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Graphene-Polyaniline Biosensor for Carbamate Pesticide Determination in Fruit Samples

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Additional information is available at the end of the chapter

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Abstract

In this study, a simple, sensitive, and low cost electrochemical biosensor for the quantitative determination of carbamate pesticides has been constructed. A composite consisting of polyaniline (PANI) and graphene oxide was electrochemically synthesised on a platinum electrode. This sensor platform was then used in the biosensor construction by electrostatic attachment of the enzyme, horseradish peroxidase (HRP) onto the surface of the Pt/GO-PANI electrode. Voltammetric results concluded that HRP immobilised on the Pt/GO-PANI composite retained its bio-electrocatalytic activity towards the reduction of H_2O_2 and was not changed during its immobilisation. The Pt/GO-PANI/HRP biosensor was then applied to successfully detect standard carbamate pesticides in a 0.1 M phosphate buffer (PB; pH = 6.8) solution. Various performance and stability parameters were evaluated for the Pt/GO-PANI/HRP biosensor, which included the optimal enzyme loading, effect of pH and long-term stability of the biosensor on its amperometric behaviour. The Pt/GO-PANI/HRP biosensor was finally applied to the detection of three carbamate pesticides of carbaryl, carbofuran, and methomyl using the enzyme inhibition method. Carbaryl, carbofuran, and methomyl analyses were amperometrically determined using spiked real samples of orange, pear, and grapes, within a concentration range of 0.01–0.3 mg/L. These results indicated that the biosensor is sensitive enough to detect carbamate pesticides in real fruit matrices. The detection limit for carbaryl, carbofuran, and methomyl in real fruit samples by amperometric method was determined to be 0.136 mg/L, 0.145 mg/L, and 0.203 mg/L, respectively. The application of the Pt/GO-PANI/HRP biosensor has demonstrated that the biosensor is sensitive enough for amperometric detection and could be a useful tool in the screening of these pesticides at low concentrations.

Keywords: Poly(2,5-dimethylaniline), Graphene, Organic phase biosensor, Carbamate pesticides, Horseradish peroxidase

1. Introduction

Pesticides are known to be very toxic compounds that pose a threat to aquatic ecosystems and human health. One such group of pesticides that have been applied in agriculture to control insects are the organophosphates and carbamates. These pesticides are of particular concern for human health since they inhibit the activity and functioning of the enzyme called acetylcholinesterase (AChE) in insects and mammals. When inhibition of AChE occurs, it may lead to respiratory paralysis and consequently death. It is therefore important for food safety and environmental protection to employ fast and effective detection technologies [1-3].

Many standard methods are available for the detection of pesticide compounds in various matrices and include gas chromatography (GC), high performance liquid chromatography (HPLC), liquid chromatography (LC) coupled to sensitive and specific detectors that includes nitrogen-phosphorous detectors (NPD), flame ionisation detectors (FID), ultraviolet detectors (UVD), diode array detector (DAD), or mass spectrometry (MS) [1, 4-5].

However, the preceding methods are known to be expensive, time-consuming, and requiring highly trained personnel to operate this equipment. Furthermore, this equipment is not always suitable for *in situ* and real-time detection of carbamate pesticides [1, 4-5].

Alternative methods that have been utilised for pesticide detection involve the use of electrochemical sensors, especially biosensors constructed with AChE in enzyme-modified electrodes. Biosensors based on the inhibition of AChE by carbamates have been successfully implemented for detection, since they provide advantages such as rapid detection, simplicity, and low cost [1, 6-7].

The development of biosensors involves one most critical step such as immobilisation, whereby the biological recognition element is associated with a physicochemical transducer [8]. Biosensor performance can be negatively affected by the immobilisation process. Therefore, intensive efforts are needed for the development of effective immobilisation methods, allowing for improvements in operational and stability storage, response time, linear range, and sensitivity, while preserving the enzyme affinity for the substrates and inhibitors [9-10].

This study involved the utilisation of graphene, modified with a conducting polymer (e.g., polyaniline) on a suitable transducer surface, for immobilisation of the enzyme during biosensor construction. Graphene is known to consist of a single layer of carbon atoms in a closely packed honeycombed two-dimensional lattice. Graphene has unique electronic, mechanical, and thermal properties that has seen this compound been extensively applied in fields such as batteries, field-effect transistors, ultrasensitive sensors, and electrochemical resonators [11-13].

However, graphene has some limitations that include poor solubility and the synthesis of graphene oxide was proposed to overcome this limitation. Graphene oxide (GO) sheets are known to be hydrophilic and offer the potential of preparing graphene film that is more processable. Secondly, the properties of GO are similar to that of graphene, which include a single atomic plane of graphite structure into which target ions, molecules, and other macromolecules can be adsorbed [11, 13].

Functionalising the GO with a conducting polymer such as polyaniline (PANI) increased the success of the sensor platform devised for this study. It is known that PANI, one of the mostly used conducting polymers in biosensor construction, has some unique properties. Some of these properties include ease of synthesis, high capacitive characteristics, low cost, supportive conducting platform for enzyme entrapment, etc. [9-10, 12]. Therefore, this study has seen the synthesis of a graphene-PANI composite film that was synthesised from graphene oxide as the starting material to tap into both the properties of graphene and PANI, but also to overcome the limitations of each of the conducting films [12].

The primary goal of the present study was therefore the construction and application of the Pt/GO-PANI/HRP biosensor that were constructed to determine the amount of carbamate pesticide compounds such as carbaryl, methomyl, and carbofuran in deciduous and citrus fruit (grapes, pears, and oranges) samples. The enzyme horseradish peroxidase (HRP) replaced the AChE usually employed in carbamate biosensor studies and was found to perform well in the enzyme inhibition studies. The results obtained for the determination of the carbamates using voltammetric (e.g., differential pulse voltammetry) analysis are discussed in this chapter.

2. Materials and methods

2.1. Chemical and reagents

Three carbamate pesticides (e.g., carbaryl, carbofuran, and methomyl) were selected for this work. Stock solutions were prepared from the 1000 mg/L certified reference materials (CRMs; Sigma-Aldrich, South Africa) of carbamate pesticides using acetonitrile organic solvent. Working standard such as 0.01, 0.1, 0.15, 0.2, and 0.3 ppm were also prepared from the stock solution and used for the spiking of real fruit samples. The enzyme horseradish peroxidase (EC 1.11.1.7 type IV from horseradish, 250–330 units/mg) was also purchased from Sigma-Aldrich (Germany). The hydrogen peroxide (30%), sulphuric acid (99 %), potassium permanganate (KMnO_4), potassium chloride (KCl), and sodium nitrate (NaNO_3) (84.99%) were all purchased from Merck, South Africa. The aniline (99.5 %), graphite (fine powder synthetic), and the following organic solvents such as *n*-hexane (96% HPLC grade), acetonitrile (HPLC grade), methanol (HPLC grade), and iso-octane (95%) were bought from Sigma-Aldrich (South Africa). All solutions were prepared with Millipore deionised water and experiments were performed at room temperature.

