



Acetylcholinesterase biosensor based on single-walled carbon nanotubes—Co phthalocyanine for organophosphorus pesticides detection

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ABSTRACT

A simple and reliable technique has been developed for the construction of an amperometric acetylcholinesterase biosensor based on screen-printed carbon electrodes. For the first time, one-step modification using single-walled carbon nanotubes and Co phthalocyanine has been proposed to decrease the working potential and to increase the signal of thiocholine oxidation. The biosensor developed made it possible to detect 5–50 ppb of paraoxon and 2–50 ppb of malaoxon with detection limits of 3 and 2 ppb, respectively (incubation 15 min). The biosensor showed high reproducibility when measurements of the substrate and inhibitor were performed (R.S.D. about 1% and 2.5%, respectively). The reliability of the inhibition measurements was confirmed by testing spiked samples of sparkling and tape waters.

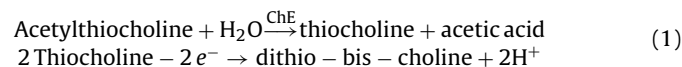
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1. Introduction

Cholinesterase biosensors have attracted considerable attention since 1990-s [1,2] due to the importance of the analytes detected which involve insecticides [3], nerve gases [4], alkaloids [5], fluorides and surfactants [6]. The main attention is focused on organic species which inhibit cholinesterase in ultra-small quantities and hence exert a high acute toxicity toward human beings. The presence of insecticides or nerve gases traces results in the irreversible suppression of cholinesterase activity followed by cessation of the nerve impulse transduction. In cholinesterase biosensors, the decay of enzyme activity is quantified as a measure of the inhibitor content by recording the biosensor signal prior to and after its contact with a sample. Most part of the research is devoted to acetylcholinesterase (AChE) preferably presented in blood serum and considered as a primary target of such agents [7] even though some other enzyme sources have been successfully applied for biosensor assembly [1,2], with recombinant enzymes isolated from mutant microorganisms [8], among them.

Electrochemical, fiber-optic and fluorometric transducers have been employed in cholinesterase sensor assembling. Among them, electrochemical devices became most popular due to inexpensive equipment and simple operation in laboratory and field conditions [1]. The amperometric detection of the AChE activity is mainly based on direct or mediated oxidation of the product of enzymatic

hydrolysis of thiocholine ester (1), an artificial analog of acetylcholine, a natural neural transmitter [9].



The direct oxidation of thiocholine (1) on bare electrodes requires rather high voltage coupled with the fouling problem of the working electrode surface. For this reason, various mediator systems have been employed, e.g., ferrocyanide [10], Prussian Blue [11], tetracyanoquinodimethane (TCNQ) [8], Co phthalocyanine [12] and carbon nanotubes (CNTs) [13]. The application of mediators significantly decreases the working potential as well as improves reproducibility and sensitivity of thiocholine detection. Most mediators are implemented in the electrode materials (carbon paste or carbon ink of screen-printed electrodes) or placed onto the surface of the working electrode. This limits the enzyme “wiring” because of the little electroactive contact area left. For this reason, CNTs are of special interest due to their ability both to form 3D-nets with high adsorptive activity toward enzymes and to establish electric contact with the electrode. These features of CNTs are most significant for oxidoreductases [14]. However, the CNTs mediation of thiocholine oxidation was also found very promising for the improvement of inhibition detection. In fact, the immobilization of multi-walled CNTs and AChE in polyelectrolyte multilayers made it possible to decrease the working potential to +150 mV and reach a high sensitivity of detection of model anticholinesterases [13]. The physical adsorption of AChE onto multi-walled CNTs allowed recording signal of thiocholine oxidation at +200 mV and mea-

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asuring picomolar concentrations of paraoxon [15]. Screen-printed electrodes covered with multi-walled CNTs were used for the quantification of the activity of human AChE in saliva and its decay in the presence of some toxicants. The concept was proved in sequential flow analysis of paraoxon with diluted human saliva as an enzyme source [16].

