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Performance of a portable biosensor for the analysis of ethion residues

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ABSTRACT

Sensitive disposable potentiometric sensors for determination of the organophosphorus pesticide (OPs), ethion and its degradation residues have been constructed. The fabricated screen printed sensors are based on multi-walled carbon nanotube–polyvinyl chloride (MWNT–PVC) composite incorporated with α-cyclodextrin (α-CD) ionophore for butyrylcholine (BuCh) determination. Butyrylcholinesterase (BuChE) activity was measured through monitoring the BuCh hydrolysis using the fabricated sensors. The electrode potential changes linearly with BuChE concentration over the range from 0.04 to 0.4 U in phosphate buffer solution. This approach can also be used to analyze ethion and its degradation products in the concentration range from 0 to 330 ng mL^{-1} by measuring the relative inhibition percentage of BuChE. From different ethion degradation products, inhibition by dioxon and monooxon were more potent than the parent pesticide. The proposed method was applied for determination of ethion in different samples with good accuracy and precision. The relative simple fabrication protocol of biosensor, high sensitivity and stability represents a promising approach for determination of environmental pollutants in field conditions.

1. Introduction

The presence of pesticide residues in food, water and soil currently represents one of the major issues for the environmental chemistry [1]. Organophosphorus compounds (OPs) with a thiophosphoryl functional group (P=S) constitute a broad class of widely used pesticides. These compounds are powerful inhibitors of cholinesterase enzymes (ChE) and effectively prevent nerve transmission by blocking breakdown of the transmitter choline (Ch) which often results in respiratory paralysis and death [2,3]. Ethion (*O,O,O′,O′*-tetraethyl *S,S′*-methylene bis(phosphoro-dithioate) is one of the widely used OPs for control of aphids, spider mites and insects on a wide variety of food, fiber, and crops [4]. High concentration (from 0.4 to 1.0 mg L^{-1}) of ethion is environmentally relevant in some Egyptian fresh water environment [5].

Official methods for the unambiguous determination of OPs are those based on mass spectrometry (MS) [6], gas chromatography (GC) [7], high-performance liquid chromatography (HPLC) [8] or thin layer chromatography [9]. However, some of these specific and sensitive analytical methods require expensive instrumentation that are inaccessible to many developed nations and require highly specialized facilities with complex sample pre-treatment. Furthermore, detection and identification of pesticide decomposition products needs additional analysis protocol those are different than the parent compounds [10]. Nowadays, there is a growing need for on-site tests in environmental analysis which is related to take the necessary corrective actions in a timely fashion and the elimination of errors associated with sampling, storage and long transportation times to the main laboratory.

Cholinesterase (ChE) biosensors are strong candidates for screening pesticide residues and are becoming more and more relevant in environmental and food analysis [11–14]. Quantification of ChE inhibitors is based on measurement of the uninhibited enzyme activity after exposure to the inhibitor. Compared with traditional methods for OPS determination [6–9], the strengths of ChE biosensors arises from the possibility of miniaturization and “in situ” measurements. In addition, owing to the biological origin of their active sites, ChE biosensors are sensitive to general toxicity, whereas other analytical techniques measure only concentration data.

Amperometric and potentiometric approaches have been used for measuring ChE activity. However, potentiometric biosensors were more preferable due to their much easier fabrication technique and measurement protocols which fit better to in situ measurements. Different substrate sensors as, acetylcholine (ACh), BuCh [15–18], and most often pH sensors [19,20] were used

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as transducers in potentiometric biosensors. ChE potentiometric sensors based on pH detection usually suffer from the lack of fast signal response with the necessity to use low buffer capacity. Amperometric ChE biosensors are usually based on measurement of the electroactive thiocholine results from enzymatic hydrolysis of acetyl- or butyryl-thiocholine [21,22]. However, their fabrications are sometimes rather complicated due to the need of immobilization and regeneration of the reactive enzyme layer.

Over the past few years, an interest has been increased in the application of simple, rapid, inexpensive disposable sensors for clinical, environmental and industrial analysis [23–25]. Screen printing seems to be one of the most promising technologies allowing sensors to be produced on a large-scale with the advantages of optimized manufacturing repeatability, long shelf-lifetime and constructing small portable devices. In addition, incorporation of nanomaterials within the sensor matrices enhances the sensor performances through improving the conductivity and transduction of the chemical signal to electrical signal. In this regard carbon nanotubes (CNTs) have attracted considerable attention due to their high electrical conductivity, strong adsorptive ability and good mechanical strength [26–28]. These features have made CNTs highly suitable in the biosensor field [29–31].

