Analytical Letters, 39: 1683–1698, 2006

ISSN 0003-2719 print/1532-236X online
DOI: 10.1080/00032710600713834

Taylor & Francis
Taylor & Francis Group

Polyaniline-Mercaptobenzothiazole Biosensor for Organophosphate and Carbamate Pesticides

Vernon S. Somerset, Michael J. Klink, Mantoa M. C. Sekota, Priscilla G. L. Baker, and Emmanuel I. Iwuoha
Sensor Research Laboratory, Department of Chemistry, University of the Western Cape, Bellville, Cape Town, South Africa

Abstract: Organophosphate and carbamate pesticides are powerful neurotoxins that impede the activity of cholinesterase enzyme leading to severe health effects. This study reports the development, characterization, and application of acetylcholinesterase (AChE) biosensors based on a gold electrode modified with mercaptobenzothiazole (MBT) self-assembled monolayer and either poly(o-methoxyaniline) (POMA) or poly(2,5-dimethoxyaniline) (PDMA) in the presence of polystyrene sulfonic acid (PSSA). The pesticide biosensors were applied in the aqueous phase detection of diazinon and carbofuran pesticides using Osteryoung square wave voltammetry (SWV) and differential pulse voltammetry (DPV) at low frequencies. The results of the study showed that up to 94% inhibition of the MBT-polyaniline-based biosensors can be achieved in sample solutions containing 1.19 ppb of these neurotoxin pesticide compounds. Both Au/MBT/PDMA-PSSA/AChE and Au/MBT/POMA-PSSA/AChE biosensors exhibited low detection limits, which were calculated using the percentage inhibition methodology. The Au/MBT/POMA-PSSA/AChE biosensor exhibited lower detection limits of 0.07 ppb for diazinon and 0.06 ppb for carbofuran than did the Au/MBT/PDMA-PSSA/AChE sensor system that had detection limits values of 0.14 ppb for diazinon and 0.11 ppb for carbofuran. The average sensitivity of the pesticide biosensor systems is 4.2 μA/ppb. A combination of the high sensor

Received 15 December 2005; accepted 20 February 2006
The authors wish to express their gratitude to the National Research Foundation (NRF), South Africa, for funding and the South African Agricultural Food, Quarantine and Inspection Services (SAAFQIS)—Analytical Services, South Stellenbosch, for the supply of pesticide standards.
Address correspondence to Emmanuel I. Iwuoha, Sensor Research Laboratory, Department of Chemistry, University of the Western Cape, Private Bag X17, Bellville, 7535 Cape Town, South Africa. E-mail: eiwuoha@uwc.ac.za

sensitivity and low detection limits means that it will be possible to deploy the polyaniline-based sensor systems as alarm devices for carbamate and organophosphate pesticides.

Keywords: Polyaniline derivatives, mercaptobenzothiazole, acetylcholinesterase, organophosphate carbamate pesticides, diazinon, carbofuran

1. INTRODUCTION

Pesticides are a form of very toxic compounds that have been shown to be responsible for many ecological and health problems but are still used intensively in agriculture, home care, medicine, and industry. The health concerns associated with pesticides have focused considerable research interest on the development of real-time detection and alarm devices such as pesticide biosensors. A range of biosensing options have been reported (Singh et al. 1999; Vakurov et al. 2004) for the detection of organophosphate and carbamate pesticides many of which are based on the principle of inhibition by cholinesterases enzymes (Pogačnik and Franko 1999; Montesinos et al. 2001; Andreescu et al. 2002). Acetylcholinesterase (AChE), also known as serine esterase, is an enzyme that plays an important role in the cholinergic transmission in mammals and insects by catalyzing the hydrolysis of acetylcholine into choline and acetic acid. The enzyme belongs to the family of hydrolase charaterized by a catalytic triad that is a coordinated structure consisting of three essential amino acids: histidine, serine and aspartic acid (Manetsch et al. 2004; Krasinski et al. 2005). The enzyme catalysis occurs when the triad's anionic binding site attracts the positively charged quaternary ammonium group of acetylcholine. The serine hydroxyl group attacks and cleaves the ester after it has been deprotonated by a neighboring histidine group in the triad (Woster 2001). However, in the presence of an inhibitor such as an organophosphate, the nucleophilic serine hydroxyl group located at the active site covalently binds to the phosphorus atom of the organophosphate. Similar reaction occurs with the carbonyl carbon of carbamates. This blocking of the triad serine inactivates the enzyme (Schulze et al. 2005; Lin et al. 2005).

Biosensors containing acetylcholinesterase have attracted much attention recently due to their application in the detection of anticholinesterase compounds or inhibitors including organophosphate and carbamate pesticides. In the presence of an organophosphate or carbamate pesticide, the normal AChE activity is altered resulting in a decrease the response signal of biosensors. This decrease in the sensor response can be related to the pesticide concentration. Various methods employing different enzyme immobilization techniques leading to the attainment of different detection limits have been reported for the construction of AchE biosensors (Singh et al. 1999; Schulze et al. 2003; Kok et al. 2004). Among these methods is the use of conducting polymers. A conducting polymer that many researchers have

successfully used in sensor development is polyaniline (PANI), which is characterized by ease of preparation, well-behaved electrochemistry, impressive signal amplification, and elimination of electrode fouling when used in biosensor applications. On the other hand, PANI has low solubility in common organic solvents and, thus, an ultimate restricted processability. Improving the solubility of PANI in organic solvents has been the focus of several studies (Palys et al. 2000; Huang et al. 2002; Chen et al. 2003). Among the methods for improving the processability of PANI is the use of sulfonic acid dopants such as *p*-toluene-sulfonic acid (TSA), dodecyl benzene-sulfonic acid (DBSA) and camphor-sulfonic acid (CSA) in PANI preparation.

