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Mercaptobenzothiazole-on-Gold Organic Phase Biosensor Systems:

4. Effect of Organic Solvents on Organophosphate and Carbamate Pesticide Determination

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1. Introduction

With an ever increasing demand for agricultural products delivered over shorter periods, we have seen the use of agricultural chemicals such as pesticides increasing in order to effectively control the damaging effects of harmful micro-organisms and insects. This has also resulted in an increase of usage of insecticides that are classified as cholinesterase (ChE) inhibitors such as organophosphates (OPs) and carbamates (CMs), to protect various crops such as bulbs, cereals, fruits, vegetables, cotton, peanuts, soybean, potato, sugar cane, coffee, alfalfa and pasture from deleterious effects. These pesticides have low environmental persistence and are highly effective as insecticides, but some exhibit potential dose-related acute and chronic toxicity in human beings by acting on the inhibition of ChE activity, followed by the accumulation of acetylcholine at cholinergic receptor sites thereby excessively stimulating the cholinergic receptors. This poses serious health effects for humans if water, processed food, fruits and vegetables with high concentrations of OPs and CMs are consumed. In children and infants it can lead to serious health effects such as eye pain, abdominal pain, convulsions, respiratory failure, paralysis and even death (Kim *et al.*, 2007; Liu *et al.*, 2008; Sbair *et al.*, 2007; Skladal *et al.*, 1997).

The toxicity of OPs and CMs are caused by their ability to bind irreversibly to the catalytic serine residue in acetylcholinesterase (AChE), which leads to inhibition of AChE that prevents nerve transmission by blocking the breakdown of the transmitter choline (Ch) (Somerset *et al.*, 2009; Liu *et al.*, 2008; Kim *et al.*, 2007; Du *et al.*, 2007; Kim *et al.*, 2000).

Since a high degree of toxicity is assigned to organophosphate (OP) and carbamate (CM) pesticide compounds, the rapid detection of these toxic chemicals in environmental samples have become increasingly important (Sbair *et al.*, 2007; Tapsoba *et al.*, 2009; Valdés-Ramírez *et al.*, 2008).

The standard methods that have been traditionally used for pesticide detection include gas chromatography (GC) with different detectors (e.g. mass spectrometry in GC-MS), high performance liquid chromatography (HPLC) and HPLC coupled with mass spectrometry (HPLC-MS) or UV detection (HPLC-UV). However, despite the precision and accuracy of these methods, analyses are restricted to laboratory facilities, are time-consuming and expensive due to its analytical cost, limiting the operation of these instruments to highly qualified laboratory personnel (Somerset *et al.*, 2009; Valdés-Ramírez *et al.*, 2008; Kim *et al.*, 2007; Gong *et al.*, 2009; Caetano and Machado, 2008).

Therefore, the need to find rapid, simple and sensitive methods for the detection of organophosphate (OP) and carbamate (CM) pesticide compounds are growing. Biosensors are considered an ideal alternative analytical tool for pesticides quantification, since they offer good selectivity, fast response, miniature size, and reproducible results. The use of amperometric AChE biosensors based on the inhibition of the AChE enzyme have shown satisfactory results for the analysis of various matrices (Frasco *et al.*, 2006; Gong *et al.*, 2009; Ion *et al.*, 2010).

In a previous paper, the application of a mercaptobenzothiazole-on-gold biosensor system for application to organophosphorous and carbamate pesticides determination has been reported. The activity of the AChE immobilized in the biosensor construction was measured by amperometry based on the detection of thiocholine produced in the enzymatic hydrolysis of acetylthiocholine as substrate. This paper further report the results obtained for the biosensor analysis of selected OPs and CMs. The aim of this work was to improve the detection limit of these pesticides with an AChE biosensor, applied to various water miscible organic solvents. The results obtained for the operation of the constructed biosensor in various aqueous-organic media are reported.