The principle aim of the present work is the determination of ethion and its degradation products using a simple potentiometric method as there is no previous electrometric method for determination of such widely used pesticide. As there is an increasing end-user demand for the use of rapid, reliable and low-cost field-based methods for the determination of toxic compounds, a fast response, disposable, robust, potentiometric BuChE biosensor for pesticide analysis was fabricated. BuChE free in solution is incubated with the pesticide sample and the decrease of the BuChE activity was used to measure the inhabitation degree of pesticide.

2. Experimental

2.1. Reagents

All reagents were of the analytical grade and bidistilled water was used throughout the experiments. Butyrylcholine iodide (BuCh) and choline chloride (Ch) were purchased from Fluka and used without further purification. Aqueous 10^{-2} mol L^{-1} solutions of BuCh and Ch were prepared in phosphate buffer solution (pH 7.0). Butyrylcholinesterase solution (BChE, EC 3.1.1.8, from equine serum, Sigma) was prepared by dissolving the appropriate amount of BuChE in phosphate buffer solution (pH 7.0). The specific enzyme activity was verified using Ellman's photometric method as modified by Gorun et al. [32]. The pesticide solution was prepared by dilution of analytical standard ethion (Fluka pestanal®) with acetonitrile.

Different cyclic macromolecules were tested as sensing ionophores including native α-, β-and γ-cyclodextrin (Sigma), heptakis (2,6-di-O-methyl)-β-cyclodextrin (Aldrich), heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin (Aldrich), 12-crown-4 (Fluka), 15-crown-5 (Fluka), calyx[4]arene and calix[8]arene (Aldrich). Sodium tetraphenylborate (NaTPB, Fluka), sodium tetrakis(4-fluorophenyl) borate (NaTFPB, Fluka) and potassium tetrakis(4-chlorophenyl) borate (KTCPB, Fluka) were used as anionic sites.

The tested electrode plasticizers were as following; *o*-nitrophenyl octyl ether (*o*-NPOE, Sigma), 2-fluorophenyl 2-nitrophenyl ether (*f*-PNPE, Fluka), dibutylphthalate (DBP, Sigma), dioctylsebacate (DOS, Avocado) and tricresylphosphate (TCP, Fluka). Poly(vinyl chloride) (PVC, relative high molecular weight, Aldrich) and graphite powder (synthetic 1–2 μm, Aldrich) were used for preparation of the printing ink. Different carbon nanomaterials including multiwall carbon nanotubes (MWCNTs, Aldrich), single wall carbon nanotubes (SWCNTs, Aldrich), functionalized multiwall carbon nanotube (FMWCNTs) [33] and carbon nanopowder (CNP, Sigma) were used.

2.2. Synthesis of ethion and its degradation products

^{14}C-ethion (**I**) labeled at carbon atom of ethyl groups was prepared using ^{14}C-ethanol according to Abdel-Gawad et al. [4]. Some of ethion degradation products have been also prepared for identification purposes including ethion monooxon (**II**), ethion dioxon (**III**) and *O*,*O*-diethyl phosphorothioate (**IV**).

2.3. Apparatus

All potentiometric measurements were carried out using Radio Shack Digital multimeter with PC interface. The pH measurements were performed using Metrohm 692-pH meter with combined pH glass electrode (6.0202.100).

2.4. Procedures

2.4.1. Sensor construction

The potentiometric bielectrode strips were fabricated by screen printing technique on a ceramic support (dimensions 5×35 mm^2) using silver- and graphite-based inks for reference and working electrodes, respectively [18]. The ion-sensing cocktail containing 0.75 mg α-CD, 0.5 mg KTCPB, 360 mg f-PNPE, 6 mL THF, 30 mg MWCNTs and 240 mg PVC as polymeric dispersant is printed on the graphite/PVC conducting track. Polymeric dispersants with MWCNTs showed good compatibility in plasticized PVC matrix. Fabricated electrodes were directly used in measurements after preconditioning in 10^{-3} mol L^{-1} BuCh solution for 10 min.

