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Sol–gel immobilized biosensor for the detection of organophosphorous pesticides: A voltammetric method

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ABSTRACT

Organophosphorous compounds are important neuroactive molecules whose presence exhibits significant analytical challenges. An acetylcholinesterase (AChE) based amperometric biosensor was developed by silica sol–gel film immobilization of the enzyme onto the carbon paste electrode. The mono enzyme biosensor was used for the determination of two organophosphorous compounds such as methyl parathion (MP) and acephate in 0.1 M phosphate buffer (pH 7.0). The substrate used was acetylthiocholine chloride (ASChCl) confirmed the formation of thiocholine and it was electrochemically oxidized giving significant increase in anodic peak current around at 0.60 V versus calomel electrode. The influence of pH, enzyme loading and substrate concentration on the response of the biosensor was investigated. The monoenzyme biosensor provided linearity to methyl parathion and acephate in the concentration range of 0.1–0.5 ppb and 50–750 ppb with an incubation time of 20 min and 4 min. The detection limits under the optimum working conditions were found to be 0.08 ppb for methyl parathion and 87 ppb for acephate. The sensor shows good operational stability 89% of its original activity for 60 successive measurements.

1. Introduction

Organophosphorous compounds are considered to be the most toxic. They are used as pesticides, insecticides and chemical war agents. The high toxicity of organophosphorous neurotoxics and their large use in modern agriculture practices has increased public concerns, health risks and the consequent contamination of water, food sources [1]. The use of any technology for detoxification of organophosphorous compounds, performed in laboratories, will need the development of analytical tools of high performance in order to control the concentration of neurotoxics.

The techniques such as gas chromatography, liquid chromatography and thin film chromatography couple with different detectors and the different types of spectroscopy are the most commonly used methods. However, these techniques are time consuming, expensive and demand a qualified and experienced staff and cannot be used for continuous monitoring. Biological techniques such as immunoassays and inhibition of cholinesterase activity by colorimetric techniques are also used for the determination of organophosphorous compounds. Immunoassays require long analysis time and pre-sample treatment which are expensive enough and these techniques are not suitable for continuous monitoring [2].

Biosensors are sensitive and can be used for pesticide determination. In these sensors, the inhibition of AChE can be measured by electrochemical techniques such as pH-shift potentiometry [3]. The main disadvantage of this technique is its strict requirement for low buffer capacity of analyte solution. This may lead to significant complexities and uncertainness in the measurement of the organophosphorous compounds. The sensitivity of pH-based analytical techniques is less than that of amperometric methods.

Many immobilization methods have been employed to fabricate biosensors with high enzyme activity and fast electro transfer rates [4]. Sol–el immobilization platforms can be preferred to other encapsulation, entrapment of sensing agents with in a polymeric matrix, since some of these procedures are tedious and results in poor stability and perturbed function, require expensive reagents. Therefore many sol–gel derived enzyme biosensors have been developed at the research level to monitor glucose, lactate, cholesterol, dopamine, H_2O_2, phenols and urea [5–11]. Recent development in the area of amperometric biosensors with sol–gel encapsulation of enzyme species as an immobilization matrix is very encouraging and offers potential advantages. These advantages include the ability of sol–gel (i) to form at low temperatures and under chemical, mechanical stability and offers negligible swelling, (ii) open to a wide variety of chemical modifications based on the inclusion of various polymer additives, redox modifiers and organically modified silanes, resulting in electrically conducting materials, (iii) to exhibit tunable pore size and pore distribution, which allows small molecules and ions to diffuse into the matrix while larger biomolecules remain trapped in

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the pores, simplicity of preparation without any kinds of modifications [12].

This work describes the construction of a simple voltammetric monoenzyme biosensor for the direct, sensitive and selective determination of methyl parathion and acephate using acetylcholinesterase by sol–gel immobilization on the surface of carbon paste electrode. The structural and molecular formulae of methyl parathion and acephate were shown in Table 1. This paper assesses experimental parameters such as pH, scan rate and enzyme loading have been investigated to evaluate the conditions for the best performance of the biosensor.

