25

Indirect Amperometric Determination of Selected Heavy Metals Based on Horseradish Peroxidase Modified Electrodes

Philiswa N. Nomngongo[1], J. Catherine Ngila[1,2] and Titus A. M. Msagati[2]
[1]*School of Chemistry, University of KwaZulu Natal*
[2]*Department of Chemical Technology, University of Johannesburg*
South Africa

1. Introduction

Due to the high toxicity of heavy metals, it is crucial to detect ultra low levels of the metals, especially in drinking water. The common techniques include spectrometric techniques such as inductively coupled plasma- atomic emission spectroscopy, ICP-AES **(**Bettinelli et al. 2000; Rahmi et al. 2007; Tuzen et al. 2008) as well as anodic stripping voltammetry (Brainina et al. 2004). Even though ICP techniques have low detection limits (ranges from parts per billion, ppb to parts per trillion, ppt (Berezhetskyy et al. 2008), however, they are unsuitable for in-situ analysis, they are expensive, sophisticated and require skilled operators. For these reasons, the development of alternative techniques such as electrochemical biosensor techniques, offer alternative methods because they are sensitive, low cost and simple to operate (Wang et al. 2009b).

Recent developments have shown the use of electrochemical biosensors as indirect methods for detection of Cd^{2+}, Cu^{2+}, Cr^{3+}, Zn^{2+}, Ni^{2+} and Pb^{2+} using urease biosensor (Ilangovan et al. 2006; Tsai et al. 2003); Cd^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+} and Pb^{2+} using alkaline phosphatase (Berezhetskyy et al. 2008); Cd^{2+}, Cu^{2+}, Zn^{2+} and Pb^{2+} by glucose oxidase (Ghica and Brett 2008); Hg^{2+} using glucose oxidase invertase and mutarose (Mohammadi et al. 2005); Cu^{2+}, Cd^{2+}, Mn^{2+} and Fe^{3+} using acetylcholinesterase (Stoytcheva 2002); and Cu^{2+}, Cd^{2+}, Zn^{2+} and Pb^{2+} by nitrate reductase (Wang et al. 2009b).

Horseradish peroxidase (HRP) biosensor has so far only been reported for detection of mercury (Han et al. 2001). This study sought to extend its application for detection of other metals such as lead, cadmium and copper. We have chosen cadmium due to its similarities with mercury with regards to toxicity as both metals, belong to the same group. In addition, we have also chosen copper and lead because of their common occurrence in environmental matrices (Pb from leaded petrol and Cu from wiring activities). Furthermore, copper is reported to show interaction with biological systems (Cecconi et al. 2002; Uriu-Adams and Keen 2005) and therefore interesting to see how it interacts with HRP enzyme.

The main aim of this present work is to investigate the inhibition of HRP enzyme by Cd, Pb and Cu, a phenomenon that can be employed for their indirect determination. Kinetic studies were done to determine the nature of enzyme inhibition (whether it is reversible or irreversible and if reversible whether it is competitive or noncompetitive). The apparent

Michealis-Menten constant (K_M^{app}) as well as maximum current (I_{max}) values in the absence and the presence of metal inhibitor were investigated. The developed biosensor was applied for determination of the Cd, Pb and Cu in tap water and landfill leachate sample.

2. Methodologies, results and discussion

2.1 Experimental reagents

All the chemicals used in this work were of analytical grade unless otherwise stated. Horseradish peroxidase (E.C. 1.11.1.7, 169 Units mg^{-1} powder, Sigma) aniline (99%), hydrochloric acid (37%), N,N-dimethylformamide (DMF), disodium hydrogen phosphate (dehydrated) and sodium dihydrogen phosphate (dehydrated) were all obtained from Sigma-Aldrich (South Africa). Cadmium and copper stock solutions (1000 ppm) were obtained from KIMIX Chemicals & Lab Supplies; and lead stock solution (1000 ppm) was obtained from Saarchem-Holpro Analytic (PTY) Ltd. Working solutions of hydrogen peroxide were prepared from 30% v/v stock solution obtained from Merck Chemical (PTY) Ltd.. Phosphate buffer (PBS, 0.1 M, pH 7.0) was used as a supporting electrolyte as per Songa et al. 2009.

3. Instrumentation

All electrochemical experiments were performed using BAS100W Electrochemical Analyzer (Bioanalytical Systems, West Lafayette, IN, USA). A 15 mL electrochemical cell consisting of Pt working electrode (A = 0.018 cm^2), Pt wire auxiliary electrode and Ag/AgCl (saturated 3 M NaCl) reference electrode. Supporting electrolyte solutions was degassed with argon gas before measurements performed at room temperature (20-25 °C). The PANI film was characterized using both Perkin Elmer Spectra 100 FT-IR Spectrometer (attenuated total reflectance, ATR) and UV-Vis Perkin Elmer Spectra spectrophotometer (PANI in DMF solution in quartz cuvette). UV photolysis of the leachate water sample was carried out by UV digester 705 equipped with a 500 W Hg lamp from Metrohm (Herisau, Switzerland). Inductively coupled plasma optical emission spectroscopy (ICP-OES) analysis of Cd, Cu, and Pb was performed using an Optima 5300 ICP-OES system (Perkin Elmer LLC, 761 Main Avenue, Norwalk, USA) equipped with AS 93plus autosampler.

4. Preparation of polyaniline (PANI) film modified electrode

Aniline was distilled before use. The platinum working electrode was first polished thoroughly with successive alumina slurries particle size of 1.0, 0.3 and 0.05 μm, and then rinsed with distilled water after each polishing step followed by 10 min sonication with ethanol and then water. The polyaniline (0.2 M aniline in 1.0 M HCl degassed in argon for 10 min) was electrochemically deposited on the platinum electrode (-200 mV to +1100 mV at 50 mVs^{-1} for 20 cycles). The PANI- modified electrode was rinsed with water before use. The modified electrode was used in subsequent biosensor fabrication.

5. Enzyme immobilization

The PANI film was reduced in PBS at a constant potential of –500 mV until the current signal reached a steady state value. This was followed by the oxidation at +0.65 V for 20 min

in the presence of HRP solution (50 µl of 2.0 mg ml^{-1} in 1.0 ml fresh PBS). During the oxidation process, the heme protein of HRP became electrostatically attached onto the PANI film (Songa et.al. 2009; Mathebe et al. 2004) .The biosensor was stored in PBS at 4 °C when not in use.

6. HRP Biosensor response to hydrogen peroxide

The response of the biosensor (Pt//PANI/HRP) to H_2O_2 was studied at pH 7.0 in PBS. Cyclic voltammetric (CV), differential pulse voltammetry (DPV) and amperometric responses of the biosensor were recorded by adding small aliquots of 0.01-0.05 M H_2O_2.

7. Determination of Cd^{2+}, Cu^{2+} and Pb^{2+} in model solutions

Amperometric measurements of HRP inhibition by cadmium, copper and lead were carried out in a cell containing 2.0 ml of 0.1 M PBS (pH 7.02) and constant concentration H_2O_2 (0.5 mM) with continuous stirring. The experiments were carried out at -0.20 V versus Ag/AgCl (3 M NaCl) and allowing the steady-state current to be attained. An appropriate volume (µl) of the inhibitor stock solution (10 ppm of each Cd^{2+}, Cu^{2+} and Pb^{2+}) was then added using a micropipette. After each experiment the enzyme electrode activity was regenerated by rinsing the electrode with distilled water.

8. Analysis of heavy metals in tap water and landfill leachate samples

Water samples were collected as follows: Tap water was collected from the Laboratory Tap at University of Western Cape, Bellville, Cape Town. Landfill leachate sample was collected from the Marrianhill landfill (Ethekwini municipal solid waste deposit). The leachate water sample was collected in polyethylene container and stored in the fridge at 4 °C.

Determination of heavy metals in tap water was achieved using standard addition method. The pH of the tap water samples was first adjusted from 8.90 to 7.04 before the analysis was carried out. The tap water sample (10 ml) was spiked with 0.1 ppm of each metal solution (Cd^{2+}, Cu^{2+} and Pb^{2+}) followed by amperometric analysis. For ICP-OES, the tap water sample was analysed without the addition of metal standards.

Leachate water sample is rich with organics; therefore prior electrochemical analysis, the organics were removed by passing the water sample through C-18 SPE column. The cartridges were first conditioned with 5 mL methanol followed by 5 mL water. The C-18 column retained the organics and the water sample containing inorganics was collected. The collected leachate water sample was spiked with 0.1 ppm of each metal solution (Cd^{2+}, Cu^{2+} and Pb^{2+}) followed by Pt/PANI/HRP biosensor analysis.