All of the above results were obtained with multi-walled CNTs that offer extended adsorption of proteins and auxiliary reagents. The use of single-walled carbon nanotubes (SWCNTs) in cholinesterase biosensors is less investigated. Being smaller and more sensitive to structural defects than multi-walled CNTs, SWCNTs form less regular layers onto the electrodes with a higher deviation of the signal measured. The formation of highly ordered SWCNTs layers was attained by self-assembling onto single-stranded DNA molecules attached to the Au electrode by terminal thiol groups [17]. The AChE was then immobilized onto the polyaniline formed polyelectrolyte complex with DNA. The biosensor was successfully applied for the determination of micro- and nanomolar concentrations of parathion and chlorpyrifos. It should be mentioned that these thionic pesticides do not inhibit AChE irreversibly and commonly they should be pre-oxidized to organophosphate analogs. In spite of its high sensitivity toward inhibitors, the assembly of the biosensors seems complicated and includes non-conductive components which could affect the electron transduction within the surface layer.

In this work, we suggest a simple and reproducible design of the AChE biosensor based on a screen-printed electrode covered with SWCNTs together with the Co phtalocyanine mediator. To the best of our knowledge, such a composition of mediators has still never been used for detecting the AChE activity and inhibition caused by insecticides.

2. Experimental

2.1. Reagents and apparatus

AChE from electric eel (687 U/mg prot.), *N*-(3-dimethylamino-propyl)-*N'*-ethylcarbodiimide chloride (EDC), 2-(*N*-morpholino)-ethanesulfonic acid (MES), *N*-hydroxysuccinimide (NHS), 2-pyridine aldioxime (2-PAM), acetylthiocholine chloride, malaoxon (2-(dimethoxyphosphorylthio) butanedioic acid diethyl ester, Pestanal, stock solution 1 mg/mL in ethanol), paraoxon (diethyl 4-nitrophenyl phosphate, 98.8% solid) were purchased from Sigma, Co phtalocyanine from Fluka, carboxylated SWCNTs from NanoLab Inc. (Waltham, MA, USA). All other reagents were of analytical grade. Electrochemical measurements were performed in PBS (0.05 M disodium phosphate +0.1 M NaCl) adjusted to pH 7.8. For electrode washing, 1.0 M NaCl solution containing 0.05 M phosphate buffer and 0.1 M ethanolamine was used. The substrate and inhibitor solutions were prepared directly prior to their use in measurements by aliquot dilution of stock solutions by bidistilled water. Buffer solutions were stored 4 °C for not more than one week.

Electrochemical measurements were performed in a non-thermostated working cell of 10 mL at ambient temperature. Voltammograms and chronoamperograms were recorded with AUTOLAB PGSTAT-12 and PGSTAT-30 potentiostats with GPES software (EcoChemie, Netherlands). Screen-printed electrodes were manufactured using screen-printer DEK-248 (DEK International) at Kazan Federal University and "Tor Vergata" University (both on polycarbonate support). The electrodes have the same geometry of the working area. Silver tracks were first printed and cured at 75 °C. Then curable graphite paste was printed onto the silver tracks to shape the counter and working electrodes. Then, Ag/AgCl ink was printed onto the area of the pseudo-reference electrode.

The insulating resistive layer was deposited and dried at 90 °C. All the materials for screen-printing were purchased from Gwent Electronic Materials Ltd. (UK) and Acheson (Italy). After drying, the electrodes had a resistance of 140–180 Ω between the electrical contact point and the working electrode area. All the potentials have been re-calculated to a standard Ag/AgCl/3.0 M KCl reference electrode to simplify the comparison with other biosensors.

2.2. AChE biosensor development

SWCNTs were pre-oxidized by concentrated HNO₃ and H₂SO₄ mixture 1:3 (v:v) for 6 h at 50 °C under sonication. After that, the SWCNTs were washed with distilled water and re-dispersed in 50% aqueous dimethylformamide. Co phtalocyanine dispersion was prepared under 10 min sonication in 50% aqueous dimethylformamide. Then, 40 μL of SWCNTs and 10 μL of Co phtalocyanine dispersion were mixed together and sonicated again for 5 min. The resulting dispersion contained 0.264 mg/mL SWCNTs and 0.2 mg/mL Co phtalocyanine. For electrode modification, 3 μL of the dispersion were placed onto the working area of screen-printed electrode and allowed to dry.