2. Experimental

2.1. Materials

All chemicals were obtained from commercial sources and used without further purification. Acetylcholinesterase (EC: 3.1.1.7, type VI-S/1.5 mg, electric eel source, 500U/1.5 mg), acetylthiocholine chloride were purchased from Sigma-Aldrich chemicals, USA. Tetraethylorthosilicate (TEOS), cetyl trimethyl ammonium bromide (CTAB), Triton-X-100 were obtained from Sigma-Aldrich chemicals co. USA. Methyl parathion and acephate were obtained from accustandard solutions company, USA. The pesticide stock solution was prepared dissolving in acetone (GR grade) solution. The graphite fine powder was procured from Lobo chemie and silicon oil (Himedia) and acetone (GR grade) was obtained from Merck specialities Pvt.Ltd. Phosphate buffer solution was prepared by mixing the appropriate quantity of 0.1 M aqueous sodium dihydrogen phosphate monohydrate and 0.1 M aqueous disodium hydrogen phosphate. All the chemicals were of analytical grade and aqueous solutions were prepared with double distilled water. The enzyme stock solution was stored at −20 °C. All stock and working solutions of chemicals were stored at 4 °C.

2.2. Apparatus

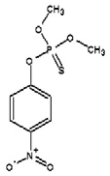
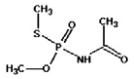
The electrochemical experiments were carried out using a model CH-660C (CH-Instruments). All the experiments were carried out in a conventional three electrode electrochemical cell. The electrode system contained a working electrode was sol–gel immobilized enzyme modified carbon paste electrode, a platinum wire as a counter electrode and saturated calomel electrode as reference electrode. All the experiments were carried out at room temperature 25 ± 2 °C.

2.3. Preparation of sol–gel immobilized enzyme modified carbon paste electrode

The carbon paste electrode was prepared as follows; 70% graphite powder and 30% silicon oil were mixed by hand to produce a homogenous a homemade carbon paste electrode and smoothed on weighing paper. A homogenous TEOS silica sol–gel was prepared by mixing 2 ml of TEOS, 1 ml of H_2O, 50 μl of 0.1 M HCl, 25 μl of 10% Triton-X-100. The mixture was stirred for 1 hr until a clear sol was formed. The sol can be stored for several months when refrigerated at −20 °C.

The 5 μl of stock sol–gel solution was vortexed with 45 μl of phosphate buffer containing 0.5 U of enzyme stock solution. The 3 μl of the enzyme sol was spread on the electrode surface. This film was allowed to polymerize at room temperature for 3–5 min. The electrode was gently washed with phosphate buffer (pH 7.0) and was used for further experimental procedure [13]. The 0.03U of enzyme was immobilized on the electrode surface.

Table.1
The structural and molecular formulae of methyl parathion and acephate.

Pesticide	Molecular formula	Structure
Methyl parathion	$C_8H_{10}O_5NPS$	
Acephate	$C_4H_{10}NO_3PS$	

3. Results and discussion

3.1. Electrochemistry of acetylthiocholine chloride (ASChCl) at the surface of AChE enzyme immobilized graphite paste electrode

The electrochemical behavior of thiocholine which was released from enzymatic hydrolysis of acetylthiocholine chloride in presence of AChE. Fig. 1 shows the cyclic voltammograms of the sensor in the presence and absence of 1 mM substrate in 0.1 M phosphate buffer (pH 7.0) at a scan rate of 10 mVs^{-1}. In the absence of substrate, the enzyme electrode gave no response and only a small background current was observed [dashed line]. When the substrate was added to the buffer solution, a relatively larger anodic current at potentials of 0.65 V was observed. In the present electrochemical approach, the electrode response was proportional to the oxidation of an electroactive species produced by the enzymatic reaction. The mechanism of the reaction has been shown in Scheme (1)[14].

The increased concentrations of substrate showed gradual increase in peak current response and finally saturated at which current values observed were limited. The calibration plot of the typical enzyme–substrate reaction was shown in Fig. 2. The kinetics of the immobilized enzyme showing diphasic in nature, i.e. at low amounts of substrate, the enzyme rate was varied with concentration (first order) and at high concentrations of substrate it reach plateau level the rate was independent of concentration (zero order). The apparent Michaelis–Menten constant was (K_m^{app}) 450 μM which was calculated from the Lineweaver–Burk equation. Analysis of the slope and intercept for the plot of reciprocal of current response (1/V) versus reciprocal of substrate concentration (1/S) allowed the determination of K_m^{app} [13].

3.2. The effect of scan rate and pH

The effect of scan rate for acetylthiocholine chloride (ASChCl) was studied at enzyme modified carbon paste electrode. It showed increase in the oxidation peak current with increase in scan rate from 5 to 50 mVs^{-1}. The graph of current (Ipa) versus square root of scan rate ($\sqrt{\nu}$) was plotted. The graph obtained resulted with good linearity Fig. 3. The correlation coefficient was 0.9384, which indicate the electrode reaction was diffusion controlled process [15–17].