2. Materials and methods

2.1 Reagents and materials

Several reagents consisting of aniline (99%), potassium dihydrogen phosphate (99+%), disodium hydrogen phosphate (98+%) and diethyl ether (99.9%) were obtained from Aldrich, Germany. The acetylthiocholine chloride (99%) was obtained from Sigma, Germany. The mercaptobenzothiazole (MBT), acetylcholinesterase (AChE, from *Electrophorus electricus*, EC 3.1.1.7; ~ 850 U/mg), acetylcholine chloride (99%) and acetone (>99.8%, pestanal) were obtained from Fluka, Germany. The hydrogen peroxide (30%) and the organic solvents ethanol (99.9%, absolute grade), acetonitrile (99.9%, pestanal grade) were purchased from Riedel-de Haën, Germany. The potassium chloride, sulphuric acid (95%), and hydrochloric acid (32%) were obtained from Merck, South Africa. Organophosphorous pesticides used in this study include chlorpyrifos, malathion and parathion-methyl. Carbamate pesticides include carbaryl, carbofuran and methomyl. The pesticide standards used in this study were purchased from Riedel-de Haën, Germany. Platinum (Pt) wires as counter electrodes were obtained from Sigma-Aldrich, South Africa. Alumina micropolish and polishing pads that were used for the polishing of the working electrode were obtained from Buehler, IL, USA (Somerset *et al.*, 2009; Somerset *et al.*, 2010a).

2.2 Instrumentation

A BAS-50/W electrochemical analyser with BAS-50/W software (Bioanalytical Systems, Lafayette, IN, USA) was used for all electrochemical protocols and data collection. Either

cyclic voltammetry (CV), Oysteryoung square wave voltammetry (OSWV), differential pulse voltammetry (DPV) or time-based amperometric modes were employed. A conventional three electrode system was employed. The working electrode was a gold disc electrode (diameter: 1.6 mm; area: $2.01 \times 10^{-2} \text{ cm}^2$; Bioanalytical Systems, Lafayette, IN, USA). Silver/silver chloride (Ag/AgCl – 3 M NaCl type) was used as the reference electrode and a platinum wire was used as auxiliary electrode (Somerset *et al.*, 2010a).

2.3 Preparation of mercaptobenzothiazole on gold electrode

Gold disc electrodes were carefully prepared for the biosensor construction. Prior to use, gold electrodes were first polished on aqueous slurries of 1 μm , 0.3 μm and 0.05 μm alumina powder. After thorough rinsing in deionised water followed by acetone, the electrodes were etched for about 5 minutes in a hot 'Piranha' solution {1:3 (v/v) 30 % H_2O_2 and concentrated H_2SO_4 } and rinsed again with copious amounts of deionised water. The polished electrodes were then cleaned electrochemically by cycling the potential scan between - 200 and + 1500 mV (vs. Ag/AgCl) in 0.05 M H_2SO_4 at the scan rate of 40 mV.s^{-1} for 10 min or until the CV characteristics for a clean Au electrode were obtained. The platinum (Pt) counter electrode was regularly cleaned before and after synthesis and in between synthesis and analysis. This involved flaming the Pt electrode in a Bunsen burner until it was white hot, followed by rinsing with copious quantities of deionised water.

The cleaned Au electrode was then immersed into an ethanol solution containing 10 mM of mercaptobenzothiazole (MBT) for 2 hours, thereby coating a self-assembled monolayer (SAM) of MBT on the gold electrode. This was followed by rinsing the SAM electrode extensively with ethanol and water and storing it in 0.1 M phosphate buffer (pH 7.2) for later use (Somerset *et al.*, 2010a).

2.4 Preparation of Au/MBT/PANI modified enzyme electrode

After preparation of the Au/MBT electrode, a polymer film layer of polyaniline (PANI) was coated on the SAM-modified electrode. A three electrode arrangement was set up in a sealed 10 ml electrochemical cell. Polyaniline (PANI) films were prepared by electropolymerisation from a 0.2 M aniline solution dissolved in 1 M hydrochloric acid (HCl) onto the previously prepared Au/MBT-modified electrode. The aniline/HCl solution was first degassed by passing argon (Ar) through the solution for approximately ten minutes and keeping the Ar blanket during electropolymerisation. Initial optimisation of the potential window for electropolymerisation was performed. The electropolymerisation was performed by scanning the potential repeatedly between - 200 mV and +1200 mV, at a scan rate of 40 mV/s (vs. Ag/AgCl) for 20 cycles. The Au/MBT-polyaniline modified electrode was then rinsed with deionised water and used as the working electrode in subsequent studies. The electrode will be referred to as Au/MBT/PANI for the gold-MBT-PANI modified electrode.

