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Analytical Methods

Electrochemical detection of carbamate pesticides in fruit and vegetables with a biosensor based on acetylcholinesterase immobilised on a composite of polyaniline–carbon nanotubes

Ivana Cesarino *, Fernando C. Moraes, Marcos R.V. Lanza, Sergio A.S. Machado

Instituto de Química de São Carlos, Universidade de São Paulo, São Carlos, São Paulo CEP 13566-590, Brazil

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ABSTRACT

A sensitive electrochemical acetylcholinesterase (AChE) biosensor was successfully developed on polyaniline (PANI) and multi-walled carbon nanotubes (MWCNTs) core–shell modified glassy carbon electrode (GC), and used to detect carbamate pesticides in fruit and vegetables (apple, broccoli and cabbage). The pesticide biosensors were applied in the detection of carbaryl and methomyl pesticides in food samples using chronoamperometry (CA). The GC/MWCNT/PANI/AChE biosensor exhibited detection limits of 1.4 and 0.95 μmol L^{-1}, respectively, for carbaryl and methomyl. These detection limits were below the allowable concentrations set by Brazilian regulation standards for the samples in which these pesticides were analysed. Reproducibility and repeatability values of 2.6% and 3.2%, respectively, were obtained in the conventional procedure. The proposed biosensor was successfully applied in the determination of carbamate pesticides in cabbage, broccoli and apple samples without any spiking procedure. The obtained results were in full agreement with those from the HPLC procedure.

1. Introduction

The continuous monitoring for low pesticide levels in food, water, and air has become a key activity in respect to human health. Such monitoring must often be carried out in situ and in real time, which demands new analytical techniques that can provide responses very quickly and at very low concentration levels. One of the most promising research fields in this area is related to enzymatic biosensors. These devices can be easily miniaturised for in situ applications which can provide fast analytical responses and are insensitive to interference from the other components of the complex matrices.

Enzymatic biosensors used to monitor the pesticides of the carbamate class are based on the inhibition of the enzymatic reaction of acetylcholinesterase (AChE) and its substrate, acetylcholine. The carbamates bind to the active estearic site of the enzyme, inhibiting biocatalytic activity by blocking the serine residue in the catalytic triad of AChE by phosphorylation or carbamylation (Singh & Agarwal, 1983).

A great number of research studies report the development of biosensors for detecting carbamates based on AChE enzymatic inhibition. Recently, Cai and Du related a biosensor based on the immobilisation of acetylcholinesterase to multi-walled carbon nanotubes for the detection of carbaryl with the detection limit reaching 4.0 μg L^{-1} (Cai & Du, 2008). Pedrosa, Caetano, Machado, and Bertotti (2008) developed an amperometric biosensor based on acetylcholinesterase (AChE) immobilised onto a self-assembled modified gold electrode. The biosensor was used in the electrochemical detection of carbamate in water with the detection limit calculated at 9.0 μg L^{-1}. In another study, Upadhyay et al. (2009) immobilised the enzyme AChE on a glassy carbon surface modified with gold–platinum bimetallic nanoparticles. This biosensor was used in the electrochemical detection of carbamates and organophosphate pesticides. Other examples of biosensors for pesticides based on the inhibition of AChE have been reported (Du, Ye, Cai, Liu, & Zhang, 2010; Viswanathan, Radecka, & Radecki, 2009) confirming that this methodology constitutes one of the most important analytical branches in environmental and foodstuff monitoring.

However, despite the extensive number of publications available on the subject, there are few studies regarding the electrochemistry of enzymes immobilised at bare electrodes surfaces. Enzymes usually have short life spans and lose activity at bare electrodes (Wen, Ye, & Zhou, 1997). To overcome such limitations, studies have focused on various strategies for enzyme immobilisation, such as adsorption, covalent linkage, entrapment, and cross-linking.

To this end, the use of nanostructured materials has attracted much attention due to unique properties such as high electrical conductivity, large surface area and the presence of functional groups that can interact with the enzyme offering excellent support for immobilisation. In this context, hybrids formed with

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* Corresponding author. Tel.: +55 16 3373 9924.
E-mail address: ivana@iqsc.usp.br (I. Cesarino).

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carbon nanotubes (CNTs) and conducting polymers have exhibited a synergic effect leading to significant enhancements in the electronic and mechanical characteristics of each single component (Agüí, Peña-Farfal, Yáñez-Sedeño, & Pingarrón, 2007).