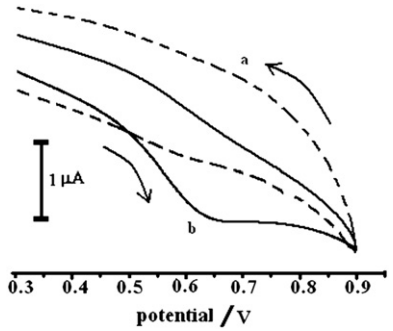


Fig. 1. Cyclic voltammogram of sol–gel immobilized acetylcholinesterase electrode in 0.1 M phosphate buffer, pH 7.0 and 0.1 M KCl (a) without substrate (dashed line), (b) with 1 mM substrate (solid line).

$$CH_3\text{-}CO\text{-}SCH_2CH_2N^+(CH_3)_3 + H_20 \xrightarrow{AChE} CH_3\text{-}COOH + (CH_3)_3N^+CH_2CH_2SH$$

$$2\,(CH_3)_3N^+CH_2CH_2SH \xrightarrow{anode} (CH_3)_3N^+CH_2CH_2S - SCH_2CH_2N^+(CH_3)_3 + 2H^+ + 2e^-$$

Scheme.1. The mechanism of the enzyme catalyzed reaction.

The effect of pH on the electrochemical response of the enzyme electrode towards the 1 mM substrate over the pH range 5.5 to 9.0 in 0.1 M phosphate buffer solution was illustrated in Fig. 4[A]. The resulting profile showed maximum sensitivity of the enzyme electrode at pH 7.0. The immobilized acetylcholinesterase enzyme electrode showed an optimum pH range of 7.0–8.0 [18]. The potential diagram was constructed by plotting the graph of anodic peak potential Epa *Vs* pH of the solution and shown in Fig. 4[B]. The pH dependence of oxidation peak potential of substrate, Ep = 0.9657 + 0.052 pH ($r^2 = 0.9905$). The graph has good linearity with a slope of 52 mV/pH, this behavior was nearly obeyed the Nernst equation for equal number of electron and proton transfer reaction [19,20].

3.3. Effect of enzyme concentration

Fig. 5 shows the effect of enzyme concentration which was used in fabrication of monoenzyme biosensor based on its response. It can be followed from the obtained data that the enzyme loading from 0.03 to 0.3U into the sol–gel matrix produced increased response with 1 mM substrate and resulted in the increased sensitivity and shorter response times. The rate of enzyme catalyzed reaction was dependent on the amount of enzyme immobilized. The lowest amount of enzyme was necessary to achieve the lowest detection limit of pesticides [21–24].

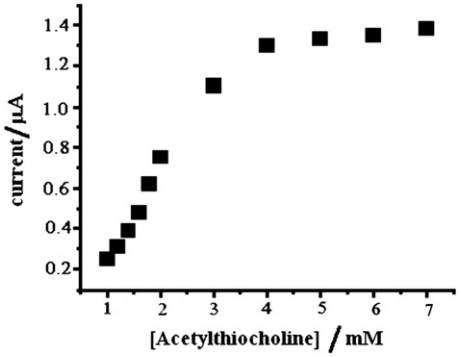


Fig. 2. Biosensor response for increasing concentration of acetylthiocholine chloride in 0.1 M phosphate buffer containing 0.1 M KCl, pH 7.0.

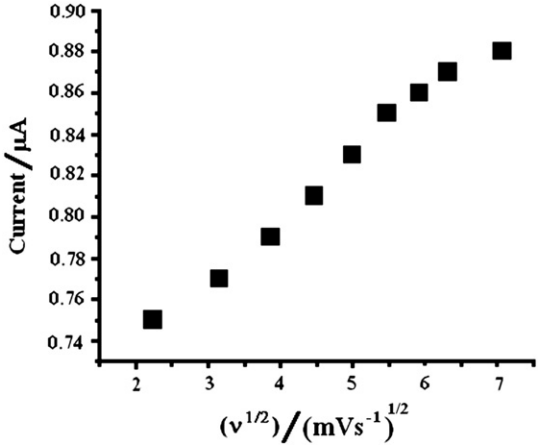


Fig. 3. The effect of square root of scan rate on anodic peak current for 1 mM ASChCl in 0.1 M phosphate buffer, pH 7.0 and 0.1 M KCl.