Following the electropolymerisation of a fresh PANI polymer film on an Au/MBT electrode, the Au/MBT/PANI electrode was transferred to a batch cell, containing 1 ml argon degassed 0.1 M phosphate buffer (pH 7.2) solution. The PANI polymer film was then reduced at a potential of - 500 mV (vs. Ag/AgCl) for approximately thirty minutes, until a steady current was achieved. Electrochemical incorporation of the enzyme acetylcholinesterase (AChE) onto the Au/MBT/PANI electrode was then performed. This involved the addition of 60 μL of AChE to the 0.1 M phosphate buffer (pH 7.2) solution.

After enzyme incorporation the Au/MBT/PANI/AChE bioelectrode was arranged vertically and then coated with a 2 μ L drop of poly(vinyl acetate) (PVAc) solution (0.3 M), prepared in acetone, and left to dry for 1 min. The resulting Au/MBT/PANI/AChE/PVAc biosensor was then ready for pesticide analysis (Somerset *et al.*, 2010a).

2.5 Inhibitory studies of AChE-based biosensors in the presence of pesticide inhibitors

A new Au/MBT/PANI/AChE/PVAc biosensor was prepared each time a new organophosphorous or carbamate pesticide was studied, including each time a new concentration of the OP and CM pesticides was evaluated. The electrochemical cell consisted of Au/MBT/PANI/AChE/PVAc bioelectrode, platinum wire and Ag/AgCl as the working, counter and reference electrode, respectively. A 1 ml test solution containing 0.1 M phosphate (0.1 M KCl, pH 7.2) solution was degassed with argon before any substrate was added and after each addition of small aliquots of 0.01 M acetylthiocholine (ATCh). Inhibition plots for each of the OP and CM pesticides detected were obtained using the percentage inhibition method. The following procedure was used. The biosensor was first placed in a stirred 1 ml of 0.1 M phosphate (0.1 M KCl, pH 7.2) solution (anaerobic conditions) and multiple additions of a standard acetylthiocholine (ATCh) substrate solution was added until a stable current and a maximum concentration of 2.4 mM were obtained. This steady state current is related to the activity of the enzyme in the biosensor when no inhibitor was present. This was followed by incubating the biosensor in anaerobic conditions for 20 min with a standard pesticide phosphate buffer-organic solvent mixture. This was followed by multiple additions of a standard ATCh substrate solution (anaerobic conditions), to a fresh 1 ml of 0.1 M phosphate (0.1 M KCl, pH 7.2) solution (anaerobic conditions) and multiple additions of a standard acetylthiocholine (ATCh) substrate solution was again added, until a stable current was obtained. The maximum concentration of acetylthiocholine (ATCh) was again 2.4 mM. The percentage inhibition was then calculated using the formula (Albareda-Sirvent *et al.*, 2001; Sotiropoulou and Chaniotakis, 2005; Wilkins *et al.*, 2000):

$$I\% = \frac{I_1 - I_2}{I_1} \times 100 \quad (1)$$

where $I\%$ is the degree of inhibition, I_1 is the steady-state current obtained in buffer solution, I_2 is the steady-state current obtained after the biosensor was incubated for 20 min in phosphate buffer-organic solvent mixture.

Cyclic, square wave and differential pulse voltammetric measurements were performed after each addition of ATCh up to a maximum concentration of 2.4 mM. Cyclic voltammetry (CV) was performed at a scan rate of 10 mV.s⁻¹ by applying a linear potential scan between - 400 mV and + 1800 mV (vs. Ag/AgCl). For some experimental runs the anodic difference square wave voltammogram (SWV) was collected in an oxidation direction only by applying a linear potential scan between - 400 mV and + 1800 mV (vs. Ag/AgCl), at a step potential of 4 mV, a frequency of 5 Hz, and a square amplitude of 50 mV.