Carbon nanotubes have been the subject of numerous investigations in chemical, physical and material science research owing to their extraordinary mechanical, chemical and electronic properties (Merkoçi et al., 2005). CNTs can be used as electrode materials with useful properties for electrochemical and bioelectrochemical applications (Moraes, Mascaro, Machado, & Brett, 2009). The ability of CNTs to promote electron-transfer reactions suggests interesting applications in the development of amperometric biosensors (Laschi, Bulukin, Palchetti, Cristea, & Mascini, 2008).

Polyaniline (PANI) is one of the most attractive conducting polymers due to its high conductivity, ease of preparation, good environmental stability, and extensive applications (Gerard, Chaubey, & Malhotra, 2002; Li, Guo, Wei, MacDiarmid, & Lelkes, 2006). The presence of an extended π-conjugated system and the three-dimensional structure of the polymer allows the adsorption of the enzyme inside composite cavities promoting significant interactions between carboxyl groups of the enzyme and amino groups present in the polymer chain (Sung & Bae, 2003).

The core–shell structure in which the CNTs are covered by a compact layer of PANI (Zhou et al., 2010), exhibits intrinsic characteristics such as a redox activity at neutral pH values (Do Nascimento, Corio, Novickis, Temperini, & Dresselhaus, 2005). The electrochemical formation of polymer layers offers significant advantages such as complete coverage of the active surface, greater control over film thickness and enhanced reproducibility (Ahuja, Mir, Kumar, & Rajesh, 2007).

The objective of this study is to develop an amperometric pesticide biosensor based on acetylcholinesterase immobilised in a core–shell structure of CNTs and polyaniline (PANI) to be used to quantify methomyl and carbaryl in liquefied apple, cabbage and broccoli samples by chronoamperometry, and to compare the obtained results with those from the HPLC technique.

2. Experimental

2.1. Instrumentation

Cyclic voltammetry (CV), square-wave voltammetry (SWV) and chronoamperometry (CA) experiments were performed in a model PGSTAT 30 Autolab Electrochemical System equipped with GPES 4.9 software (Eco Chemie, Utrecht, The Netherlands). The morphology of the composite surface was characterised using a Field-Emission Gun Scanning Electron Microscope (FEG-SEM) and the images were recorded using a FEG-Zeiss model Supra 35VP (Zeiss, Germany).

The electrochemical cell was assembled in a conventional one-compartment three-electrode design: the working electrode was a glassy carbon disk modified by the core–shell multi-walled carbon nanotubes (MWCNTs) and PANI composite in which the acetylcholinesterase was immobilised; the Ag/AgCl/KCl (3.0 mol L^{-1}) was used as the reference electrode; and a Pt plate was used as the auxiliary electrode. A PTFE cover provided the access to the electrodes and to the degassing facility. All experiments were conducted at a controlled temperature of 25 °C.

2.2. Methodologies

The chronoamperometric (CA) experiments were performed by polarising the working electrode at 0.0 V for 5 s, followed by a potential step to +0.30 V for 60 s in a 0.2 mol L^{-1} phosphate buffer solution (PBS) at pH 7.0. The square-wave voltammetry (SWV) experiments were carried out using the following parameters, initially optimised: frequency of 10 Hz, pulse amplitude of 70 mV, step potential of 2 mV, in a 0.2 mol L^{-1} phosphate buffer solution (PBS) at pH 7.0.

The analytical methodology employed to investigate enzyme inhibition by carbamate consisted of the addition of different amounts of the pesticide in the electrochemical cell containing 0.2 mol L^{-1} PBS and 4.0×10^{-5} mol L^{-1} acetylthiocholine chloride (AChC), the substrate for the enzymatic reaction. After each carbamate addition, the biosensor was immersed in the electrolyte and an induction time of 10 min was imposed to allow the competitive adsorption in the active enzymatic sites to reach equilibrium. After this, the amount of thiocholine generated in the enzymatic reaction was evaluated by CA or by SWV. After each determination, electrode activity was restored by immersion in PBS without carbamate for 10 min.

2.3. Chemicals and solutions

All solutions were prepared with water purified in a Millipore Milli-Q system (resistivity ⩾18 MΩ cm). Aniline (Sigma–Aldrich, Germany) purification was carried out by distillation at 140 °C, using metallic zinc as a catalyst.