Carbodiimide binding was used to immobilize AChE. Briefly, 3 μL of 15 mM NHS and 3 mL of 8.7 mM EDC in MES buffer were spread on the electrode surface modified with SWCNTs – Co phtalocyanine and left for 30 min. After that, the electrodes were rinsed with distilled water and dried at ambient temperature. Then 3 μL of the AChE solution of a certain concentration in a 0.005 M phosphate buffer, pH 7.2, were placed onto the electrode surface and dried. Prior to use, the electrodes were washed with a PBS buffer containing ethanolamine to saturate activated carboxylic groups of SWCNTs which did not bind the enzyme. The AChE biosensors could be stored at 4 °C without any loss of enzyme activity for at least 3 months.

2.3. Signal and inhibition measurement

Prior to its first use, the AChE biosensor as-prepared was washed with PBS and placed in the working cell with 10 mL of PBS. Then the potential was cycled in the DC mode between 0 and 0.8 V at 50 mV s⁻¹ (20 cycles). This suppresses a possible influence of readily oxidizable impurities on the background current and accelerates measurements of the substrate signal. After that, the biosensor was placed into the new PBS solution and polarized at 0.05 V. After stabilization of the current, acetylthiocholine solution was injected and the steady-state current recorded within 1 min. The biosensor was washed with distilled water and PBS in between the measurements. For inhibition measurement, the AChE biosensor was incubated in the pesticide solution and the oxidation current was measured once again at the same concentration of the substrate. The concentration of the stock solutions of pesticides was determined by HPLC. The consecutive dilutions were prepared not more than two hours prior to use.

The relative decay in the current (2) was calculated as the degree of inhibition I%.

$$I\% = \frac{I_0 - I}{I_0} \times 100\%, \quad (2)$$

where *I*₀ and *I* are the currents of thiocholine oxidation measured prior to and after the contact of the biosensor with inhibitor, respectively. The biosensor was recovered by washing in the 3 mM 2-PAM solution in PBS containing 0.1 M monoethanolamine. The use of common carbon materials does not provide full recovery of AChE activity after inhibition measurements because part of enzyme adsorbed on the support is not accessible for the re-activator. The immobilization scheme suggested in this work presumes the enzyme to be covalently attached to terminal groups of SWCNTs.

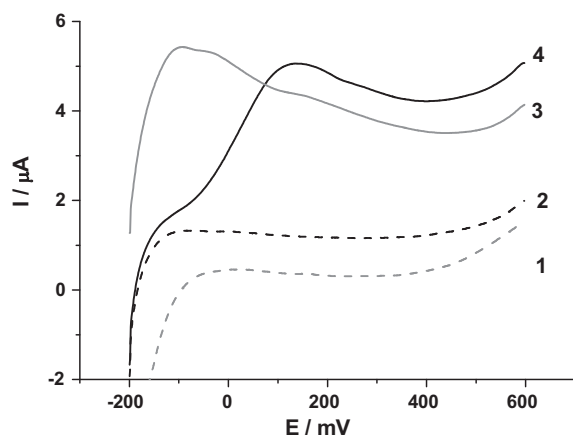


Fig. 1. Voltammograms recorded with AChE biosensors based on SWCNTs – Co phthalocyanine (1, 3) and SWCNTs (2, 4) in PBS (1, 2) and in 1.0 mM acetylthiocholine (3, 4). Scan rate 50 mV s^{-1} . Surface layer was obtained by deposition of the suspension contained 0.79 mg of SWCNTs and (if mentioned) 0.60 Co phthalocyanine per electrode.

Hence there are much less steric hindrances for both inhibition and re-activation of AChE. This makes the reactivation much more effective in comparison with conventional immobilization protocols.

3. Results and discussion

3.1. AChE biosensor performance

The cyclic voltammograms recorded with AChE biosensor are presented in Fig. 1. No resolved peaks relating to SWCNTs or Co phthalocyanine were observed in the absence of the enzyme substrate. Moreover, the background current recorded with the biosensor based on the SWCNTs modified electrode was slightly higher than that obtained in the presence of SWCNTs – Co phthalocyanine. This could be due to the difference in specific surface square and charge separation affected by components of mediator system.

The addition of acetylthiocholine produced a highly broadened peak on CVs in the area from 0 to 300 mV (SWCNTs) and from –200 to 400 mV (modification by SWCNTs – Co phthalocyanine). An attachment of Co phthalocyanine not only reduces the working potential but also increases maximal shift of the current against the background curve (compare curves 1 with 3 and 2 with 4 on Fig. 1).