2.2. Apparatus

2.2.1. Voltammetric measurements

Electrochemical measurements were conducted in a three electrode electrochemical cell under controlled temperature (25°C). The working electrode was Pt (diameter = 1.6 mm). The reference electrode was comprised of an Ag/AgCl in 3 M NaCl system, and a Pt wire was used

as the auxiliary electrode. All the electrochemical measurements were performed with an Epsilon electrochemical analyser (BASi Instruments, 2701 Kent Ave, West Lafayette, IN 47906, USA), utilising cyclic voltammetry (CV) and differential pulse voltammetry (DPV) modes. Electrocatalytic responses of the Pt/GO-PANI/HRP biosensor to H_2O_2 substrate were investigated by amperometric mode in the presence and absence of carbaryl, carbamate, and methomyl standards in the test solutions [14-15].

2.3. Preparation of graphene oxide and polyaniline mixture

Approximately 2.01 g of graphite powder, 1.03 g of NaNO_3 , and 4.02 g of KMnO_4 were weighed and dissolved in 100 mL of concentrated sulphuric acid (H_2SO_4) solution and the resulting mixture was stirred vigorously for 7 hours at room temperature. Subsequently, 250 mL of 5% H_2SO_4 aqueous solution was added and the solution was kept at 98°C for 2 hours. Temperature was reduced to room temperature and the 10 mL of 30% H_2O_2 was slowly added and the reaction was further stirred for 2 hours. A light brown graphene oxide (GO) precipitate was obtained by washing it with 0.1 M H_2SO_4 solution and then distilled water until the pH of the supernatant was neutral [16].

The precipitate obtained was transferred to a glass vial to prepare the 1 g GO precipitate for mixing with the aniline monomer, before electrosynthesis of the graphene oxide-aniline mixture was performed. A 10 mL aqueous solution of 0.5 M aniline in 1 M H_2SO_4 solution was prepared separately and then transferred to the glass vial containing the GO precipitate. The mixture was allowed to mix for approximately 5–10 minutes using a sonicator bath. Electrosynthesis of the graphene oxide-polyaniline (GO-PANI) film was obtained by scanning the potential repeatedly between -0.2 V and $+1.1\text{ V}$ (vs. Ag/AgCl) for 10 cycles at a scan rate of 40 mV/s . The obtained GO-PANI polymer film was then characterised using CV, DPV measurements, including UV-Vis and FTIR spectrometry (results not reported here). The modified constructed electrode was referred to as Pt/GO-PANI and stored in 0.1 M phosphate buffer (pH 7.2) solution at 4°C .

2.4. HRP biosensor construction

The Pt/GO-PANI electrode was stored in phosphate buffer (PB) solution at 4°C , when not in use. This was followed by enzyme incorporation to obtain the horseradish peroxidase (HRP) biosensor. The biosensor was constructed, using the prepared Pt/GO-PANI electrode that was transferred to a batch cell, containing a 1 mL solution of argon degassed 0.1 M phosphate buffer (pH 6.8). The GO-PANI film was next reduced at a potential of -500 mV (vs. Ag/AgCl) until a steady current was achieved (approximately 30 minutes). Afterwards, the electrode was transferred to a second batch cell containing 50 mL of 2 mg/L of HRP in a 0.1 M phosphate buffer (pH 6.8) solution. This solution was also argon degassed, before enzyme immobilisation through covalent binding to the GO-PANI film was performed. This was achieved by oxidation of the PANI film in the presence of HRP at a potential of $+700\text{ mV}$ (vs. Ag/AgCl) until a steady current was achieved (approximately 40 minutes) [10]. The achievement of a steady state current was an indication that bulk electrolysis was complete and the polymer film was charged and ready for enzyme attachment.

Following enzyme attachment, the constructed Pt/GO-PANI/HRP biosensor was thoroughly rinsed with double-distilled water, in order to remove any unbound enzyme followed by storage in the working 0.1 M phosphate buffer (pH 6.8) solution at 4°C.

2.5. Stability and reproducibility studies

The Pt/GO-PANI/HRP biosensor was further evaluated at different 7 day intervals, using the same specific constructed biosensor. A test solution consisting 1 ml of 0.1 M phosphate buffer, 0.1 M KCl (pH = 6.8) solution was degassed before any H₂O₂ as substrate was added. The Pt/GO-PANI/HRP biosensor was then evaluated using a 1 ml test solution to which small aliquots of H₂O₂ as substrate was added and peak current collected. This procedure was repeated for one month on 7 day intervals, on the 7th, 14th, 21st, and 28th day after the specific sensor was constructed. The peak current collected were then compared to determine the stability of the biosensor constructed [9, 17].

The reproducibility of the Pt/GO-PANI/HRP biosensor was also evaluated by the construction of 5 HRP biosensors, for which the sensing of H₂O₂ as substrate was evaluated.

3. Electrochemical detection

3.1. Determination of the limit of detection

The limit of detection of the Pt/GO-PANI/HRP biosensor was evaluated by performing 10 replicate measurements in a 0.1 M phosphate buffer, 0.1 M KCl (pH = 6.8) solution, or on any one of the analyte (standard pesticide) solutions at the lowest working concentration. This was followed by the construction of a calibration graph of current (*I_p*) versus saline phosphate buffer (PBS) or analyte concentration for which the slope and the linear range was then determined. The limit of detection (LOD) was then calculated with the following equation:

$$LOD = \frac{3 \cdot s}{m}$$

In which *s* is the standard deviation of the 10 replicate measurements in PBS, or on any one of the analyte (standard pesticide) solutions at the lowest working concentration? The variable *m* represents the slope of the calibration graph in the linear range, which was also used to estimate the sensitivity of the measurements performed [9, 18].

3.2. Amperometric detection of carbamate pesticides

The detection of the carbamate pesticides (e.g., carbaryl, carbofuran, and methomyl) was performed using the percentage inhibition method. During this procedure, the Pt/GO-PANI/HRP biosensor was placed in a stirred 1 mL of 0.1 M phosphate (0.1 M KCl, pH 6.8) solution (anaerobic conditions), followed by multiple additions of a standard hydrogen

peroxide (H_2O_2) substrate solution, until no relative change in current response was obtained. No increase in peak current during the catalytic monitoring of the HRP biosensor to substrate addition indicated that substrate saturation was reached.

The biosensor was thereafter rinsed with double-distilled water and incubated in a carbaryl solution of specific concentration and the HRP enzyme was exposed for 20 minutes to the chosen pesticide concentration in a 0.1 M phosphate (0.1 M KCl, pH 6.8) solution. After pesticide exposure, the HRP biosensor was thoroughly rinsed with deionised water and placed into a freshly stirred 1 mL of 0.1 M phosphate (0.1 M KCl, pH 6.8) solution.