An amount of approximately 1.0 g of MWCNT (90% purity, Sigma–Aldrich, Germany), synthesised by thermal chemical vapour deposition, was mixed with 500 mL of a 1:3 mixture of HNO_3/H_2SO_4 for 12 h, in order to promote its functionalisation (Moraes, Cabral, Mascaro, & Machado, 2011). This was then filtered through a 0.45 μm Millipore nylon filter membrane. The resulting MWCNT was continuously washed using distilled water until the pH of the filtrate was neutral, and then dried overnight in a vacuum oven at 120 °C.

A 10 mg/mL solution of acetylcholinesterase enzyme from bovine erythrocyte (Type XII-S, 426 unit mg^{-1} of solid, Sigma–Aldrich, Germany) was prepared in 0.2 mol L^{-1} PBS pH 7.0. Acetylthiocholine chloride, carbaryl and methomyl were purchased from Sigma–Aldrich.

2.4. Preparation of the AChE biosensor

Prior to modification, the GC electrode surface was polished with 0.05 μm alumina slurries, rinsed thoroughly with double distilled water, sonicated for 3 min in alcohol followed by 3 min in water and then dried in air. The GC electrode was immersed in a solution containing 500 μL of aniline and 7.0 mg of MWCNT diluted in 25.0 mL of 0.5 mol L^{-1} of H_2SO_4 solution and using the cyclic voltammetry the hybrid was electropolymerised between −0.2 and +0.8 V *vs.* Ag/AgCl with a scan rate of 50 mV s^{-1}. The GC electrode containing the PANI/MWCNT film was removed from the solution and, after drying at a controlled temperature of 25 °C, 7.0 μL of AChE was dropped on the electrode surface. The biosensor was dried at a controlled temperature and then immersed in PBS prior to use.

2.5. Preparation of the fruit and vegetables samples and analysis of carbamate

For the analysis of carbaryl in the apple samples, four apples (from the local market) were cut into small pieces, 168.0 g of these pieces were weighed and mixed in a blender with 0.2 mol L^{-1} PBS, pH 7.0. The CA analysis of carbaryl was performed directly in the extract of the sample using the standard addition method. For the analysis of methomyl in cabbage, a bundle of leaves was cut and mixed in a blender with 0.2 mol L^{-1} PBS, pH 7.0, resulting in a final volume of 1.0 L. To prepare the samples of broccoli, 117.7 g of broccoli were weighed and mixed in a blender with 0.2 mol L^{-1} PBS, pH 7.0. The CA analysis of methomyl was carried out directly in the extracts of the cabbage and broccoli samples using the standard addition method.

2.6. HPLC measurements

The analytical curves were obtained by the standard addition method and measurements were performed in triplicate. For carbaryl, the mobile phase consisted of a mixture of acetonitrile and water 70:30 (v/v), with a flow rate of 1 mL min^{-1} under isocratic conditions. The chromatographic column was a C18 (250 × 4.6 mm) Shimadzu Shim–Pack CLC–ODS (Kyoto, Japan). The monitored wavelength was 220 nm. For methomyl, the flow rate and the chromatographic column were the same as those used for the carbaryl analysis; however the mobile phase consisted of a mixture of acetonitrile and water 50:50 (v/v) and the monitored wavelength was 230 nm. The analysis of carbamates in cabbage, broccoli and apple using the HPLC procedure was performed in the extracts of the samples described in Section 2.4. However, before analysis, the extracts were centrifuged at 3600 rpm for 15 min and the supernatant liquids were filtered using a PTFE syringe filter, 33 mm × 0.22 μm.

3. Results and discussion

3.1. Development and characterisation of the MWCNT/PANI/AChE biosensor

The consecutive cyclic voltammograms for the electropolymerisation of the PANI/MWCNT composite on the GC electrode from a 25 mL support electrolyte solution containing 0.5 mol L^{-1} H_2SO_4 + 500 μL of aniline and 7.0 mg of MWCNT with a scan rate of 50 mV s^{-1} showed a well-defined oxidation peak at +0.24 V (due to the polymerisation of aniline forming emeraldine salt) and this peak increase with the cyclisation. Aiming to achieve a composite film that: (a) completely covers the electrode surface with core–shell PANI/MWCNT, and (b) is sufficiently thin to avoid loss of catalytic activity by increasing the electron transfer resistance, a number of 25 voltammetric cycles in the electrodeposition step was found to be the optimum procedure. The efficiencies of such coverage were tested by the amperometric responses of the biosensor.