For ICP-OES analysis, the leachate samples were filtered with 0.45 µm pore size filter before they were subjected to UV digester. This procedure was done in order to destroy all dissolved organic matter in the landfill leachate sample. A UV digester 705 equipped with a 500 W Hg lamp from Metrohm was used. The quartz vessels were arranged concentrically around the Hg lamp with a distance of 2.5 cm. Ten mL of leachate samples were placed in quartz vessels and 100 µL H_2O_2 was added to each sample. The solution was irradiated with UV light for about 2 hour. The leachate water sample was then analyzed by ICP-OES.

9. Results and discussion

9.1 Electrosynthesis of PANI film

Multiscan voltammetry of Pt/PANI electrode was performed (result not shown). The redox peak currents increased with increasing scan rate while the peak potentials showed slight increase in positive potential. These observations shows that the polymer is electroactive and the peak currents are diffusion controlled **(**Mathebe et al. 2004). In order to calculate surface concentration of the PANI film, Γ^*_{PANI}, Brown-Anson equation (1) (Bard & Faulkner 2000) was used.

$$I_P = \frac{n^2 F^2 \Gamma^*_{PAN} A}{4RT} v \tag{1}$$

where n is the number of electrons (n = 2) transferred, F is the Faraday constant (96584 C mol^{-1}), Γ^*_{PANI} is the surface concentration of the PANI film (mol cm^{-2}), A is the surface area of the electrode (0.0177 cm^2), v is the scan rate (V s^{-1}), R is the gas constant (8.314 J mol K^{-1}), and T is the absolute temperature of the system (298 K). A graph of peak current versus scan rate was obtained and the slope of the curve was used to calculate the surface concentration of the PANI film. The surface concentration was found to be 7.8×10^{-7} mol cm^{-2}. The surface concentration obtained in our study was comparable to that reported by Mathebe et al. 2004 (1.85×10^{-7} mol cm^{-2}).

The Randles-Sevcik equation (2) was used to calculate the diffusion coefficient of the electrons within the polymer (Gau et al. 2005).

$$i_p = 2.69\times10^5 n^{3/2} A D_e^{1/2} C v^{1/2} \tag{2}$$

where i_p is the peak current (A), n is the number of electrons appearing in half-reaction for the redox couple, A is the area of the electrode (cm^2), D is the diffusion coefficient (cm^2/s), C is the concentration (mol/cm^3) and v is scan rate (V/s). Equation 2 was used to plot peak current versus the square root of the scan rate and the slope of the linear regression was used to estimate the diffusion coefficient of the electrons within the polymer (D_e) as 4.07×10^{-8} cm^2 s^{-1}.

10. Spectroscopic characterization of polyaniline

The absorption spectrum of PANI (dissolved in DMF) shows two characteristic absorption peaks at 340 nm and 660 nm. The first absorption peak was assigned to π–π^* transition of the benzenoid rings and the second was attributed to the transition of benzenoid rings into quinoid rings **(**Laska & Widlarz 2005; Kan et al. 2006). The results (UV-Vis characterization of PANI) obtained in this study are in close agreement with the literature values (Laska &Widlarz 2005; Kan et al. 2006; Singh et al. 2008; Mazeikiene et al. 2007; Kang, Neoh & Tan 1998).

The FTIR absorption band at 3325 cm^{-1} was assigned to N-H stretching of the amine group of polyaniline (spectrum not shown). The peaks at 1596 and 1493 cm^{-1} which are characteristics of, polyaniline, were most likely due to the C=C stretching of quinoid and benzenoid groups, respectively (Lakshmi et al. 2009; Kim et al. 2001).

11. Cyclic Voltammetric (CV) and Differential Pulse Voltammetric (DPV) response of Pt/PANI/HRP biosensor to hydrogen peroxide

The electrochemical behaviour of Pt/PANI/HRP electrode in the absence and presence of H_2O_2 in PBS (0.1 M, pH 7.02) was studied using CV and DPV. Figure 1 shows CV (A) and DPV(B) of Pt/PANI/HRP electrode in different concentrations of H_2O_2 (0-6.9 mM) at scan rate of 10 mV s^{-1} and 20 mV s^{-1} for CV and DPV, respectively. The value used in this study for the optimum concentration of HRP was as per Ndangili et al. (2009) and Songa et al. (2009). The effect of pH on HRP electrode response was investigated by CV in the pH ranges from 5.5 to 8.5 in the presence of 1.0 mM H_2O_2. The HRP electrode response current achieved a maximum value at pH 7.0. Therefore, in order to obtain maximum sensitivity, 0.1 M PBS solution of pH 7.0 was used throughout this study.

As expected, in the absence of H_2O_2, no significant current was observed. However increasing the amount of H_2O_2 showed increased cathodic peak current intensity due to the reduction of H_2O_2. In order to confirm whether the change in current intensity observed was due to the enzymatic catalytic reduction of H_2O_2, control experiments in the absence of HRP were carried out. At both the bare electrode (Pt) and polymer modified surface (Pt//PANI) no H_2O_2 reduction current was observed at -200 mV. This is because, the reduction reaction of H_2O_2 at both electrodes in the absence of HRP, is very slow and usually occurs at higher potentials. The difference in the observations made for the control experiments (Pt and Pt/PANI) compared to that for Pt/PANI/HRP confirms that the increase in the cathodic current was due to the direct electron transfer between the HRP molecules and the electrode (Wang & Wang 2004). Moreover, PANI provides a suitable platform for the immobilization of HRP on the platinum electrode surface and it also mediates in electron transfer between HRP and the electrode (Gerard et al. 2002). Thus the reduction peak is an indication of the electrocatalytic activity of the enzyme on H_2O_2 (Sun et al. 2004).

Figure 2 shows the possible mechanism of electric transduction between the platinum electrode, PANI and HRP enzyme active site combined with electrocatalytic reduction process of H_2O_2 by HRP. It can be seen from figure 2 that H_2O_2 is reduced by HRP to form water and in turn HRP gets oxidized to form Compound I. The latter is converted to HRP through the formation of intermediate (Compound II) via a two-electron reduction step (Iwuoha et al. 1997). The reduction of Compound I is due to the direct electron transfer that takes place between the PANI modified electrode and the enzyme (Liu & Ju 2002).

The relatively low potential value for H_2O_2 reduction (-200 mV) in the presence of HRP, ensures minimal risk of interfering reactions of other electroactive species in solution as well as low background current and noise levels (Tong et al. 2007; Wang &Wang 2004).

12. Amperometric responses of Pt/PAN/HRP to H_2O_2

Amperometric responses of Pt/PANI/HRP biosensor were investigated by consecutively increasing the concentration of H_2O_2 at a working potential of -200 mV. Figure 3 presents a typical steady state current-time plots obtained with the fabricated biosensor upon successive additions of 10 µL of 0.001 M H_2O_2 into 2.0 mL PBS with the calibration plot as an inset. It was observed that, upon the addition of H_2O_2 into the PBS, the reduction current rises sharply to reach the steady state value. In addition, the biosensor attained 95% steady state current within 5 seconds after each addition of 10 µL 0.010 M H_2O_2. This observation