The anodic difference differential pulse voltammogram (ADPV) was collected in an oxidation direction only by applying a linear potential scan between - 400 mV and + 1800

mV (vs. Ag/AgCl), at a scan rate of 10 mV.s^{-1} and a pulse amplitude of 50 mV. The sample width, pulse width and pulse period were 17 ms, 50 ms and 200 ms, respectively (Somerset *et al.*, 2010a).

2.6 Assessment of organic solvent influence on biosensor operation

The response of the Au/MBT/PANI/AChE/PVAc biosensor was further evaluated in the presence of various organic-aqueous solvent mixtures. The biosensor response was first measured in a 0.1 M phosphate buffer, KCl (pH 7.2) solution, in the presence of a fixed concentration of ATCh. The biosensor was thereafter incubated for 20 minutes in an aqueous-solvent mixture or the pure organic solvent. The response of the Au/MBT/PANI/AChE/PVAc biosensor was then again measured in a 0.1 M phosphate buffer, KCl (pH 7.2) solution, in the presence of a fixed concentration of ATCh. The results for the calibration curves obtained after successive addition of the substrate ATCh to 0.1 M phosphate buffer, KCl (pH 7.2) solutions before and after incubation of the AChE biosensor in the polar organic-aqueous solvent mixtures were then evaluated and characterised (Somerset, 2007b).

3. Results and discussion

3.1 Effect of polar organic solvents on amperometric behaviour of biosensor

Research conducted by other researchers has shown that organic solvents can induce extensive changes in the activity and specificity of an enzyme. This is due to the enzyme's structure and reactivity that depends on several non-covalent interactions in the biocatalyst, which includes hydrogen bonding, ionic, hydrophobic, and van der Waals interactions. Enzymes have further evolved to maintain their structural stability in aqueous medium, but organic solvents are known to disrupt the abovementioned forces of interaction in the enzyme, causing changes in the kinetic and thermodynamic behaviour of the enzyme. Any changes that occur in solvent hydrophobicity, dielectric constant and water content of the reaction medium, affect the ability of enzymes to use their free energy of binding with a substrate, leading to changes in substrate specificity and reactivity. The solvents media that can be used for biosensing can be classified into two groups, i.e. anhydrous organic media and water-containing media. When anhydrous organic media are employed, it refers to pure solvents or a mixture of pure organic solvents that may be polar or non-polar in nature (Iwuoha *et al.*, 1997; Dordick, 1992).

Contrary, water-containing organic media consist of micro-aqueous systems, water-organic solvent mixtures, water and immiscible organic solvent biphasic systems and reverse micellar solutions. The term micro-aqueous reaction media are associated with the non-polar organic solvents that are immiscible with water. Enzymes generally require essential water of hydration for activity, therefore it is essential that non-polar solvents be saturated with water before they are used as reaction media for biosensing. These systems will contain a water content that is very insignificant when compared to the water content of organic solvents, and it depends on the ability of the solvent to absorb water. Since the so-called anhydrous aqueous systems require a minimum amount of water for enzyme activity, it is more suitable to refer to such a system as a micro-aqueous solution. Polar organic solvents can be used as systems that contain some amount of water. The hydration of polar solvents

ensures that the flexibility, structure and local dielectric constant of the enzyme redox site environment, stay as much as possible, unaltered. If the effect of increased solvent polarity occurs, it will weaken the electrostatic forces in the enzyme, which will lead to water partitioning out of the enzyme into the bulk solvent. For this reason, biosensors exhibit much greater reactivity in the presence of polar organic solvents that contain some amount of water (Iwuoha *et al.*, 1997; Chatterjee and Russell, 1992; Borzeix *et al.*, 1992; Somerset, 2010b).

3.2 Voltammetric characterisation in a 90% aqueous-organic solvent mixture

In Figure 1 the individual results obtained for the inhibition of the AChE enzyme in the Au/MBT/PANI/AChE/PVAc biosensor after 20 minutes of incubation in a 90% water-organic solvent mixture is shown.