The morphological characteristics of composites were examined by FEG-SEM. The corresponding micrographs were presented in Fig. 1. For comparison, the MWCNT morphology can be observed in Fig. 1A, in accordance with those already presented in the literature (Hernández-Pérez et al., 2008; Moraes, Golinelli, Mascaro, & Machado, 2010). The complete coverage of the MWCNT with PANI is evident in Fig. 1B, pointing to the core–shell arrangement of the composite (Zhou et al., 2010). The disposition of such nanostructures on the GC electrode surfaces generates cavities, indicated by the arrows in Fig. 1C, in which the biomolecules, as enzymes, can be properly immobilised. Such tridimensional structures, plenty of nitrogen groups from PANI, are responsible for the biocompatibility between the composite and the biomolecules.

As described in the Section 2, AChE immobilisation on the composite surface was carried out by dropping 7.0 μL of the buffer solution containing the enzyme on the electrode surface, which was further dried at a controlled temperature and immersed in PBS prior to use. No cross-linking agent (gluteraldehyde or diimide) was necessary. This constitutes a real contribution from the composite surface to the efficiency of the biosensor since the cross-linking agents frequently bond themselves to the active sites of enzymes, thus inhibiting their activity. The anchorage of proteins in the porous composite surface is indicated in Fig. 1D by the arrows. The amorphous material bonded to the PANI nitrogen groups is associated to the AChE.

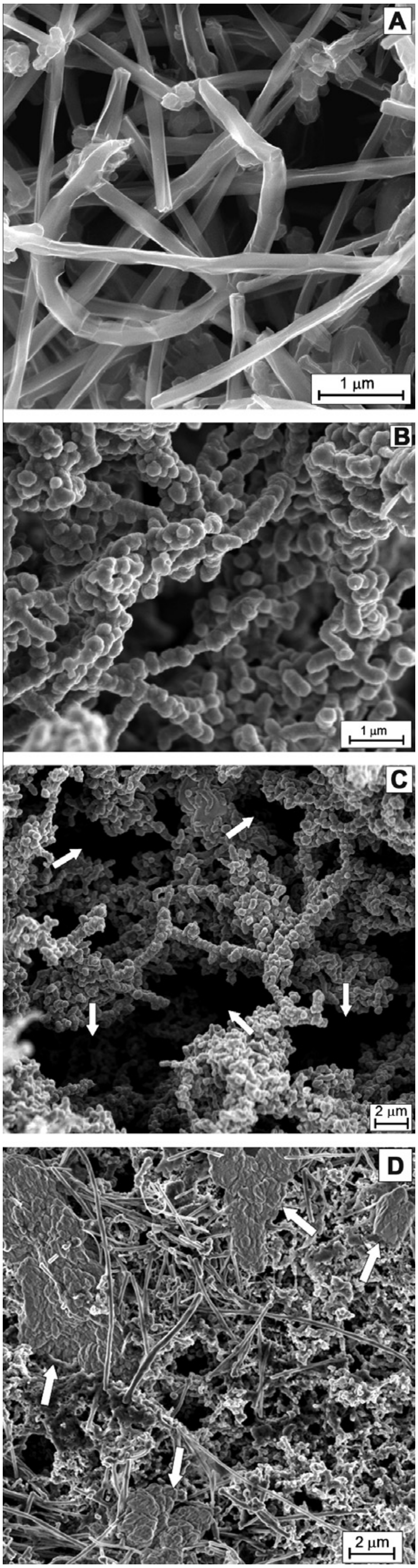

Fig. 1. FEG-SEM micrographs for: (A) MWCNT (magnitude of 45,000×); (B) PANI/MWCNT core–shell (magnitude of 35,000×); (C) PANI/MWCNT cavities (magnitude of 12,000×) and (D) the anchorage of proteins in the porous of the PANI/MWCNT composite surface (magnitude of 10,000×).

3.2. Electrochemical response of the MWCNT/PANI/AChE biosensor

To evaluate the effect of the carbon nanotubes on the hybrid film, SWV experiments were carried out using a frequency of 10 Hz, a pulse amplitude of 70 mV, and a step potential of 2 mV in 0.2 mol L^{-1} phosphate buffer (pH 7.0) containing 20.0 μmol L^{-1} of acetylthiocholine chloride (AChC), the substrate of the enzyme. The biosensors evaluated were fabricated both in the presence of carbon nanotubes (GC/MWCNT/PANI/AChE) and in the absence of carbon nanotubes (GC/PANI/AChE).