Another method that is known to improve the solubility of PANI is the polymerization of functionalized aniline, e.g., *2*-methoxyaniline (*o*-anisidine). The functional groups present in the units of PANI polymer chain cause a decrease in the stiffness of the polymer chain resulting in better solubility. For example, aniline substituted with two methoxy groups, *2,5*-dimethoxyaniline (DMA), has been reported to produce a very soluble polymer, poly(*2,5*-dimethoxyaniline) (PDMA), with a conductivity similar to that of PANI. Also in comparison to PANI, PDMA shows redox transitions from leucoemeraldine to its fully oxidized state at much lower potentials, which are 0.70 V vs. Ag/AgCl for PANI and 0.27 V vs. Ag/AgCl for PDMA (Palys et al. 2000; Huang et al. 2002; Chen et al. 2003).

In this study a biosensor for organophosphate and carbamate detection was constructed with gold electrode coated with a mercaptobenzothiazole (MBT) self-assembled monolayer (SAM) and a polyaniline derivative poly(*o*-methoxyaniline, POMA) or poly(*2,5*-dimethoxyaniline, PDMA) polymer film, on which AchE was immobilized. A thiol SAM of mercaptobenzothiazole (MBT) was used since thiol monolayers are known to influence the properties or topological structure of chemically or electrochemically synthesized conducting polymers as shown by Mazur and Krysinski (2001). Electropolymerization was performed in acid solutions containing poly(styrene sulfonic acid) (PSSA) as stabilizer. This was followed by the immobilization of the enzyme AChE in the polymeric composite to form the Au/MBT/POMA-PSSA/AChE or Au/MBT/PDMA-PSSA/AChE biosensors.

2. EXPERIMENTAL

2.1. Materials

Poly(*4*-styrene sulfonic acid) (PSSA), *2,5*-dimethoxyaniline (DMA), potassium dihydrogen phosphate (99+%), and disodium hydrogen phosphate (98+%) were obtained from Aldrich. Fluka supplied the mercaptobenzothiazole (MBT), acetylcholinesterase (AChE, from *Electrophorus electricus*, EC 3.1.1.7) and acetylcholine chloride (99%). Merck's sulfuric acid (95%), hydrochloric acid (32%), and ethanol (absolute −99.9%) were used

in the experiments. All electrochemical measurements were carried out in phosphate buffered saline (PBS) solution (0.1 M phosphate, 0.1 M KCl, pH 7.2). Electrochemical protocols were performed with a BAS-50/W electrochemical analyzer using cyclic voltammetry (CV), Osteryoung square wave voltammetry (SWV) or differential pulse voltammetry (DPV) amperometric modes. A conventional three electrode system was employed: BAS 1.6 mm diameter Au disc working electrode, BAS 3 M NaCl-type Ag/AgCl reference electrode, and a platinum wire auxiliary electrode.

2.2. Preparation of AChE Biosensor

Prior to use, gold disc electrode was first etched for about 5 min in a hot Piranha solution [1:3 (v/v) 30% H_2O_2 and concentrated H_2SO_4]. It was then polished on aqueous slurries of 1, 0.3, and 0.05 micron alumina powder. After thorough rinsing with deionized water followed by acetone, the electrodes were cleaned electrochemically by cycling between -200 and 1500 mV in 0.05 M H_2SO_4 at a scan rate of 10 mV/s for 10 min or until the CV characteristics for a clean Au electrode were obtained. The self-assembled monolayer (SAM) of mercaptobenzothiazole (MBT) was formed by immersing the cleaned Au electrode into an ethanolic solution of 10 mM of MBT for 2 h. The Au/MBT SAM electrode was rinsed with copious amount of ethanol and water and stored in 0.1 M phosphate buffer (pH 7.2) solution for later use. For the in situ deposition of poly(o-methoxyaniline) or poly(2,5-dimethoxyaniline) on the SAM, the gold-MBT electrode was immersed into a freshly prepared 10 cm^3 monomer solution of mixed 0.1 M *2,5*-dimethoxyaniline (DMA) [or 0.1 M *o*-methoxyaniline (OMA)] in aqueous 1 M HCl as well as 2 cm^3 of poly(4-styrene sulfonic acid) (PSSA). The resultant Au/MBT/PDMA-PSSA (or Au/MBT/POMA-PSSA) polymer film was then reduced in 0.1 M phosphate buffer solution, pH 7.2 at -500 mV for 20 min. This was followed by oxidation in the presence of 1 mg mL^{-1} AChE at +650 mV vs. Ag/AgCl for 1500 s, sample interval of 500 ms, and a sensitivity of 1×10^{-6} A V^{-1}. During this oxidation process the enzyme becomes electrostatically attached to the Au/MBT/PDMA-PSSA (or Au/MBT/POMA-PSSA) polymer surface to give an electrode profile represented by Au/MBT/PDMA-PSSA/AChE (Au/MBT/POMA-PSSA/AChE).

2.3. Determination of Organophosphate and Carbamate Pesticides

The Au/MBT/POMA-PSSA/AChE biosensor was placed in a 0.1 M phosphate buffer (0.1 M KCl, pH 7.2) solution to which 20 μL aliquots of 0.01 M acetylcholine (ACh) were added. Voltammetric measurements (CV, SWV, and DPV) were performed after each addition of the acetylcholine

up to a maximum concentration of 1.1 mM. Then a 0.16 ppb acetone solution of either organophosphate or carbamate pesticide was added to the cell solution with voltammetric measurements made after each addition. The decrease in current due to the addition of pesticide solution was recorded.

3. RESULTS AND DISCUSSION

3.1. Characterization of Au/MBT Electrode

A novel approach was used for the preparation of the gold electrode before polymer layers were deposited. The gold electrode surface was first modified with a thiol self-assembling monolayer (SAM) of mercaptobenzothiazole (MBT). The thiol monolayer was meant to control both the polymer deposition and its selectivity on the electrode surface (Mazur and Krysinski 2001). Figure 1 shows the cyclic voltammogram (CV) of a clean Au electrode (I) in comparison with the same electrode coated with MBT monolayer (II). The voltammograms show that there is a change in the sensitivity of the Au electrode surface characterized by a decrease in E_p and current when MBT was coated on the electrode surface. This behavior has been interpreted for similar systems, as being indicative of the formation of a closely packed MBT monolayer on Au surface (Wang et al., 2000). Mercaptobenzothiazole thus provides a molecular template upon which the polymer