Then the experiments with AChE biosensor were performed in chronoamperometric mode. For this reason, the working potential was chosen to be higher than –100 mV suggested by cyclic voltammetry. The potential of +50 mV was specified to reach zero background current and maximal stability of the signal. This is significantly lower than that obtained with the electrodes covered with multi-walled CNTs [13,15]. The appropriate curves (Fig. 2) confirm a high and fast response reached within 5–7 s after the substrate injection.

The experiments with the same amount of Co phthalocyanine dispersed onto screen-printed electrode showed lower mechanical stability of the surface layer and higher potential of thiocholine oxidation (about 250 mV) in comparison with those obtained for SWCNTs and SWCNTs – Co phthalocyanine. This might be due to rather low temperature of surface drying. Polycarbonate support of screen-printed electrodes does not allow sintering the mediator particles which preserve mobility onto the sensor surface and are partially leaching under measurement conditions. This value corresponds well with literary data [12] describing the application of screen-printed electrodes covered with Co phthalocyanine and AChE from various sources implemented in polymeric matrix.

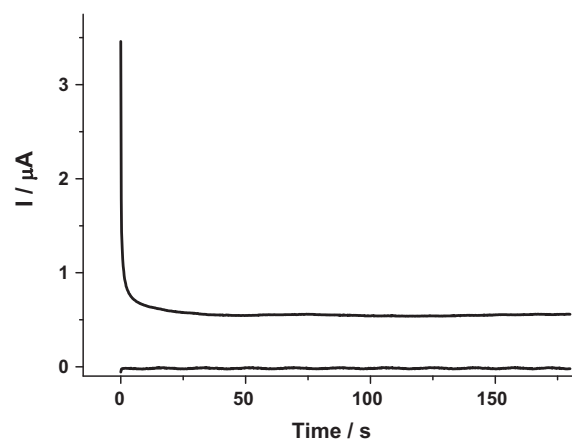


Fig. 2. Chronoamperogram of background signal (1) and 1.0 mM acetylthiocholine (2) in PBS, with working potential of 50 mV.

Also, the activity of mediator systems decreased in the series of measurement with repeated scanning the potential in the same substrate solution. Some of the problems related to the instability of Co phthalocyanine can be solved by introduction of the mediator in the carbon paste used to manufacture screen-printed electrode. However, in this format it would be much more complicated to reach an intimate contact between all the partners of the electron transfer chain.

Thus, the results obtained make it possible to conclude that the use of SWCNTs and Co phthalocyanine has synergic effect increasing the efficiency of each part of electrocatalyst. This can be related to the even distribution of Co phthalocyanine along SWCNTs or additional electrostatic interactions stabilizing redox forms of Co in the complex.

The biosensor based on SWCNTs – Co phthalocyanine mediation system makes it possible to detect 0.02–1.0 mM of acetylthiocholine with the limit of detection of $5 \mu\text{M}$ (Fig. 3). In addition to regression curve, the concentration dependence of the residuals was obtained by subtraction of the linear trend from average values of the signal. The shift did not exceed 0.05 mA with the maximal deviation at 0.2 mM acetylthiocholine. This corresponded to R.S.D. of 10%. At higher concentrations, these values decreased to 1.2–1.3%. All the data correspond to three repetitions of signal measurements.

The apparent Michaelis constant (K_m)_{app} was calculated from linear piece of the curve in the plots of $1/I$ against $1/C$ where I is the biosensor signal and C acetylthiocholine concentration. The obtained value of $0.070 \pm 0.012 \text{ mM}$ is similar to that reported for free enzyme (0.1 mM [18,19]). For AChE immobilized in various matrices, the (K_m)_{app} values are commonly higher indicating steric limitations of enzyme – substrate interactions. Thus, the following (K_m)_{app} values were reported for different immobilization protocols of AChE onto screen-printed electrodes: 0.67 mM (entrapment in a PVA-SBQ polymer [20]), 0.32 mM (sol-gel immobilization [20]), 0.45 mM (affinity immobilization via nitrilotriacetic acid [20]), 0.08 mM (cross-linking with glutaraldehyde [21]), and 0.22 mM (affinity immobilization with concanavalin A [18]). Low value of (K_m)_{app} confirms the advantage of SWCNTs – Co phthalocyanine matrix which both provides mediation of the thiocholine oxidation and avoids steric hindrance of an enzymatic substrate hydrolysis.