In the SWV profiles presented in Fig. 2A the GC/MWCNT/PANI/AChE electrode (open square) showed a well-defined oxidation peak at +0.025 V. This electrochemical process has been associated to thiocholine oxidation generating the respective dimer, ditihio-bis-choline (Liu, Riechers, Mellen, & Lin, 2005; Rotariu, Zamfir, & Bala, 2010), as shown in the following reactions:

$$(CH_3)_3N^+CH_2CH_2SCOCH_3 + H_2O \rightarrow (CH_3)_3N^+CH_2CH_2SH + CH_3COOH$$

$$2(CH_3)_3N^+CH_2CH_2SH \rightarrow (CH_3)_3N^+CH_2CH_2S{-}SCH_2CH_2N^+(CH_3)_3 + 2e^-$$

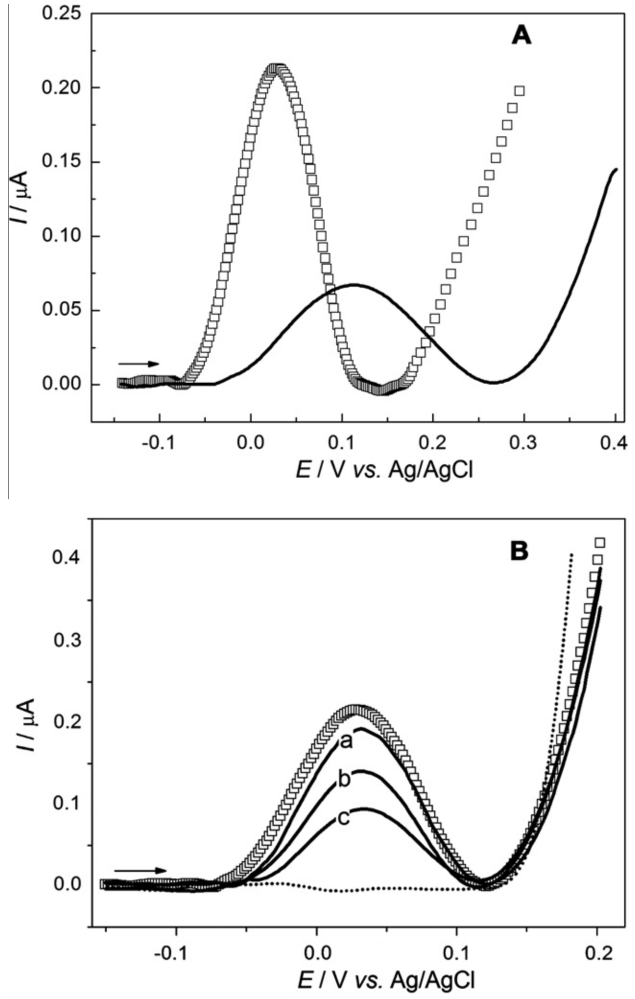

Fig. 2. Square wave voltammograms in 0.2 mol L^{-1} PBS (pH 7.0) containing 20.0 μmol L^{-1} of AChC for (A) GC/MWCNT/PANI/AChE electrode (open square), GC/PANI/AChE electrode (solid line) and (B) for GC/PANI/MWCNT/AChE electrode in absence (dotted line) of AChC, presence (open square) and after addition of (a) 30, (b) 40, (c) 50 mol L^{-1} of carbaryl (solid line), with an incubation time of 10 min.

The effect of the MWCNT on the hybrid film can be observed when comparing the GC/MWCNT/PANI/AChE electrode and the biosensor fabricated in the absence of MWCNT. For the GC/PANI/AChE electrode (solid line in Fig. 2A) the dithio-bis-choline oxidation peak occurred at +0.125 V, i.e. the biosensor prepared with carbon nanotubes (GC/MWCNT/PANI/AChE electrode) shifted in 100 mV the peak potential to more negative values, and also increased the current peak value by approximately three times. In this sense, the presence of MWCNT improved the electrochemical response of the enzymatic product and acted as an electrocatalyst.

The amount of enzyme dropped over the top of the electrode was optimised in the range of 1.0–5.0 units mg^{-1}. The current peak increased up to 3.0 unit mg^{-1} of the enzyme on the biosensor. For values greater than 3.0 unit mg^{-1} the current peak remained constant. Thus, 3.0 unit mg^{-1} was chosen as the optimal value.

The biosensor developed above was tested by immersion in a solution containing 0.2 mol L^{-1} phosphate buffer (pH 7.0) and 20.0 μmol L^{-1} of acetylthiocholine chloride (AChC). The SWV responses were collected in Fig. 2B, after the exposition of the enzyme to its substrate (open square) and only to the PBS (dotted line).