3.4. Measurement of organophosphorous pesticides

AChE sensors have been used to carry out inhibition studies with the two pesticides: methyl parathion and acephate. Sol–gel immobilization plot form used could provide wider concentration range of pesticide detection sensitive to ppb level. The detection was based on

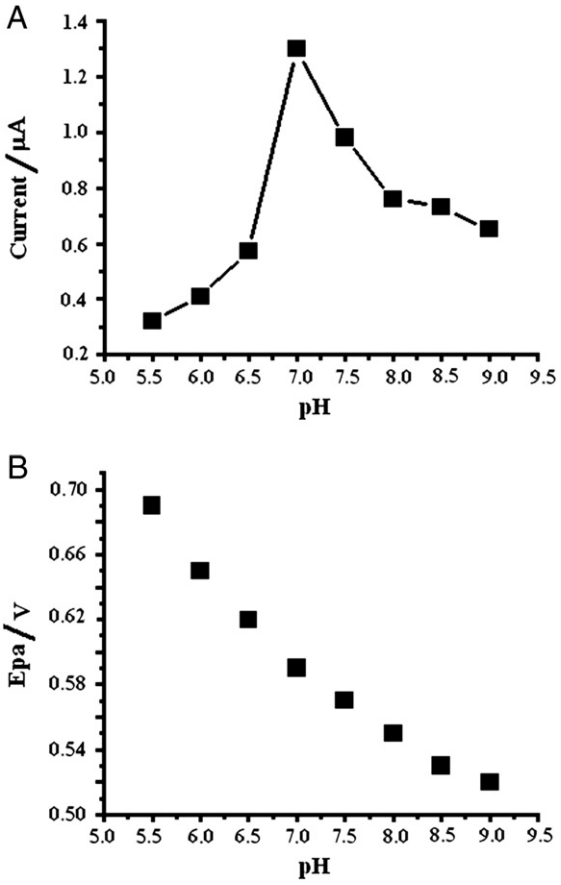


Fig. 4. [A] Effect of pH on the enzyme electrode response. [B] Graph of different pH vs Epa to 1 mM ASChCl.

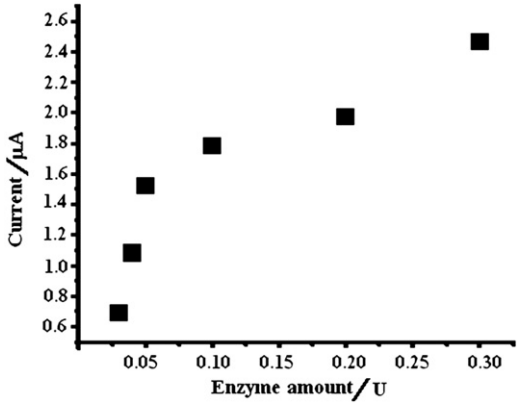


Fig. 5. Biosensor response for increasing concentration of enzyme on the surface of the electrode in 0.1 M phosphate buffer containing 0.1 M KCl, pH 7.0.

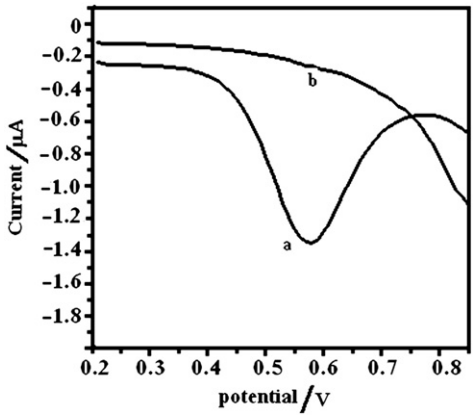


Fig. 6. Differential pulse voltammogram of (a) substrate alone, (b) with pesticide solution.

the measurement of current response of the substrate 1 mM ASChCl by placing the biosensor in a buffered solution (0.1 M phosphate buffer and 0.1 M KCl at pH 7.0). The response of the biosensor was measured as the current increases in relation with the base current when the substrate was added to the buffer (I_i). After that, the biosensor was placed for 1 min in contact with the sample containing the pesticides, which inhibit the AChE and the same measurement was repeated (I_F). The percentage of inhibition and residual enzyme activity were calculated as follow: [25]

$$\text{Inhibition } \% \text{ (I\%)} = [(I_i - I_F)/I_i] \times 100$$
$$\text{Residual enzyme activity } \% \text{ (REA \%)} = [I_F/I_i] \times 100$$

Methyl parathion and acephate pesticides are known to inhibit the reaction between AChE and acetylthiocholine chloride, hence they have been used widely as toxic reference to test acetylcholinesterase biosensors.