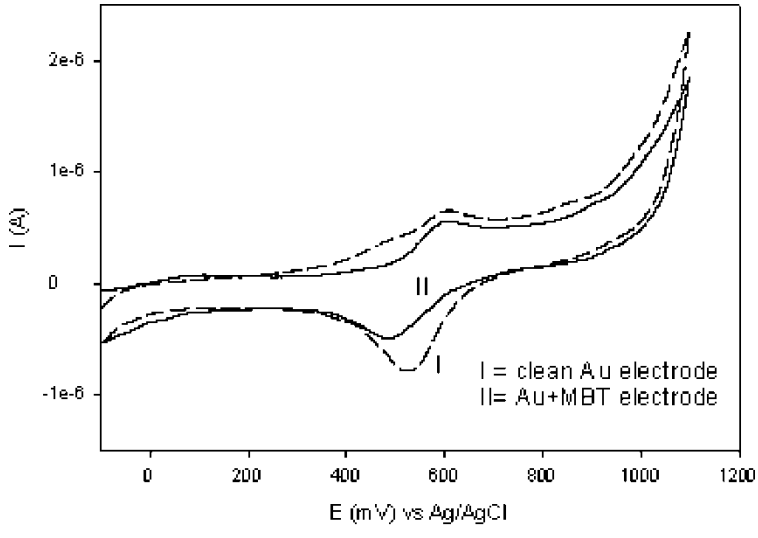

Figure 1. Cyclic voltammograms of (I) clean Au electrode and (II) Au/MBT-self-assembled monolayer in 0.1 M phosphate buffer (pH 7.2), from −100 mV to +1100 mV at a scan rate of 10 mV/s.

synthesis can be performed. The surface concentration of the MBT film on the gold electrode was estimated from a plot of peak current (I_p) against scan rate (ν) in accordance with Brown–Anson analysis (Bard and Faulkner 2001; Mathebe et al. 2004) using Eq. (1):

$$I_p = \frac{n^2 \cdot F^2 \cdot A \cdot \Gamma_{MBT} \cdot \nu}{4 \cdot R \cdot T} \tag{1}$$

where n represents the number of electrons transferred, F is the Faraday constant (96,584 C mol^{-1}), Γ_{MBT} is the surface concentration of the MBT film (mol·cm^{-2}), A is the surface area of the electrode (0.0177 cm^2), ν is the scan rate (V·s^{-1}), R is the gas constant (8.314 J mol K^{-1}), and T is the temperature of the system (298 K). The Γ_{MBT} value was estimated to be 1.228×10^{-8} mol cm^{-2}. This value compares very well with a value of 2.8×10^{-10} mol cm^{-2} calculated by Wan et al. (2002).

3.2. Electropolymerization of Polyaniline Derivatives on the Au/MBT Electrode

3.2.1. Formation of Poly(*2,5*-dimethoxyaniline) (PDMA)

Electrodeposition of poly(*2,5*-dimethoxyaniline) (PDMA) was carried out in a solution containing 0.1 M *2,5*-dimethoxyaniline, 1 M HCl, and 2 cm^3 PSSA.

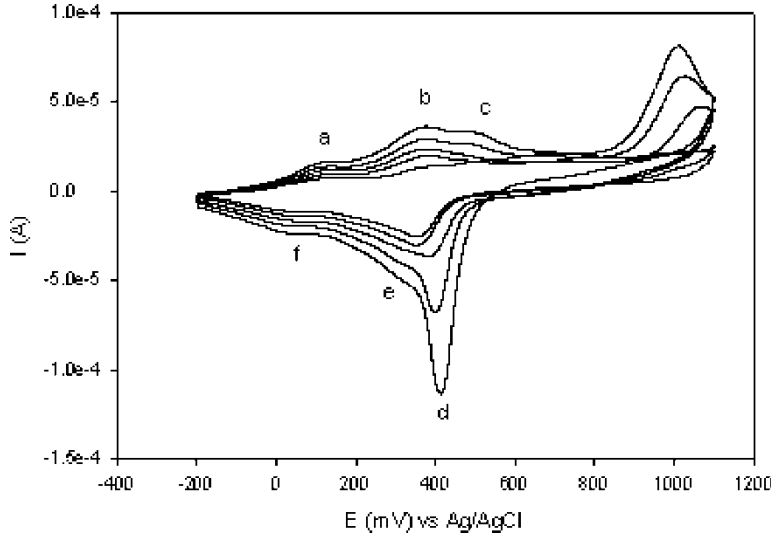

Figure 2. Electrosynthesis of PDMA-PSSA film in 1 M HCl on Au/MBT-SAM electrode at 40 mV/s from −200 to +1100 mV.

The PDMA cyclic voltammograms (CVs) grown at 40 mV/s are shown in Fig. 2. Three sets of redox couples can be seen in Fig. 2, which are a/f, b/e, and c/d. The anodic peaks occur at +115.3 (a), +380.2 (b) and +506.3 mV (c). Cathodic peak were observed at +409.6 mV (d), +304.5 mV (e) and at +27.0 mV (f). The redox couples of PDMA in Fig. 2 can be assigned to a/f and c/d, while redox couple b/e is probably due to impurities such as benzoquinone. Previous studies (Palys et al., 2000; Huang et al. 2002) attribute the redox transitions of PDMA to the transition from the leucoemeraldine to emeraldine base forms, followed by the transition from the emeraldine to the pernigraniline base. Multiscan voltammetry resulted in an increase in the redox peaks which indicates the formation of conducting polymer at the Au/MBT surface. It also provides further evidence that the Au/MBT surface is conductive.