The electrochemical potential for the dimerisation reaction depends strongly on the catalytic properties of the electrode surface. To avoid the action of several species that can undergo electrochemical oxidation at positive potential values, it is convenient that thiocholine dimerisation occurs at nearly 0.0 V (*vs.* Ag/AgCl). Occasionally mediators are used to shift the potential towards less positive values. As can be observed in Fig. 2B, in this study no mediator had to be used to obtain a dimerisation potential close to 0.0 V. This observed effect is due to the extraordinary catalytic property of MWCNTs.

It is possible to observe that, after the addition of growing amounts of carbaryl (solid line in Fig. 2B) and an incubation time of 10 min, the SWV response presented peak currents correspondingly lower, although at the same potential as that obtained for thiocholine oxidation. This is associated to the reversible competitive adsorption of carbaryl on the active enzyme site, inhibiting its activity (Du, Chen, Cai, & Zhang, 2007). Therefore, this phenomenon can be used to quantify the amount of carbaryl in the electrolyte.

Due to the reversible character of carbamates inhibition, the biosensor activity can be restored, after use, by simple immersion in PBS during 10 min. Further tests showed that the enzyme activity recovers its initial value, reproducing the same thiocholine oxidation measurement obtained without inhibition.

3.3. Analytical performance of the GC/MWCNT/PANI/AChE biosensor

With the voltammetric behaviour of the biosensor well settled, chronoamperometric experiments were performed, varying the carbaryl concentration, aiming to build up the calibration curves, as described in the Section 2. The variation of pesticide concentration in the electrolyte, from 9.9 to 49.6 μmol L^{-1}, promoted increasing inhibition in the diffusion currents of the chronoamperograms, similar to those demonstrated for SWV peak currents presented in Fig. 3A. The percentage of inhibition, calculated as previously reported (Caetano & Machado, 2008), exhibits a linear behaviour with the pesticide concentration, following the equation: $\%I = 3.75 + 1.10[\text{carbaryl}]$, with a correlation coefficient of 0.999 ($n = 5$). A limit of detection (LOD) of 1.4 μmol L^{-1} (0.28 mg kg^{-1}) was determined using the 3σ/slope ratio, where σ is the standard deviation of the diffusion current values of 10 chronoamperograms of the blank, as recommended by IUPAC (Blanc, González-Casado, Navalón, & Vílchez, 2000; Currie, 1999). The same procedure was applied to the variation of methomyl concentration (Fig. 3B) and the linear response range was found

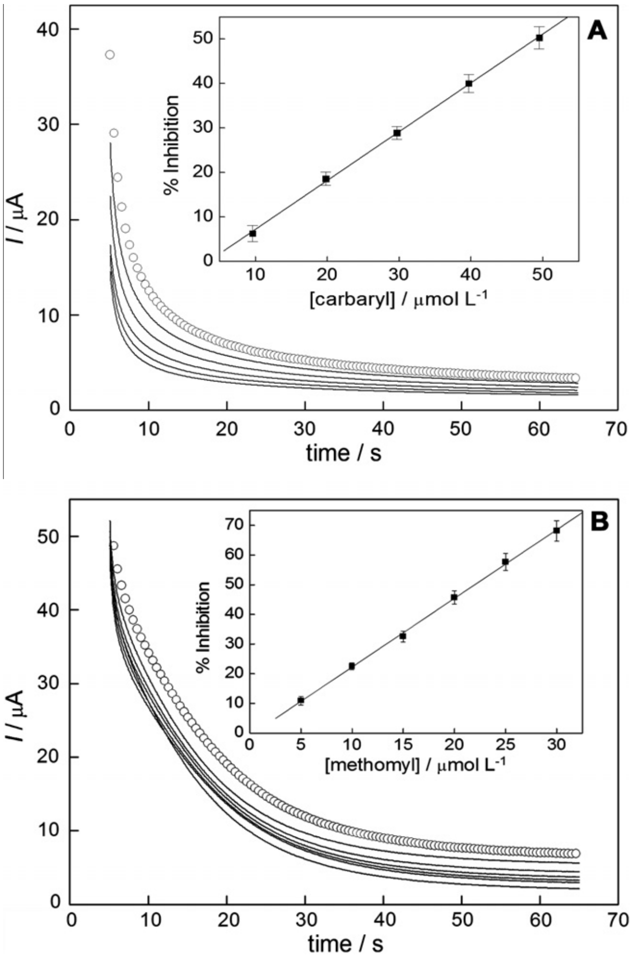

Fig. 3. Chronoamperograms using GC/PANI/MWCNT/AChE electrode in 0.2 mol L^{-1} PBS (pH 7.0) containing 4.0×10^{-5} mol L^{-1} of AChC for (A) carbaryl and (B) methomyl in the absence (○) and presence (solid line) of the pesticides. Inset: linear dependence of percentage of inhibition with the concentrations of carbaryl (A) and methomyl (B).