Quantitative analysis of individual pesticides was carried out according to the above procedure. Fig. 6 shows the DPV voltammogram for substrate alone and pesticide solution where complete inhibition occurred. Calibration plots based on the dependence of the % inhibition on concentration were linear and shown in Fig. 7[A] and [B] for methyl parathion and acephate respectively. The detection limit and limit of quantification values were 0.08 ppb, 87 ppb and 0.28 ppb, 292 ppb for methyl parathion and acephate respectively. When compared the above two pesticides methyl parathion was more toxic than acephate because it has less DL value than acephate. Thus, these results clearly indicate that the proposed electrochemical method of analysis was reliable for the determination of individual pesticides. The calibration plot of % residual enzyme activity with incubation time for methyl parathion and acephatewas shown in Fig. 8[A] and [B]. When the concentration of pesticide increases the residual enzyme activity of the enzyme decreases with time. Methyl parathion and acephate was shown in Fig. 9[A] and [B]. Determination of DL and QL were carried by using the following expression [15,26–29].

$$\text{DL} = 3S_b/S$$
$$\text{QL} = 10S_b/S$$

Where S_b is standard deviation, S is the slope of the working curve, DL is the detection limit, QL is the quantification limit. Table 2 shows the various parameters determined for methyl parathion and acephate.

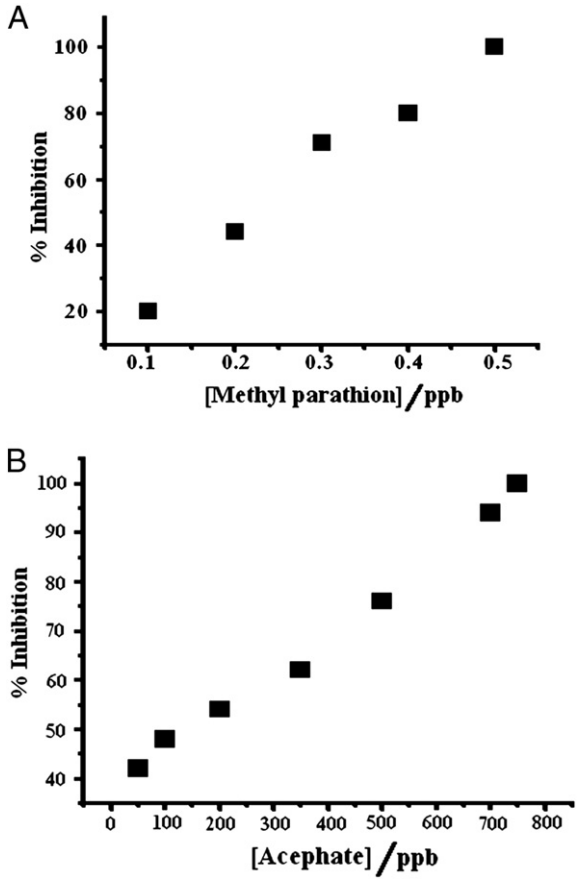


Fig. 7. Inhibition plots of [A] methyl parathion after 20 min incubation time, [B] acephate after 4 min incubation time with the measurement conditions in 100 mM phosphate buffer/KCl.