3.2.2 Formation of Poly(*o*-methoxyaniline) (POMA)

Electrodeposition of poly(*o*-methoxyaniline) (POMA) was carried out as described for PDMA except that a potential scan rate of 20 mV/s was used. The CV of POMA electrodeposition (Fig. 3) shows prominent anodic and cathodic peaks at +377 mV (a) and +324.2 mV (b), respectively, for polyaniline transitions. The redox states of POMA are determined by the relationship between the reduced amine and the oxidized imine groups in the polymer backbone. The result is that the polymer can exist in three possible oxidation states, namely, leucoemeraldine, emeraldine, and pernigraniline (Tripathy

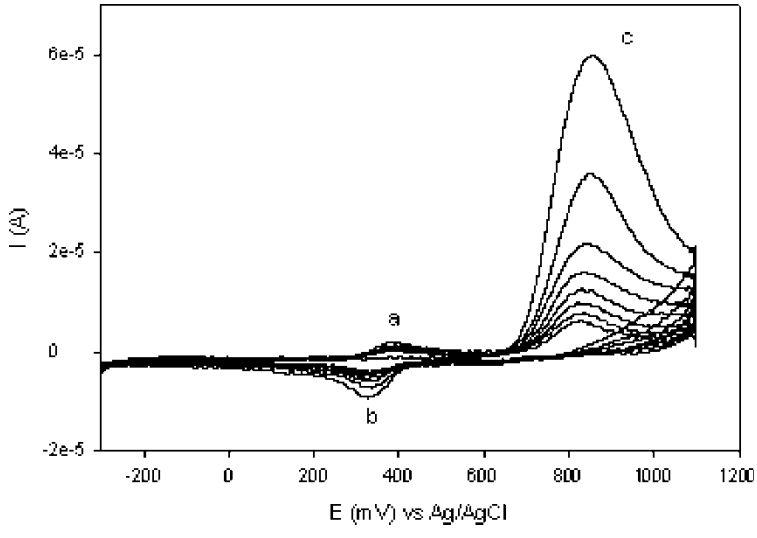

Figure 3. Electrosynthesis of POMA-PSSA film in 1 M HCl on Au/MBT-SAM electrode at 20 mV/s from −500 to +1100 mV.

et al. 2002; Laska 2004). In accordance with previous studies (Palys et al. 2000; Huang et al. 2002) the redox peaks can be attributed to the following transitions: fully reduced leucoemeraldine (b) → emeraldine radical cation (a) → fully oxidized pernigraniline dication. Multi-cycle CV results show an increase in the redox peak currents, indicating the growth of conducting polymer film with every cycle completed. The formal potential for the redox couple (a/b) was calculated to be +350.6 mV.

3.3. Reactivity of Au/MBT/PDMA(or POMA)-PSSA/AChE to Acetylcholine

Figure 4 is a schematic representation of the reactions of the Au/MBT/PDMA-PSSA/AChE biosensor system. The figure shows that acetylcholine hydroxylation produces acetic acid and choline, and the former is reduced to acetaldehyde through an electron exchange process mediated by PDMA and mercaptobenzathiazole. Evidence for the reduction of acetic acid to acetaldehyde was collected by studying the electrochemical behavior of enzyme-free Au/MBT/POMA-PSSA and Au/MBT/PDMA-PSSA polymer films in the presence of acetic acid. It can be seen from Fig. 5(a) that the cyclic voltammetric cathodic peak current increases as the concentration of acetic acid increases. This increase in the reduction current was confirmed with the DPV results shown in Fig. 5(b) Similar results were obtained with enzyme-free Au/MBT/PDMA-PSSA polymer electrode.

3.4. The Response of Au/MBT/POMA-PSSA/AChE to Acetylcholine

The cyclic voltammograms of Au/MBT/POMA-PSSA/AChE in the cell solution containing acetylcholine substrate under anaerobic conditions are

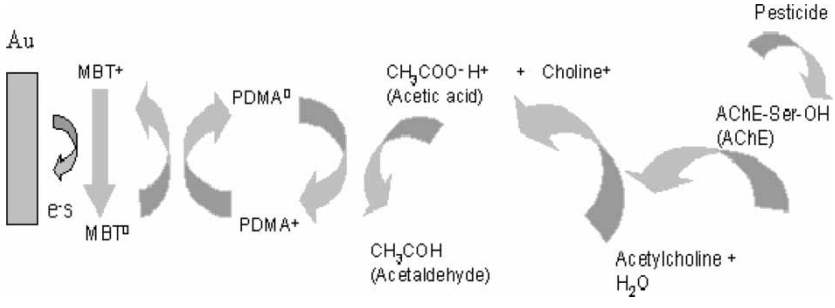

Figure 4. A schematic representation of the Au/MBT/PDMA-PSSA/AChE biosensor reaction occurring at the gold electrode, with the pesticide inhibitor effect also indicated.