From the results shown in Figure 1 it was observed that the highest decrease in the catalytic activity of AChE was observed when the biosensor was exposed to acetonitrile, while the

Fig. 1. Results obtained for the inhibition of the AChE enzyme activity in the Au/MBT/PANI/AChE/PVAc biosensor after 20 minutes of incubation in 3 different 90% water-organic solvent mixtures. The maximum ATCh substrate concentration was 2.0 mM during the biosensor evaluation.

lowest decrease was observed in acetone. Results on the use of organic solvents in biocatalysis research have shown that enzymes have a high activity in hydrophobic solvents that have a log P value greater than 4. On the other hand, enzyme activity will be low in hydrophilic solvents with a log P value less than 2, where P is the octanol/water partition coefficient of a specific organic solvent. The log P values of the polar solvents used in this study are - 0.33, - 0.23 and - 0.24 for acetonitrile, acetone and ethanol respectively. The results for the log P values of the three solvents shown in Figure 1 are less than 2, therefore it was expected that the AChE activity will not be high since the solvents are hydrophilic. The results obtained thus indicate that the effect of adding 10% water to keep the active centre of the AChE enzyme hydrated during the biosensor studies, improved the enzyme activity. The best results of 10% inhibition of the AChE catalytic activity was obtained for acetone (Iwuoha *et al.* 1997; Konash and Magner, 2006; Somerset, 2010b).

Analysis of the voltammetric results (not shown here) for the inhibition studies on a 90% acetone-aqueous organic solvent mixture, revealed that relatively similar anodic peak data was observed after exposure of the AChE biosensor to the solvent mixture. In the case of the cyclic voltammetry (CV) results, a good decrease in the anodic current at a potential of approximately + 1250 mV (vs. Ag/AgCl) was observed. Further evaluation of the differential pulse voltammetry (DPV) responses of the Au/MBT/PANI/AChE/PVAc biosensor, confirmed the anodic peak results at + 1195.3 mV (vs. Ag/AgCl). Although a slight shift in the peak potential (E_p) occurred, the magnitude of the anodic current was relatively similar (Somerset, 2010b).

3.3 Voltammetric characterisation in a 95% aqueous-organic solvent mixture

The AChE enzyme activity in the Au/MBT/PANI/AChE/PVAc biosensor was then studied in a 90% water-organic solvent mixture, with the results shown in Figure 2.

The results shown in Figure 2 indicate that higher percentage inhibition results were obtained for the evaluation of the AChE enzyme activity in 95% water-organic solvent mixtures. The inhibition effect was the highest for acetonitrile used as solvent, with the decreasing order of inhibition as acetonitrile > acetone > ethanol for the 3 solvent mixtures evaluated. The results further indicate that the decrease of the water content to 5% in the aqueous-organic solvent mixtures, result in a considerable reduced AChE enzyme response for the constructed biosensor. When 5% water-organic solvent mixtures were used, the lowest inhibition observed was 10% for acetone, while for the 10% water-organic solvent mixtures, the lowest inhibition observed were 33% for ethanol. This can also be attributed to the fact that less water was used in the water-organic solvent mixtures under discussion, indicating that the hydration of the enzyme influences the catalytic ability of the enzyme.

In Figure 3 the differential pulse voltammetric (DPV) responses for the inhibiting effect of a 95% ethanol-aqueous mixture on the enzyme AChE activity in the Au/MBT/PANI/AChE/PVAc biosensor is shown. A shorter potential window between + 600 and + 1100 mV is shown in order to highlight the effect of the inhibition observed.

Evaluation of the results shown in Figure 3 clearly shows the inhibiting effect of the 95% ethanol-aqueous organic solvent mixture on the catalytic activity of the enzyme AChE, with the decreasing anodic peak current observed. At a potential of approximately + 847.6 mV (vs. Ag/AgCl) the difference in anodic current was of 2 orders magnitude after exposure to the ethanol-aqueous organic solvent mixture with reduced water content, compared to the results in the previous section (Somerset, 2010b).

Fig. 2. Results obtained for the inhibition of AChE enzyme activity in the Au/MBT/PANI/AChE/PVAc biosensor after 20 minutes of incubation in 3 different 95% water-organic solvent mixtures. The maximum ATCh substrate concentration was 2.0 mM during the biosensor evaluation.