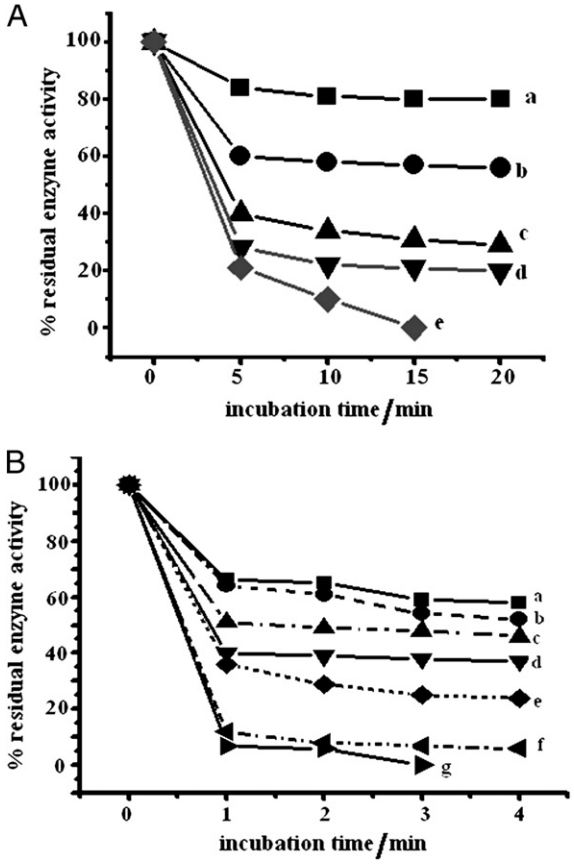


Fig. 8. The effect of incubation time at various inhibitor concentrations on the activity of immobilized enzyme in 0.1 M phosphate buffer/KCl, pH 7.0. [A] For methyl parathion: (a) 0.1 ppb, (b) 0.2 ppb, (c) 0.3 ppb, (d) 0.4 ppb, (e) 0.5 ppb. [B] For acephate: (a) 50 ppb, (b) 100 ppb, (c) 200 ppb, (d) 350 ppb, (e) 500 ppb, (f) 700 ppb, (g) 750 ppb.

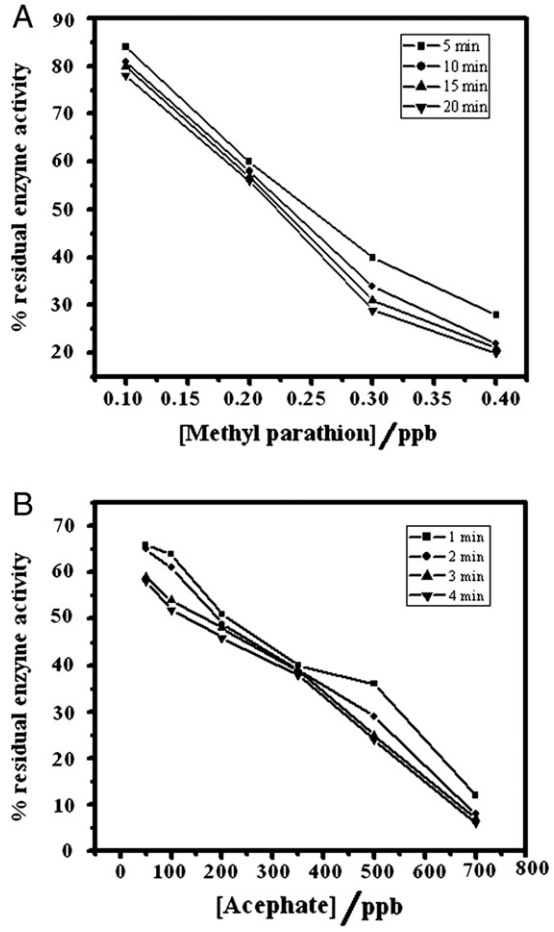


Fig. 9. The variation of residual enzyme activity with different inhibitor concentrations with time in 0.1 M phosphate buffer/KCl, pH 7.0. [A] For methyl parathion, [B] for acephate.

The robustness of the developed method was evaluated by studying the concept of repeatability (new electrode, same standard solution, same day, same analyst, *n* is number of assays) and reproducibility (new electrode, new standard solution, different days, different analyst, *n* is number of assays) of the biosensor towards the inhibition of pesticides [26,27]. To study this experiment the chosen concentration of the stock solutions of methyl parathion and acephate were 0.4 ppb and 600 ppb. The results obtained for the developed procedure towards the inhibition of both the pesticides was reproducible, because there was no significant difference between the RSD values of the both the pesticides. The results were shown in Table 2. The long term stability of the AChE biosensor was investigated under the storage conditions (at 4° C), it was noticed that the activity of immobilized AChE was stable up to 1 month.

Table.2
The various parameters determined for methyl parathion and acephate.

Parameters	Methyl parathion	Acephate
Response time (min)	1	1
Incubation time (min)	20	4
Linear range (ppb)	0.1 – 0.5	50 – 750
Intercept of calibration curve (10^{-7}A)	4.2	37.66
Slope of calibration curve (10^{-7}A / μgL-1)	196	0.08
Correlation coefficient	0.9884	0.9955
Standard deviation	5.4894	2.3418
Detection limit (DL) (ppb)	0.08	87
Quantification limit (QL) (ppb)	0.28	292
Repeatability (%RSD) ($n=3$)	2.85	3.27
Reproducibility (%RSD) ($n=3$)	3.73	4.09

n = number of assays.