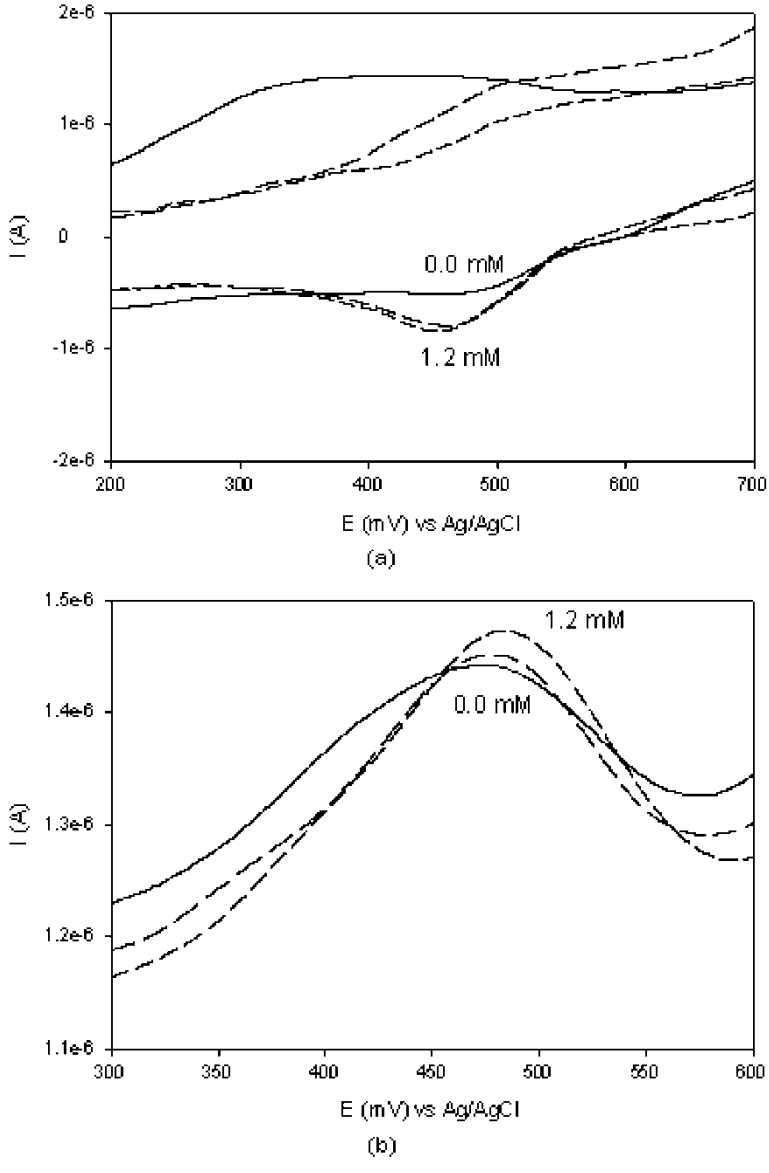

Figure 5. (a) Cyclic voltammogrammetric (10 mV/s) and (b) differential pulse voltammetric (5 Hz) responses of Au/MBT/POMA-PSSA polymer film to acetic acid.

shown in Fig. 6. The figure shows clear anodic response of the biosensor for up to 1.10 mM acetylcholine concentration. In the absence of acetylcholine, the anodic peak ($E_{p,a}$) and the cathodic peak ($E_{p,c}$) potentials were found to be +438.5 and +461.5 mV, respectively, with a formal potential ($E^{\circ\prime}$) of

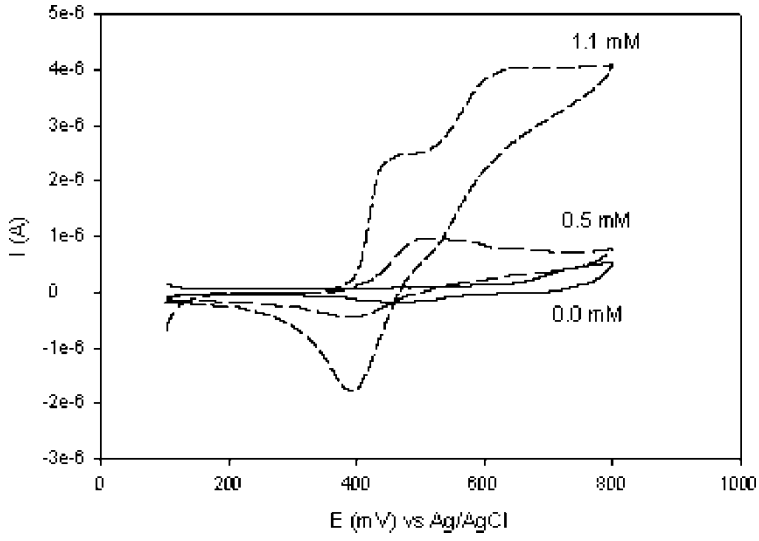

Figure 6. Cyclic voltammetric responses of Au/MBT/POMA-PSSA/AChE biosensor to acetylcholine in 0.1 M phosphate buffer, KCl (pH 7.2), with the potential scanned between +200 to +800 mV at a scan rate of 5 mV/s.

+456.0 mV. At a concentration of 0.5 mM acetylcholine the biosensor had $E_{p,a} = +515.9$ mV, $E_{p,c} = +389.2$ mV, and $E^{\circ\prime} = +452.5$ mV. For a 1.10 mM acetylcholine, it was found that $E_{p,a} = +470.9$ mV, $E_{p,c} = +392.1$ mV, and $E^{\circ\prime} = +431.5$ mV. Similar results were obtained when the PDMA biosensor variant was used.

The cyclic voltammetric responses of the biosensors to acetylcholine were validated with SWV results. The SWV of the Au/MBT/PDMA-PSSA/AChE biosensor measured at 5 Hz are shown in Fig. 7. These SWVs were recorded under anaerobic conditions and the net (difference between forward and reverse waves) responses were plotted. The SWV responses of the biosensors follow the same pattern discussed for cyclic voltammetry. Acetylcholine concentrations of 0.2 mM (a), 0.6 mM (b), and 1.1 mM (c) gave anodic peak potential values of +624.9 mV, +686.6 mV, and +676.3 mV, respectively. These SWV results are similar to the values calculated using cyclic voltammetry.

3.5. Detection of an Organophosphate and Carbamate Pesticides

Figure 8 contains the anodic SWV responses of Au/MBT/POMA-PSSA/AChE biosensor to diazinon pesticide in cell solution containing 1.1 mM acetylcholine alone (a), and 1.1 mM acetylcholine plus: 0.18 ppb (b), 0.51 ppb (c), and 1.19 ppb (d) diazinon. There is an anodic shift in the square wave peak potential associated with the inhibition of the sensor

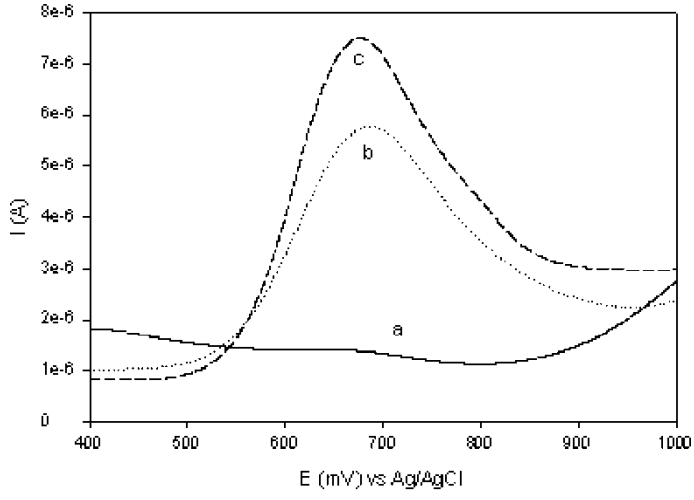

Figure 7. Square wave voltammograms of Au/MBT/PDMA-PSSA/AChE biosensor to acetylcholine in 0.1 M phosphate buffer, KCl (pH 7.2), with the potential scanned between +400 to +1000 mV at a frequency of 5 Hz.