Thereafter, the Pt/GO-PANI/HRP biosensor was again exposed to sequential addition of H_2O_2 substrate solution in a 0.1 M phosphate (0.1 M KCl, pH 6.8) solution. This represented the biosensor current response after enzyme inhibition was performed, which was evident in the reduced biosensor current responses obtained.

This procedure was repeated for several different concentrations of carbaryl (followed by carbofuran and methomyl), to obtain a standard calibration curve. The percentage inhibition ($I\%$) for each concentration of carbaryl inhibition of the enzyme was determined using the formula in Eqn 1:

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

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 in buffer solution after the biosensor was exposed to carbaryl standard solution [10].

The anodic difference differential pulse voltammogram (DPV) responses were collected in an oxidation direction also, by applying a linear potential scan between -0.40 V and -1.0 V (vs. Ag/AgCl). The DPV responses were performed at a scan rate of 10 mV/s and a pulse amplitude of 0.50 V. The sample width, pulse width, and pulse period were 17 ms, 50 ms, and 200 ms, respectively [9, 18].

4. Results and discussion

4.1. Optimisation of solution pH for Pt/GO-PANI/HRP biosensor

After successful construction of the Pt/GO-PANI/HRP biosensor, evaluation of the biosensor was performed in the pH range from 5.0 to 7.2, to evaluate and determine the optimum current response for the constructed biosensor. A fresh biosensor was constructed and evaluated at each of the pH values evaluated from 5.0 to 7.2. The results obtained are displayed in Figure 1.

The optimum pH of the Pt/GO-PANI/HRP biosensor was determined as follows. A series of 0.1 M phosphate buffer, 0.1 M KCl solutions were prepared at different pH values of 5.0, 5.5,

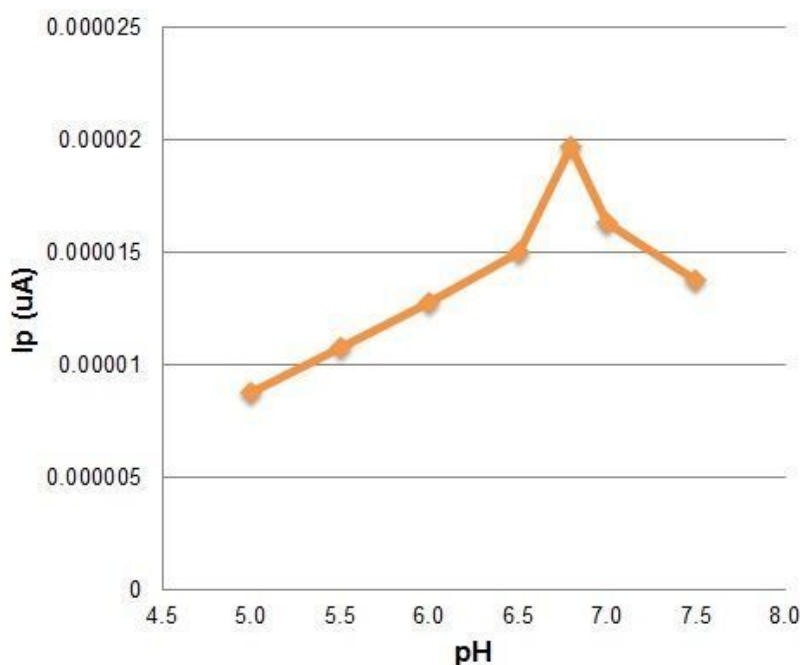


Figure 1. Results obtained for the optimisation of the pH of the Pt/GO-PANI/HRP biosensor evaluated in 0.1 M phosphate buffer, 0.1 M KCl solution with the addition of H_2O_2 as substrate.

6.0, 6.5, 6.8, 7.0, and 7.5. An aliquot of 1 ml test solution containing 0.1 M phosphate buffer, 0.1 M KCl solution was degassed with argon before any substrate was added [9].

The Pt/GO-PANI/HRP biosensor was then evaluated in the 1 ml test solution by sequential addition of the $1 \mu\text{M}$ H_2O_2 substrate to the test solution. After degassing, the maximum current response of the biosensor was then obtained at the different pH values after a total of 0.8–1.2 μM of the H_2O_2 substrate was added. In Figure 1, the results obtained have shown that the optimum peak current response was obtained at pH = 6.8. This pH was then used in all subsequent Pt/GO-PANI/HRP biosensor investigations.

4.2. Voltammetric characterisation of Pt/GO-PANI/HRP biosensor

The differential pulse voltammetric (DPV) responses of the Pt/GO-PANI/HRP biosensor for the analysis of standard carbamates pesticide samples, incubated in acetonitrile-saline phosphate buffer (pH = 6.8; 0.1 M KCl) solution, were recorded for each of the three different pesticides investigated.

In Figure 2 the optimum DPV responses for the Pt/GO-PANI/HRP biosensor to sequential hydrogen peroxide (H_2O_2) substrate addition up to 1.2 μM is shown. The results shown are for the substrate addition (before pesticide exposure) and the respective responses obtained after incubation in different carbaryl pesticide concentrations.

Figure 2 shows a decrease in the maximum cathodic current obtained after incubation of the biosensor in a 0.01 mg/L carbaryl standard solution, when the Pt/GO-PANI/HRP biosensor was subjected to successive additions of H_2O_2 as substrate. A similar decrease was shown when