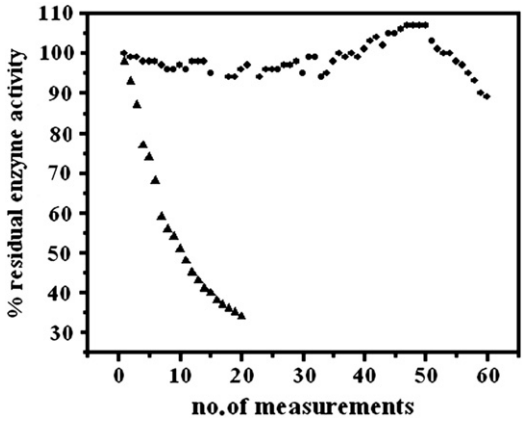


Fig. 10. Dependence of residual activity of the enzyme in the presence of 1 mM substrate with successive number of measurements: (a) in the absence, (ii) in the presence of 100 ppb of inhibitor.

3.5. Biosensor response characteristics

Fig. 10 shows the operational stability of the biosensor was also confirmed by examining its repeated response to 1 mM ASChCl for 1 hr. The enzyme electrode shows good operational stability retaining 89% of its original activity with 60 successive measurements with substrate alone. The activity of the entrapped acetylcholinesterase in the sol–gel film was stable and there was no decrease up to 50 measurements in the absence of inhibitor. The enzyme electrode in the presence of inhibitor could not retain its activity instead dropped to 34% of its original value after 20 measurements.

4. Conclusions

The research described in this paper resulted in voltammetric method for the determination of organophosphorous pesticides. Biosensor prepared by using simple sol–gel technology to immobilize the acetylcholinesterase takes minimal preparation time. The detection limit of the AChE sensor was directly related to the capacity of the pesticide to inhibit AChE. The new sensors are of single use and can be produced at low cost. The AChE biosensors possess distinct advantages, including monitoring of hydrophobic substrates and relative ease of enzyme immobilization.

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References

[1] H. Tavakoli, H. Ghourchian, Mono-enzyme biosensor for the detection of organophosphorous compounds, J. Iran. Chem. Soc. 7 (2010) 322–332.
[2] Nicole Jaffrezic-Renault, New trends in biosensors for organophosphorus pesticides, Sensors 1 (2001) 60–74.
[3] F. Arduini, F. Ricci, C.S. Tauta, D. Moscone, A. Amine, G. Palleschi, Detection of carbamic and organophosphorous pesticides in water samples using a cholinesterase biosensor based on Prussian Blue-modified screen-printed electrode, Anal. Chim. Acta 580 (2006) 155–162.
[4] Valber A. Pedrosa, Josiame ca etano, Sergio A.S. Machado, Renato S. Freire, Mauro Bertotti, Acetylcholinesterase immobilization on 3-mercaptopropionic acid self assembled monolayer for determination of pesticides, Electroanalysis 19 (2007) 1415–1420.
[5] S. Sampth, O. Lev, Anal. Chem. 68 (1996) 2015–2021.
[6] T.M. Park, E.I. Lcouoha, M.R. Smyth, R. Freoney, A.J. Mcshane, Sol–gel based amperometric biosensor incorporating an osmium redox polymer as mediator for detection of L-lactate, Talanta 44 (1997) 973–978.
[7] T. Yao, K. Takashima, Amperometric biosensor with a composite membrane of sol–gel derived enzyme film and electrochemically generated poly(1,2-diaminobenzene) film, Biosens. Bioelectron. 13 (1998) 67–73.