response by diazinon. The shift can be explained as resulting from the blocking of the serine hydroxyl group by the covalently bound phosphate group, which invariably reduces the overall charge of the catalytic active site (Krasinski et al. 2005). Similar results were obtained for the carbamate pesticide, carbofuran

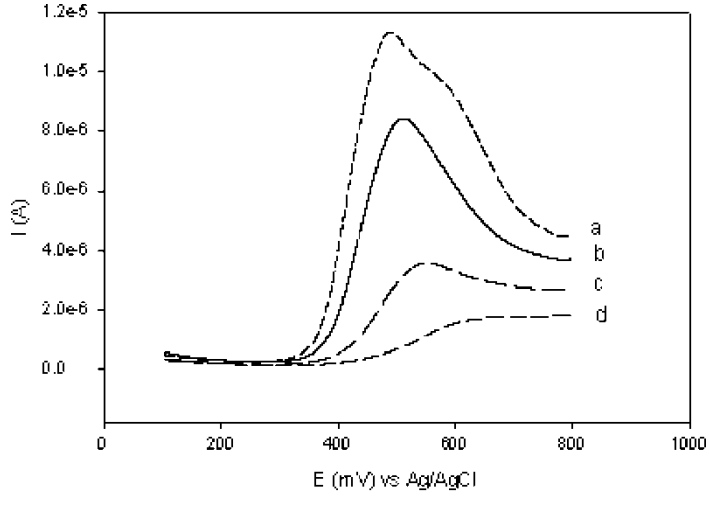

Figure 8. Square wave voltammograms of Au/MBT/POMA-PSSA/AChE biosensor responses to diazinon in 1.10 mM acetylcholine (0.1 M phosphate buffer, KCl, pH 7.2) at a frequency of 5 Hz.

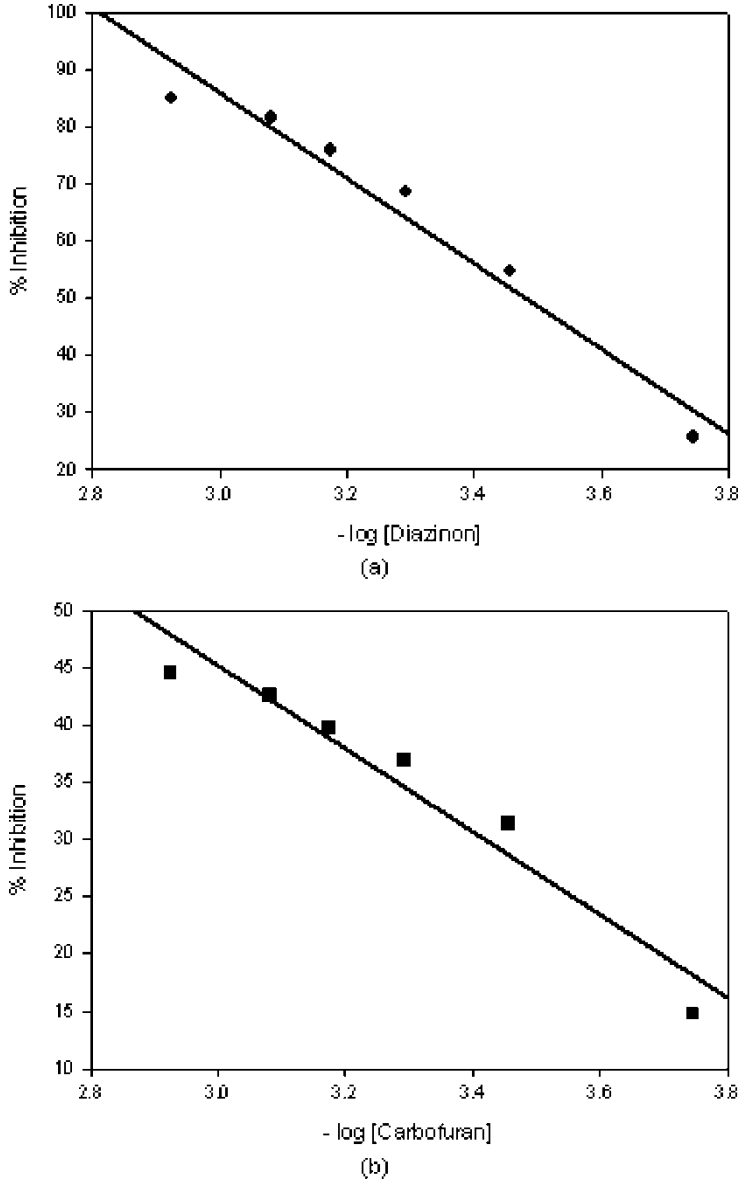

Figure 9. The percentage inhibition plot of (a) diazinon and (b) carbofuran for Au/MBT/POMA-PSSA/AChE biosensor in 1.10 mM acetylcholine (0.1 M phosphate buffer, KCl, pH 7.2).

(results not shown). The percentage inhibition of the biosensor can be calculated with Eq. (2):

$$I\% = \frac{(I_1 - I_2)}{I_1} \times 100, \tag{2}$$

where $I\%$ is the degree of inhibition, I_1 is the response to acetylcholine, and I_2 is the current due to pesticide (Albareda-Sirvent et al. 2001). Analysis of both diazinon and carbofuran data showed that when the enzyme electrode was exposed to diazinon concentration of 1.19 ppb, 85% inhibition of the biosensor occurred while carbofuran of similar concentration gave only about 50% inhibition. Plots of the percentage inhibition of the biosensor as a function of pesticide concentration (as shown in Fig. 9) allowed the determination of the theoretical detection limits of the sensor, which were calculated as 0.07 ppb for diazinon and 0.06 ppb for carbofuran.

