphite paste, redox current follows decrease. The decrease in peak current may be attributed to the decrease of conductivity of the electrode because of the increase in amount of less conductive iron oxide and decrease in more conductive graphite powder, in the paste. Therefore, based on the maximum current response, 25% (w/w) IOMGPE was used for the subsequent electrochemical study.

3.5. Differential pulse voltammetric investigation

Differential pulse voltammetry is a powerful electro-analytical technique that can be used in both electro-kinetic and quantitative determination of redox couples immobilized on the surface of electrode. Because of high speed, fine sensitivity and excellent background current separation of differential pulse voltammetry (DPV), it is used to investigate the electrochemical behavior of chlorpyrifos at the surface of IOMGPE. The differential pulse voltammograms obtained for different concentrations of chlorpyrifos in 0.1 M phosphate buffer of pH 7.5 at IOMGPE are presented in figure-6. For 100µM concentrated pesticide solution a well defined sharp peak at about 133mV has been observed with modified sensor. The observed shift in potential toward positive direction, accompanied by peak current enhancement is clear evidence for the catalytic effect of iron-oxide toward chlorpyrifos oxidation, confirming the previous result obtained by cyclic voltammometry.

3.5.1 .Effect of pulse amplitude

A plot between the peak current at different pulse amplitude as a function of pulse amplitude is presented in figure-4. A substantial increase in peak current observed up to 25mV and further increase in pulse amplitude results decrease in peak current. Also, the response of current signal observed unstable and non-reproducible. Accordingly, the pulse amplitude of 25mV is selected as the optimum value to carry out the subsequent experiment.

3.5.2. Effect of step potential

The effect of DPV step potential (ΔE), which determines the potential change between two data points in the experiment, was also investigated. Step potential values in the range of 1- 6 mV at a frequency of 15Hz and pulse amplitude of 25mV, were applied for the oxidation of 2.5mM chlorpyrifos using the modified sensor. The peak current increase nearly linearly as the DPV step potential increase up to 4mV and then the peak current declined slightly as is evident from figure-5. Hence differential pulse step potential of 4mV was chosen as optimal condition for the subsequent experiment.

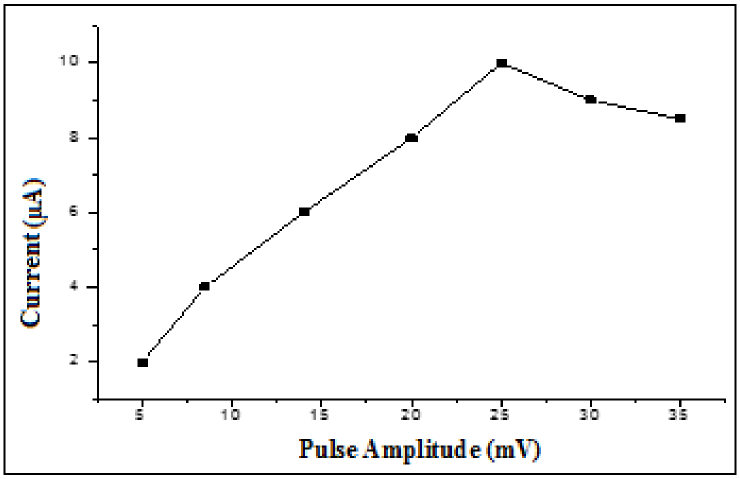


Figure 4. The effect of pulse amplitude on peak current of 2.5mM chlorpyrifos in 0.1 M PBS at pH 7.5 using IOMGPE; at15 Hz frequency and 4 mV step potential.