2.4.2. Sensor calibration

The developed sensors were calibrated by immersing the bielectrode strip in BuCh solutions covering the concentration range from 10^{-7} to 10^{-2} mol L^{-1} [34]. Potential readings were recorded after stabilization and plotted against BuCh concentration.

2.4.3. Measurement of BuChE activity

BuChE activity was determined using the developed sensor under the optimum electrode matrix composition. Aliquots of BuChE solutions were added to the electrochemical measuring cell containing 9.0 mL phosphate buffer (pH 7.0) and 1.0 mL of 10^{-3} mol L^{-1} BuCh solution. The enzyme activity was estimated by monitoring the change of BuCh electrode potential within the reaction time. Calibration curve were constructed by plotting the initial rate ($\Delta E/\Delta t$, determined by drawing a tangent of the first linear part of potential-time curve) against BuChE concentration.

2.4.4. Measurement of inhibition by pesticide

It was almost the same as enzyme activity where 0.4 U of BuChE was incubated with different pesticide concentrations for 30 min and the remaining enzymatic activity was measured. For each concentration, 5 replicates were obtained and the mean value of the inhibition degree (*I* %, calculated from the residual enzyme activity) was represented against the pesticide concentration.

2.5. Samples analysis

2.5.1. Commercial ethion sample

Commercial ethion sample (ENDO, HELB for Chemical and Pesticide Production, Cairo, Egypt, assigned 50% ethion) was purchased from local stores. Sample was diluted with acetonitrile and analyzed under optimized conditions. A chromatograph with

a flame photometric detector (FPD) was used as official method for ethion determination.

2.5.2. Determination of ethion residues in water samples

Ethion residues in spiked water samples (1.0 mg L^{-1}) were extracted with dichloromethane, chloroform and hexane as described in details elsewhere [35]. Ethion residues in both aqueous layer (corresponding to degradation product (**IV**)) and organic layer (for the parent pesticide and its oxons) were estimated by the proposed procedure in comparison to radioassy protocol at the Radioisotope Department, Atomic Energy Authority, Egypt, using a Packard liquid-Scintillation Spectrometer (Model TRI-CARB 2300 TR).

2.5.3. Determination of ethion residues in soybean oil

Soybean seeds (var. Crawford) were cultivated under normal field conditions in a field area. Irrigation, fertilization and soil management were conducted as practiced in the field [36]. Aqueous ethion solution (4 mg/plant each time) was topically applied with a micropipette on healthy leaves of plants twice (21 days apart) just before the flowering stage. Soybean pods were collected 30 days after the second spraying with ethion (harvest time) and the oil was extracted from its dry seeds by n-hexane in Soxhlet apparatus for 12 h. The obtained crude oil (20 mL) was partitioned between 50 mL acetonitrile and 10 mL hexane three times to remove the oil where the ethion residues were distributed in acetonitrile layer. After dryness, the obtained residue was dissolved in appropriate volume of acetonitrile and analyzed with the fabricated sensor and radioassy procedures.

3. Results and discussion

Biosensor technologies are efficient tools for detection of pollutants and OPs pesticides [11,12,37,38]. Herein, a simple disposable potentiometric sensor was developed for determination of ethion. Simple fabrication protocols were conducted without the need of enzyme immobilization or regeneration. Under normal conditions, BuChE hydrolyzes BuCh to Ch and butyric acid [2,3] and the enzymatic activity can be monitored with the fabricated sensor. Pesticide inhibits BuChE causing variation of the enzymatic reaction rate correlated with the pesticide concentration.