The carbofuran responses of Au/MBT/PDMA-PSSA/AChE biosensor in 1.10 mM acetylcholine (0.1 M phosphate buffer, KCl, pH 7.2) was studied by DPV and the results are shown for 0.22–1.19 ppb carbofuran in Fig. 10. For this biosensor, a carbofuran concentration of 1.19 ppb gave a 94% inhibition. The corresponding result for diazinon is 91%. From the percentage inhibition plots shown in Fig. 11, the detection limits of the

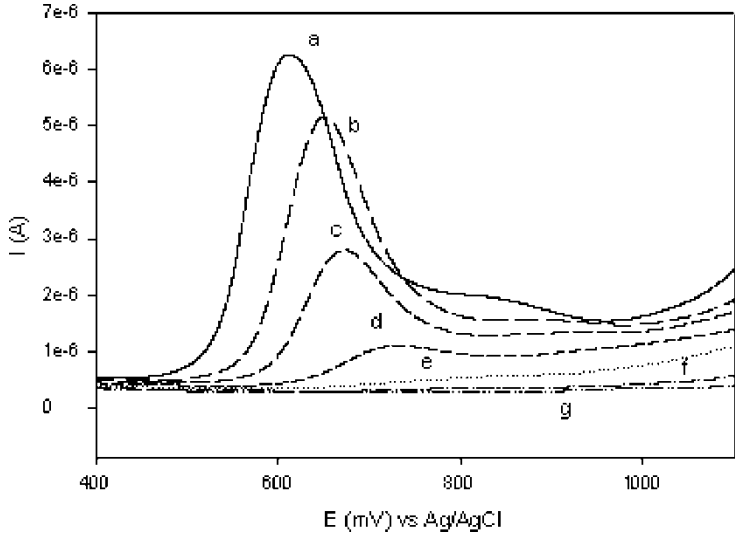

Figure 10. Differential pulse voltammetric responses of Au/MBT/POMA-PSSA/AChE biosensor to carbofuran in (a) 1.10 mM acetylcholine alone; and 1.10 mM acetylcholine plus (b) 0.22 ppb, (c) 0.65 ppb, (d) 1.03 ppb, (e) 1.15 ppb, (f) 1.17 ppb, and (g) 1.19 ppb. Experimental conditions are 0.1 M phosphate buffer, KCl, pH 7.2 at a frequency of 5 Hz.

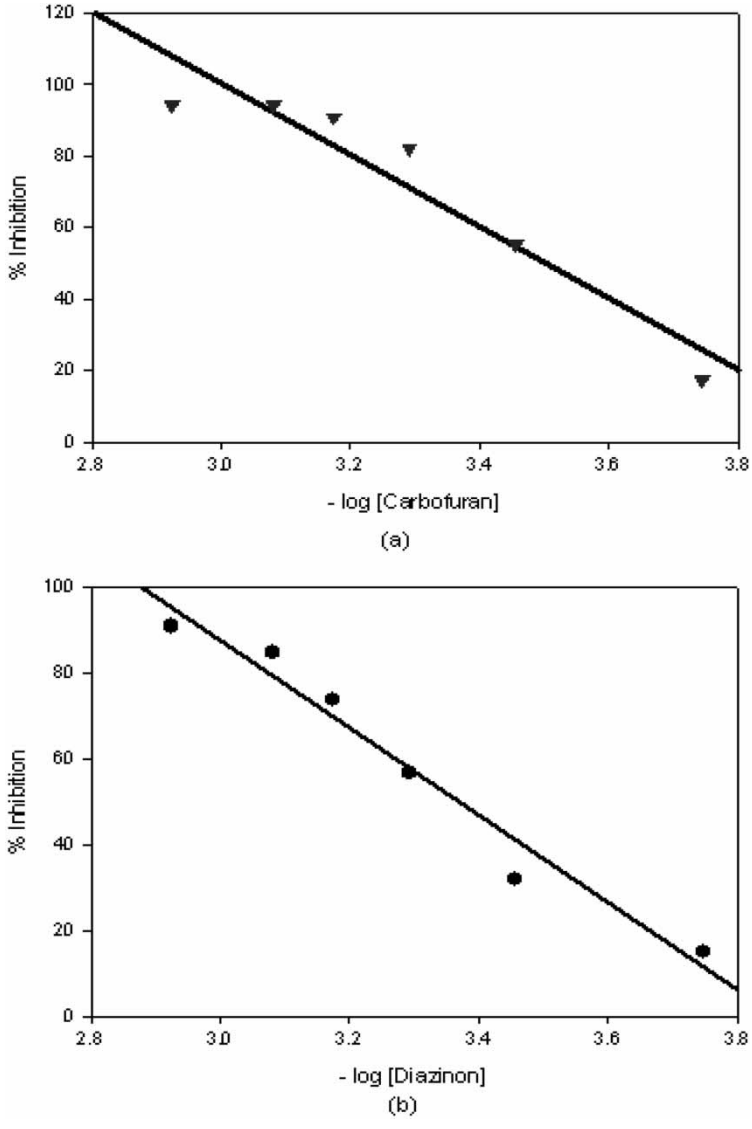

Figure 11. The inhibition plot of percentage inhibition vs.−log (Carbofuran) pesticide concentration in (a) and vs.−log (Diazinon) in (b) as determined with the Au/MBT/PDMA-PSSA/AChE/ACh biosensor in 0.1 M phosphate buffer, KCl (pH 7.2) solution.

Au/MBT/PDMA-PSSA/AChE biosensor are 0.11 ppb for carbofuran and 0.14 ppb for diazinon.

4. CONCLUSIONS

Organophosphates and carbamates are nonpersistent pesticides but they are powerful neurotoxins. The achievement of between 85% and 94% inhibition of the Au/MBT/PDMA-PSSA/AChE and Au/MBT/POMA-PSSA/AChE biosensors, respectively by 1.19 ppb of these neurotoxins attests to their potency. Of importance is the 200 mV shift in redox peak potential of the polyaniline-based biosensor systems in sample solutions containing 1.19 ppb carbofuran. The potential shifts imply that the carbamyl or phosphoryl bonds formed with the active site serine hydroxyl group drastically reduced the charge density of the active site and alters the electrocatalytic behavior of the sensor. Low detection limits were calculated for the pesticide bioelectrodes using the percentage inhibition methodology. Different detection limits values were obtained for POMA-containing (0.07 ppb for diazinon and 0.06 ppb for carbofuran) and PDMA-containing (0.14 ppb for diazinon and 0.11 ppb for carbofuran) biosensors. By having one order of magnitude lower in its detection limit values, the POMA-containing biosensor can be used in cases were the pesticide concentrations are known to be very low. This study suggests that the polyaniline-based sensor system could be deployed as an alarm device for the two pesticides.