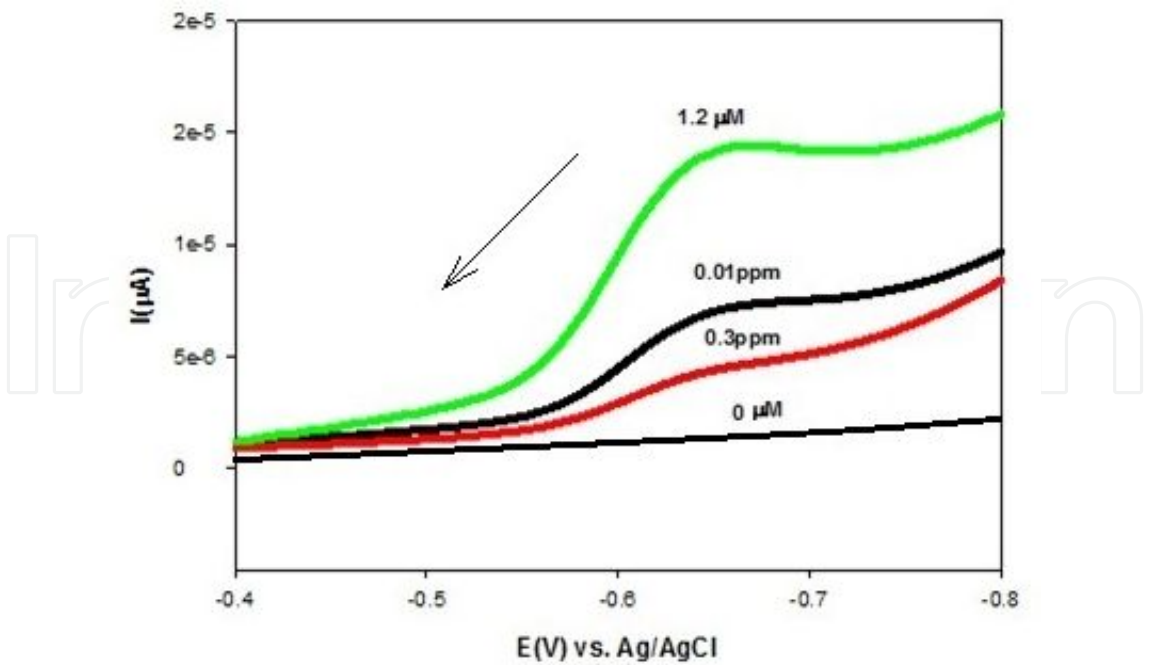


Figure 2. Results for the Pt/GO-PANI/HRP biosensor responses to an optimum 1.2 μM H_2O_2 substrate addition before incubation, and responses obtained after incubation in different carbaryl pesticide concentrations of 0.01 ppm ($n = 3$; RSD = 4.67%) and 0.3 ppm ($n = 3$; RSD = 6.92%), respectively.

the Pt/GO-PANI/HRP biosensor was incubated in a 0.3 mg/L carbaryl standard solution. It was observed that the maximum cathodic current obtained decreased as the carbaryl pesticide concentration was increased.

The results obtained for the stability of the Pt/GO-PANI/HRP biosensor have shown that a gradual and steady decrease in peak current (not shown here) was observed over time. The decrease in peak current was more apparent after 28 days. The relative standard deviations (RSD) were found to be 10.3%.

Similarly, the results obtained for the reproducibility of the Pt/GO-PANI/HRP biosensor have shown that the HRP enzyme immobilised in the GO-PANI matrix was relatively stable. The five sensors evaluated have shown a relative standard deviation (RSD) of 6.5% for the evaluation of H_2O_2 as substrate.

Figure 3 shows the calibration curves of peak current versus substrate concentration for the successive addition of H_2O_2 as substrate in the absence of carbaryl pesticide, followed by measured responses after exposure to 0.01 and 0.3 mg/L carbaryl pesticide solutions.

The results obtained for the responses of the Pt/GO-PANI/HRP biosensor to carbaryl pesticide inhibition have shown clear trends. It was firstly observed that the highest peak current responses were obtained in the absence of pesticide exposure (Figure 3). This was followed by a decrease in peak current responses observed as the carbaryl pesticide concentration was increased from 0.01 to 0.3 mg/L, with the lowest peak current responses observed for the highest pesticide concentration of 0.3 mg/L evaluated in Figure 3.

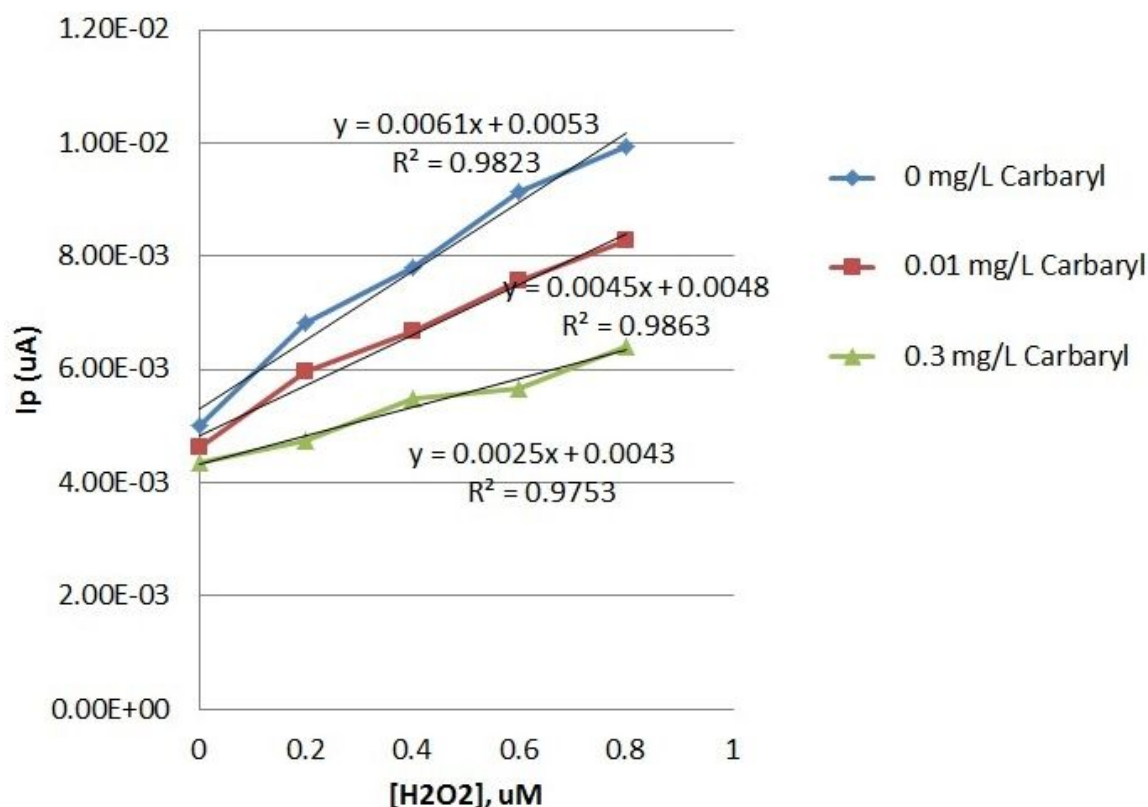


Figure 3. Results for the combined calibration plots of the Pt/GO-PANI/HRP biosensor responses to H₂O₂ substrate addition in the absence of carbaryl, followed by the addition of different carbaryl concentrations of 0.01 mg/L ($n = 3$; RSD = 10.4%) and 0.3 mg/L ($n = 3$; RSD = 17.3%), respectively.

The responses of the Pt/GO-PANI/HRP biosensor to 1.2 μ M of the substrate H₂O₂ added before incubation, followed by the respective responses obtained after incubation in different carbofuran pesticide concentrations, are shown in Figure 4.

The first result indicated that a shift in cathodic peak potential was obtained after 1.2 μ M of the H₂O₂ substrate was added to the Pt/GO-PANI/HRP biosensor. Carbofuran pesticide standard solutions had a similar behaviour as for carbaryl as pesticide when the HRP biosensor was incubated and evaluated. However, the results obtained have shown that the cathodic peak potentials were obtained at different potentials after substrate addition to the biosensor, compared to the carbaryl investigation.

The results in Figure 4 further showed that not only was a peak potential shift observed for carbofuran pesticide evaluation, but the carbofuran behaviour in the Pt/GO-PANI/HRP biosensor further showed a smaller decrease in cathodic peak current obtained in comparison to the results obtained for carbaryl.