High operational stability is one of the most important requirements to meat biosensors developed for inhibition measurements. The average response toward 1.0 mM acetylthiocholine was $2.20 \pm 0.02 \mu\text{A}$ ($n = 9$). No significant trend in the nine consec-

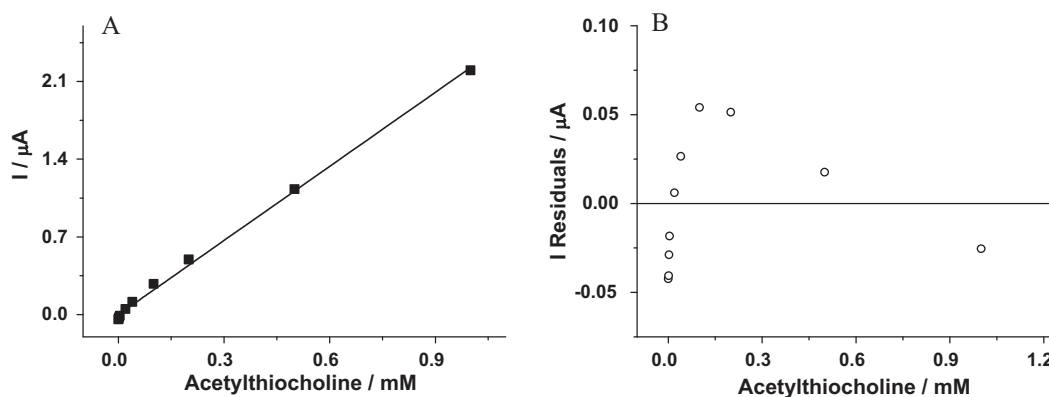


Fig. 3. Acetylthiocholine calibration curve (A) and its residuals analysis (B). Points on the curve correspond to average values of three repetitive chronoamperometric measurements.

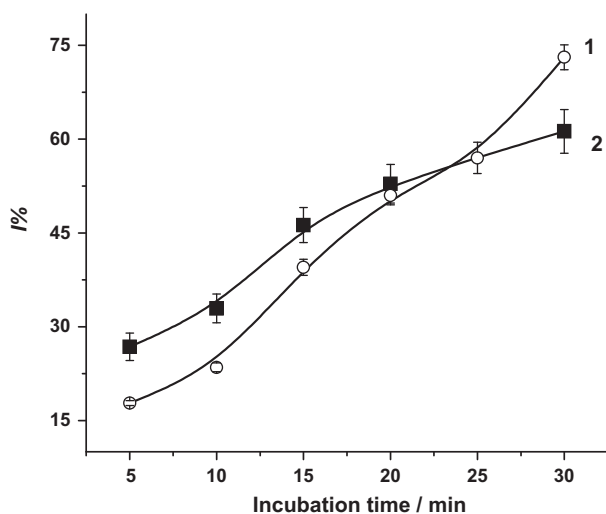


Fig. 4. The dependence of the inhibition ($I\%$) on the incubation period, 1–30 ppb of paraoxon, 2–10 ppb of malaoxon.

utive measurements of the signal with the same AChE biosensor from the same substrate solution was observed. Deviation of the background current in the absence of the substrate, i.e. in the measurements in PBS did not exceed 0.5% for 30 min of continuous measurements. The reproducibility of the current recorded in the presence of 1.0 mM acetylthiocholine for six different biosensors produced in the same lot toward 1.0 mM acetylthiocholine was $2.10 \pm 0.18 \mu\text{A}$ (8.6%). An ultra-fast response (5–6 s) indicates no diffusional limitations of enzymatic reaction. This is also confirmed by the measurement of alternating acetylthiocholine concentrations (0.5–1.0 mM) which did not exhibit any changes in at least six measurement cycles. The lifetime of the biosensor stored in dry conditions at 4 °C was at least 3 months. After that, both the signal and its sensitivity toward insecticides became lower.

Table 1

Analytical characteristics of insecticide determination with AChE biosensor based on screen-printed electrode modified with SWCNTs – Co phthalocyanine mediator, $I\% = a + b \times C_i$ (ppb), inhibition 15 min.