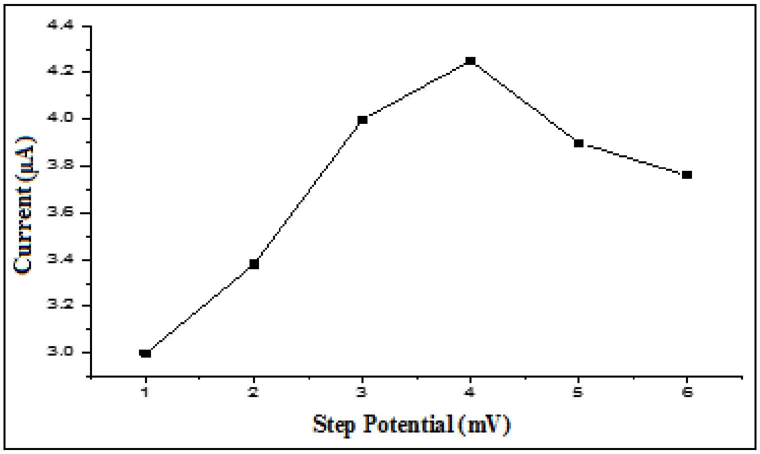


Figure 5. The effect of step potential on the peak current of 2.5 mM chlorpyrifos in 0.1 M PBS at 7.5pH; frequency 15 Hz, and pulse amplitude 25 mV.

3.6. Calibration plot of Chlorpyrifos

In order to see the practical utility of the developed method for quantitative analysis of chlorpyrifos, differential pulse voltametric technique has been employed. This is because the observed current signals are better defined even at its low concentrations as compared to that of cyclic voltammetric method. Keeping the optimal differential pulse parameters in practical use the differential pulse volammograms at different low concentrations of Chlorpyrifos are accessible as in figure-6. The peak current signals have observed a linear increase with rise in concentration of analyte in the studied range i.e. between 1μM to 100μM. From the plot between the anodic peak current and concentration of analyte, a standard calibration curve has been framed and indicated as in inset of figure-6. The linear regression equation with correlation coefficient **(R^2)** and standard deviation **(S)** are given as below.

$i_{pa}(\mu A) = 0.587 + 0.0925\ C\ (\mu M)$

$R^2 = 0.9979\ (n = 9)$

S = 0.071.

Deviations from linearity have been observed for higher concentrations of the pestiside. These could be accounted in terms of some other phenomenon like the adsorption of either chlorpyrifos or any of its oxidation products or both, on the sensor/ analyte interface. The limit of detection for chlorpyrifos was calculated by measuring the DPV of modified electrode without chlorpyrifos in its nine operations along with the standard deviation from same measurements. Accordingly, the magnitude of standard deviation from these nine measurements is 8.6×10^{-8}. The limit of detection (LoD) was calculated based on signal to noise ratio (S/N) of three i.e. LoD = $\frac{3S}{m}$ (Del-Carlo et al., 2002; Guzsvány et al., 2011; Guziejewski et al., 2012). The calculated value of LoD of chlorpyrifos is 2.8×10^{-6}M.

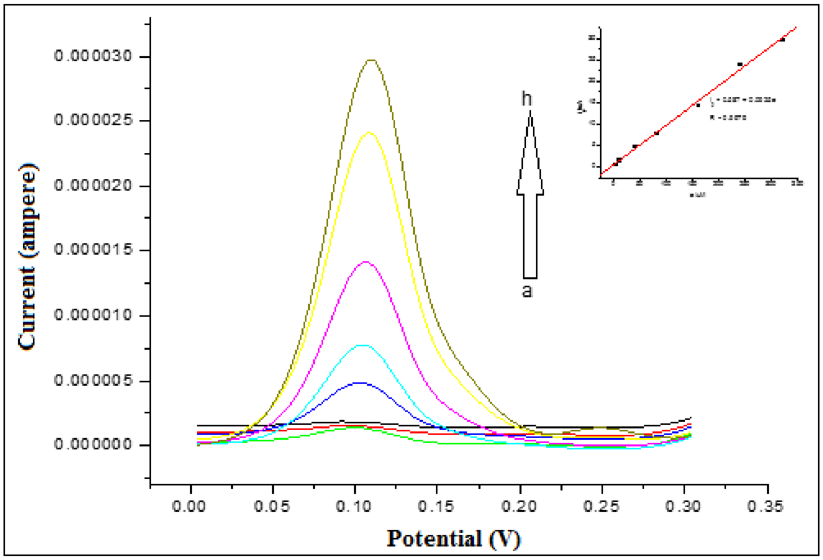


Figure 6. DPV of chlorpyrifos for different concentrations (from a - h; 1.0, 4.0, 6.0, 10.0, 20.0, 40.0, 60.0, and 100.0 μ M) using 50 mV/s (background subtracted).