The combined Lineweaver-Burk plots for the Pt/GO-PANI/HRP biosensor responses to 1.2 μ M of the substrate H₂O₂ added before incubation, followed by the respective responses obtained after incubation in different carbofuran pesticide concentrations are shown in Figure 5.

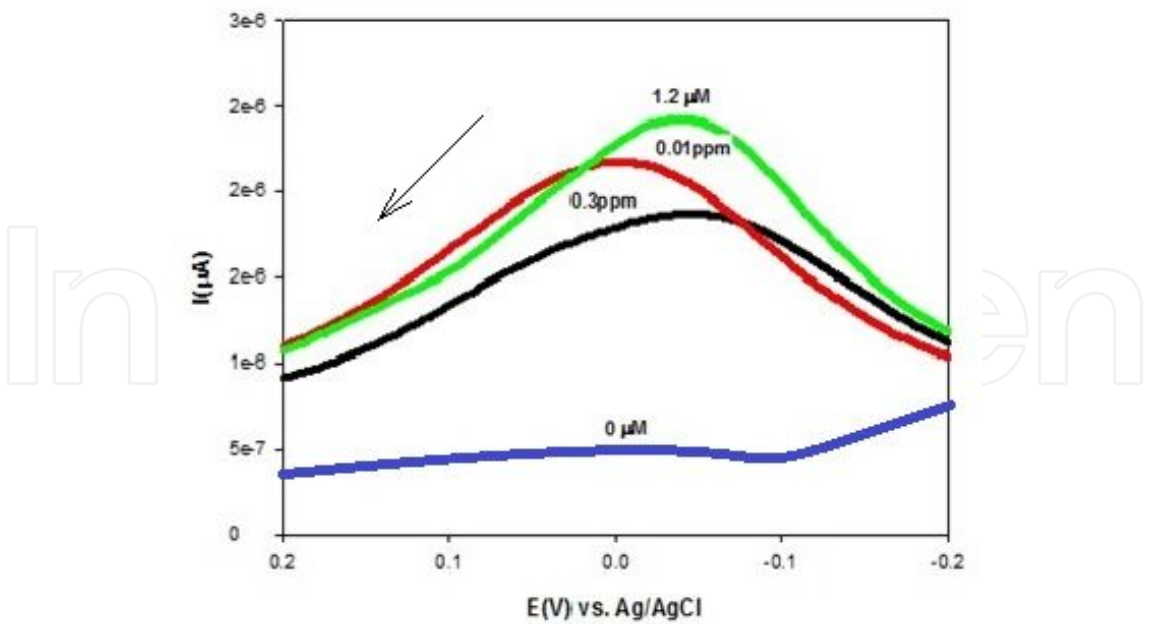


Figure 4. Results for the Pt/GO-PANI/HRP biosensor responses to an optimum 1.2 μM H_2O_2 substrate addition before incubation, and responses obtained after incubation in different carbofuran pesticide concentrations of 0.01 ppm ($n=3$; RSD = 8.35%) and 0.3 ppm ($n=3$; RSD = 10.4%), respectively.

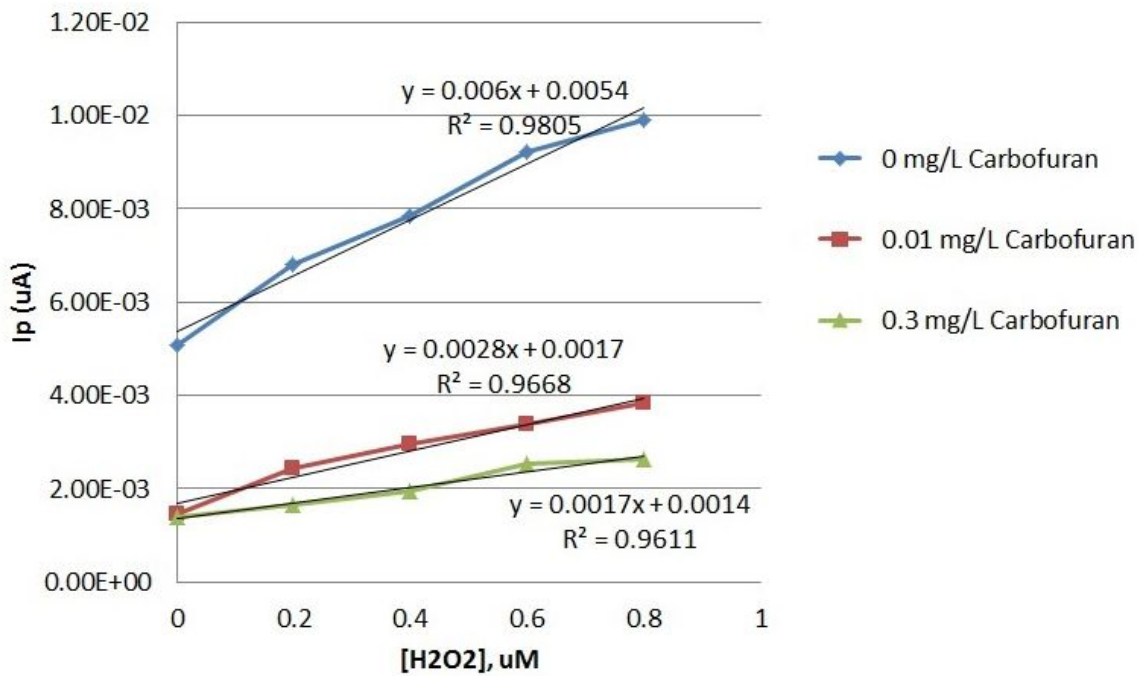


Figure 5. Results for the combined calibration plots of the Pt/GO-PANI/HRP biosensor responses to H_2O_2 substrate addition in the absence of carbofuran, followed by the addition of different carbofuran concentrations of 0.01 mg/L ($n=3$; RSD = 31.5%) and 0.3 mg/L ($n=3$; RSD = 38.9%), respectively.

The results in Figure 5 show the calibration curves of current versus substrate concentration for the successive addition of H_2O_2 , clearly showing a decrease in the cathodic current after incubation of the biosensor in a 0.01 mg/L and 0.3 mg/L carbofuran standard solutions, respectively.

The Pt/GO-PANI/HRP biosensor results for the 0.01 mg/L and 0.3 mg/L carbofuran standard solutions showed that a relatively small difference in the peak current responses of the biosensor was observed. This was a very important observation made, especially since the difference in pesticide concentration evaluated was rather tenfold bigger.

Next, the Pt/GO-PANI/HRP biosensor was evaluated against methomyl as pesticide (Figure 6). The results obtained for methomyl also showed the same behaviour as observed for carbaryl and carbofuran pesticides standard solutions. Figure 6 represents the DPV responses for the Pt/GO-PANI/HRP biosensor to 1.0 μM substrate (H_2O_2) before incubation and the respective responses obtained after incubation in 0.01 mg/L and 0.3 mg/L methomyl pesticide concentrations.

