Talanta 83 (2010) 357–363

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Disposable potentiometric sensors for monitoring cholinesterase activity

Elmorsy Khaled [a,*], H.N.A. Hassan [a], Gehad G. Mohamed [b], Fahem A. Ragab [c], Aly Eldin A. Seleim [c]

[a] Microanalysis Laboratory, National Research Center, Dokki, Giza, Egypt
[b] Chemistry Department, Faculty of Science, Cairo University, Giza 12613, Egypt
[c] Critical Care Department, Faculty of Medicine, Cairo University, Giza, Egypt

ARTICLE INFO

Article history:
Received 26 July 2010
Received in revised form
16 September 2010
Accepted 18 September 2010
Available online 24 September 2010

Keywords:
Disposable screen-printed sensor
Potentiometry
β-Cyclodextrin
Butyrylcholine
Cholinesterase
Malathion

ABSTRACT

A highly sensitive disposable screen-printed butyrylcholine (BuCh) potentiometric sensor, based on heptakis (2,3,6-tri-o-methyl)-β-cyclodextrin (β-CD) as ionophore, was developed for butyrylcholinesterase (BuChE) activity monitoring. The proposed sensors have been characterized and optimized according to the constituents of homemade printing carbon ink including β-CD, anionic sites, and plasticizer. The fabricated sensor showed Nernstian responses from 10^{-6} to 10^{-2} mol L^{-1} with detection limit of 8×10^{-7} mol L^{-1}, fast response time (1.6 s) and adequate shelf-life (6 months). Improved selectivity towards BuCh with minimal interference from choline (Ch) was achieved and the sensor was used for determination of 0.06–1.25 U mL^{-1} BuChE. The developed disposable sensors have been successfully applied for real-time intoxication monitoring through assaying cholinesterases (ChEs) activity in human serum. Determination of organophosphate pesticide was conducted by measuring their inhibition of BuChE with successful assaying of malathion in insecticide samples with high accuracy and precision.

1. Introduction

Cholinesterases (ChEs) are among the most important enzymes needed for the proper functioning of the nervous systems of human, other vertebrates, and insects. The role of acetylcholinesterase (AChE) in living organisms is the catalytic hydrolysis of the neurotransmitter acetylcholine (ACh) into choline (Ch) and acetic acid. Organophosphates (OPs) are widely used in the agriculture around the world as pesticides. These neurotoxic compounds, which are structurally similar to the nerve gases soman and sarin, irreversibly inhibit AChE resulting in the accumulation of ACh which interferes with muscular responses and in vital organs produces serious symptoms and eventually death [1,2]. Classical analytical techniques for OPs determination are gas chromatography (GC), high performance liquid chromatography (HPLC) and thin layer chromatography (TLC) coupled with different detectors and spectral techniques [3–5]. Although these methods are very sensitive, they are not adapted for in situ and real-time detection of pesticides as they are time consuming, involve expensive apparatus and require skilled technicians.

In contrast, ChEs sensors attract great attention due to their ability to detect trace amounts of anticholinesterase compounds in environment [6–12]. In design of ChEs electrochemical sensors, either potentiometric or amperometric signal transducers are utilized. Amperometric biosensors are based on measurement of products formed as a result of enzymatic hydrolysis; in this case, artificial substrates (acetyl- or butryl-thiocholine) are used, and the resulting electroactive thiocholine can be measured amperometrically on different electrodes [13–17]. However, fabrications of the working electrodes are sometimes rather complicated and time consuming with poor reproducibility.

Simple ChEs potentiometric sensors were based on ACh [18], BuCh [19,20] selective electrodes, and most often pH sensors [21–25]. The main disadvantages of the pH-shift based method, which monitor the pH changes during the enzymatic reaction, is the strong requirement for low buffer capacity (to avoid proton consumption) and multiple fabrication steps including enzyme immobilization and reactivation.

As the demand for point-of-care testing and on-spot monitoring in clinical, environmental and industrial analysis increases, both practical and economic interests have been driven the development of various kinds of disposal electrochemical sensors based on screen-printing technology [26–28]. Screen-printed electrodes (SPEs) have been used for the potentiometric determination of various species using different commercial printing inks [29–31]; however the ink compositions are usually unknown in many respects and some of the ink components may interfere with the electrochemical measurements [32]. Homemade printing inks (prepared by mixing of carbon powder, plasticizer and binding material) were optimized and successfully applied for fabrication of disposable potentiometric electrodes [33,34].

* Corresponding author. Tel.: ++2 0103781777; fax: +2 0233370931.
E-mail address: elmorsykhaled@yahoo.com (E. Khaled).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.09.020

In our previous work [35], the conventional carbon paste electrodes (CPEs) and PVC electrodes were used for flow injection analysis (FIA) potentiometric determination of choline esters (CEs). The objective of the present study was to develop simple and inexpensive disposable potentiometric sensors for the rapid, sensitive, and accurate determination of ChEs activity and anticholinesterase insecticide.