3.1. Optimal BuCh sensor matrices compositions

Screen printing technology has increasingly been used for mass production of inexpensive, reproducible and disposable electrochemical sensors [23–25]. Poisoning of the traditional PVC and carbon paste electrode surfaces by proteins and other contaminants, besides their size, limited their widespread applications for on-site monitoring; therefore, the use of disposable sensors was of choice. Due to the critical role of the matrix composition on the sensor sensitivity to BuCh, comprehensive studies were carried out to elucidate the influence of the nature and content of sensing ionophore, ionic additives, nature and content of the carbon nanomaterial, and plasticizer. In preliminary experiments, nine different cyclic compounds were tested as sensing material including α-, β- and γ-CD, 2,3-di-O-methyl-β-CD, 2,3,6-tri-O-methyl-β-CD, 15-crown-5, 12-crown-4, calyx[4]arene and calix[8]arene. From different tested ionophores, α-CD showed the best performance towards BuCh with minimal interference of Ch (Nernstian slope values were 56.0 ± 2.5 and 36.0 ± 1.0 mV $decade^{-1}$ in the concentration range from 10^{-6} to 10^{-2} mol L^{-1} for BuCh and Ch, respectively). Moreover, the content of α-CD within the electrode matrix was varied from 0 to 2.5 mg and incorporation of 0.75 mg of the selected ionophore was sufficient to obtain reasonable cationic slope of 58.0 ± 1.2 mV $decade^{-1}$.

Cyclodextrins behave as neutral carrier ionophores; therefore CD sensors function only in the presence of ionic additives [39]. From different anionic sites, KTCPB gave the best electrode characteristic (Nernstian slope was 59.6 ± 1.9 mV $decade^{-1}$). Furthermore, different amounts of KTCPB (from 0 to 5 mg) were added to the electrode matrix and 0.5 mg was selected.

The influence of the membrane plasticizer on electrode matrices incorporated with α-CD ionophore and KTCPB as ionic sites, was studied using five plasticizers having different dielectric constant, namely *f*-PNPE, *o*-NPOE, TCP, DOS and DBP (ε=50, 24, 17.6, 5.2 and 4.7, respectively). Application of the less polar plasticizers produced electrode with lower Nernstian slopes (53.8 ± 2.6, 51.2 ± 3.1, and 50.6 ± 3.35 mV $decade^{-1}$, for TCP, DBP and DOS, respectively). On the other hand, *o*-NPOE and *f*-PNPE improved the electrode sensitivity (slope values were 56.8 ± 1.7 and 59.3 ± 1.2 mV $decade^{-1}$) which may be attributed to the presence of aromatic rings within the plasticizer structure can enhance the solubility of the ionophore and the formed BuCh–α-CD inclusion complex within the electrode matrix [40].

Incorporation of nanomaterials in the electrode matrix improves the conductivity and transduction of the chemical signal to electrical signal, which in turn improved the dynamic working range and electrode response time [41,42]. Different carbon nanomaterials were added to the electrode matrix including MWCNTs, FMWCNTs, SWCNTs, and carbon nanopowder. The obtained results revealed the superiority of MWCNTs and SWCNTs (Nernstian slopes were 63.8 ± 1.1 and 59.5 ± 2.1 mV $decade^{-1}$, respectively) compared with other tested carbon nanomaterials. Contrary, FMWCNTs showed lower slope values (46.9 ± 2.6 mV $decade^{-1}$) which may be attributed to the presence of carboxylic group that disturb the dispersion of CNTs within the PVC matrix and cause adsorption of some species on the electrode surface. Furthermore, different amounts of MWCNTs (ranging from 0 to 270 mg) were added to the electrode matrix, and incorporation of 30 mg was the most suitable.

3.2. Electrode performance

Performances of the fabricated SPEs were evaluated according to the IUPAC recommendation [34] and compared traditional coated graphite electrode (CGEs) were prepared with the same electrode matrix (Table 1). Screen printed electrodes modified with MWCNTs showed the best performance; indicated were the most sensitive compared with other electrodes (Nernstian slope and detection limit values were 64.8 ± 1.1 mV $decade^{-1}$ and 3.0×10^{-7} mol L^{-1}, respectively).

As the aim of the fabricated sensors is to follow the BuChE activity through monitoring the change of BuCh concentration (BuCh), thus, evaluation and selecting of the electrode will much depend on the electrode response time and the potential stability. Response time of the fabricated sensors was tested by measuring the time required to achieve a steady state potential (within

Table 1
Analytical performances[a] of various butyrylcholine sensors.

Sensors	SPE/CNT	SPE	CGE/CNT
Concentration range (mol L^{-1})	10^{-6}–10^{-2}	10^{-6}–10^{-2}	10^{-5}–10^{-2}
Slope (mV $decade^{-1}$)	64.8 ± 1.1	60.8 ± 0.9	58.9 ± 1.8
R	0.9997	0.9996	0.9986
LOD (mol L^{-1})	3.0×10^{-7}	1.0×10^{-6}	1.7×10^{-6}
Response time (s)	<2	2	5
Preconditioning time (min)	10	10	60
Shelf lifetime (week)	24	24	4

[a] Results are average of five different calibrations.