[8] P.C. Pandey, S. Upadhyay, I. Tiwari, G. Singh, V.S. Tripathi, A novel ferrocene encapsulated palladium-linked ormosil-based electrocatalytic dopamine biosensor, Sens. Actuators, B 75 (2001) 48–55.
[9] T.M. Park, Amperometric determination of hydrogen peroxide by utilizing a sol–gel-derived biosensor incorporating an osmium redox polymer as mediator, Anal. Lett. 32 (1999) 287–298.
[10] J. Li, L.S. china, N.K. Goh, S.N. Tan, Silica sol–gel immobilized amperometric biosensor for the determination of phenolic compounds, Anal. Chim. Acta 362 (1998) 203–211.
[11] P.C. Pandey, G. Singh, Tetraphenylborate doped polyaniline based novel pH sensor and solid-state urea biosensor, Talanta 55 (2001) 773–782.
[12] J. Lin, C.W. Brown, Sol–gel glass as a matrix for chemical and biochemical sensing, Trends Anal. Chem. 16 (1997) 200–211.
[13] K. Anitha, S. VenkataMohan, S. Jayarama Reddy, Development of acetylcholinesterase silica sol–gel immobilized biosensor—an application towards oxydemeton methyl detection, Biosens. Bioelectron. 20 (2004) 848–856.
[14] G.S. Nunes, D. Barcelo, B.S. Grabaric, J.M. Diazcruz, M.L. Ribeiro, Evaluation of a highly sensitive amperometric biosensor with low cholinesterase charge immobilized on a chemically modified carbon paste electrode for trace determination of carbamates in fruit, vegetable and water samples, Anal. Chim. Acta 399 (1999) 37–49.
[15] Umesh Chandra, B.E. Kumara Swamy, Ongera Gilbert, B.S. Sherigara, Voltammetric resolution of dopamine in the presence of ascorbic acid and uric acid at poly (calmagite) film coated carbon paste electrode, Electrochem. Acta 55 (2010) 7166–7174.
[16] Ongera Gilbert, B.E. Kumara Swamy, Umesh Chandra, B.S. Sherigara, Simultaneous detection of dopamine and ascorbic acid using polyglycine modified carbon paste electrode: a cyclic voltammetric study, J. Electroanal. Chem. 636 (2009) 80–85.
[17] S. Chithravathi, B.E. Kumara Swamy, Umesh Cahndra, G.P. Mamatha, B.S. Sherigara, Electrocatalytic oxidation of sodium levothyroxine with phenyl hydrazine as a mediator at carbon paste electrode: a cyclic voltammetric study, J. Electroanal. Chem. 645 (2010) 10–15.
[18] N. Mionelto, J.-L. Marty, Karube, Acetylcholinesterase in organic solvents for the detection of pesticides: biosensor application, Biosens. Bioelectron. 9 (1994) 463–470.
[19] B.D. Jones, J.D. Ingle Jr., Evaluation of immobilized redox indicators as reversible, in situ redox sensors for determining Fe(III)-reducing conditions in environmental samples, Talanta 55 (2001) 699–714.
[20] M.C. Shen, H.C. Gheng, V.S. Vasantha, Preparation and electrocatalytic properties of the TBO/nafion chemically-modified electrodes, J. Electroanal. Chem. 588 (2006) 235–243.
[21] E. Suprun, G. Evtugun, H. Budnikov, F. Ricci, D. Moscone, G. Palleschi, Acetylcholinesterase sensor based on screen-printed carbon electrode modified with prussian blue, Anal. Bioanal. Chem. 383 (2005) 597–604.
[22] S. Sotiropoulou, N.A. Chaniotakis, Lowering the detection limit of the acetylcholinesterase biosensor using a nanoporous carbon matrix, Anal. Chim. Acta. 530 (2005) 199–204.
[23] A. Ivanov, G. Evtugyn, H. Budnikov, Riccif, D. Moscone, G. Palleschi, Cholinesterase sensors based on screen-printed electrodes for detection of organophosphorus and carbamic pesticides, Anal. Bioanal. Chem. 377 (2003) 624–631.
[24] G.A. Evtugyn, H.C. Budnikov, E.B. Nikolskaya, Sensitivity and selectivity of electrochemical enzyme sensors for inhibitor determination, Talanta 46 (1998) 465–484.
[25] Alian Hildebrandt, Jordi Ribas, Ramon Bragos, Jean - Louis Marty, Marius Tresanchez, Silvia Lacorte, Development of a portable biosensor for screening neurotoxic agents in water samples, Talanta 75 (2008) 1208–1213.
[26] T. Madhusudana Reddy, S.J. Reddy, Differential pulse adsorptive stripping voltammetric determination of nifedipine and nimodipine in pharmaceutical formulations, urine, and serum samples by using a clay-modified carbon-paste electrode, Anal. Lett. 37 (2004) 2079–2098.
[27] T. Madhusudana Reddy, M. Sreedhar, S.J. Reddy, Voltametric behavior of Cefixime and Cefpodoxime Proxetil and determination in pharmaceutical formulations and urine, J. Pharm. Biomed. Anal. 31 (2003) 811–818.
[28] Djenaine De Souza, Sergio A.S. Machado, Electroanalytical method for determination of the pesticide dichlorvos using gold-disk microelectrodes, Anal. Bioanal. Chem. 382 (2005) 1720–1725.
[29] Rajesh.N. Hegde, B.E. Kumara Swamy, Nagaraj.P. Shetti, Sharanappa.T. Nandibewoor, Electro-oxidation and determination of gabapentin at gold electrode, J. Electroanal. Chem. 635 (2009) 51–57.