2. Experimental

2.1. Reagents

All reagents were of the analytical grade and bidistilled water was used throughout the experiments. Various CEs including; acetylcholine bromide (ACh, Acros Organics); acetylthiocholine iodide (AtCh, Fluka); acetylmethylcholine chloride (AmCh, Aldrich); butrylcholine iodide (BuCh, Fluka) and choline chloride (Ch, Fluka) were used without further purification. AChE (from electrophorus electricus electric eel, 426 U mg^{-1}) and BuChE (from equine serum, 165 U mg^{-1}) were purchased from Sigma. Analytical standard malathion was purchased from Fluka.

β-CD derivatives including; native β-CD (**I**, Sigma), heptakis (2,6-di-o-methyl)-β-CD (**II**, Aldrich), heptakis (2,3,6-tri-o-methyl)-β-CD (**III**, Aldrich) and 2-hydroxypropyl-β-CD (**IV**, Aldrich) were used as sensing ionophores. Sodium tetraphenylborate (NaTPB, Fluka), sodium tetrakis (4-fluorophenyl) borate (NaTFPB, Fluka), potassium tetrakis (4-chlorophenyl) borate (KTCPB, Fluka), phosphotungstic acid (PTA, Sigma), phosphomolybdic acid (PMA, Sigma), sodium dodecylsulfate (SDS, Fluka) or reineckate ammonium salt (RAS, Sigma) were used as anionic sites.

The tested electrode plasticizers were *o*-nitrophenyloctylether (*o*-NPOE, Sigma), 2-fluorophenyl-2-nitrophenylether (FPNPE, Fluka), dibutylphthalate (DBP, Sigma), dioctylphthalate (DOP, BDH), dioctylsebacate (DOS, Avocado) and tricresylphosphate (TCP, Fluka). Polyvinylchloride (PVC, relative high molecular weight, Aldrich) and graphite powder (synthetic 1–2 μm, Aldrich) were used for printing ink preparation.

2.2. Samples

ChEs activity was measured in human serum obtained from suspected patients admitted to ICU of the National Center for Clinical and Environmental Toxicology, Cairo University Hospitals, Egypt. The blood samples were centrifuged at 4000 rpm for 3 min and the supernatants (serum) were assayed for ChEs activity using the developed procedures and the official Ellman's reaction [36].

Commercial pesticide samples, Malathion Coupra (Kafr El-Zayat Pesticide and Chemicals Company, 57% malathion) and Prioderm lotion (0.5% (w/v) malathion in ethanol base, Pharco Pharmaceutical Company, Alexandria, Egypt,) were purchased from local stores. A chromatograph with a flame photometric detector (FPD) was used as official method for malathion determination.

2.3. Apparatus

All potentiometric measurements were carried out using RadioShack Digital multimeter with PC interface. pH measurements were done 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 in arrays of six couples consisting of the working and reference electrodes following the procedures described elsewhere [33,34]. Ag/AgCl pseudo-reference electrode was firstly printed using homemade ink prepared by mixing 0.9 g Ag/AgCl mixture (65:35%) with 0.8 g of 8% PVC solution (in acetone–cyclohexanone mixture), and cured at 60 °C for 30 min. The working electrodes were printed using carbon ink containing 7.5 mg β-CD, 12 mg KTCPB, 0.45 g *o*-NPOE, 1.25 g of 8% PVC solution and 0.75 g carbon powder. The printed electrodes were cured at 50 °C for 30 min and kept dry at 4 °C. A layer of an insulator was then placed onto the printed electrodes, leaving a defined rectangular shaped (5 mm × 5 mm) working area and a similar area on the other side for the electrical contact. Fabricated electrodes were used directly in measurements after two calibrations which served as a preconditioning process.

2.4.2. Sensor calibration

The developed sensors were calibrated by immersing the bielectrode strip in 10 mL of 10^{-7} to 10^{-2} mol L^{-1} CEs solutions. The potential readings were recorded after stabilization and plotted against the target analyte concentration (log [CEs]).

2.4.3. Measurement of BuChE activity

BuChE activity was determined using the developed sensor under the optimum conditions. Aliquots of the enzyme solutions, containing 0.625–12.5 U, were added to 10 mL of 10^{-4} mol L^{-1} BuCh solution in PBS at pH 7.3. The enzyme activity was estimated by monitoring the change in the BuCh electrode potential within the reaction time.

2.4.4. Measurement of inhibition by malathion

It was almost the same as enzyme activity where 2.5 U BuChE was added to 10 mL of 10^{-4} mol L^{-1} BuCh solution containing malathion at various concentrations. The enzyme activity was estimated by measuring the change of electrode potential after 5 min of enzyme injection. The degree of inhibition was measured using the following equation [20]:

$$I\% = \left(\frac{E_{\text{Pesticide}} - E_{\text{Enzyme}}}{E_{\text{Baseline}} - E_{\text{Enzyme}}} \right) \times 100$$

where E_{Baseline} was the measured potential before injection of BuChE, $E_{\text{Pesticide}}$ and E_{Enzyme} were the potentials measured after 5 min of enzyme addition into PBS with and without malathion, respectively.