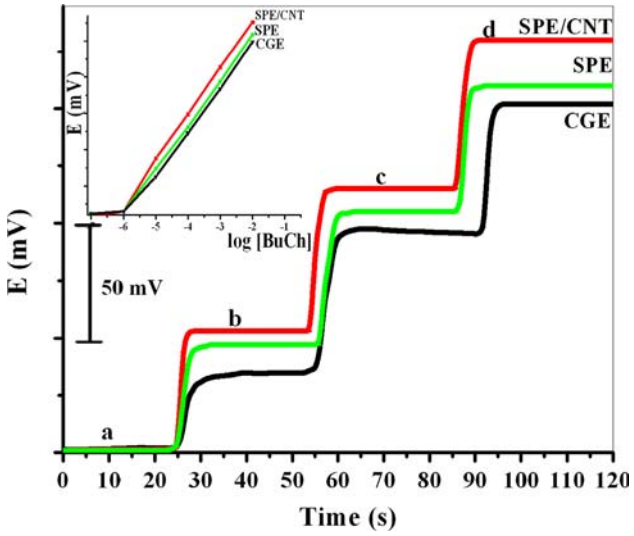

Fig. 1. Dynamic response of different BuCh sensors: (a) 1×10^{-6}; (b) 1×10^{-5}; (c) 1×10^{-4} and (d) 1×10^{-3} mol L^{-1} BuCh solutions.

$\pm$ 1 mV) after sudden increase in the BuCh concentration from 1×10^{-6} to 1×10^{-3} mol L^{-1} (Fig. 1). SPEs incorporated with MWCNTs showed spontaneous response (less than 2 s) compared with other electrodes, which can be explained on the basis of the synergistic effect between MWCNT and α-CD within the electrode matrix. In addition, introduction of carbon nanotubes as ion-to-electron transducer has much improved the electrode potential stability.

One disturbing drawback of solid contact electrodes was the potential drift and the poor adhesion of the electroactive membrane to the metal substrate [43,44]. Such phenomena, can be explained on the basis of water and ions penetration through the sensing membrane forming undefined layer between sensing membrane and conductor. In the present electrodes, the sensing membrane contain the same polymer matrix of the conducting track, therefore, during printing, co-polymerization of the two PVC matrices (sensitive membrane and conducting track) will prevent formation of such internal water layer and thus improve the electrode potential stability. SPEs showed high potential stability of SPEs as 10 min preconditioning time was sufficient to get stable potential compared with 60 min for CGEs (Table 1). In addition, incorporation of MWCNTs also makes an essential role to enhance the hydrophobicity of the membrane, which contributes to the more stable potential signal by elimination of undesirable water layer at the interface [45].

Operational lifetimes of the fabricated screen printed electrodes were tested by performing day-to-day calibration; SPEs showed useful shelf lifetime at 4 °C of 24 weeks during which the Nernstian slopes did not change significantly ($\pm$ 1 mV decade^{-1}). Even though the use of the SPEs allows a single use of the biosensor, it can be reliably applied up to 20 times without significant losses of the sensitivity.

When the BuChE activity is determined using the BuCh-sensor, the electrode selectivity, especially interference from the reaction product (Ch), should be considered. Interference evaluation was performed using matched potential method (MPM) in order to assess the effect of interferents [46]. The developed sensors were highly selective to BuCh as $-\log K_{BuCh,Ch}$ was 3.50 indicating the low Ch interference. Moreover, the developed sensors were highly selective; as many organic compounds (glycine, caffeine, citrate, maltose, sucrose, and starch) as well as inorganic cations (Na^+, K^+, Li^+, Ca^{2+}, Mg^{2+}, and NH_4^+) and anions (Cl, Br, nitrate, acetate and sulfate) did not show significant interference.