Insecticide	<i>a</i>	<i>b</i>	<i>R</i>	Limit of detection (ppb)	Concentration range (ppb)
Paraoxon	0.70 ± 1.25	1.05 ± 0.05	0.9898	2.0	5–50
Malaoxon	9.50 ± 1.90	2.85 ± 0.20	0.9685	0.2	2–50

3.2. Inhibition measurements

3.2.1. Insecticide determination

Organophosphorus pesticides exert irreversible inhibition due to the formation of covalent bonds between the serine hydroxyl group of an enzyme active site and the organophosphate moiety. The most sensitive protocol of the measurement of irreversible inhibition assumes the incubation of the biosensor in an insecticide solution followed by the substrate addition and signal measurement [22]. The inhibition ($I\%$) quantified as the relative decay of the current recorded (see (1)) does not depend on the substrate concentration. For this reason, all the measurements were performed with 1.0 mM acetylthiocholine corresponding to the upper limit of the calibration curve and the best reproducibility of about 0.8%. Paraoxon and malaoxon were chosen as model inhibitors. The first is often used for the characterization of cholinesterase biosensors whereas the latter can be considered a metabolite of malathion, i.e. a common insecticide. The sensitivity toward inhibitors increased with decreasing amounts of AChE taken for immobilization. In all the experiments, the real activity of enzyme aliquots was estimated by spectrophotometric Ellman method [23]. Thus, the inhibition of 20 ppb malaoxon was found to be 72% for 0.010 U of AChE per electrode and 58% for 0.075 U. For other experiments, 0.018 U of AChE per electrode were chosen as a compromise between the sensitivity toward inhibitors and the signal value sufficient for its accurate quantification. Carbodiimide binding of AChE to terminal carboxylic groups of SWCNTs provided the formation of stable and well reproducible adduct with covalently attached enzyme. This was confirmed by high reproducibility of the response toward substrate/inhibitor. No leaching or spontaneous inactivation was observed in all the measurements.

The inhibition degree of AChE regularly increases with incubation period (Fig. 4). The calibration curves of paraoxon and malaoxon were obtained for 15 min incubation. In general, the dependence obtained is fitted by logistic function with the saturation at 50–60% inhibition. The initial piece of the curve can be approximated by linear regression. The appropriate characteristics of regression are summarized in Table 1. The bimolecular inhibition constant of paraoxon was reported to be higher than that of malaoxon ($2.9 \times 10^5 \text{ M}^{-1} \text{ min}^{-1}$ and $1.1 \times 10^5 \text{ M}^{-1} \text{ min}^{-1}$ for native

Table 2

Measurement of insecticides in spiked tape water of Kazan city and sparkling water available on local market ("Volzhanka") with the AChE biosensor based on the SWCNTs – Co phthalocyanine mediator system. Inhibition 15 min.

Insecticide	Water source	Inhibitor added (ppb)	Inhibitor found (ppb)	Recovery (%)
Malaoxon	Tape water (Kazan City)	5	6.2 ± 0.2	123 ± 4
Paraoxon	Sparkling water "Volzhanka"	30	35 ± 2	117 ± 6
	Tape water (Kazan City)	30	34.3 ± 0.3	114 ± 1

AChE [24]). However, the immobilization and hydrophobicity of the enzyme environment can significantly alter the kinetic parameters of inhibition [25]. The LOD values of pesticide determination were derived from the accuracy of signal measurements achieved for two consecutive measurements performed with the same biosensor prior to and after incubation step. For 9% inhibition criterion, the LOD values are equal to 3 ppb of paraoxon and 2 ppb of malaoxon.

The reproducibility of the inhibition with standard solutions of 10 ppb malaoxon prepared in PBS was 2.5% ($I\%$ 43.6 ± 1.1%, $n=6$), paraoxon 2.1 ($I\%$ 42.7 ± 0.9). In a set of six biosensors the appropriate values are 5.5 and 4.5%, respectively. Thus, the biosensor developed showed high reproducibility of the inhibition measurements. Each biosensor makes it possible to perform up to five measurements by enzyme reactivation with 2-PAM solution. For this purpose, after inhibition measurements the biosensors were immediately put into the 2-PAM solution for 15 min and then washed with bidistilled water and PBS. In the series of measurements, a slight decrease in the signal of AChE biosensor was observed by about 2–5% of its initial value in each measurement cycle. Meanwhile the inhibition referred to reduced initial signal remained the same. The limit in the number of consecutive measurements is related to an increase in the deviation of $I\%$ which increased to 7–10% by the fifth measurement.