Table 1. The recovery results of chlorpyrifos from its aqueous solutions using the IOMGPE.

Sample	***Concentration of Chlorpyrifos solution***		***Recovery%***
	In use (µM)	***Found (µM)[a]***	
1	5	4.94	98.8
2	15	14.38	95.9
3	25	24.11	96.4
4	35	32.50	92.9
5	45	44.93	99.8
6	50	48.12	96.2

Note: [a]The average of five runs.

3.7. Recovery Test

Solution of different concentrations up to 50µM, were prepared by using the dilution method from 2.5mM stock solution of chlorpyrifos. In order to determine the quantity of the pesticide, each sample of its aqueous solution was operated through five repetitions using differential pulse voltamommetry and the average concentration calculated by using the standard calibration curve, from these repetitions have been reported in table 1. The recovery results of the pesticide from in-use aqueous solutions are presenting an admirable resemblance, which justify the accuracy and workability of the present sensor.

Table 2. Comparison of the proposed method with earlier reported methods for the voltammetric determination of Chlorpyrifos.

Method Applied	***Linear range (ppm)***	***LOD (µg/L)***
Immunochromatography (Hua et al., 2010)	50-250	132.91
β-SPR biosensor system (Mauriz et al., 2006)	-	45-64
[BMIM][BF4]-MWCNT gel-modified CP electrode (Zamfir et al., 2011)	3.505-350.5	1.402
dsCT-DNA/PANI-PVS/ITO (Prabhakar et al., 2008;Gahlaut et al., 2012)	0.5-200	0.50
BSA/anti-chlorpyrifos/GS-MB/ GNPs/GCE **(**Shaidarova et al., 2001; Papp et al., 2011)	1-500	0.10
Present work	**1 -100**	**0.982**

3.8. Comparison with other methods

The application of the proposed method in the voltammetric determination of chlorpyrifos has been compared with recently reported methods and the results are summarized in table 2. It is evident from the table that the newly developed electrochemical sensor based on IOMGPE provides a comparable analytical performance and lower magnitude for limit of detection than most of the reported electrodes. In addition, the IOMGPE offers simple preparation, easy handling and low cost as compared with other electrodes. Therefore, the proposed method can be effectively applied for the determination of chlorpyrifos in water solutions/ extracts of fresh fruits and vegetables etc.

3.9. Stability and reproducibility of IOMGPE

The reproducibility of the fabricated electrode was investigated by cyclic voltammetry. The relative standard deviation (RSD) obtained for four successive determinations of 2.5mM chlorpyrifos at IOMGPE was 3.42%, which showed that the electrode provides good reproducibility. After two weeks of repeated use, no significant difference in the response of the electrode was observed which indicate the stability of the electrode. After storing in the laboratory at room temperature for about two months, a slight decrease in the response of the peak current was observed, suggesting acceptable storage stability. Hence the relative stability of the IOMGPE is good and reproducibility up to 98% after two months.

4. CONCLUSION

In this study a simple, fast, reproducible procedure has been used for developing of Fe_3O_4 modified graphite paste electrode for determination of chlorpyrifos pesticide in aqueous solutions. It was found that the redox peak current of chlorpyrifos has improved significantly and the oxidation peak shifted towards less positive potentials at the IOMGPE as compared to graphite paste electrode which suggests that IOMGPE displays excellent electro-catalytic property towards chlorpyrifos oxidation. The obtained results showed that the present sensor has better analytical performance than the bare graphite paste in the chlorpyrifos determination. LOD of chlorpyrifos was 2.8×10^{-6} M; the average recovery was found 96.6 %.

5. ACKNOWLEDGEMENTS

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