3.3. Determination of BuChE activity

The remarkable sensitivity and selectivity of the fabricated BuCh–SPEs suggest its application as an indicator electrode for monitoring the BuChE activity. According to the enzymatic action of BuChE, BuCh was hydrolyzed to Ch, therefore the BuCh concentration at the phase boundary is decreased and electrode potential decreased (Fig. 2). The minimum electrode potential will be reached when the BuCh ions at the sample–membrane interface are consumed by the enzyme and the reaction product governs the electrode response [47].

BuChE activity was greatly influenced by the pH of the reaction solution. The optimum pH value was examined by measuring the enzyme activity using the BuCh-sensor in phosphate buffer solutions at different pH values (from 6 to 9). BuChE enzyme and its substrate (BuCh) were dissolved in phosphate buffer solution and the measuring cell volume was completed with buffer at the same corresponding pH value. Preliminary experiments have shown that higher sensitivity can be achieved in the pH range 7–8 and pH 7.0 was selected. Calibration curves were constructed at the optimum conditions by plotting the initial rate ($\Delta E/\Delta t$, determined by drawing a tangent of the first linear part of potential-time curve) against the BuChE concentration. The linear equation was $\Delta E/\Delta t = 0.004–0.500$ [BuChE, U], $r = 0.9968$ in the concentration range from 0 to 0.4 U mL^{-1}.

3.4. Determination of ethion

The mechanism of irreversible cholinesterase inhibition by OPs is based on phosphorylation of the ChE active site [48]. The effect of incubation time on the enzyme activity was performed in order to achieve the highest sensitivity. Ethion displayed increasing inhibition of BuChE with increasing the inhibition time (Fig. 3); the relative inhibition degree increased from 15% at zero time to 60% after 10 min, and 82% after 30 min. The inhibition curve trended to a stable value with longer incubation time; therefore incubation for 30 min was selected to compromise between the sensitivity and analysis time.

Upon construction, the fabricated sensors were used for measuring the remained BuChE activity after incubation with different ethion concentrations for 30 min (Fig. 4). Under the optimal experimental conditions, the relative inhibition (I %) was proportional to ethion in the concentration range from 0 to 330 ng mL^{-1} and the regression

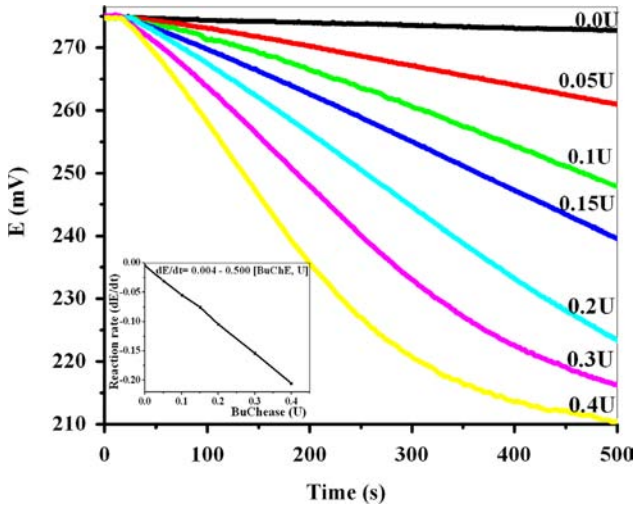

Fig. 2. Determination of BuChE activity using the fabricated BuCh–SPEs sensor. Experimental conditions: 9.0 mL phosphate buffer (pH 7.0) and 1.0 mL of 10^{-3} mol L^{-1} BuCh solution.

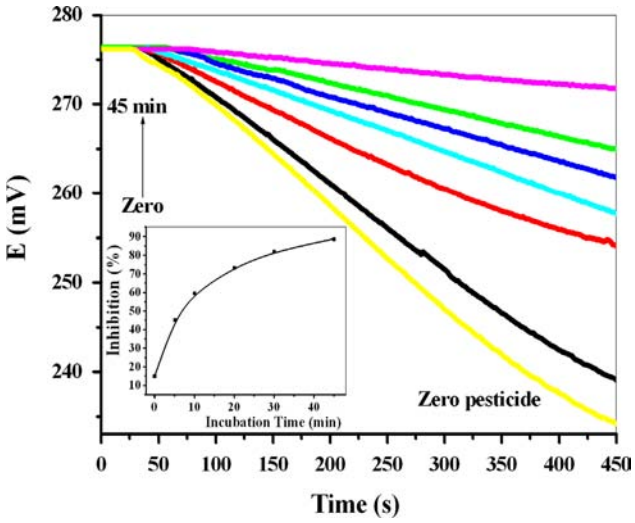

Fig. 3. Inhibition effect on butyrylcholinesterase by ethion monitored by BuCh–SPE as a function of the incubation time. Experimental conditions: the measuring cell contained 9.0 mL phosphate buffer (pH 7.0) and 1.0 mL of 10^{-3} mol L^{-1} BuCh and ethion samples were incubated with 0.4 U BuChE.