The characteristics of malaoxon determination are comparable with those reported for other AChE biosensors. Thus, the use of recombinant AChEs from various sources immobilized on screen-printed electrodes with TCNQ made it possible to discriminate paraoxon and malaoxon in binary mixtures [25]. The estimated 2 ppb LOD was calculated from the signal errors reported. Flow-injection analysis of malaoxon with AChE from electric eel and some recombinant enzymes showed the LOD of malaoxon from 0.6 to 1.2 ppb [26]. The enzymes were entrapped in PVC bearing styrylpyridinium groups.

For paraoxon, the use of CNTs in combination with AChE entrapped in polyelectrolyte multilayers made it possible to reach much lower limit of detection (0.4 pM, or 0.1 ppb) [13]. Screen-printed electrode with multi-walled CNTs showed the LOD of 0.15 ppb for a longer incubation period (30 min.) [15]. Meanwhile the use of similar screen-printed electrodes with TCNQ and AChE immobilized in hydrogel showed much lower sensitivity toward paraoxon (22 ppb) [8].

3.2.2. Real sample measurements

Tap and sparkling water were spiked with malaoxon and paraoxon. The samples were diluted with PBS and then its inhibition was measured in the conditions described above. The results are presented in Table 2.

The results indicate some overestimation of the insecticide content. The AChE activity is rather sensitive toward ionic strength and pH of the media. Probably, some losses of the activity can be related to the use of the samples with very low content of salts (less than 1 mg per liter in sparkling water). Among total content of salts this might be due to insufficient amounts of alkali-earth metals, well known effectors of AChE. Changes in pH are less probable due to the use of PBS for sample dilution.

In blank experiment, the samples tested showed inhibition of about 3–4%. Taking into account the deviation of the inhibition obtained the reliability of the insecticide detection can be considered satisfactory. For malaoxon, the overestimation of its content by 30% in the samples of tape waters was reported with other AChE biosensors as well [27]. The accuracy of the measurement can be further improved by increasing the buffer capacity of the sample tested and the removal of dissolved carbon dioxide from sparkling water.

4. Conclusion

The approach to the development of AChE biosensor suggested in this work provides a simple and reliable alternative to a more sophisticated multi-layer deposition of regular layers of mediators and the enzyme. Due to the one-stage deposition of SWCNTs and Co phthalocyanine followed by carbodiimide binding of the enzyme, high efficiency of the electron transfer was reached together with minimal steric hindrance of the AChE active site. The use of SWCNTs provided directed coordination of protein molecule at the terminal carboxylic groups of oxidized SWCNTs. Thus the enzyme is little raised over the surface of a transducer. Co phthalocyanine, a second mediator, shows its advantages as effective and specific electrocatalyst of thiocholine oxidation as recently reported in literature [28]. As a results, the biosensor is the first one which coupled the SWCNTs and Co phthalocyanine for AChE activity monitoring characterised by high sensitivity toward AChE irreversible inhibitors, fast response and efficient re-activation of inhibited enzyme by 2-PAM. Even though the use of the screen-printed electrode allows a single use of the biosensor, it can be reliably applied up to ten times with no losses of the sensitivity toward the insecticides considered.

In comparison with other AChE sensors based on CNTs, the newly proposed combination of SWCNTs and Co phthalocyanine provides the lowest working potential and hence minimal interferences from electrochemically active components of the sample tested. The potential chosen (+50 mV) can be lowered down to –100 mV if the measurement is performed by DC voltammetry and this is the lowest working potential ever used for these purposes. The LODs achieved (3 and 2 ppb for paraoxon and malaoxon, respectively) are sufficient for the detection of dangerous amounts of insecticides in fresh waters, confirming the potentiality of AChE to be used as screening method for toxic compounds such as organophosphorous insecticides [29]. The comparison of the inhibition constants [24] determined for the native enzyme with the sensitivity (slope of calibration curves, Table 1) of paraoxon and malaoxon determination made it possible to conclude that some non-enzymatic factors affected the inhibition quantification. This might be due to hydrophobic effects caused by interactions of side-walls of SWCNTs with phenolic fragments of paraoxon or general changes in hydrophobic–hydrophilic balance onto the electrode interface. In the future, these factors can be used for specific changes of the selectivity toward individual pesticides, e.g., by implementation of surfactants or, vice versa, inclusion of polar (ionized) functional groups in the polymeric matrix used for enzyme immobilization.

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