3. Results and discussion

Screen printing technology has increasingly been used for the mass production of inexpensive, reproducible and disposable electrochemical sensors for application in pharmaceutical, biomedical, and environmental fields [26–28]. Poisoning of PVC and CPEs surfaces by proteins, besides their size limited their widespread applications in clinical monitoring; therefore, the use of disposable sensors was of choice.

β-CD based homemade carbon inks, with well defined constituents, were prepared and optimized for fabrication of BuCh disposable sensors. Comprehensive studies were carried out to elucidate the influence of ionophore, anionic sites and plasticizer, to select the optimal electrode composition possessing the most favorable electroanalytical performance prior to their application for ChEs activity measurement.

3.1. Optimal BuCh sensor matrices compositions

Four β-CD derivatives were incorporated in the printing carbon ink and the performances of the fabricated screen-printed carbon electrodes (SPCEs) towards different BuCh were tested. β-CD ionophore **III** higher sensitivity than other tested

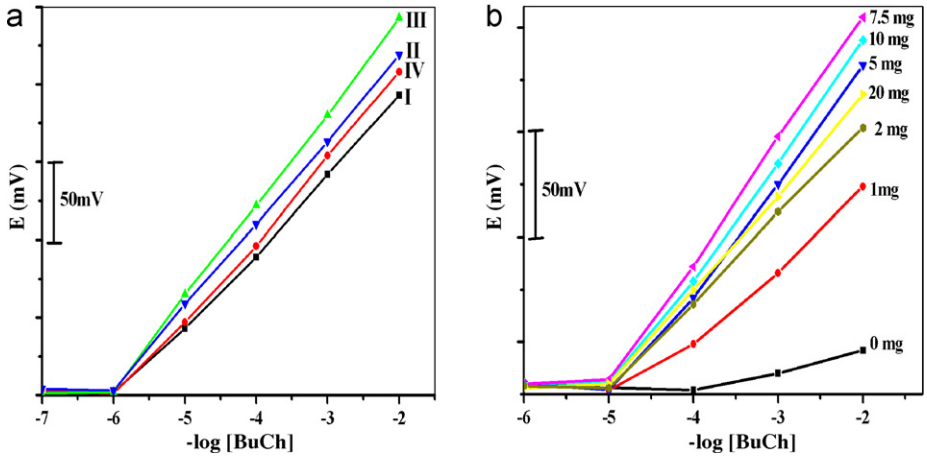

Fig. 1. Performance characteristics of BuCh sensors: (a) containing different β-CD ionophores and (b) different contents of β-CD **III**.

ionophores as the slope values were $50.1 \pm 0.7, 53.6 \pm 2.0, 56.3 \pm 2.8$ and 52.5 ± 3.5 mV decade^{-1} for the ionophores **I–IV**, respectively (Fig. 1a). Moreover, β-CD **III** content was changed in the electrode matrix from 0 to 20 mg and the electrode performances were evaluated. It was found that electrodes free of β-CD showed insignificant response (about 17.5 ± 1.0 mV decade^{-1}) and the addition of 7.5 mg of the ionophore was sufficient to obtain reasonable cationic slope of 59.6 ± 1.4 mV decade^{-1}. Further increase of the ionophore content resulted in decrease of the electrode response which may be attributed to the strike hindrance at the electrode surface (Fig. 1b).

It is well known that lipophilic ionic sites promote the interfacial ion-exchange kinetics and decrease the bulk resistance by providing mobile ionic sites in the electrode matrix [37,38]. β-CDs behave as neutral carrier ionophores and their ISEs functional only when anionic sites are incorporated. It was found that incorporation of ionic sites improved the electrode sensitivity by extent depend on the ionic site lipophilicity. Addition of KTCPB to the electrode matrix exhibited the highest slope value (61 ± 1.1 mV decade^{-1}) compared with other ionic sites. Furthermore, the amount of KTCPB was changed from 0 to 15 mg and addition of 12 mg was selected.

The influence of the plasticizer on the performance of BuCh sensors modified with β-CD ionophore **III** and KTCPB as ionic sites was studied using six plasticizers having different dielectric constant, namely; FPNPE, o-NPOE, TCP, DOS, DBP and DOP (ε = 50, 24, 17.6, 5.2, 4.7 and 3.8, respectively). Plasticizer selection was crucial for appropriate sensor performance (Fig. 2), the application of the less polar plasticizers decreased the sensitivity which might be attributed to less solvation of the ionophore and the formed BuCh–β-CD complex (slope values were 52.9 ± 2.7, 48.6 ± 1.5, 47.1 ± 2.0 and 46.3 ± 1.9 mV decade^{-1} in the concentration range from 10^{-5} to 10^{-2} mol L^{-1} for TCP, DOP, DBP and DOS, respectively). Higher sensitivity was observed for electrodes containing high polar aromatic plasticizers; o-NPOE and FPNPE as the slope values were 61.3 ± 0.9 and 58.7 ± 1.4 mV decade^{-1}, respectively.