Fig. 3. Results for the DPV responses (before and after) of the Au/MBT/PANI/AChE/PVAc biosensor in a 0.1 M phosphate buffer, KCl (pH 7.2) solution in the presence of a 2 mM ATCh solution.

3.4 Voltammetric characterisation in pure organic solvent

The third investigation focussed on the use of the pure organic solvent, compared to the aqueous-organic solvent mixtures with 5% and 10% water content. Figure 4 shows the individual results obtained for the inhibition of AChE activity in the Au/MBT/PANI/AChE/PVAc biosensor after 20 minutes of incubation in the 3 different pure organic solvents.

Fig. 4. Inhibition results for AChE enzyme activity in the Au/MBT/PANI/AChE/PVAc biosensor after 20 minutes of incubation in the pure organic solvents, with the ATCh substrate concentration at 2.0 mM.

The results obtained for the exposure of the enzyme to pure organic solvents are shown in Figure 4. Analysis of the results has shown that the highest inhibiting effect on the AChE enzyme activity was experienced when the enzyme was exposed to the pure organic solvents. The individual inhibition results obtained were 93% in acetonitrile, 96% in acetone and 77% in ethanol. Comparison of these results to that obtained for the 90% and 95% aqueous-organic solvent mixtures in the previous sections, indicate a definite decrease in AChE enzyme activity in the absence of water. In the case of ethanol as polar organic solvent, it was observed that the best results were obtained in using this solvent, therefore the Au/MBT/PANI/AChE/PVAc biosensor assay of the OP and CM pesticides were further evaluated using this solvent.

A summary of the results obtained for the percentage inhibition investigation of the AChE enzyme activity in different aqueous-polar solvent mixtures, are shown in Table 1.

Polar organic solvent	Log <i>P</i>	% Inhibition of enzyme, AChE, in different solvent mixtures		
		90% aqueous-solvent mixture	95% aqueous-solvent mixture	100% pure solvent
acetonitrile	- 0.33	41	63	93
acetone	- 0.23	10	47	96
ethanol	- 0.24	18	33	77

Table 1. Summary of results obtained for the percentage inhibition investigation of the AChE enzyme activity in the different aqueous-polar solvent mixtures investigated.

The results obtained in Table 1 indicate that aqueous-solvent mixtures of acetone and ethanol with water gave the best results and the smallest degree of inhibition of the AChE biosensor and are in line with the investigations reported by Evtugyn *et al.* (1998) on the presence of water and in the enzyme’s active centre (Somerset, 2010b).

3.5 Inhibition results for standard samples evaluated

Following the study of the AChE activity in the different aqueous-polar organic solvents, inhibition data were collected for each of two organophosphorus and carbamate pesticide standard samples, respectively. The results obtained for the inhibition studies are shown in Table 2.

Biosensor inhibition results					
		Organophosphates		Carbamates	
[pesticide], ppb	- log [pesticide]	% I (chlorpyrifos)	% I (malathion)	% I (carbaryl)	% I (methomyl)
0.60	0.222	43.42	31.96	18.07	36.15
1.00	0.000	51.71	41.55	25.30	44.80
2.00	-0.301	60.00	48.90	36.09	56.20
5.00	-0.699	67.49	57.43	48.16	63.84
7.00	-0.845	73.80	67.50	58.97	72.37
10.00	-1.000	80.61	74.59	67.70	79.59

Table 2. Percentage inhibition results obtained for six different concentrations of two organophosphorus and carbamate pesticide standard concentrations investigated, using the Au/MBT/PANI/AChE/PVAc biosensor.

Analysis of the results show in Table 2, indicate that the highest percentage inhibition (%I) of 81% was obtained for chlorpyrifos at a concentration of 10.0 ppb, while the lowest %I of 18% was obtained for carbaryl at a concentration of 0.6 ppb.

Inhibition plots for the results shown in Table 2 were plotted and are shown in Figure 5. The inhibition plots were obtained using the percentage inhibition method described in section 2.5.

Fig. 5. Graphs of percentage inhibition vs. $-\log [\text{pesticide}]$ results for two different organophosphorous pesticides in (a) and two different carbamate pesticides in (b). Results were obtained with the use of the Au/MBT/PANI/AChE/PVAc biosensor for six different pesticide concentrations evaluated.