REFERENCES

Albareda-Sirvent, M., Merkoçi, A., and Alegret, S. 2001. Pesticide determination in tap water and juice samples using disposable amperometric biosensors made using thick-film technology. *Anal. Chim. Acta*, 442: 35–44.

Andreescu, S., Noguer, T., Magearu, V., and Marty, J.-L. 2002. Screen-printed electrode based on AChE for the detecton of pesticides in presensce of organic solvents. *Talanta*, 57: 169–176.

Bard, A.J. and Faulkner, L.R. 2001. *Electrochemical Methods. Fundamentals and Applications*, 2nd Ed. J. Wiley & Sons, Inc: New York, USA.

Chen, S.-S., Wen, T.-C., and Gopalan, A. 2003. Electrosynthesis and characterization of a conducting copolymer having S–S links. *Synth. Metals*, 132: 133–143.

Huang, L.-M., Wen, T.-C., and Gopalan, A. 2002. In situ UV-visible spectroelectrochemical studies on electrochromic behavior of poly(2,5-dimethoxy aniline). *Synth. Metals*, 130: 155–163.

Kok, F.N. and Hasirci, V. 2004. Determination of binary pesticide mixtures by an acetylcholinesterase–choline oxidase biosensor. *Biosens. Bioelectron.*, 19: 661–665.

Krasinski, A., Radic, Z., Manetsch, R., Raushel, J., Taylor, P., Sharpless, K.B., and Kolb, H.C. 2005. In situ selection of lead compounds by click chemistry: Target-guided optimization of acetylcholinesterase inhibitors. *J. Am. Chem. Soc.*, 127: 6686–6692.

Laska, J. 2004. Conformations of polyaniline in polymer blends. *J. Molecul. Struct.*, 701: 13–18.

Lin, G., Lee, Y.-R., Liu, Y.-C., and Wu, Y.-G. 2005. Ortho effects for inhibition mechanisms of butyrylcholinesterase by *o*-substituted phenyl *N*-butyl carbamates and comparison with acetylcholinesterase, cholesterol esterase, and lipase. *Chem. Res. Toxicol.*, 18: 1124–1131.

Manetsch, R., Krasinski, A., Radic, Z., Raushel, J., Taylor, P., Sharpless, K.B., and Kolb, H.C. 2004. In situ click chemistry: Enzyme inhibitors made to their own specifications. *J. Am. Chem. Soc.*, 126: 12809–12818.

Mathebe, N.G.R., Morrin, A., and Iwuoha, E.I. 2004. Electrochemistry and scanning electron microscopy of polyaniline/peroxidase-based biosensor. *Talanta*, 64: 115–120.

Mazur, M. and Krysiński, P. 2001. Bulk- and surface-initiated chemical in situ polymerisation of 2,5-dimethoxyaniline and 2-methoxyaniline on thiol-coated gold electrodes. *Electrochim. Acta*, 46: 3963–3971.

Montesinos, T., Pérez-Munguia, S., Valdez, F., and Marty, J.-L. 2001. Disposable cholinesterase biosensor for the detection of pesticides in water-miscible organic solvents. *Anal. Chim. Acta*, 431: 231–237.

Palys, B., Kudelski, A., Stankiewicz, A., and Jackowska, K. 2000. Influence of anions on formation and electroacitivity of poly-2,5-dimethoxyaniline. *Synth. Metals*, 108: 111–119.

Pogačnik, L. and Franko, M. 1999. Determination of organophosphate and carbamate pesticides in spiked samples of tap water and fruit juices by a biosensor with photothermal detection. *Biosens. Bioelectron.*, 14: 569–578.

Schulze, H., Vorlova, S., Villatte, F., Bachmann, T.T., and Schmid, R.D. 2003. Design of acetylcholinesterases for biosensor applications. *Biosens. Bioelectron.*, 18: 201–209.

Schulze, H., Muench, S.B., Villatte, F., Schmid, R.D., and Bachmann, T.T. 2005. Insecticide detection through protein engineering of nippostrongylus brasiliensis acetylcholinesterase B. *Anal. Chem.*, 77: 5823–5830.

Singh, A.K., Flounders, A.W., Volponi, J.V., Ashley, C.S., Wally, K., and Schoeniger, J.S. 1999. Development of sensors for direct detection of organophosphates Part I: immobilization, characterization and stabilization of acetylcholinesterase and organophosphate hydrolase on silica supports. *Biosens. Bioelectron.*, 14: 703–713.

Tripathy, S.K., Kumar, J., and Nalwa, H.S. 2002. *Handbook of Polyelectrolytes and Their Applications. Volume 2. Polyelectrolytes, Their Characterization and Polyelectrolyte Solutions*. American Scientific Publishers: Los Angeles, California USA.

Vakurov, A., Simpson, C.E., Daly, C.L., Gibson, T.D., and Millner, P.A. 2004. Acetylecholinesterase-based biosensor electrodes for organophosphate pesticide detection II. Immobilization and stabilization of acetylecholinesterase. *Biosens. Bioelectron.*, (In press).

Wan, Q. and Yang, N. 2002. The direct electrochemistry of folic acid at a 2-mercaptobenzothiazole self-assembled gold electrode. *J. Electroanal. Chem.*, 527: 131–136.

Wang, J., Zeng, B., Fang, C., and Zhou, X. 2000. Electrochemical characteristics of 2-mercaptobenzothiazole self-assembled monolayer on gold. *Anal. Sci.*, 16: 457–461.

Woster, P.M. 2001. *Pharmaceutical Biochemistry*. HarperCollins Publishers Inc.: New York, NY USA.