3.2. Electrode performance

The potentiometric response characteristics of the developed sensors, at the optimal matrix composition, were evaluated according to the IUPAC recommendation [39]. The fabricated sensors displayed Nernstian cationic responses towards the different CE derivatives with sensitivities depending on the nature of the side chain substitution in CE molecule (Table 1). The highest electrode response was towards BuCh (the slope was 61.2 ± 0.5 mV decade^{-1} with LOD 0.8×10^{-7} mol L^{-1}). On the other hand, the sensors susceptibility to Ch was minimal (the slope was 26.3 ± 1.4 mV decade^{-1} in the concentration range from 10^{-4} to 10^{-2} mol L^{-1}) indicating the possibility of electrode application for monitoring BuCh enzymatic reactions.

For analytical applications of a novel sensor, the fast response with stable potential readings is of critical importance, especially when monitoring kinetic or enzymatic reactions. The developed sensors possessed a fast response of 1.6 s with stable and reproducible potential readings.

The fabrication reproducibility was investigated by measuring the performances of sensors fabricated within the same batch and from different batches. The average slope values of 10 BuCh electrodes within the same batch were 61.2 ± 0.5 mV decade^{-1} with standard potential 554 ± 10.0 mV; while the corresponding values between three different batches were 59.0 ± 2.3 mV decade^{-1} and 548 ± 8.1 mV, respectively. Furthermore, the storage stability was quite good, after fabrication of SPCEs; they were kept in a storage box at 4 °C and directly used for potentiometric measurements after two calibrations which served as a preconditioning process. The SPCEs showed shelf-time of 6 months after printing without significant change in slopes of the calibration graphs, and the same strip can be used for more than 10 calibrations.

As the fabricated electrodes will be used for monitoring the enzymatic reaction, the working pH range must be tested. The influ-

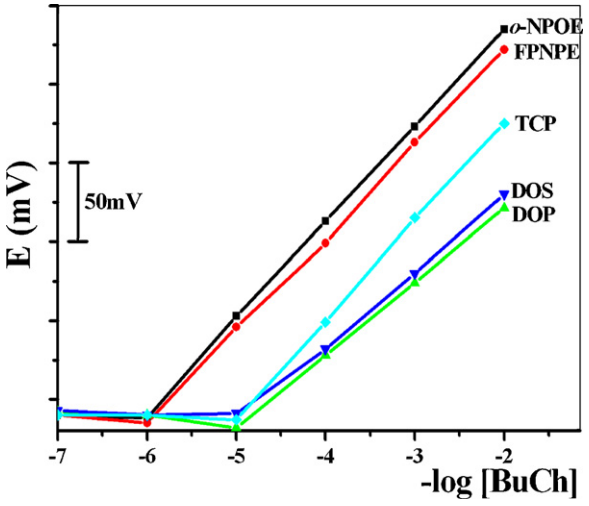

Fig. 2. Effect of plasticizer type on BuCh sensors containing 7.5 mg β-CD III and 12 mg KTCPB as ionic sites.

Table 1
Electrochemical performance of CEs-SPCEs sensors modified with β-CD ionophores.

Electrode performance	SPCE				
	BuCh	ACh	AtCh	AmCh	Ch
Concentration range (mol L^{-1})	10^{-6} to 10^{-2}	10^{-5} to 10^{-2}	10^{-5} to 10^{-2}	10^{-6} to 10^{-2}	10^{-4} to 10^{-2}
Slope (mV decade^{-1})	61.2 ± 0.5	58.3 ± 2.7	59.1 ± 1.3	53.2 ± 0.7	25.4 ± 2.6
r	0.9982	0.9988	0.9991	0.9990	0.9944
LOD (mol L^{-1})	8.0×10^{-7}	10^{-5}	8.0×10^{-6}	10^{-6}	10^{-4}
Response time (s)	1.6	1.6	1.6	1.6	1.6
Shelf-lifetime (month)	6	6	6	6	6
Working pH range	4–9	4–9	4–9	4–9	4–9

ence of the pH was investigated in the pH range from 2 to 12 and stable Nernstian responses were achieved in the range from 4 to 9.

ChEs catalyze hydrolysis of CEs into Ch and the corresponding carboxylic acid, so the magnitude of Ch interference and electrode selectivity are of critical importance. Interference evaluation was performed using matched potential method (MPM) in order to assess the effect of interferents [40,41]. The developed sensors were highly selective; organic compounds (glycine, caffeine, citrate, maltose, sucrose, and starch) as well as inorganic cations (Na^+, K^+, Li^+, Ca^{2+}, Mg^{2+}, and NH_4^+) did not show significant interference and the obtained selectivity coefficients were similar to that obtained with the corresponding CPEs [35]. More emphases were done on Ch interference and the developed sensors were highly selective to BuCh as $-\log K_{BuCh,Ch}$ was 2.88 indicating the low Ch interference (up to 60-fold of Ch will not interfere), while on the other hand, the higher Ch interference was found in case of Ach sensors; the corresponding value of $-\log K_{ACh,Ch}$ was 0.67. When using ACh as substrate, the interference from Ch should be considered and application of BuCh as enzyme substrate was preferred.