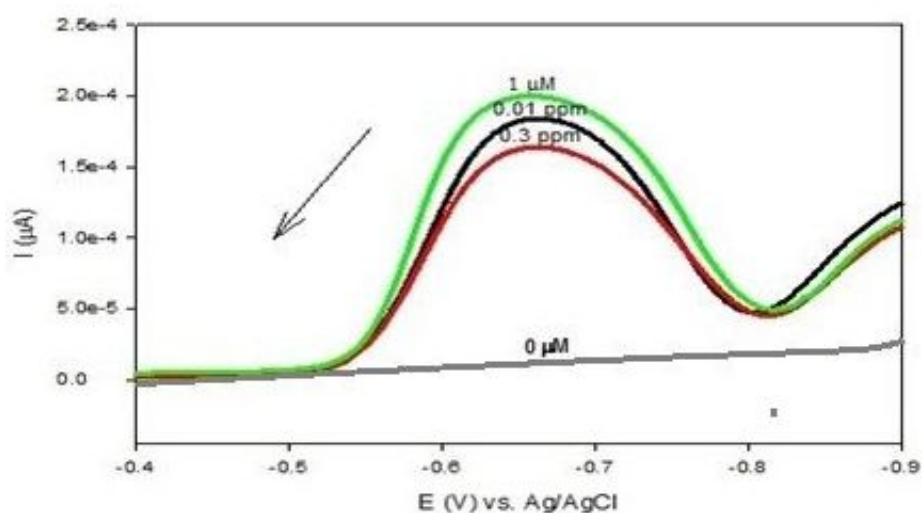


Figure 6. Results for the Pt/GO-PANI/HRP biosensor responses to an optimum 1.0 μM H_2O_2 substrate addition before incubation, and responses obtained after incubation in different methomyl pesticide concentrations of 0.01 ppm ($n = 3$; RSD = 3.86%) and 0.3 ppm ($n = 3$; RSD = 9.25%), respectively.

Analysis of the results in Figure 6 has shown a relatively small decrease in the cathodic current after incubation of the biosensor in a 0.01 mg/L methomyl standard solution, compared to the peak current results obtained for the biosensor response in H_2O_2 substrate solution.

Figure 7 confirmed the results obtained in Figure 6. The Pt/GO-PANI/HRP biosensor evaluation has confirmed the methomyl pesticide results to be similar to that of carbaryl and carbofuran, indicating that the lowest peak current responses were observed for the highest pesticide concentration evaluated in Figure 7.

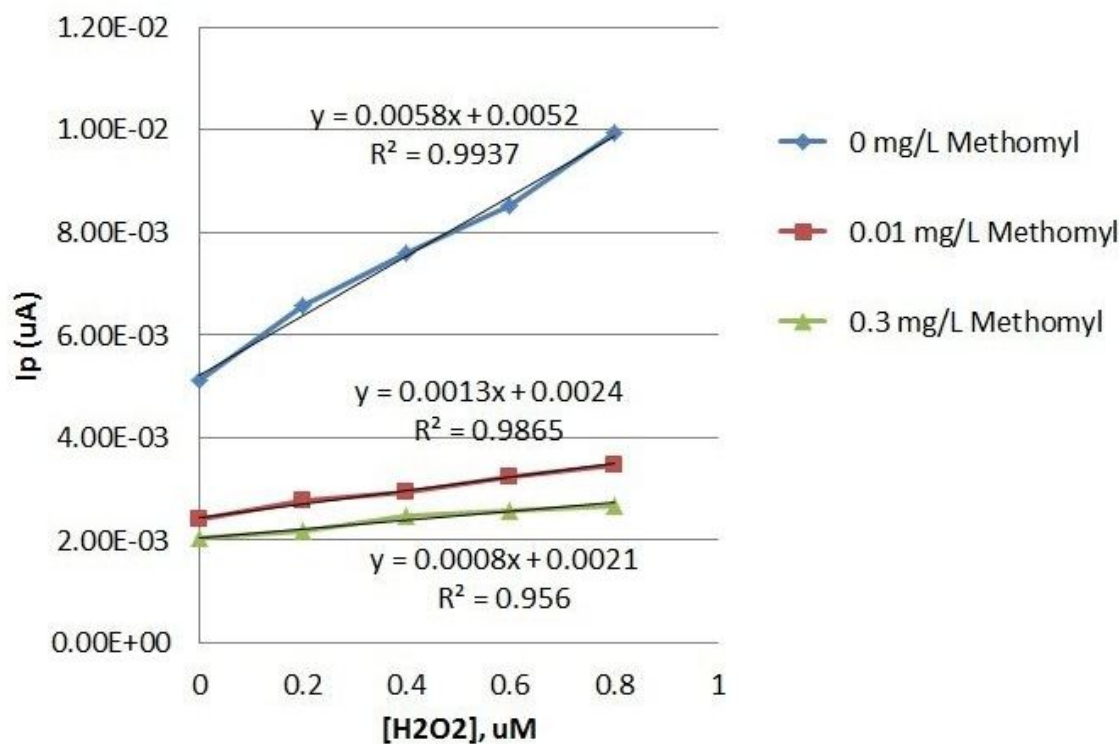


Figure 7. Results for the combined calibration plots of the Pt/GO-PANI/HRP biosensor responses to H₂O₂ substrate addition in the absence of methomyl, followed by the addition of different methomyl concentrations of 0.01 mg/L (*n* = 3; RSD = 32.5%) and 0.3 mg/L (*n* = 3; RSD = 39.8%), respectively.

4.3. Inhibition studies of standard carbamate pesticide samples

The Pt/GO-PANI/HRP biosensor was further evaluated using the percentage inhibition method described in Section 2.5.

Analyses of the results in Figure 8 have shown that the HRP biosensor obtained different results for each of the carbamate pesticides evaluated. A steady increase in the percentage inhibition was observed as the individual concentrations for each of the respective carbamate pesticides was increased (see each individual graph). The highest percentage inhibition was observed for carbaryl (58%), followed by methomyl (52%), and the least for carbofuran (35%) [9, 18].

Analysis of the percentage inhibition results in Figure 8 have also shown that for carbaryl, the highest percentage inhibitions were obtained ranging from 39% to 58% over pesticide concentrations ranging from 0.01 to 0.3 ppm (or mg/L). In the case of methomyl, the percentage inhibitions ranged between 28% to 52%, while in the case of carbofuran it ranged between 16% to 35% over the same concentration range.

The Pt/GO-PANI/HRP biosensor evaluation has further shown that although HRP can replace acetylcholinesterase (AChE) as enzyme in the evaluation of carbamate pesticides, reduced percentage results were obtained for HRP as enzyme [9, 18].