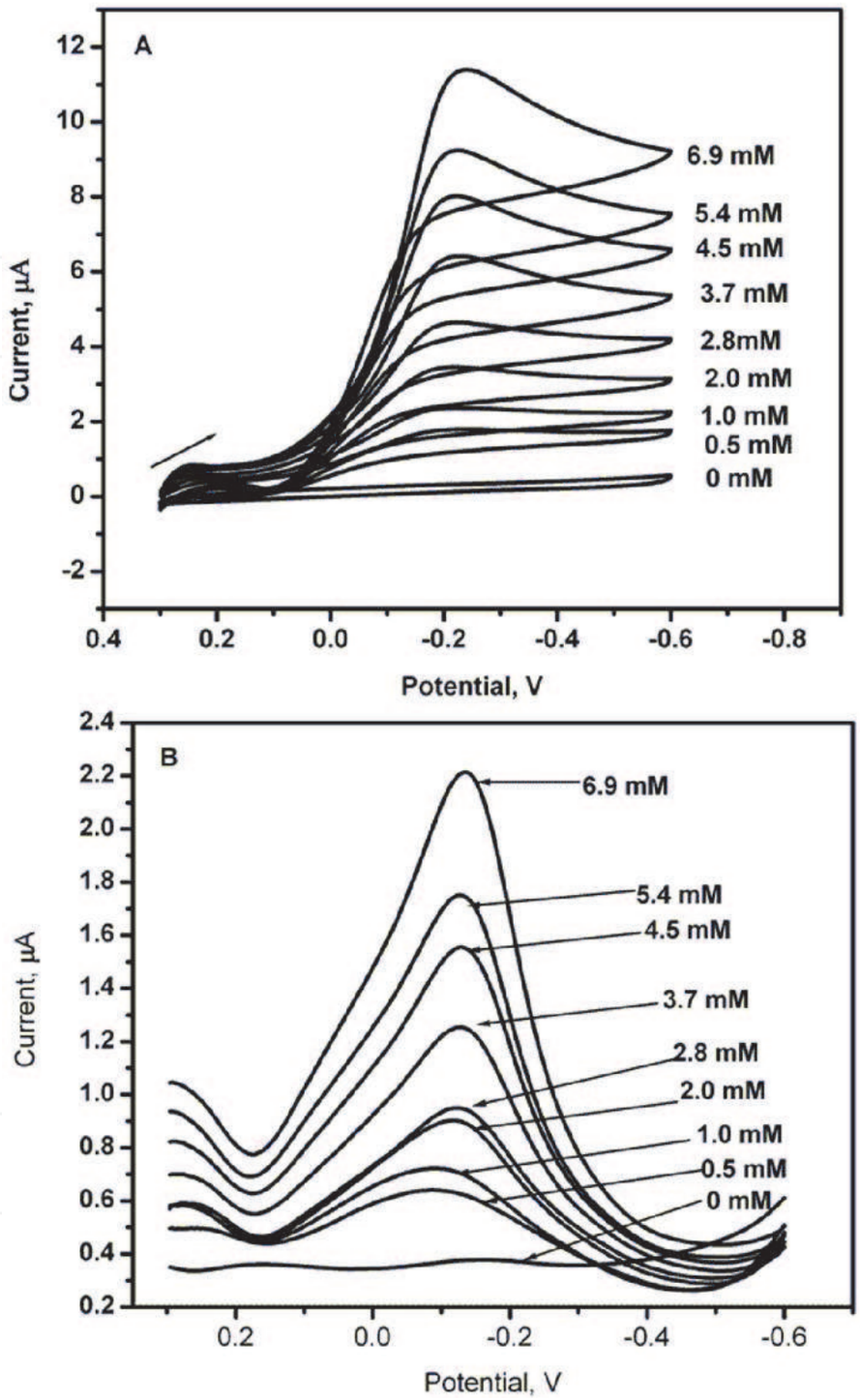


Fig. 1. Cyclic voltammograms and (B) Differential pulse voltammograms for the response of the biosensors (Pt/PANI/HRP) to different concentrations of H_2O_2 ranging from 0.5 to 6.9 mM made up in 0.1 M PBS (pH 7.02). CV experiments: scan rate, 10 mV/s; DPV experimental conditions were: scan rate 20 mV s^{-1} pulse width: 50 msec and pulse amplitude: 20 mV. Arrow (→) indicate direction of potential scan

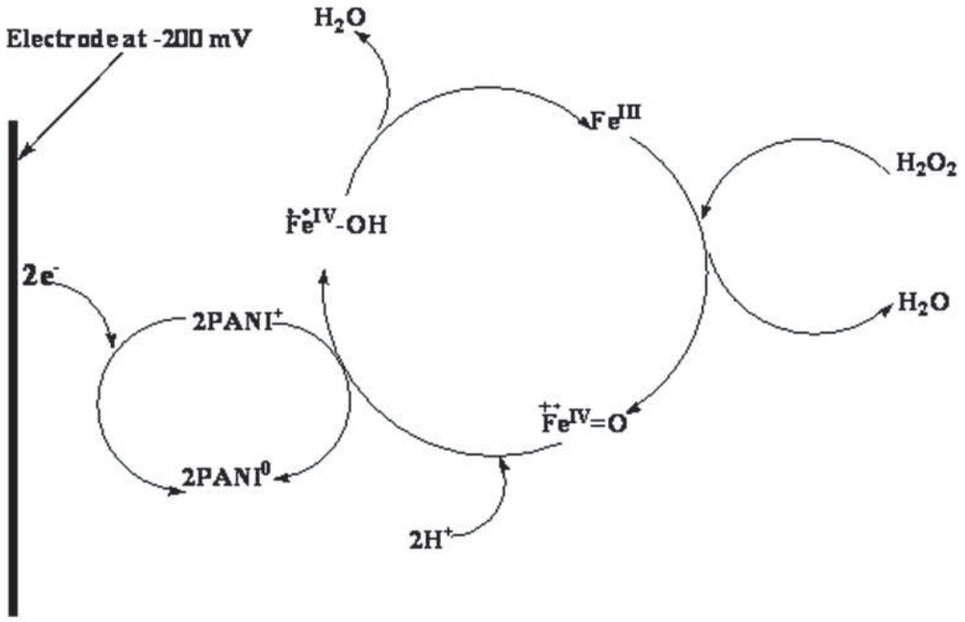


Fig. 2. Mechanism of electric transduction between the platinum electrode, PANI and HRP enzyme active site.

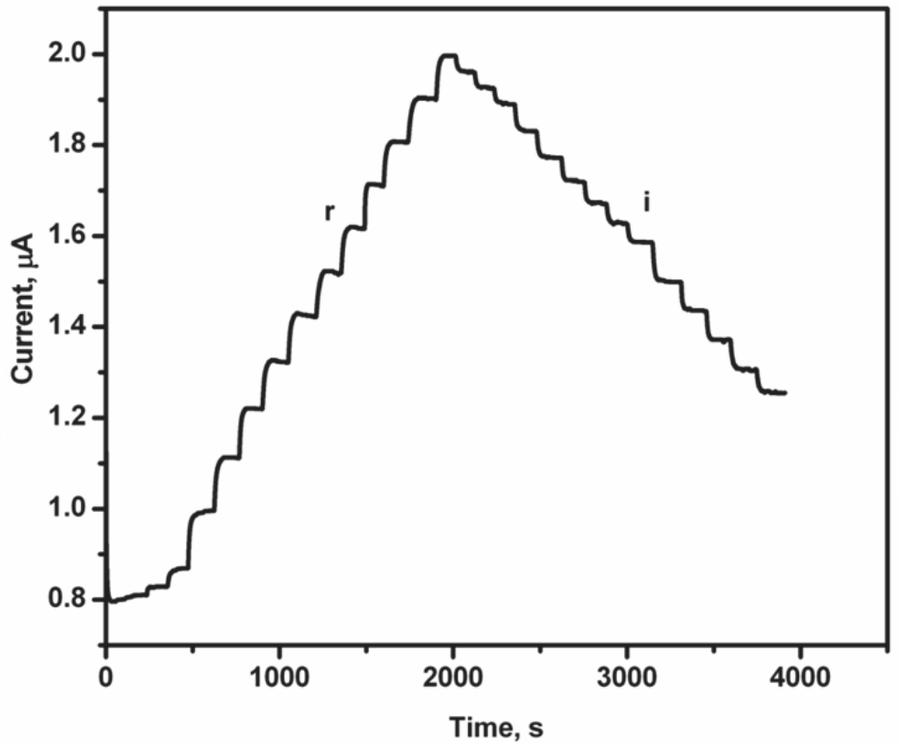


Fig. 3. Amperometric responses of Pt/PANI/HRP to successive additions of 10 μL 0.05 mM of hydrogen peroxide (inset shows the calibration curve). Potential: −0.2 V; supporting electrolyte: 0.1 M PBS (pH 7.02).

implied a fast response of the fabricated biosensor.The response currents at the biosensor were linear to H_2O_2 in the range from 0.05 to 3.17 mM with a correlation coefficient of 0.9991 (n = 18); sensitivity of 1.75 μA mM^{-1}; and a detection limit of 36.8 nM (0.0368 μM) (estimated at a signal-to-noise ratio of 3).

13. Kinetics of Pt/PANI/HRP electrode

A plot (not included) of the reciprocal of current versus the reciprocal of H_2O_2 concentration (Lineweaver–Burk plot) showed a linear relationship implying a kinetic behavior (Liu and Ju 2002; Wang, et al. 2009a). Using equation 3, Michaelis–Menten constant of the enzyme could be calculated as per previous studies (Liu, and Ju 2002; Mathebe, Morrin, and Iwuoha 2004):

$$\frac{1}{I} = \frac{1}{I_{\max}} + \left(\frac{K'_M}{I_{\max}[H_2O_2]} \right) \tag{3}$$

where I is the observed response current, $I_{\max}$ is the maximum steady state current that can be attained for the system (Pt//PAN/HRP biosensor), K_M^{app} is Michaelis–Menten constant and [H_2O_2] is the concentration of H_2O_2 in the bulk solution. The *slope (*1.18 μA^{-1}mM*)* and the *y-intercept* (0.958 μA^{-1}) of the Lineweaver–Burk plot are equal to $I_{\max}$ and $\frac{K_M^{app}}{I_{\max}}$, respectively. The values of $I_{\max}$ and K_M^{app} were estimated as 1.04 μA and 1.23 mM, respectively. The K_M^{app} value obtained in our study was relatively better (lower) than previous studies (Wang et al. 2009a; Liu & Ju 2002; Ndangili et al. 2009. Since K_M^{app} is inversely proportional to the affinity of the enzyme for the substrate (Wang et al. 2009; Ansari et al. 2009), based on the value 1.23 mM it can be concluded that Pt/PANI/HRP electrode exhibited high affinity towards H_2O_2. The differences in the values obtained by different workers indicate that the affinity between H_2O_2 and HRP is plainly dependent on the immobilizing materials and procedure used (Wang et al. 2009).