3.3. Determination of ChEs activity

Both BuChE and AChE enzymes belong to the same structural class of proteins, the esterase/lipase family. They are serine hydrolases that share substantial structural similarities, but differ in substrate specificities. The substrate relative reaction rate values of BuChE are equal to 1.0 for BuCh, 0.5 for buterylthiocholine, and 0.4 for both ACh and AtCh [42–44].

After successful application of the developed SPCEs for CEs determination with low interference from Ch, these sensors will be used as an indicator electrode for monitoring the ChEs enzymatic reactions. As the CEs concentration decreases due to ChE activity, the corresponding electrode potential decreases parallel, and hence the enzymatic reaction rate can be estimated from the potential–time curve.

The optimum substrate concentration possessing the maximum enzyme activity was tested using BuCh solutions of different concentrations (from 10^{-5} to 10^{-3} mol L^{-1}) with addition of 20 U of BuChE enzyme. It was found that 10^{-4} mol L^{-1} solution showed the best enzymatic reaction rate.

Each enzyme has its own optimum working pH range, beyond which the enzyme activity severely decreased or even ceased. The optimum pH was examined by operating the enzymatic reaction at different pH values range from 6 to 9, and pH 7.3 gave the maximum reaction rate. To sustain the obtained results, the enzyme activity was determined without PBS, and it was concluded that the enzymatic reaction rate increased by about 7-folds than that in absence of buffer (Fig. 3).

For construction of the calibration curve, three strategies were followed; plotting the initial rate potential–time ($\Delta E/\Delta T$), the enzymatic reaction velocity, or measuring the potential change at a fixed time (ΔE) against enzyme concentration. According to the first strategy, the slope values of the linear part of potential–time curve (Fig. 3b) were plotted against the BuChE concentration (Fig. 3c), the linear equation was: $\Delta E/\Delta T = -2.013 - 61.33$ [BuChE], $r = -0.9994$ in the concentration range from 0.06 to 1.25 U.

The second strategy [19] was based on calculation of the reacted BuCh amount estimated from the calibration curve of the BuCh–SPCE obtained in Section 3.2, followed plotting the reacted amount against time (Fig. 4a). The initial velocity of the enzymatic reaction was calculated from the slope of the linear parts and plotted against the corresponding BuChE concentration (Fig. 4b). The linear equation was: the reaction velocity = −0.013 + 0.91 [BuChE], $r = 0.9978$.

For spot and field measurement, alternative simpler calculation procedures can be suggested by measuring the potential change after a fixed time of enzyme addition against enzyme concentration. Detailed experimental results revealed that there was a linear dependence of the potential difference (ΔE) with the enzyme concentration and 2 min was sufficient to get reasonable sensitivity and

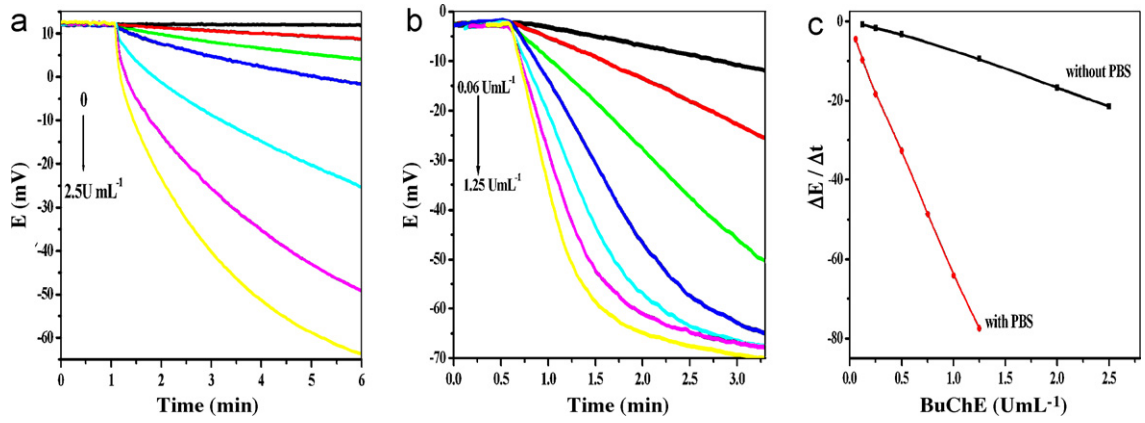

Fig. 3. Determination of enzyme activity of BuChE using 10 mL of 10^{-4} mol L^{-1} BuCh solution and BuCh–SPCEs. (a) Without PBS; (b) in PBS pH 7.3; (c) Relationship between reaction rate ($\Delta E/\Delta T$) and enzyme concentration.