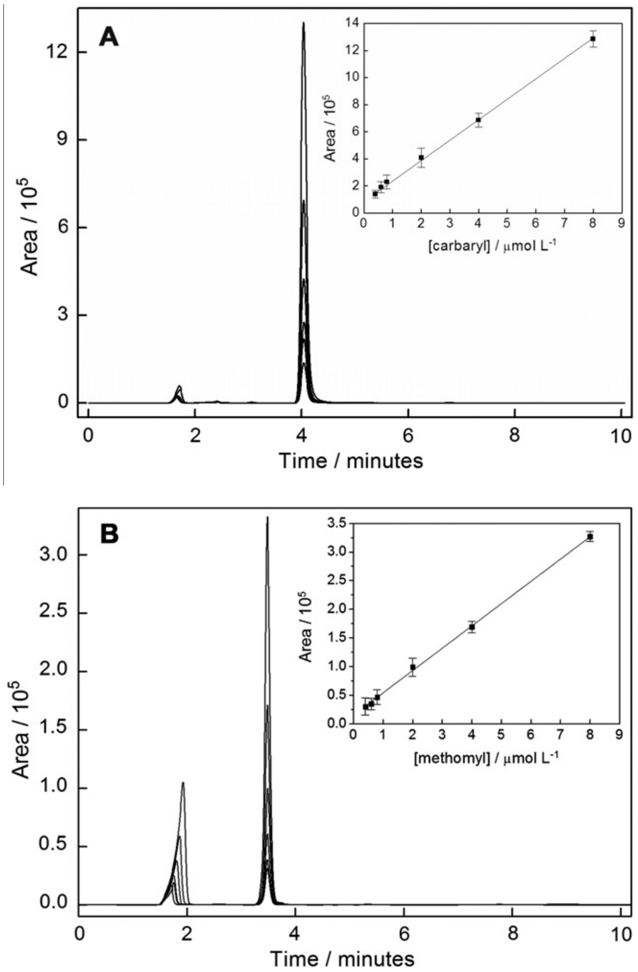

Fig. 4. Chromatograms for different concentrations of (A) carbaryl and (B) methomyl. Insert: the analytical curve obtained by the areas of the chromatographic peaks.

to be between 4.9 and 29.2 µmol L^{-1}, with a linear relationship of $\%I = 0.91 + 2.32$[methomyl], a correlation factor of 0.998 ($n = 6$), and LOD of 0.95 µmol L^{-1} (0.15 mg kg^{-1}). Such values of the LOD are adequate to monitor contaminations with such pesticides according to the National Health Surveillance Agency (ANVISA) in Brazil, which establishes a maximum residue limit of 3.0 mg kg^{-1} for methomyl in cabbage and broccoli and 2.0 mg kg^{-1} for carbaryl in apples (Agência Nacional de Vigilância Sanitária).

Aiming to perform reproducibility and repeatability tests, the electrochemical measurements with thiocholine were performed 10 times each in the conventional procedure, given reproducibility and repeatability values of 2.6% and 3.2%, respectively.

In order to test the biosensor stability, it was stored in 0.20 mol L^{-1} PBS (pH 7.0) at 4 °C and used daily in experiments similar to those described above, for up to 60 days. After 2 months the electrode loses only 8.5% of its activity, which represents very reasonable stability for this kind of biosensor.

3.4. Comparison with the results obtained by high-performance liquid chromatography

It is well known that biosensors show low selectivity for a given class of carbamates or organophosphorous pesticides. In this way, during the development of the analytical procedure, the use of HPLC is convenient to identify the nature of a particular pesticide and allows the consequent monitoring in the target crop by the biosensor.