14. Reproducibility, repeatability and stability of Pt/PAN/HRP biosensor

To evaluate the reproducibility of the HRP based biosensor, six biosensors were prepared under similar conditions separately. The amperometric responses of each biosensor to successive additions of H_2O_2 (0.05 to 0.30 mM) were recorded and gave a relative standard deviation (RSD) of 3.8% (n = 6). The precision (repeatability) of a selected biosensor was investigated by recording the response current using 0.05 mM H_2O_2 for replicate experiments (n = 10). The biosensor showed a high precision with a %RSD of 2.1%. The stability of the Pt/PANI/HRP electrode (stored in 0.1 M PBS at 4 °C in between measurements) was tested every two days by amperometric measurements in the presence of 0.05 mM H_2O_2. After two weeks, the biosensor retained > 95% of its initial response, indicating good stability. The latter implies that the HRP molecules were firmly immobilized in the PANI films which provided a biocompatible microenvironment.

15. Detection of heavy metals (Cd^{2+}, Cu^{2+} and Pb^{2+}) in model solutions

Heavy metals such as Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+} and Pb^{2+} as well as some organic and inorganic compounds such as sodium azide, cyanide, L–cystine, dichromate, ethylenethiourea, hydroxylamine, sulfide, vanadate, p–aminobenzoic acid are well-known inhibitors of horseradish peroxidase (Zollner 1993). Figure 4 (I) shows amperometric responses of Pt/PANI/HRP to successive additions of 0.05 mM H_2O_2 (denoted by r) followed by addition of Cd^{2+} (denoted by i). It can be observed that the current for the electrocatalytic reduction of H_2O_2 decreases upon the addition of Cd^{2+}. This observation clearly showed that the catalytic activity of HRP at Pt/PANI electrode is inhibited by Cd^{2+} by binding to the enzymes active sites. The inhibition of HRP activity was analyzed by monitoring the current decrease at -0.20 V using the following procedure: H_2O_2 was added to PBS (electrolyte solution) and the current response was recorded. After the stabilization of the steady-state current response, Cd^{2+} was added, leading to an immediate decrease in the biosensor response.

The other two heavy metal ions (Pb^{2+} and Cu^{2+}) showed the same behaviour. Calibration plots for the determination of the metals in the range 0.05 to 60.8 ppb were obtained. The linear ranges were established as 4.76-55.3 ppb for Cd^{2+} and Pb^{2+} while that for Cu^{2+} was 2.38-52.8 ppb. Limits of detection (LOD= $\frac{3\times SD}{m}$) and limits of quantification (LOQ= $\frac{10\times SD}{m}$)

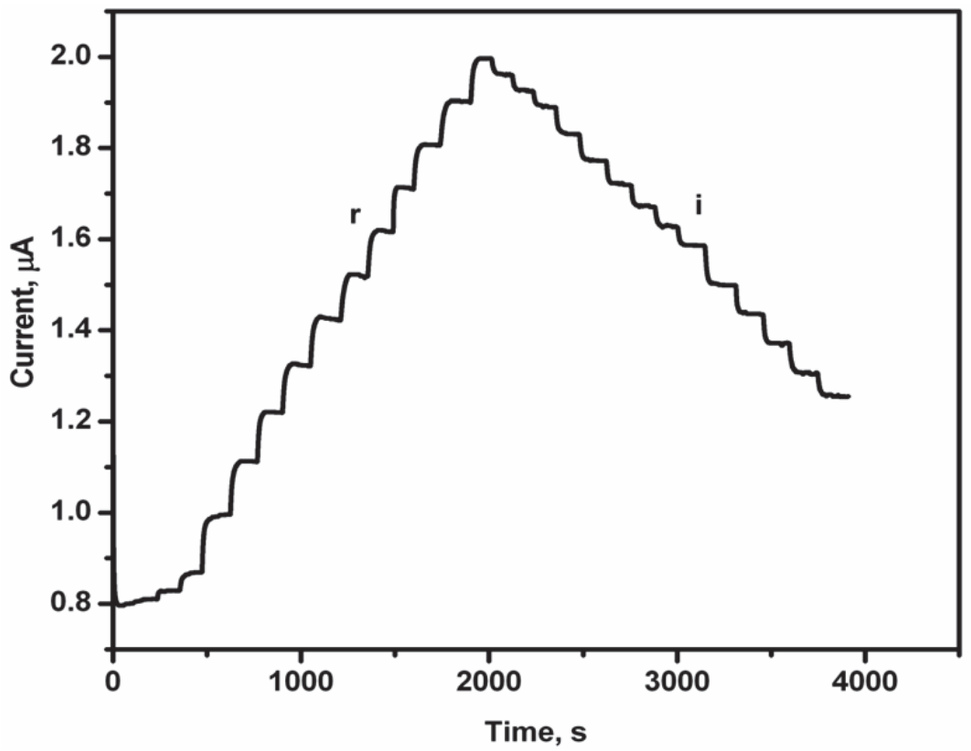


Fig. 4. Typical amperometric responses of Pt/PANI/HRP biosensor to successive additions of 0.05 mM hydrogen peroxide (r) and Cadmium (i). Applied potential: −0.20 V; supporting electrolyte: 0.1 M PBS (pH 7.02).

were calculated and their values are presented in Table 1. Where SD is standard deviation of the blank signal (n = 8) obtained in PBS (SD = 2.4×10^{-4} μA) and m is the slope of the calibration curve. The low LOD and LOQ values confirmed good sensitivity of the proposed biosensor method for the determination of heavy metals. Table 6 shows a summary of analytical response and linear regression characteristics of calibration curves for heavy metals. The inhibition of heavy metals to HRP enzyme is reversible, and the biosensor can be reused (beyond 10 runs) after rinsing with double distilled water.

Metal ion	Linear range/ppb	Slope/μAppb^{-1}	Correlation Coefficients (R^2)	LOD/ppb	LOQ/ppb
Cd^{2+}	4.76-55.3	7.945×10^{-3}	0.9985	0.091	0.30
Pb^{2+}	4.76-40.5	2.100×10^{-2}	0.9995	0.033	0.12
Cu^{2+}	2.38-52.8	7.230×10^{-3}	0.9990	0.10	0.33

Table 1. A summary of analytical characteristics and regression parameters for calibration curves for determination of heavy metals.

16. Inhibition studies

The values of the steady state current in the absence (I_0) and in the presence (I_i) of an inhibitor were determined from the recorded amperograms in Figure 4. The inhibition percentage ($I\%$) was calculated from the following expression (Nwosu et al. 1992):

$$I\% = \frac{I_0 - I_i}{I_0} \times 100\% \tag{4}$$

The $I\%$ values obtained were used to compare the inhibitory effects of the different metal ions on the activity of the immobilized HRP. Inhibition plots showing the effects of heavy metals on the activity of the immobilized HRP are presented in Figure 5. The order of inhibition was found to increase from Pb^{2+} (32.8 %), Cu^{2+} (43.4 %) to Cd^{2+} (51.1 %) in the presence of 0.95 mM H_2O_2. It should be noted that the higher the $I\%$ value the higher the degree of enzyme inhibition by the heavy metal.

There are two approaches that can be used for inhibition studies; incubation method and direct method (without incubation) and in this study the latter was employed. It was observed that when using the direct method, it is difficult to attain the concentration that causes 50% inhibition (IC_{50}) (Songa et al. 2009); this worked reasonably well for Cd^{2+} (51.1%) with the value for IC_{50} calculated as 3.13 ppm but was not successful for Pb^{2+} and Cu^{2+}. Therefore unlike Cd^{2+}, in order to attain the 50% inhibition (IC_{50} value) for Pb^{2+} and Cu^{2+}, either a higher concentration of the metal can be used or the incubation method can be employed.