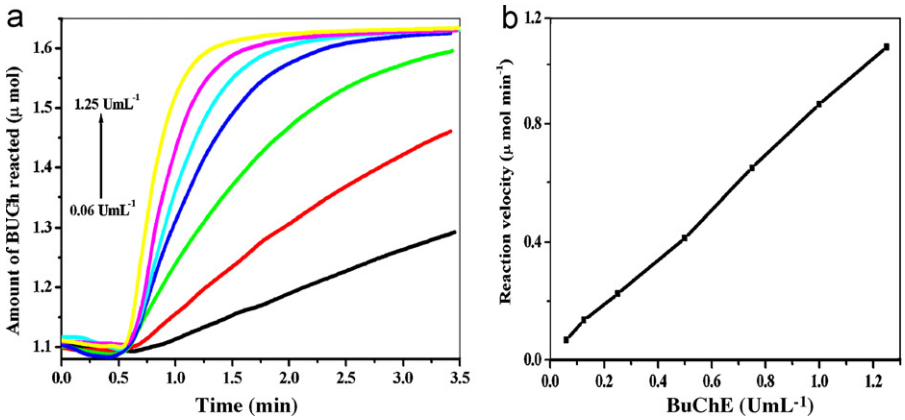

Fig. 4. Determination of enzyme activity of BuChE using 10 mL of 10^{-4} mol L^{-1} BuCh solution and BuCh–SPCEs. (a) Time-course of the amount of BuCh reacted after addition of BuChE and (b) relationship between initial velocity and enzyme concentration.

reproducibility of measurement. The linear equation in the concentration range 0–0.5 U BuChE mL^{-1} was $\Delta E = 0.26–90.3$ [BuChE], $r = 0.9948$.

For comparison, AChE activity was also determined using the fabricated SPCEs sensor and ACh as reaction substrate. According to the first strategy, the calibration graph was linear in the concentration range 1.25–10.0 U AChE mL^{-1} with linear equation was: $\Delta E/\Delta T = 0.276–0.70$ [AChE], $r = -0.9998$ (Fig. 5). The sensitivity the BuChE method was about 14 times more sensitive than that for AChE, which may be explained on the basis of the higher electrode sensitivity towards the reaction substrate (BuCh) with lower susceptible interference of the reaction product (Ch) compared to the ACh procedure.

3.4. Determination of anticholinesterases (OPs)

OPs are irreversible inhibitors of ChEs as they block the active site of enzyme through a nucleophilic attack producing a serine phosphorester via phosphorylation [45]. Such inhibition affect can be suggested as a base for indirect procedures for Ops determination using of the fabricated ChEs sensors. Fig. 6 shows the potential–time curve after addition of the BuChE to 10^{-4} mol L^{-1} BuCh solutions containing various concentrations of malathion. As the concentration of malathion was increased, more enzyme was inhibited and the rate electrode potential change became smaller. Calibration graph obtained by plotting the inhibition against malathion concentration was linear in the range 0–120 μg mL^{-1}.

More sensitivity of the inhibition reaction can be achieved via incubation the BuChE with malathion, followed by addition of the inhibited enzyme to the BuCh solutions. The effect of incubation time was investigated by incubation of 2.5 U BuChE with 20 μg mL^{-1} of malathion for 15, 30, 60, 90 or 120 min followed by measuring the BuChE activity. Results indicated that inhibition is almost completed within the first 30 min; later there is no significant effect. Under the optimal experimental conditions, calibration graph was linear in the concentration range from 0 to 30 μg mL^{-1} with linear equation: $I\% = 0.261 + 1.68$ [malathion], $r = 0.9998$.

3.5. Sample analysis

The developed disposable sensors were applied for assaying ChEs activity in human serum under the optimum conditions. Results (Table 2) showed non-significant difference between the proposed and the official methods [36]. In addition, malathion was determined in commercial insecticide and pharmaceutical preparations under the optimized inhibition procedure. The obtained results (Table 3) indicated that the developed SPCEs are applicable for the malathion determination with simple and reproducible procedures.

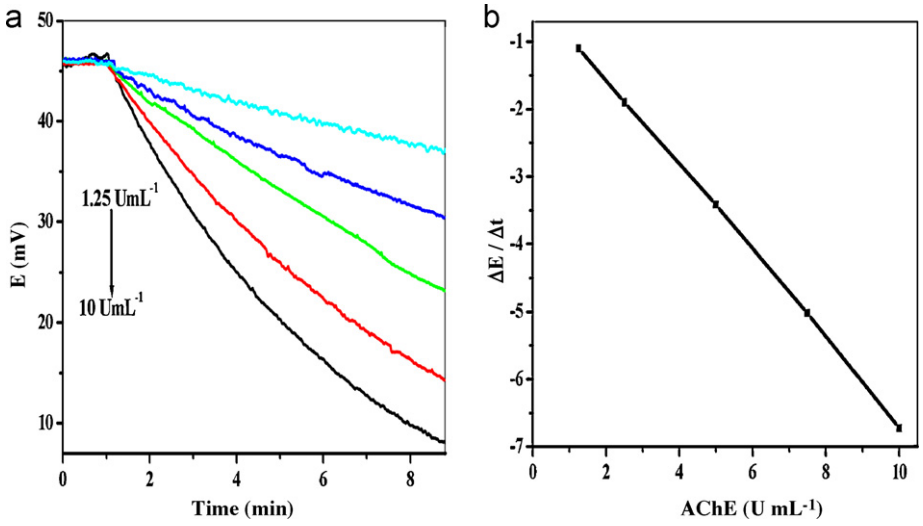

Fig. 5. Determination of enzyme activity of AChE using 10 mL of 10^{-4} mol L^{-1} ACh solution and ACh–SPCEs. (a) Time-course of the electrode potential and (b) relationship between reaction rate ($\Delta E/\Delta T$) and enzyme concentration.