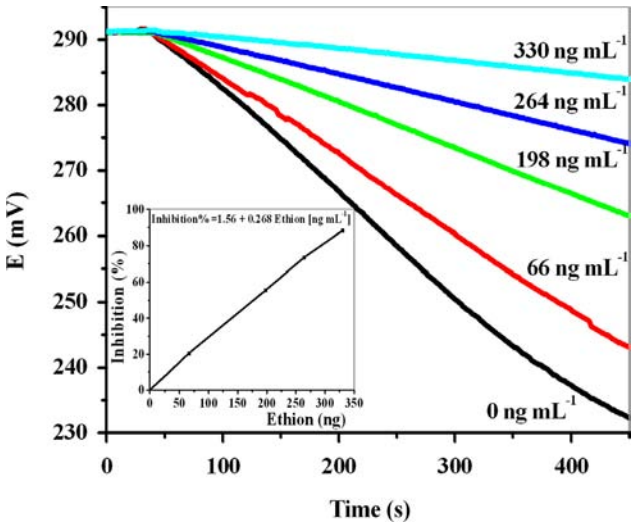

Fig. 4. Inhibition of BuChE by ethion. Experimental conditions: measuring cell contained 9.0 mL phosphate buffer (pH 7.0) and 1.0 mL of 10^{-3} mol L^{-1} BuCh solution. Ethion samples were incubated with 0.4 U BuChE for 30 min.

equation was $I\,\% = 1.56 + 0.268$ Ethion [ng mL^{-1}] with the correlation coefficients of 0.9991 and detection limit 22.0 ng mL^{-1} (calculated on the basis of 3δ). For the developed sensors, five runs (at fixed BuChE and ethion concentrations) were performed on 4 different days, in order to evaluate the reproducibility of the results. Average recoveries for 200 ng mL^{-1} of ethion were $99.2 \pm 3.5\%$ and $98.8 \pm 4.8\%$, respectively, indicating acceptable precision and reproducibility of the method.

3.5. Determination of ethion degradation products

As they are degraded, OPs slowly leave behind residues in food and water sources, which can become more concentrated as they move up the food chain and some of these degradation products are even more toxic than the parent compounds [49,50]. Cholinesterase inhibition percentages are different for various insecticides at the same concentration levels, moreover, for the same formulae, the inhibition percentages of OPs commercial form (P=S) are lower compared with the oxidized form (P=O) which are quickly obtained in nature [51].

Synthesis and characterization of ethion and its degradation products (either ^{14}C-ethion or unlabeled ethion) were reported in details elsewhere [4,35] and revealed the presence of the parent compound together with three metabolites; ethion monooxon (**II**), ethion dioxon (**III**) and *O,O*-diethyl phosphorothioate (**IV**). In the present work, the acute toxicity of these degradation products compared to the parent pesticide was estimated. Certain BuChE concentration was incubated with different ethion or its degradation products (**I–IV**) for 30 min followed by measuring the residual BuChE activity. The inhibition percentages were linearly related to OPs in different concentration ranges and sensitivities depending on the nature of pesticide used (Fig. 5 and Table 2). From the slopes of these calibration curves, it can be concluded that the acute toxicity was in following order: **III** > **II** > **I** > **IV**. If the four OPs coexist, the competitive inhibition may happen in the enzymatic reaction, and the competitive ability is also in the same order of selectivity.

3.6. Sample analysis

To further demonstrate the practicality of the proposed analysis protocol based on BuChE inhibition, ethion residues in soybean oil, water samples and ethion commercial sample were detected. After appropriate dilution with acetonitrile, samples were incubated with BuChE according to the recommended procedures. The obtained results were in agreement with the official radioassay and flame photometric chromatographic measurements (Table 3).