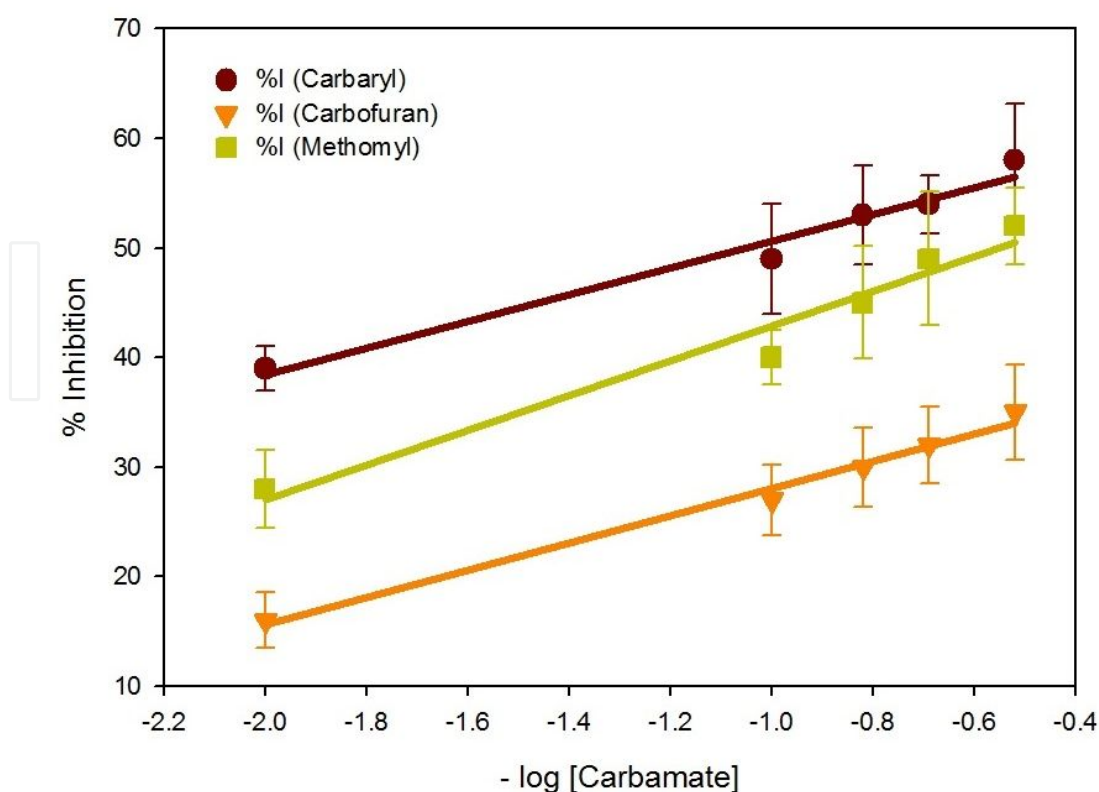


Figure 8. Graphs for the percentage inhibition vs. log [carbamate pesticide] for all three different carbamate pesticides investigated are shown. Inhibition results were collected with the application of the Pt/PDMA-PVS/HRP biosensor.

4.4. Carbaryl, carbofuran, and methomyl pesticide analysis in real samples

The applicability of the HRP biosensor for carbamate pesticide evaluation was further demonstrated by evaluating three different fruit samples such as pear, grapes, and oranges for the determination of carbaryl, carbamate, and methomyl concentrations in spiked samples.

In this work, the method applied for the sample preparation was a liquid–liquid extraction of the pear, grape, and orange samples. The method of standard addition was used to determine the concentrations of carbaryl, carbofuran, and methomyl in these fruit samples.

In this regard, the Pt/GO-PANI/HRP biosensor was immersed in an electrochemical cell containing a fresh 1 ml of 0.1 M phosphate buffer (0.1 M KCl; pH = 6.8) solution and a 1000 μ L aliquot of fruit sample was added to the PBS solution, after which the amperometric responses of the biosensor was measured.

The same procedure was then used to evaluate the fruit samples spiked with carbaryl, carbofuran, and methomyl standard solutions, respectively. A known concentration of analyte was added to the extracted fruit sample solution in order to account for any impurities in the calibration of extracted samples. The concentrations of carbaryl, carbofuran, and methomyl in the samples were then determined by extrapolation.

Figure 9 shows the calibration curves of peak current vs. carbamate concentration for the determination of carbaryl, carbofuran, and methomyl in real fruit samples.

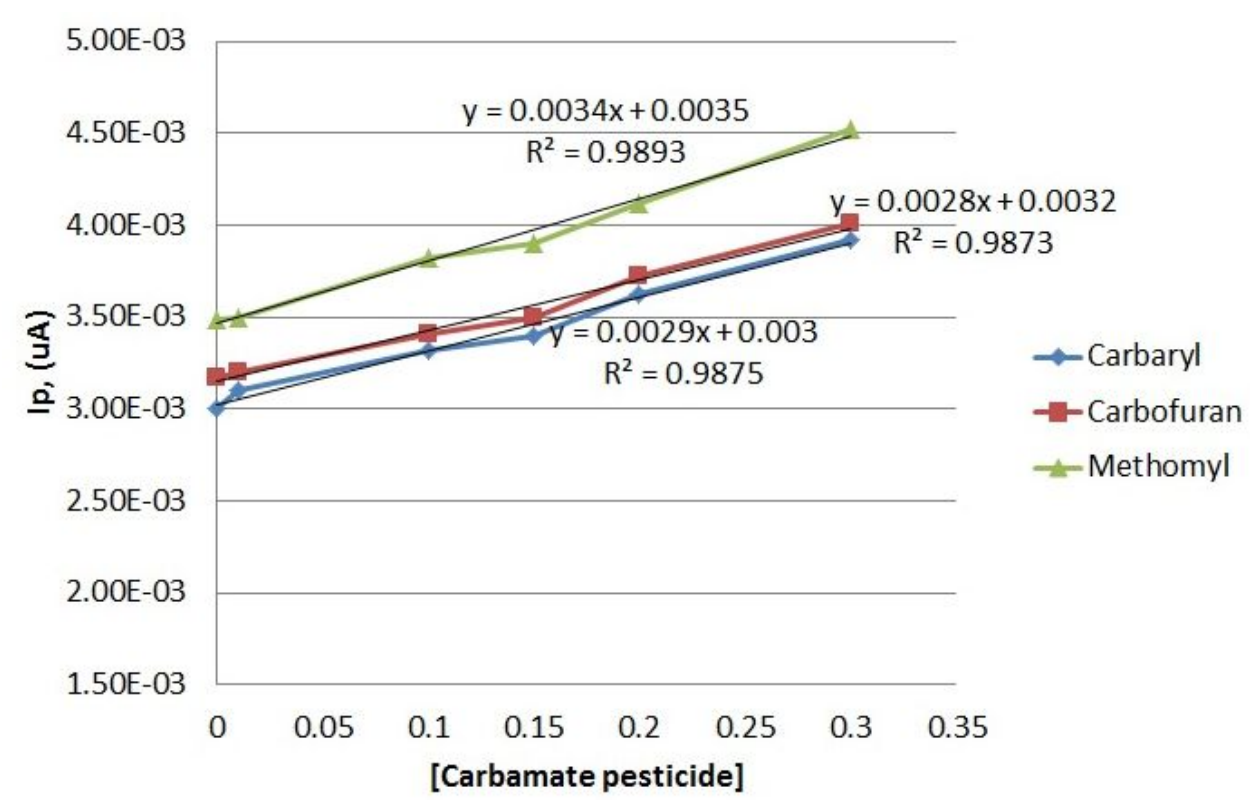


Figure 9. Analytical calibration curves and results obtained for the determination of carbaryl, carbofuran, and methomyl concentrations in real fruit samples by the method of standard addition ($n = 4$).