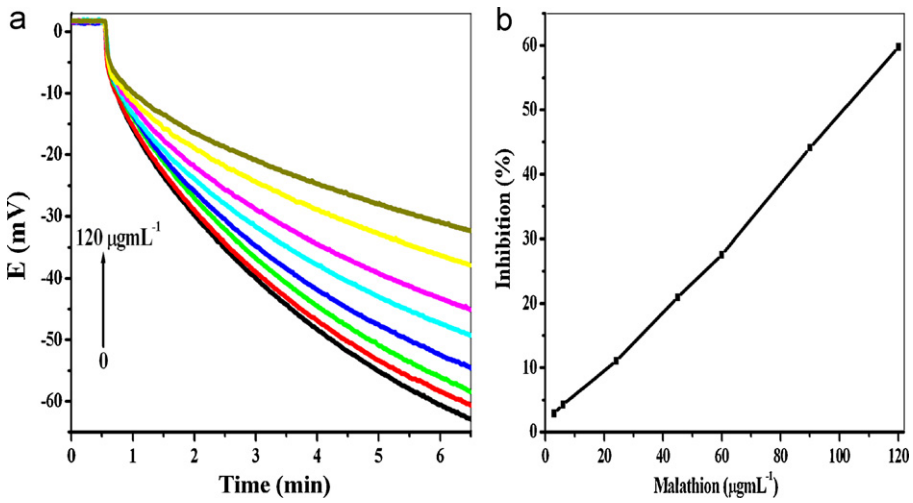

Fig. 6. Inhibition of enzyme reaction by malathion. (a) Potential–time curve of BuCh sensor in presence of 2.5 U BuChE, 10 mL of 10^{-4} mol L^{-1} BuCh solution in PBS at pH 7.3 and different malathion concentrations; (b) calibration curve for malathion.

Table 2
ChEs quantification in human serum.

Sample	Official (photometric)		Developed (potentiometric)	
	U mL^{-1}	RSD	U mL^{-1}	RSD
1	5.64	0.04	5.52	0.06
2	6.71	0.06	6.60	0.07
3	7.12	0.08	6.98	0.06
4	4.55	0.04	4.42	0.05
5	4.21	0.02	4.17	0.03

Table 3
Determination of malathion in commercial insecticide and pharmaceutical preparations.

Sample	Taken (μg mL^{-1})	Found	
		Official (HPLC)	Developed (potentiometric)
Malathion Coupra, 57%	6.0	5.82 ± 0.04	5.79 ± 0.06
Prioderm lotion	8.0	7.91 ± 0.05	7.78 ± 0.09

4. Conclusion

The present work demonstrates the application of potentiometric butyrylcholine sensor based on β-CD for fast and sensitive assay of cholinesterases activity and organophosphate pesticides. The sensing element based on screen-printed carbon material permits mass fabrication of the disposable electrodes with fast response time of about 1.6 s and adequate shelf-life (6 months). The unique characteristic of the sensor resides in the fact there is no need for enzyme immobilization or regeneration which greatly simplifies the fabrication procedures, lowers the production costs and improves the electrode lifetime.

The developed SPCEs have been successfully applied in the field of clinical diagnosis of intoxication through measuring the ChEs activity in blood samples of the suspected individuals with good accuracy and precision. The responses of the fabricated electrodes to commercial pesticides formulations indicate that they can be used as suitable pesticide alarms or screening devices to reduce the number of expensive assays and capable of detecting the environment contamination with simple portable measuring system.

Acknowledgement

Authors acknowledge the support from the project 8030501 NRC.