Analysis of the results in Figure 5 (a) indicate that in the case of the organophosphorous pesticides, higher percentage inhibition results were obtained for chlorpyrifos compared to malathion over the 6 different concentrations evaluated. For the results in Figure 5 (b) it was observed that carbaryl had higher percentage inhibition results compared to methomyl, in evaluation of the carbamate pesticides. Comparison of the results for the four different pesticide samples investigated, shows that the highest overall percentage inhibition was obtained for the chlorpyrifos OP pesticide evaluated.

The data of the inhibition plots were further analysed in order to obtain results for the sensitivity, detection limits and regression coefficients that are shown in Table 3.

Pesticide	Organophosphorous pesticides		
	Sensitivity (%I/decade)	Detection limit (nM)	Regression coefficient (R ²)
Chlorpyrifos	-26.68	0.028	0.997
Malathion	-35.24	0.189	0.998
Pesticide	Carbamate pesticides		
	Sensitivity (%I/decade)	Detection limit (nM)	Regression coefficient (R ²)
Carbaryl	-21.92	0.880	0.996
Methomyl	-21.04	0.111	0.995

Table 3. Results for the different analytical parameters calculated from the inhibition plot data of the Au/MBT/PANI/AChE/PVAc biosensor detection of standard OP and CM pesticide solutions (n = 2).

The results in Table 3 indicate that the lowest sensitivity was obtained for methomyl as pesticide, with the sensitivity of carbaryl relatively close to that result. A very good

sensitivity was also obtained for chlorpyrifos and this pesticide delivered the lowest detection limit of 0.028 nanoMolar (nM). Comparison of the detection limits for the four OP and CM pesticides evaluated have shown that the detection limit decreases from carbaryl > malathion > methomyl > chlorpyrifos. The application of the Au/MBT/PANI/AChE/PVAc biosensor delivered very good results for analysis of the pesticides in a 90% aqueous-ethanol solvent mixture.

Furthermore, the detection limits of the Au/MBT/PANI/AChE/PVAc biosensor also compare favourably with the detection limits of 1.91×10^{-8} M for paraoxon and 1.24×10^{-9} M for chlorpyrifos ethyl oxon obtained in 5% aqueous-acetonitrile solvent mixture, obtained with the SPCE/PVA-SbQ/AChE biosensor constructed by Dutta *et al.* (2008).

4. Conclusions

The results described in this chapter have successfully demonstrated the construction and use of an Au/MBT/PANI/AChE/PVAc thick-film biosensor for the detection of organophosphorous and carbamate pesticides in various polar organic solvents of acetonitrile, acetone and ethanol. This study has also shown that self-assembled monolayers can be applied in thick film biosensor construction and that the poly(vinyl acetate) film does not interfere with the PANI-AChE electrocatalytic activity towards thiocholine. Furthermore, very good detection limits for the standard OP and CM pesticide standard samples were obtained with the Au/MBT/PANI/AChE/PVAc biosensor. Application of the constructed biosensor to aqueous-polar solvent mixtures of acetone and ethanol with water gave the best results and the smallest degree of inhibition of the AChE enzyme activity. The results also clearly indicated that the presence of water has a considerable effect on the functioning of the AChE-biosensor, highlighting the importance of the presence of water in the enzyme's active centre, particularly for organic phase enzyme electrodes. The results for the detection limit values for the individual organophosphate pesticides were 0.028 nM (chlorpyrifos) and 0.189 nM (malathion). The detection limit values for the individual carbamate pesticides were 0.880 nM (carbaryl) and 0.111 nM (methomyl).

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This book provides recent information on various analytical procedures and techniques, representing strategies for reliability, specificity, selectivity and sensitivity improvements in pesticides analysis. The volume covers three main topics: current trends in sample preparation, selective and sensitive chromatographic detection and determination of pesticide residues in food and environmental samples, and the application of biological (immunoassays-and biosensors-based) methods in pesticides analysis as an alternative to the chromatographic methods for "in situ" and "on line" pesticides quantification. Intended as electronic edition, providing immediate "open access" to its content, the book is easy to follow and will be of interest to professionals involved in pesticides analysis.

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