The calibration curve for carbaryl (Figure 9) determination in pear fruit samples was found to be relatively linear within a concentration range of 0–0.3 mg/L and with a very good correlation coefficient ($r^2 = 0.9875$; $n = 4$). The carbaryl detection limits (LOD) in pear fruit sample achieved by this method was 0.136 mg/L. The biosensor reproducibility for successive measurements was good with a R.S.D. value of 3.5%. The concentration of carbaryl in replicate measurements ($n = 4$) of pear samples was found to be 0.08 mg/L.

The calibration curve for carbofuran (Figure 9) was also found to be linear within a concentration range of 0–0.3 mg/L and with a very good correlation coefficient ($r^2 = 0.9873$; $n = 4$). Carbofuran was analysed in orange fruit samples and the detection limit (LOD) achieved by this method was found to be 0.145 mg/L, which is slightly less than for carbaryl. The concentration of carbofuran in replicate measurements ($n = 4$) of orange samples was found to be 0.05 mg/L.

The calibration curve and results for methomyl determination in grape samples is also displayed in Figure 9. This curve was again found to be linear within a concentration range of 0–0.3 mg/L and with a very good correlation coefficient ($r^2 = 0.9893$; $n = 4$). The methomyl detection limits (LOD) in grape fruit sample achieved by this method was 0.203 mg/L. The

concentration of methomyl in replicate measurements ($n = 4$) of grape samples was found to be 0.05 mg/L.

The results reported for the LOD values in this study compared relatively well to previous studies done by this research group for the evaluation of carbamate pesticides [9, 18-19].

A summary of the results obtained for the application of the Pt/GO-PANI/HRP biosensor to real fruit samples analysis is shown in Table 1.

Pesticide Evaluated	Matrix	Added (mg/L)	Detected (mg/L)	%Recovery
Carbofuran	Pears	0.01	0.0053	53.0
		0.10	0.0599	59.9
	Grapes	0.01	0.0048	48.0
		0.10	0.0601	60.1
	Oranges	0.01	0.0051	51.0
		0.10	0.0608	60.8
Carbaryl	Pears	0.01	0.0080	80.2
		0.10	0.0801	80.1
	Grapes	0.01	0.0080	80.6
		0.10	0.0799	79.9
	Oranges	0.01	0.0070	70.3
		0.10	0.0699	69.9
Methomyl	Pears	0.01	0.0050	50.4
		0.10	0.0601	60.1
	Grapes	0.01	0.0051	51.7
		0.10	0.0608	60.8
	Oranges	0.01	0.0050	50.3
		0.10	0.0599	59.9

Table 1. The results obtained for the application of the Pt/GO-PANI/HRP biosensor in the evaluation and analysis of carbofuran, carbaryl, and methomyl concentrations in pear, grape, and orange samples, respectively.

Analysis of the results in Table 1 has shown very interesting trends for the application of the Pt/GO-PANI/HRP biosensor to the determination of carbamate pesticides in fruit samples.

Analysis of the percentage recoveries for the spiked samples (Table 1) have shown variability between the different fruits and pesticides evaluated. In the case of carbofuran results, it was found that for the lower spiked concentration of 0.01 mg/L, the highest percentage recovery was obtained in the grapes, while the lowest percentage recovery was observed in the pears. Evaluation of the high carbofuran concentration of 0.10 mg/L has shown that the lowest percentage recovery was obtained in the pears, with the slightly higher recovery obtained in the oranges.

Evaluation of the carbaryl results has shown overall higher percentage recoveries, compared to the results of carbofuran and methomyl. For the low 0.01 mg/L concentration, the percentage recoveries ranged between 70.3% (oranges) to 80.6% (grapes). In the case of the high 0.10 mg/L concentration, the recoveries ranged between 69.9% (oranges) and 80.1% (pears).

The percentage recovery results for methomyl showed the lowest percentage recoveries compared to the other carbamates evaluated. For the low 0.01 mg/L concentration, the percentage recoveries ranged between 50.3% (oranges) and 51.7% (grapes). In the case of the high 0.10 mg/L concentration, the recoveries ranged between 59.9% (oranges) and 60.8% (grapes). Future work will investigate and compare the results obtained for a Pt/GO-PANI/AChE biosensor.

The results obtained for the analysis of real fruit samples (Table 1), using the Pt/GO-PANI/AChE biosensor, have further highlighted some difficulties obtained with the recoveries. In fact, the relatively lower recoveries in real samples obtained for carbofuran (50–60%), carbaryl (70–80%), and methomyl (50–60%) indicate that some matrix effects may also have hampered better results. It further highlights the difficulty of organic phase biosensor determination of carbamate in fruit samples, indicating that some matrix effects should be further investigated in future biosensor construction and investigations.

5. Conclusion

The results for work done in this study have shown that the Pt/GO-PANI/HRP biosensor was successfully developed for the detection and quantification of carbamates such as carbaryl, carbofuran, and methomyl in real fruit samples. The principle of the detection was based on the reduced biosensor response measurements, which occurred as a result of the catalytic activity of HRP, immobilised on a GO-PANI matrix immobilised on a platinum electrode. The effect of the inhibition was found to be increasing with increasing concentrations of the carbamates utilised in the biosensor inhibition studies. An incubation period of 20 min was applied and the response of the HRP biosensor was measured before and after incubation in each of the three carbamates investigated. The percentage inhibition results showed that the Pt/GO-PANI/HRP biosensor was more inhibited by the carbofuran pesticide exposure, and the least inhibited in the carbaryl pesticide. Due to the sensitivity of HRP biosensor for these carbamates, the development of this method is a way forward for the analysis of carbaryl, carbofuran, and methomyl at residue levels such as those occurring in the environment. The Pt/GO-PANI/HRP biosensors further demonstrated a detection limit of 0.136 mg/L for carbaryl determination, followed by 0.145 mg/L for carbofuran and 0.203 mg/L for methomyl determination in real fruit samples. The Pt/GO-PANI/HRP biosensor evaluation has further shown that although HRP can replace acetylcholinesterase (AChE) as enzyme in the evaluation of carbamate pesticides, reduced percentage inhibition results were obtained for HRP as enzyme. This result may also have affected the percentage recovery results obtained for the determination of carbaryl, carbofuran, and methomyl in spiked pear, orange, and grape samples. It further highlights the difficulty of organic phase biosensor determination of carbamate in fruit

samples, indicating that some matrix effects should be further investigated in future biosensor construction and investigations.

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