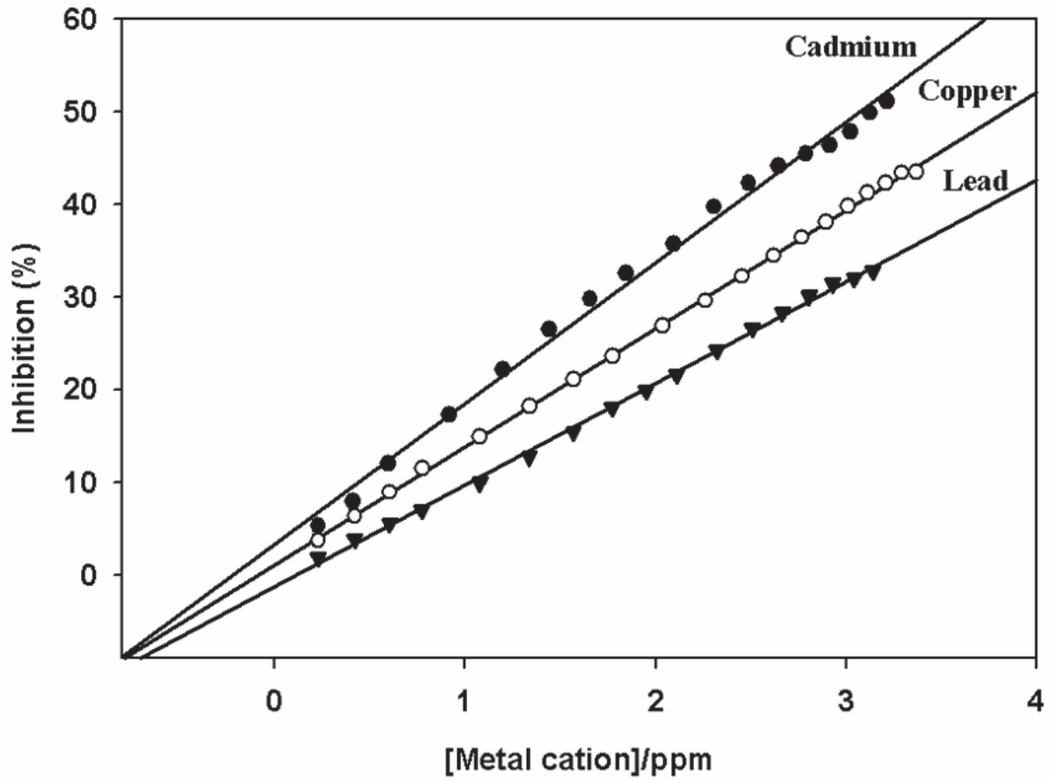


Fig. 5. Plots showing the Inhibition of Pt/PANI/HRP biosensor by heavy metals; Cd^{2+}, Cu^{2+} and Pb^{2+} .

After successfully obtaining the IC_{50} value for Cd^{2+} using direct approach, the results from the latter approach were used to investigate the inhibition kinetics and the type of inhibition (competitive or non-competitive).

17. Investigation of inhibition kinetics and mechanism

The amperometric response of the biosensor to various H_2O_2 concentrations (in 0.1 M PBS, pH 7.02) in the absence (0 ppm Cd^{2+}) and presence (3.13 ppm Cd^{2+}, IC_{50}) of an inhibitor was recorded at -200 mV (Figure 6A). The response of the biosensor to various H_2O_2 concentrations in the presence of an inhibitor was done as follows; HRP electrode was first incubated in PBS containing 3.13 ppm Cd^{2+} for 20 min followed by the successive addition of different concentrations of H_2O_2. A fast response of the biosensor to the additions of different concentrations of H_2O_2 was observed in the absence of Cd^{2+} whereas the presence of 3.13 ppm Cd^{2+} gave a slow response. Studies suggest that during incubation, Cd^{2+} slowly binds to the enzyme and leads to gradual conformational changes (Keyhani et al. 2003, **Tayefi-Nasrabadi et al. 2006**). Therefore, it can be suggested that the difference in background current could be due to the conformational changes of HRP. Figure 6B shows the Lineweaver–Burk plots for enzymatic reactions. The *slope* and the *y-intercept* values of the linear plots were used to calculate apparent Michealis-Menten constants K_M^{app} and maximum current (I_{max}) in the absence and the presence of Cd^{2+} (Table 2).

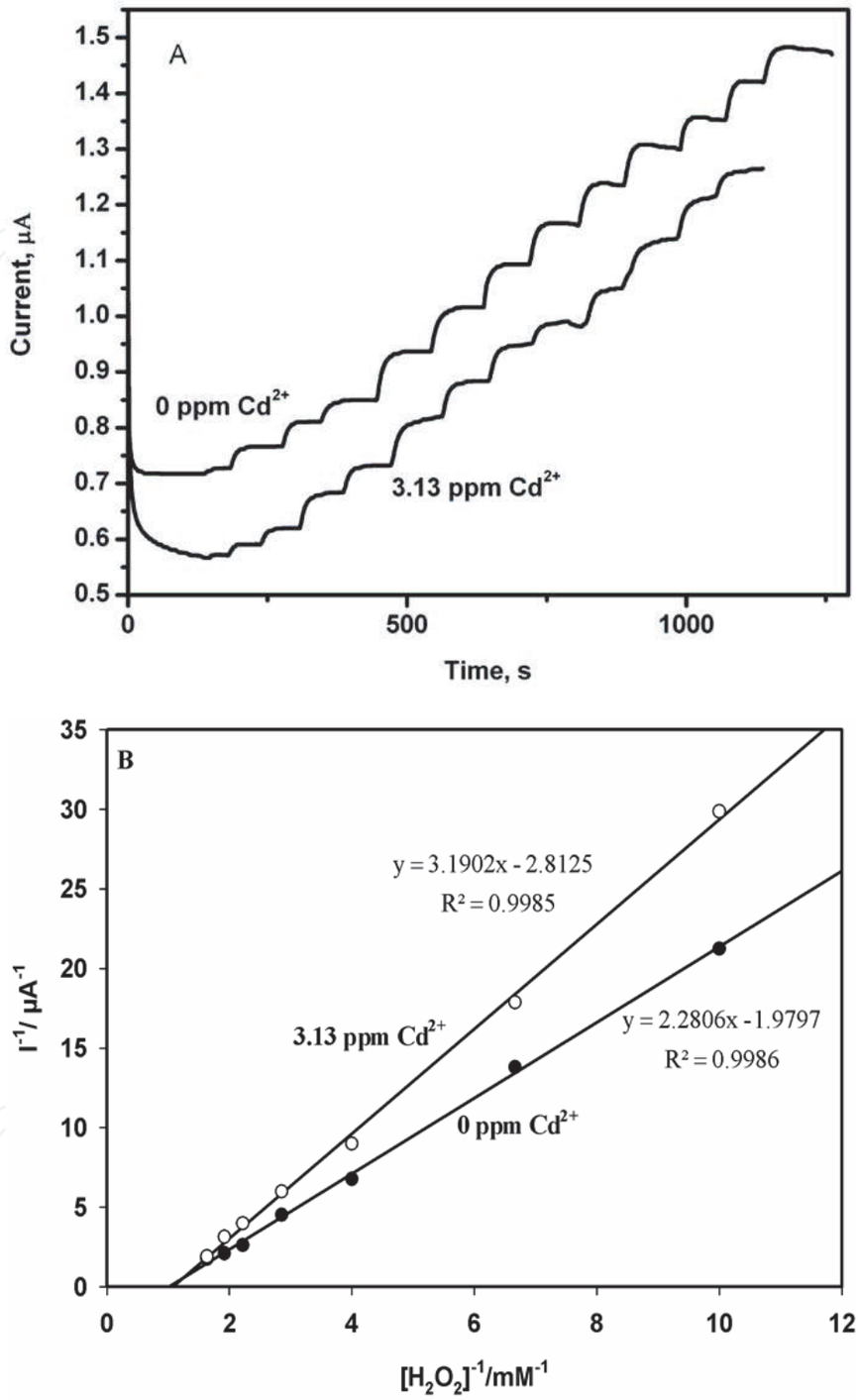
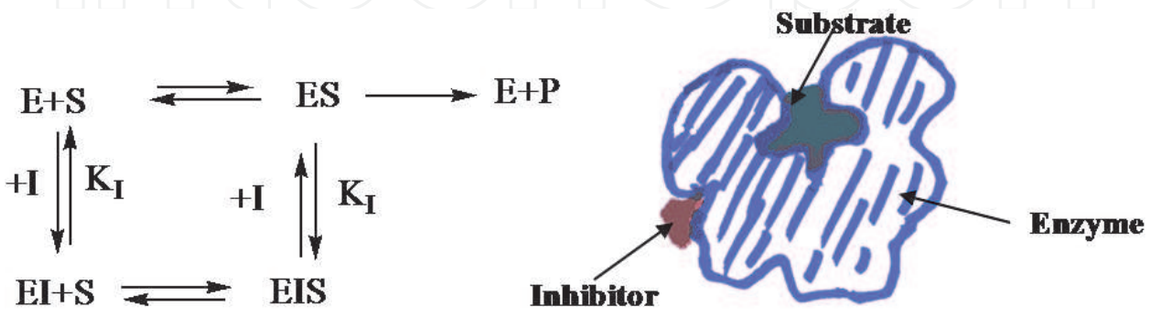


Fig. 6. (A) Pt/PANI/HRP biosensor response to successive additions of H_2O_2 in the absence and presence of heavy metals, at applied potential of -0.20 V. (B) Lineweaver- bulk plot for HRP response to H_2O_2 in the absence and presence of heavy metals.