References

[1] F.R. Coulson, A.D. Fryer, Pharmacol. Ther. 98 (2003) 59.
[2] K. Boonyapisit, K.H.J. Kaminski, R.L. Ruff, Am. J. Med. 106 (1999) 97.
[3] J. Sherma, Anal. Chem. 65 (1993) 40R.
[4] G. Durand, N. de Bertrand, D. Barcelo, J. Chromatogr. 562 (1991) 507.
[5] R. Jeannot, E. Sauvard, Analusis 27 (1999) 271.
[6] S. Andreescu, J.L. Marty, Biomol. Eng. 23 (2006) 1.
[7] M. Trojanowicz, M.L. Hiuchman, Trends Anal. Chem. 15 (1996) 38.
[8] A.P. Periasamy, Y. Umasankar, S.M. Chen, Sensors 9 (2009) 4034.
[9] S. Sole, A. Merkoci, S. Alegret, Crit. Rev. Anal. Chem. 33 (2003) 89.
[10] A. Mulchandani, W. Chen, P. Mulchandani, J. Wang, K.R. Rogers, Biosens. Bioelectron. 16 (2001) 225.
[11] M.J. Dennison, A.P.F. Turner, Biotechnol. Adv. 13 (1995) 1.
[12] M. Trojanowicz, Electroanalysis 14 (2002) 1311.
[13] N. Mionetto, J.L. Marty, I. Karube, Biosens. Bioelectron. 9 (1994) 463.
[14] H.C. Budnikov, T.P. Medyantseva, S.S. Babkina, J. Electroanal. Chem. 310 (1991) 49.
[15] A.L. Hart, W.A. Collier, D. Janssen, Biosens. Bioelectron. 12 (1997) 645.
[16] P. Skladal, M. Fiala, J. Krejci, Int. J. Environ. Anal. Chem. 65 (1996) 139.
[17] Q. Deng, S. Dong, Analyst 121 (1996) 1123.
[18] G. Baumm, F.B. Ward, Anal. Chem. 43 (1971) 947.
[19] T. Imato, N. Ishibashi, Biosens. Bioelectron. 10 (1995) 435.
[20] J. Ding, W. Qin, Electroanalysis 21 (2009) 2030.
[21] R.E. Gyurcsanyi, Z. Vagfoldi, K. Toth, G. Nagy, Electroanalysis 11 (1999) 712.
[22] S.V. Dzyadevych, V.N. Arkhypova, C. Martelet, N. Jaffrezic-Renault, J.M. Chovelon, A.V. Elskaya, A.P. Soldatkina, Electroanalysis 16 (2004) 1873.
[23] B. Liu, Y.H. Yang, Z.Y. Wu, H. Wang, G.L. Shen, R.Q. Yu, Sens. Actuators B 104 (2005) 186.
[24] K. Duttaa, D. Bhattacharyaya, A. Mukherjeeb, S.J. Setfordc, A.P.F. Turnerc, P. Sarkar, Ecotoxicol. Environ. Safety 69 (2008) 556.
[25] J. Zhang, A. Luo, P. Liu, S. Wei, G. Wang, S. Wei, Anal. Sci. 25 (2009) 511.
[26] J.P. Hart, S.A. Wring, Trends Anal. Chem. 16 (1997) 89.
[27] J.P. Hart, A. Crew, E. Crouch, K.C. Honeychurch, R.M. Pemberton, Anal. Lett. 37 (2004) 789.
[28] O.D. Renedo, M.A.A. Lomillo, M.J.A. Martinez, Talanta 73 (2007) 202.
[29] L. Tymecki, E. Zwierkowska, S. Glab, R. Koncki, Sens. Actuators B 96 (2003) 482.
[30] L. Tymecki, M. Jakubowska, S. Achmatowicz, R. Koncki, S. Glab, Anal. Lett. 34 (2001) 71.
[31] R. Koncki, S. Glab, J. Dziwulska, I. Palchetti, M. Mascini, Anal. Chim. Acta 385 (1999) 451.
[32] J. Wang, B. Tian, V.B. Nascimento, L. Angnes, Electrochim. Acta 43 (1998) 3459.
[33] E. Khaled, H.N.A. Hassan, A. Girgis, R. Metelka, Talanta 77 (2008) 737.
[34] E. Khaled, H.N.A. Hassan, G.G. Mohamed, A.A. Seleim, Drug Test. Anal. 2 (2010), doi:10.1002/dta.159.
[35] E. Khaled, H.N.A. Hassan, G.G. Mohamed, F.A. Ragab, A.A. Seleim, Int. J. Electrochem. Sci. 5 (2010) 448.
[36] G.L. Ellman, D.K. Courtney, V. Andres, R.M. Featherstone, Biochem. Pharmacol. 7 (1961) 88.
[37] U. Schaller, E. Bakker, U.E. Spichiger, E. Pretsch, Anal. Chem. 66 (1994) 391.
[38] U. Schaller, E. Bakker, E. Pretsch, Anal. Chem. 67 (1995) 3123.

[39] R.P. Buck, E. Lindner, Pure Appl. Chem. 66 (1994) 2527.
[40] Y. Umezawa, P. Buhlmann, K. Umezawa, K. Tohda, S. Amemiya, Pure Appl. Chem. 72 (2000) 1851.
[41] K. Tohda, D. Dragoe, M. Shibata, Y. Umezawa, Anal. Sci. 17 (2001) 733.
[42] Z. Kovarik, Z. Radic, H.A. Berman, V. Simeon-Rudolf, E. Reiner, P. Talylor, Biochem. J. 373 (2003) 33.
[43] L. Savini, A. Gaeta, C. Fattorusso, B. Catalanotti, G. Campiani, L. Chiasserini, C. Pellerano, E. Novellino, D. McKissic, A. Saxena, J. Med. Chem. 46 (2003) 1.
[44] R.M. Blong, E. Bedows, O. Lockridge, Biochem. J. 327 (1997) 747.
[45] V. Pavlov, Y. Xiao, I. Willner, Nano Lett. 5 (2005) 649.