4. Conclusion

This study proposes a novel disposable biosensor for determination of ethion and its degradation products based on inhibition of butyrylcholinesterase enzyme. The fabricated screen printed electrodes, incorporated with α-CD and carbon nanotube, showed high sensitivity and selectivity towards butyrylcholine with spontaneous response time and long shelf-lifetime. The presence of MWCNTs improved the electron transfer and electrode performance. The developed BuChE biosensor provides a simple and reliable alternative to sophisticated enzyme immobilization and

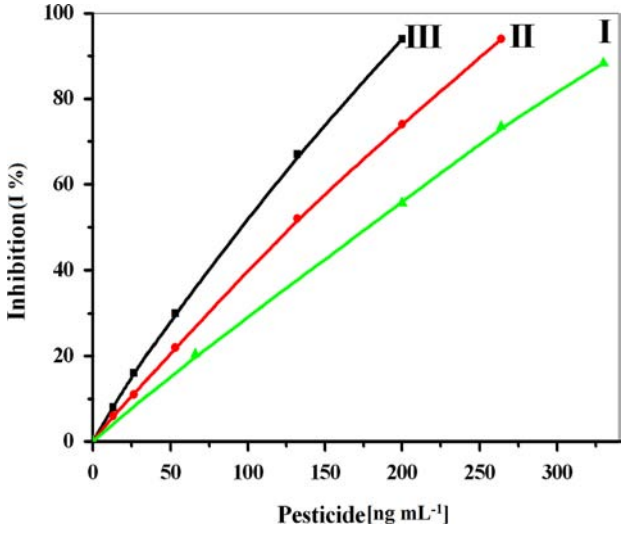

Fig. 5. Calibration curves for different ethion derivatives: (I) ethion; (II) ethion monooxon and (III) ethion dioxon. Experimental conditions: measuring cell contained 9.0 mL phosphate buffer (pH 7.0) and 1.0 mL of 10^{-3} mol L^{-1} BuCh solution. Pesticide samples were incubated with 0.4 U BuChE for 30 min.

Table 2
Coefficients of calibration curves for ethion and its degradation products ($I\ \% = a + b \times$ pesticide concentration), detection limits and concentration range determined with butyrylcholinesterase screen printed biosensor.

	Ethion (**I**)	Ethion monooxon (**II**)	Ethion dioxon (**III**)	Diethyl phosphorothioate (**IV**)
Concentration range	0–330 ng mL^{-1}	0–265 ng mL^{-1}	0–200 ng mL^{-1}	0–60 μg mL^{-1}
Slope (b)	0.268 ± 0.006	0.357 ± 0.008	0.469 ± 0.015	1.420 ± 0.030
Intercept (a)	1.48 ± 0.13	1.80 ± 0.11	2.66 ± 0.15	0.49 ± 0.11
Correlation coefficient	0.9992	0.9988	0.9981	0.9992
Detection limit (3δ)	22.0 ng mL^{-1}	15.0 ng mL^{-1}	10.0 ng mL^{-1}	4.0 μg mL^{-1}

Table 3
Measurement of ethion in commercial formulation, water sample and soybean oil using butyrylcholinesterase biosensor.

Sample	Found (ppm)		
	Official	Developed (potentiometric)	Recovery (%)
Ethion, ENDO, 50%	3.80 ± 0.20[a]	3.88 ± 0.18	102.1
Soybean oil	1.93 ± 0.03[b]	2.02 ± 0.06	104.7
Water sample			
Organic layer	0.625 ± 0.04[b]	0.640 ± 0.03	102.4
Aqueous layer	0.107 ± 0.01[b]	0.100 ± 0.04	93.4

[a] Values according to chromatographic measurements.
[b] Values according to radioassay measurements.

reactivation protocols. Even though the use of the screen-printed electrode allows a single use of the biosensor, it can be reliably applied up to 20 times without losses of the sensitivity toward the insecticides considered.

Based on relative degree of enzyme inhibition, the proposed sensor can be used for detection of ethion and its degradation products in oil and water samples. The fabricated biosensors are attractive and can be used as simple and direct screening devices for monitoring detoxification processes available to unskilled users with significant decrease in cost per analysis to complement or replace the classical analytical methods.

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