Kinetic parameters	*0 ppm Cd^{2+}*	*3.125 ppm Cd^{2+}*
Slope μA^{-1}mM	2.28	3.19
y-intercept μA^{-1}	1.98	2.81
K_M^{app} /mM	1.16	1.13
I_{max} /μA	0.505	0.356

Table 2. Apparent Michealis-Menten constants (K_M^{app}) and I_{max} in the absence and the presence of Cd^{2+} (3.125 ppm).

The K_M^{app} values for Cd^{2+} in the absence (1.16 mM) and the presence (1.13 mM) of the inhibitor were not significantly different (based on statistical t-test, n =3, p = 0.05), whereas the I_{max} values decreased. The decrease in I_{max} indicates that the type of inhibition in this study is reversible and also non-competitive inhibition and the inhibition constant was found to be 27.8 μM (Amine et al. 2006; Stoytcheva 2002). Non-competitive inhibition occurs when the inhibitor binds to both the enzyme and enzyme-substrate complex. The possible mechanism of non-competitive inhibition can be seen in Scheme 1 (Amine et al. 2006); where E represents HRP enzyme, S represents H_2O_2, ES represents Compound I containing an oxyferryl centre with the iron in the ferryl state ($Fe^{IV}=O$), and a porphyrin π cation radical; EIS presents HRP-heavy metal-H_2O_2 complex; I represents heavy metal cation and P represents H_2O.



Scheme 1. Mechanisms for reversible non-competitive enzyme inhibition.

18. Selectivity of Pt/PAN/HRP biosensor

In order to show the selectivity of Pt/PANI/HRP biosensor, the response of possible interferences in tap water (drinking water) components on the determination of heavy metals were investigated. A range of cations were measured to examine if they interfered with the determination of heavy metal cations. The effect of possible interferences in drinking water such as Mg^{2+}, Zn^{2+}, Ca^{2+}, K^{+}, Fe^{3+} and Na^{+} was investigated by Pt/PANI/HRP biosensor under the same working conditions. Table 3 presents the possible interferences tested with the biosensor. It can be observed that most cations had minimal effect (<5%) on the determination of heavy metals apart from Fe^{3+} which also inhibit HRP activity. However, at the same concentration (1.0 ppm), the degree of inhibition by interferent is still less than that of the analyte cations

Interferent	Concentration (ppm) Added	%Inhibition
Fe^{3+}	1.0	7.46
Fe^{2+}	1.0	2.01
Zn^{2+}	1.0	1.86
Mg^{2+}	1.0	2.43
Na^{+}	1.0	2.58
K^{+}	1.0	2.06
Ca^{2+}	1.0	2.56

Table 3. Interference studies showing the effects of cations on the response of Cd^{2+}, Pb^{2+} and Cu^{2+}.

Apart from the degree of inhibition of the interfering species, the selectivity of the biosensor was also evaluated by two methods; mixed and separate solutions (Stefan et al. 2001; Macca & Wang 1995) with respect to Cd^{2+}, Cu^{2+} and Pb^{2+}. For the mixed solution method, the concentration of the interfering cation was 1.0 ppm and the concentration of the analyte was 0.5 ppm. In the case of separate solution method, the concentration of the analyte and that of interfering cation were equal (1.0 ppm). Amperometric selectivity coefficients and response ratio were calculated using equations (5) and (6) for mixed solutions and separate solutions (Macca & Wang 1995), respectively.

$$K_{i,j}^{amp} = \left(\frac{\Delta I_j}{\Delta I_t - \Delta I_j} \right) \times \frac{c_i}{c_j} \tag{5}$$

$$R_{i,j} = \frac{\Delta I_j}{\Delta I_i} \tag{6}$$

where ΔI_t, ΔI_j and ΔI_i are current values recorded for mixed solution, interfering cation and analyte of interest, respectively; c_j and c_i are concentrations of the interfering cation and

the analyte, respectively. The $K_{i,j}^{amp}$ and $R_{i,j}$ values in Table 4 suggest that Fe^{3+} is a strong interferent while the other cations species are relatively not interfering with the determination of cadmium, copper and lead. Therefore, the results suggest that Pt/PANI/HRP electrode can be used for the determination of cadmium, copper and lead in the presence of other cations except Fe^{3+}.

Table 4. Interference studies showing the effects of cations on the response of Cd^{2+}, Pb^{2+} and Cu^{2+} using mixed (amperometric selectivity coefficients, $K_{i,j}^{amp}$) and separate (response ratio, $R_{i,j}$) solution methods.

Interferent, j	Amperometric selectivity coefficient, $K_{i,j}^{amp}$			Response ratio, $R_{i,j}$		
	Cd	Cu	Pb	Cd	Cu	Pb
Fe^{3+}	0.80	1.33	2.28	0.56	0.74	0.89
Fe^{2+}	4.06×10^{-3}	4.72×10^{-3}	4.50×10^{-3}	1.10×10^{-2}	1.35×10^{-2}	1.61×10^{-2}
Zn^{2+}	5.69×10^{-3}	6.63×10^{-3}	6.31×10^{-3}	1.42×10^{-2}	1.42×10^{-2}	2.25×10^{-2}
Mg^{2+}	3.64×10^{-3}	4.23×10^{-3}	4.03×10^{-3}	9.12×10^{-3}	1.21×10^{-2}	1.24×10^{-2}
Na^{+}	3.64×10^{-3}	4.23×10^{-3}	4.03×10^{-3}	6.15×10^{-3}	1.12×10^{-2}	1.44×10^{-2}
K^{+}	4.15×10^{-3}	4.15×10^{-3}	3.95×10^{-3}	8.95×10^{-3}	1.19×10^{-2}	1.42×10^{-2}
Ca^{2+}	3.66×10^{-3}	3.66×10^{-3}	3.49×10^{-3}	7.90×10^{-3}	1.05×10^{-2}	1.25×10^{-2}

19. Application of Pt/PANI/HRP for analysis heavy metals in tap water and landfill leachate samples

The performance of the biosensor (Pt/PANI/HRP) was tested using both tap water and landfill leachate water samples. The latter were collected from Sensor Lab in the University of Western Cape (Cape Town, South Africa) and Marrianhill landfill in Durban (South Africa). The quantification of Cd^{2+}, Pb^{2+} and Cu^{2+} in water samples was achieved by employing the standard addition method. For tap water sample, the sample preparation was not required. This is because the possible interferences that are normally present drinking water samples did not have much effect on the catalytic activity of immobilized HRP. The procedure involved initially measuring the response of the biosensor after subsequent additions of 0.05 mM H_2O_2 followed by metal-spiked tap water (0.1 ppm metal ion solution), into the PBS solution containing H_2O_2 (0.5 mM). On addition of the water sample, current intensity was observed to decrease and the magnitude of the decrease was proportional to the amount of metal spiked. The decrease in the response current was most likely due to the inhibition of the enzyme by the metal ions. A possible dilution effect due to increased volume of added tap water was checked by setting a control experiment where deionized water was used instead of metal spiked tap water. The inhibition effect was not observed with the deionized water. In order to double-check the effect of metal inhibition, H_2O_2 was added into the mixture and resulted in an increase in current intensity. Similar

observations have been made by Han et al. (2001); Stoytcheva (2002) and Ghica & Brett (2008).

The concentrations of Cd^{2+}, Pb^{2+} and Cu^{2+} (0.4507 ppb Cd^{2+}, 0.2201 ppb Pb^{2+}, 41.77 ppb Cu^{2+}) in the tap water sample (analysed in triplicate for each metal) were calculated from the calibration curves and the results are presented in Table 5.

	Tap water sample		Landfill leachate water sample	
Cation	**Amperometric Biosensor**	**ICP-OES**	**Amperometric Biosensor**	**ICP-OES**
Cd^{2+}	0.46 ± 0.004	0.34 ± 0.05	ND	ND
Pb^{2+}	0.22 ± 0.0008	ND	ND	ND
Cu^{2+}	41.8 ± 0.07	41.5 ± 0.2	14.6 ± 0.09	14.0 ± 0.03

Concentrations were determined in ppb; ND: not detected, ± standard error ($SE_{\bar{x}} = \frac{s}{\sqrt{n}}$, where s is the sample standard deviation and n is the size of the sample).

Table 5. Comparison between the two analytical techniques: Amperometric biosensor and ICP-OES for determination of heavy metals in Tap water sample.