For this reason, HPLC experiments were performed to analyse carbaryl in apple samples, and methomyl in cabbage and broccoli samples, in order to compare the chromatographic results with those from the biosensor measurements. Aliquots of 20 µL of acetonitrile (analytical grade) spiked with different carbaryl or methomyl amounts were injected into the chromatograph. Fig. 4A reports the results obtained for carbaryl and Fig. 4B for methomyl. The retention times were found to be 4.0 and 3.5 min for carbaryl and methomyl, respectively. The inset in Fig. 4A shows the relationship between peak areas and carbaryl concentration. A well-defined linear relationship was observed in the range of 0.4–8.0 µmol L^{-1} with a slope of 1.46 and a correlation coefficient of 0.999. For methomyl (inset Fig. 4B), the linear response was found to be in the same concentration range as that of carbaryl, with a slope of 0.38 and a correlation coefficient of 0.999.

3.5. Analysis of carbamates pesticides in fruit and vegetables

The developed biosensor was used for the quantification of carbaryl in apple samples acquired locally. Carbaryl determinations were performed in triplicate, without any further spiking procedure, using the standard addition method. Recoveries between 97.3% and 103.4% of carbaryl from apple samples were obtained

Table 1
Determination of carbaryl in the apple sample.

Repetition	Carbaryl (μmol L^{-1})		
	Proposedbiosensor	HPLC	E_r (%)*
1	1.65	1.59	3.6
2	1.72		7.6
3	1.68		5.4
Mean ± SD	1.68 ± 0.10**		

* E_r = detected proposed biosensor *vs.* detected HPLC.
** $\bar{x} \pm t_{(n-1)} s/\sqrt{n}$.

Table 2
Determination of methomyl in the broccoli and cabbage samples.

Repetition	Methomyl in the cabbage sample (μmol L^{-1})			Methomyl in the broccoli sample (μmol L^{-1})		
	Proposed biosensor	HPLC	E_r*(%)	Proposed biosensor	HPLC	E_r*(%)
1	2.42	2.22	8.3	1.80		2.8
2	2.31		3.9	1.83	1.75	4.4
3	2.25		1.3	1.92		8.8
Mean ± SD	2.33 ± 0.22**			1.85 ± 0.15**		

* E_r = detected proposed biosensor *vs.* detected HPLC.
** $\bar{x} \pm t_{(n-1)} s/\sqrt{n}$.

for 9.9, 19.8 and 29.7 μmol L^{-1} carbaryl additions, and were extrapolated to the resulting straight line. The results obtained for the samples were compared with those for the same samples using HPLC and the calibration curve in Table 1. According to the Student's *t*-test, there were no significant differences between the HPLC and the biosensor methods at a 95% confidence level. This result indicates that the GC/MWCNT/PANI/AChE electrode can be used for the determination of carbaryl in apple samples. The average value of carbaryl detected in the apple samples by the proposed biosensor was 1.68 μmol L^{-1} (0.34 mg kg^{-1}) and this value is smaller than the maximum residue level of carbaryl allowed by the National Health Surveillance Agency in apple samples, as discussed earlier (2.0 mg kg^{-1}).

The GC/MWCNT/PANI/AChE electrode was also applied, using the same methodology as used for methomyl determination in broccoli and cabbage samples. Recoveries between 94.6% and 101.2% and 95.4% and 101.9% methomyl from cabbage and broccoli, respectively, were obtained. The content of pesticides found in samples without any spiking, using the standard addition method for 4.9, 9.8, 14.7 μmol L^{-1} methomyl are presented in Table 2. The *t*-test yielded no significant differences between the HPLC and the proposed analytical method at a 95% confidence level. This result attests the accuracy of the AChE biosensor for the determination of methomyl in cabbage and broccoli samples. The average concentrations of methomyl detected in cabbage (0.38 mg kg^{-1}) and broccoli (0.30 mg kg^{-1}) samples are lower than the maximum residue level of methomyl permitted by Brazilian law, as discussed earlier (3.0 mg kg^{-1}).

4. Conclusions

The GC electrode modified with PANI/MWCNT as a core–shell structure was successfully applied for the immobilisation of the acetylcholinesterase enzyme and the determination of carbamate pesticides in fruit and vegetables. The results obtained by the proposed methodology for carbaryl in apple, and methomyl in cabbage and broccoli, were quite similar to those obtained using the HPLC procedure. Although the acetylcholinesterase biosensors are known presenting low selectivity for carbamate, which make then inappropriate for screening procedure for pesticides, they are quite sensitive. The amperometric GC/MWCNT/PANI/AChE biosensor was demonstrated to be a convenient methodology to quantify pesticides in foods, presenting long-term stability and not requiring the use of mediators (phthalocyanine) or cross-linking agents (glutaraldehide), which is promising for future applications in the area of biosensors.

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