The values obtained were compared against the allowed MCLs by USEPA in drinking water. The MCLs are given as 5 ppb, 15 ppb and 1300 ppb for cadmium, lead and copper, respectively. The World Health Organization (WHO 2004) on the other hand has given the guideline values for cadmium, lead and copper in drinking water as 3.0 ppb, 10 ppb and 2000 ppb, respectively. It can be seen that the concentrations of Cd^{2+}, Pb^{2+} and Cu^{2+} obtained in this study are much lower than the USEPA and WHO guideline values, implying that the drinking water was not contaminated and therefore safe for human consumption.

In the case of landfill leachate water sample, sample preparation was performed. Solid phase extraction (amperometric biosensor) and UV digestion (ICP-OES) were used to minimize organic interferences. After removal of organics the same procedure that was applied for tap water sample was followed. Summary of the concentrations found by the fabricated biosensor are presented in Table 5.

Results of heavy metals obtained by amperometric biosensor and ICP-OES were compared statistically by Student t-test (two-tailed). At 95% confidence interval, the results by the two analytical techniques for the determination of Cu^{2+} were not significantly different ($p = 0.035 < 0.05$). However, the two methods differed significantly for the determination of cadmium, at 95% confidence interval ($P = 0.116 > 0.05$). The reason for this discrepancy could be the difference in limits of detection (LOD) capabilities by the two techniques. The detection limit for cadmium was 2.70 ppb with the ICP-OES and 0.091 ppb (91 ppt) with the biosensor. Thus the detection limit for Cd^{2+} obtained with the biosensor is lower by a magnitude of 30 to that by ICP-OES. It should be noted that the concentration of Cd^{2+} in the tap water sample was less than the LOD of ICP-OES (LOD = 2.70 ppb cited from ICP-OES operation manual). This explains the poor precision when the tap water sample was analyzed by ICP-OES in triplicate (3 repeated runs gave 0.41, 0.25 and 0.35 ppb). Lead on the other hand, was not

detected probably because its level was lower than 90 ppb (conc. of Pb^{2+} was 0.22 ppb by the biosensor). It can be concluded that the Pt/PANI/HRP biosensor technique is suitable for determination of ultra trace levels of heavy metals in drinking water.
The amount of copper in landfill leachate samples (Table 5) showed good correlation between the results obtained with the biosensor and the standard technique. Applying the Student t test, it was possible to verify that the averages obtained by the both methods are not significantly different at a confidence level of 95% ($P = 0.009 < 0.05$). With both methods, cadmium and lead were not detected.

20. Conclusions

An inhibition amperometric biosensor for the determination of selected heavy metals was fabricated on the base of the inhibition to horseradish peroxidase, which was immobilized on platinum-polyaniline electrode. Inhibition of HRP activity by heavy metals followed noncompetitive reversible mechanism. The HRP biosensor exhibited fast response, high sensitivity towards the determination of heavy metals (LOD of 0.091, 0.033 and 0.10 ppb for Cd^{2+}, Pb^{2+} and Cu^{2+}, respectively). The fabricated biosensor was applied for the determination of heavy metals in real samples (landfill leachate and tap water). The evaluation of the amperometric biosensor measurements against the standard technique (ICP-OES technique) verified the suitability of biosensor for rapid analysis of heavy metals. Moreover, the amperometric biosensor requires minimal sample preparation as compared to the tiresome sample pretreatment procedures required prior to metal determination by the conventional ICP-OES method.

21. Acknowledgement

The authors are grateful for NRF funding through grant-holder Dr Anna Soares. They also acknowledges the Sensor Lab at University of Western Cape where part of the work for this study was carried out as well as NRF sponsorships through grant-holder Prof E I Iwuoha

22. References

Amine, A., Mohammadi, H., Bourais I. & Palleschi G. (2006). Enzyme inhibition-based biosensors for food safety and environmental monitoring. *Biosensors and Bioelectronics* 21: 1405–1423.

Ansari, A. A., Solanki, P. R. & Malhotra, B. D. (2009). Hydrogen peroxide sensor based on horseradish peroxidase immobilized nanostructured cerium oxide film. *Journal of Biotechnology* 142: 179–184.

Baba, A., Tian, S., Stefani, F., Xia, C., Wang, Z., Advincula, R. C., Johannsmann, D. & Knoll, W. (2004). Electropolymerisation and doping/dedoping properties of polyaniline thin films as studied by electrochemical-surface plasmon spectroscopy and by the quartz crystal microbalance. *Journal of Electroanalycal Chemistry.* 562: 95–103.

Bard, A. J. & Faulkner, L. R. (2000). *Electrochemical Methods: Fundamentals and Applications*, 2nd ed., New York: J. Wiley and Sons.

Berezhetskyy, A. L., Sosovska, O. F., Durrieu, C., Chovelon, J-M., Dzyadevych, S. V. & Tran-Minh, C. (2008). Alkaline phosphotase conductometric biosensor for heavy metal ions determination. *ITBM-RBM* 29: 136-140.

Bettinelli, M., Beone, G. M. Spezia, S. & Baffi, C. (2000). Determination of heavy metals in soils and sediments by microwave-assisted digestion and inductively coupled plasma optical emission spectrometry analysis. *Analytica Chimica Acta* 424: 289–296.

Brainina, K. Z., Stozhko, N. Y., Belysheva, G. M., Nzhevatova, O. V., Kolyadina, L. I., Cremisini, C. & Galletti, M. (2004). Determination of heavy metals in wine by anodic stripping voltammetry with thick film modified electrode. *Analytica Chimica Acta* 514: 227-234

Cecconi, I., Scaloni, A., Rastelli, G., Moroni, M., Vilardo, P. G., Costantino, L., Cappiello, M., Garland, D., Carper, D. Petrash, J. M., Corso A. D. & Mura, U. (2002). Oxidative Modification of Aldose Reductase Induced by Copper Ion: Definition of the metal–protein interaction mechanism. *Journal of Biological Chemistry* 277: 42017-42027.

Gau, V., Ma, S-H., Wang, H., Tsukuda, J., Kible, J. & Haake, D. A. (2005). Electrochemical molecular analysis without nucleic acid amplification. *Methods* 37: 73–83.

Gerard, M., Chaubey, A. & Malhotra, B. D. (2002). Application of conducting polymers to biosensors: Review. *Biosensors and Bioelectronics* 17: 345-359.

Ghica, M. E. & Brett, C. M. A. (2008). Glucose oxidase inhibition in poly(neutral red) mediated enzyme biosensors for heavy metal determination. *Microchimica Acta* 163: 185-193.

Giardi, M. T., Koblızek, M. & Masojıdek, J. (2001). Photosystem II-based biosensors for the detection of pollutants. *Biosensors and Bioelectronics* 16: 1027–1033.

Han, S., Zhu, M., Yuan, Z. & Li, X. (2001). A methylene blue- mediated enzyme electrode for the determination of trace mercury (II), mercury (I), methylmercury, and mercury-glutathione complex. *Biosensors and Bioelectronics* 16: 9-16.

Ilangovan, R., Kranstanov, D. A., Zachariah, C. & Elizabeth, R. (2006). Enzyme based biosensor for heavy metals ions determination. *Biotechnology & Biotechnological Equipment* 184-189.

Kang, E. T., Neoh, K. G. & Tan, K. L. (1998). Polyaniline: A polymer with many interesting intrinsic redox states. *Progress in Polymer Science* 23: 211-324.

Kan, J., Zhou, S., Zhang, Y. & Patel, M. (2006.) Synthesis and characterization of polyaniline nanoparticles in the presence of magnetic field and samarium chloride. *European Polymer Journal* 42: 2004–2012.

Kenawy, I. M. M., Hafez, M. A. H., Akl, M. A. & Lashein, R. R. (2000). Determination by AAS of some trace heavy metal ions in some natural and biological samples after their preconcentration using newly chemically modified chloromethylated polystyrene-PAN ion exchanger. *Analytical Science* 16: 493-500.

Kim, B-J., S-G. Oh, M-G. Han & S-S. Im. (2001). Synthesis and characterization of polyaniline nanoparticles in SDS micellar solutions. *Synthetic Metals* 122: 297–304.

Lakshmi, G. B. V. S., Dhillon, A. Siddiqui, A. M., Zulfequar, M. & Avasthi, D. K. (2009). RF-plasma polymerization and characterization of polyaniline. *European Polymer Journal* 45: 2873–2877.

Lapkowski. M. (1990). Electrochemical synthesis of linear polyaniline in aqueous solutions *Synthetic Metals* 35 : 169-182.

Laska, J. & Widlarz, J. (2005). Spectroscopic and structural characterization of low molecular weight fractions of polyaniline. *Polymer* 46: 1485–1495.

Liu, S-Q. & Ju, H-X. (2002). Renewable reagentless hydrogen peroxide sensor based on direct electron transfer of horseradish peroxidase immobilized on colloidal gold-modified electrode. *Analytical Biochemistry* 307: 110–116.

Luo, X., Killard, A. J., Morrin, A. & M. R. Smyth. (2006). Enhancement of a conducting polymer-based biosensor using carbon nanotube-doped polyaniline. *Analytica Chimica Acta* 575: 39–44.

Malitesta, C. & Guascito, M. R. (2005). Heavy metal determination by biosensors based on immobilized by electropolymerisation. *Biosensors and Bioelectronics* 20: 1643-1647.

Martha, S. K., Hariprakash, B., Gaffoo, S. A., Trivedi, D. C. & A. K Shukla. (2006). A low-cost lead-acid battery with high specific-energy. *Journal Chemical Science* 118: 93–98.

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

Mazeikiene, R.; Tomkute, V., Kuodis, Z., Niaura, G. and Malinauskas, A. (2007). Raman spectroelectrochemical study of polyaniline and sulfonated polyaniline of different pH. *Vibrational Spectroscopy* 44: 201-208.

Mohammadi, H., Amine, A., Cosnier S., & Mousty, C. (2005). Mercury-enzyme inhibition assays with an amperometric sucrose biosensor based on a trienzymatic –clay matrix. *Anal. Chim. Acta* 543: 143-149.

Mu, S., Chen, C. & Wang. J. (1997). The kinetic behaviour for the electrochemical polymerization of aniline in aqueous solution. *Synthetic Metals* 88: 249-254.

Ndangili, P. M., Waryo, T. T., Muchindu, M., Baker, P. G. L., Ngila, J. C. & Iwuoha, E. I. (2009). Ferrocenium hexafluorophosphate-induced nanofibrillarity of polyaniline–polyvinyl sulfonate electropolymer and application in an amperometric enzyme biosensor. *Electrochimica Acta* 55: 4267–4273.

Pournaghi-Azar, M. H. & Habibi, B. (2007). Electropolymerisation of aniline in acid media on the bare and chemically pre-treated aluminum electrodes: A comparative characterization of the polyaniline deposited electrodes. *Electrochim. Acta* 52: 4222–4230.

Prechthai, T., Parkpian, P. & Visvanathan C. (2008). Assessment of heavy metal contamination and its mobilization from municipal solid waste open dumping site. *Journal of Hazardous Material* 156: 86-94.

Rahmi, D., Zhu, Y., Fujimori E., Umemura, T. & Haraguchi H. (2007). Multielement determination of trace metals in seawater by ICP-MS with the aid of with aid of down-sized chelating resin packed microcolumn for preconcentration. *Talanta* 72: 600-606.

Ruzgas, T., Gorton, L., Emneus, J. & Marko-Varga G. (1995). Kinetic models of horseradish peroxidase action on a graphite electrode. *Journal of Electroanalytical. Chemistry*391: 41-49.

Seo, K-D., Lee, K-P, Gopalan, A. I., Chung, S. J., Lim, Y. T. & Choi S-H. (2007). Horseradish Peroxidase (HRP) Immobilized Poly(aniline-co-*m*-amino the electrocatalytic behaviour of the enzyme phenol) film electrodes–fabrication and evaluation as hydrogen peroxide sensor. *Sensors* 7: 719-729.

Singh, K., A. Ohlan, R. K. Kotnala, A. K. Bakhshi & Dhawan, S. K. (2008). Diectric and magnetic properties of conducting ferromagnetic composite of peolyaniline with γ-Fe_2O_3 nanoparticles. *Material Chemistry and Physics.* 112: 651-658.

Songa, E. A.; Arotiba, O. A., Owino, J. H. O., Jahed, N., Baker, P. G. L. & Iwuoha E. I. (2009). Electrochemical detection of glyphosate herbicide using horseradish peroxidase immobilized on sulfonated polymer matrix. *Bioelectrochemitry* 75: 117-123.

Stoytcheva, M., (2002). Electrochemical Evaluation of the Kinetic Parameters of a Heterogeneous Enzyme Reaction in Presence of Metal Ions. *Electroanalysis* 14: 923-927.

Sun, D., Cai, C., Li, X., Xing W. & Lu, T. (2004). Direct electrochemistry and bioelectrocatalysis of horseradish peroxidase immobilized on active carbon. *J. electroanalytical Chemistry* 566: 415-421.

Tayefi-Nasrabadi, H., Keyhani, E. & Keyhani J. (2006). Conformational changes and activity alterations induced by nickel ion in horseradish peroxidase. *Biochimie* 88: 1183–1197.

Tong, Z.; Yuan, R., Chai. Y., Chen, S., Xie, Y. (2007). Amperometric biosensor for hydrogen peroxide based on Hemoglobin/DNA/Poly-2,6-pyridinediamine modified gold electrode. *Thin Solid Films* 515: 8054–8058.

Tsai, H-C., Doong, R-A. Chiang, H. C. and Chen K. T. (2003). Sol-gel derived urease-based optical biosensor for rapid determination of heavy metals. *Analytica Chimica Acta* 481: 75-84.

Tuzen, M., Sayji, K. O. & Soylak, M. (2008). Solid phase extraction of heavy metal ions in environmental samples on multiwalled carbon nanotubes. *Journal of Hazardous Materials* 152: 632-639.

Uriu-Adams, J. Y. & Keen, C. L. (2005). Copper, oxidative stress, and human health. *Molecular Aspects of Medicine* 26: 268–298.

USEPA (1991), "USEPA Maximum contaminant level goals and national primary drinking water regulations for lead and copper; final rule", Federal Register, Vol. 56 110, 26460-9, 40 CFR Parts 141 and 142.

Wang, L. & Wang, E. 2004. Novel hydrogen peroxide sensor based on horseradish peroxidase immobilized on colloidal Au modified ITO electrode. *Electrochemical Communication* 6: 225–229.

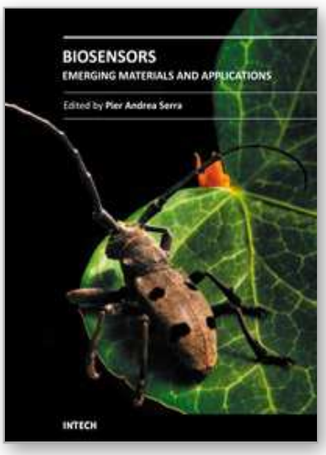
Wang, P., Li, S. & Kan, J. (2009a). A hydrogen peroxide biosensor based on polyaniline/FTO. *Sensors and Actuators* B 137: 662–668.

Wang, X-J., Xia, S-Q., Zhao, J-F., Zhao, H-N. & Renault, N. J. (2009b). Inhibitive Determination of heavy metal ions by conductometric nitrate reductase biosensor. *Chemical Research in Chinese Universities* 25: 443-44.

World Health Organization (WHO) (2004), Guidelines for Drinking Water Quality, 3rd ed., Vol. 2, World Health Organization (WHO), Geneva.

Yin, H., Ai, S., Shi, W. & Zhu, L. (2009). A novel hydrogen peroxide biosensor based on horseradish peroxidase immobilized on gold nanoparticles–silk fibroin modified glassy carbon electrode and direct electrochemistry of horseradish peroxidase. *Sens. Actuators* B 137: 747–753.

Zhang, L. and Lian J. (2007). Electrochemical synthesis of copolymer of aniline and o-aminophenol and its use to the electrocatalytic oxidation of ascorbic acid. *J. Electroanalytical Chemitry* 611: 51–59.



Biosensors - Emerging Materials and Applications
Edited by Prof. Pier Andrea Serra

ISBN 978-953-307-328-6
Hard cover, 630 pages
Publisher InTech
Published online 18, July, 2011
Published in print edition July, 2011

A biosensor is a detecting device that combines a transducer with a biologically sensitive and selective component. Biosensors can measure compounds present in the environment, chemical processes, food and human body at low cost if compared with traditional analytical techniques. This book covers a wide range of aspects and issues related to biosensor technology, bringing together researchers from 19 different countries. The book consists of 27 chapters written by 106 authors and divided in three sections: Biosensors Technology and Materials, Biosensors for Health and Biosensors for Environment and Biosecurity.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Philiswa N. Nomngongo, J. Catherine Ngila1, and Titus A. M. Msagati (2011). Indirect Amperometric Determination of Selected Heavy Metals Based on Horseradish Peroxidase Modified Electrodes, Biosensors - Emerging Materials and Applications, Prof. Pier Andrea Serra (Ed.), ISBN: 978-953-307-328-6, InTech, Available from: http://www.intechopen.com/books/biosensors-emerging-materials-and-applications/indirect-amperometric-determination-of-selected-heavy-metals-based-on-horseradish-peroxidase-modifie

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen