Inhibitive Determination of Metal Ions Using a Horseradish Peroxidase Amperometric Biosensor

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1. Introduction

The development of electro-analytical methods for the determination of mercury, lead, cadmium and various other trace metals in acidic media or at different pH values are not new and for that reason, the investigation of alternative techniques have been ongoing and especially to find mercury-free electrodes (Ugo et al., 1995). Stripping voltammetry has been widely used for trace metal analysis with mercury as the working electrode due to its remarkable analytical properties. However, due to the toxicity of mercury and the human health risk that it poses (bioaccumulation in the food chain), there have been insistent efforts to remove the use of mercury completely. Electroanalysis has therefore seen the use of mercury-free sensors, while much attention has been dedicated to the development of such sensors over the last decade (Hwang et al., 2008; Sonthalia et al., 2004). Several heavy metals create environmental and human health concerns when elevated concentrations of these metals are present in the environment. In this regard, lead (Pb) and mercury (Hg) and more increasingly cadmium (Cd) heavy metals are of prime environmental concern, since they are significant for environmental surveillance, food control, occupational medicine, toxicology and hygiene (Ensafi and Zarei, 2000). Lead (Pb) is furthermore constantly monitored in natural and drinking water due to the harmful effects that are often manifested in young children (Zen et al., 2002). It is also known that several trace metals are regarded as essential micro-nutrients and play an integral role in the life processes of living organisms. In contrast, metals such as aluminium, silver, cadmium, gold, lead and mercury play no biological role in living organisms and lead to toxicity

and adverse human health effects when present (Somerset et al., 2010a; Estevez-Hernandez et al., 2007; Honeychurch et al., 2002).

The simultaneous analysis of metal ions is typically performed with inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), X-ray fluorescence spectrometry (XRF), or atomic absorption spectrometry (AAS). These are well established methods that are characterised by low detection limits, but these methods require expensive instrumentation and trained personnel and cannot be used for field and on-site measurements. On the other hand, anodic stripping voltammetry (ASV) is one of the most favourable techniques for the determination of heavy metal ions due to its low cost, high sensitivity, easy operation and the ability of analysing element speciation (Li et al., 2010; Somerset et al., 2010b).

In this chapter the use of a conducting polymer modified platinum surface on which horseradish perioxidase (HRP) has been immobilised was investigated as an alternative transducer platform for the amperometric analysis of Hg^{2+}, Pb^{2+} and Cd^{2+} ions in aqueous solutions. The results obtained for the quantitative analysis of the metal ions included the detection limit, linear range, sensitivity and R.S.D. for individual metal ions and are discussed in this chapter. Results for the biosensor storage stability and response reproducibility were also investigated and reported.

2. Materials and methods

2.1. Chemicals

The reagents aniline (99%), 2,2´-dithiodianiline (98%), potassium dihydrogen phosphate (99%), hydrogen peroxide (30%), disodium hydrogen phosphate (98%) and diethyl ether (99.9%) were obtained from Sigma-Aldrich, Germany. The enzyme peroxidase (EC 1.11.1.7 type IV from horseradish) was also purchased from Sigma-Aldrich, Germany. The potassium chloride, sulphuric acid (95%), ethanol (98%) and hydrochloric acid (32%) were obtained from Merck, South Africa. The standards for cadmium (Cd), lead (Pd) and mercury (Hg) were purchased as atomic adsorption standard solutions (1000 mg/l) and purchased from Sigma-Aldrich, Germany.

2.2. Apparatus

Electrochemical protocols were performed with a PalmSens portable potentiostat / galvanostat, with the PSTrace program and accessories (PalmSens® Instruments BV, 3992 BZ Houten, the Netherlands). The portable potentiostat was interfaced with a microcomputer controlled by PS 2.1 software for data acquisition and experimental control.

A conventional three-electrode system was employed consisting of a platinum (Pt) working electrode, a BAS 3 M NaCl-type Ag/AgCl reference electrode, and a platinum wire auxiliary electrode (Somerset et al., 2010a).

2.3. Construction of Pt/PANI/HRP biosensor

2.3.1. Electrosynthesis of polymer film on platinum electrode

During electropolymerisation a three-electrode cell with a 10 ml capacity was utilised. Electropolymerisation from a 0.2 M aniline solution dissolved in a 1 M hydrochloric acid (HCl) solution onto a thoroughly cleaned and polished Pt electrode was performed. The aniline / HCl solution was first degassed by passing argon (Ar) through the solution, followed by electropolymerisation to obtain a smooth polymer coated electrode surface. During electropolymerisation the potential was repeatedly scanned from − 200 mV to + 1200 mV, at a scan rate of 40 mV/s. This was done for 20 voltammetric cycles, to ensure a relatively thick polymer film was obtained for enzyme immobilisation (Mathebe et al., 2004; Somerset et al., 2009; Somerset et al., 2010a; Nomngongo et al., 2011).

2.3.2. Enzyme immobilisation and biosensor preparation

Electropolymerisation of a fresh PANI polymer film on a Pt electrode was followed by activation of the polymer film for enzyme attachment. The Pt/PANI electrode was transferred to a batch cell, containing 1 ml of a 0.1 M phosphate buffer (pH 6.8) solution, degassed with argon. The fresh PANI polymer film was first reduced at a potential of - 500 mV (vs. Ag/AgCl) until a steady current was achieved. The Pt/PANI bioelectrode was next transferred to a 0.1 M phosphate buffer (pH 6.8) solution, containing HRP (2 mg/ml in 1.0 ml fresh buffer solution). The enzyme solution was then argon degassed, after which enzyme immobilisation onto the Pt/PANI bioelectrode was achieved by oxidation of the PANI film in the presence of HRP at a potential of + 400 mV (vs. Ag/AgCl), until steady-state current was achieved. The resulting biosensor will be referred to as Pt/PANI/HRP biosensor (Morrin et al., 2003; Mathebe et al., 2004; Somerset et al., 2009; Nomngongo et al., 2011).

2.4. Biosensor response evaluation

2.4.1. Voltammetric measurements

Cyclic and differential pulse voltammetry measurements were used to monitor the responses of the Pt/PANI/HRP biosensor towards hydrogen peroxide (H_2O_2) as substrate (Morrin et al., 2005; Mathebe et al., 2004; Nomngongo et al., 2011). Cyclic voltammetry (CV) was performed at a slow scan rate of 10 mV/s in order to study the catalytic oxidation of H_2O_2 by applying a potential scan between + 400 mV and - 1200 mV (vs. Ag/AgCl). Furthermore, to differentiate between the voltammetric responses, the cyclic voltammogram was first recorded in the absence of the substrate, followed by analysis in the presence of H_2O_2 as substrate. This was achieved by sequential addition of 1 mM of H_2O_2 solution to the 1 ml of 0.1 M phosphate buffer (PB) solution, degassed with argon that was repeated after each addition of the substrate (Chen and Gu, 2008; Nomngongo et al., 2011).

Differential pulse voltammetry (DPV) immediately followed the CV analysis in the same solution mentioned in the previous paragraph. The cathodic difference differential pulse voltammogram (DPV) was collected in the reduction direction only by scanning the potential between

+ 400 mV and - 1200 mV (vs. Ag/AgCl), at a potential step of 20 mV and a pulse amplitude of 20 mV. As for the CV analysis, the DPV measurements were first obtained in the absence of the substrate, followed by analysis in the presence of H_2O_2 as substrate (Nomngongo et al., 2011).

2.4.2. Inhibition response measurements

The electrochemical cell prepared for biosensor inhibition measurements consisted of the Pt/ PANI/HRP bioelectrode, a platinum wire and Ag/AgCl as the working, counter and refer- ence electrode, respectively. Inhibition measurements were performed in a 1 ml test solution containing 0.1 M PB solution that was degassed with argon before any substrate was added and after each addition of small aliquots of 1 mM H_2O_2 solution.

Inhibition plots for each of the heavy metals studied (e.g. Cd^{2+}, Pb^{2+}, and Hg^{2+}) were obtained using the percentage inhibition method. This procedure involved the study of the Pt/ PANI/HRP biosensor in the presence of H_2O_2 solution first, followed by exposure to sequential additions of the heavy metal solutions. The heavy metal concentrations evaluated during se- quential addition were 0.001 ppb, 0.005 ppb and 0.01 ppb for each of Cd^{2+}, Pb^{2+}, and Hg^{2+}.

For the inhibition studies, the Pt/PANI/HRP biosensor was first placed in a stirred 1 ml of 0.1 M PB solution (anaerobic conditions) and multiple additions of a standard peroxide sub- strate solution was added until a stable current and a maximum concentration of 6 mM were obtained. This steady state current was related to the activity of the biosensor with no inhibitor present. In the second phase of the inhibition studies, the biosensor was transferred to a fresh 1 ml of 0.1 M PB solution (anaerobic conditions) and multiple additions of a stand- ard heavy metal solution (e.g. Cd^{2+}, Pb^{2+}, and Hg^{2+}) was again added, until a stable current was obtained (Nomngongo et al., 2011).

The percentage inhibition was then calculated using the formula (Somerset et al., 2007; Guascito et al., 2008; Nomngongo et al., 2011):

$$I\% = \frac{I_1 - I_2}{I_1} \times 100\% \tag{1}$$

where $I\%$ is the degree of inhibition, I_1 is the steady-state current obtained in buffer solution with no heavy metal ion present, while I_2 is the steady-state current obtained after the bio- sensor was exposed to sequential additions of the separate heavy metal ions of Cd^{2+}, Pb^{2+}, and Hg^{2+} respectively.

3. Results and discussion

3.1. Cyclic voltammetric characterisation of PANI electropolymerisation

In Figure 1, the cyclic voltammogram (CV) for the electropolymerisation of polyaniline (PANI) on a Pt electrode is shown, which was obtained by cycling the potential between − 200 and + 1100 mV at a scan rate of 40 mV/s.
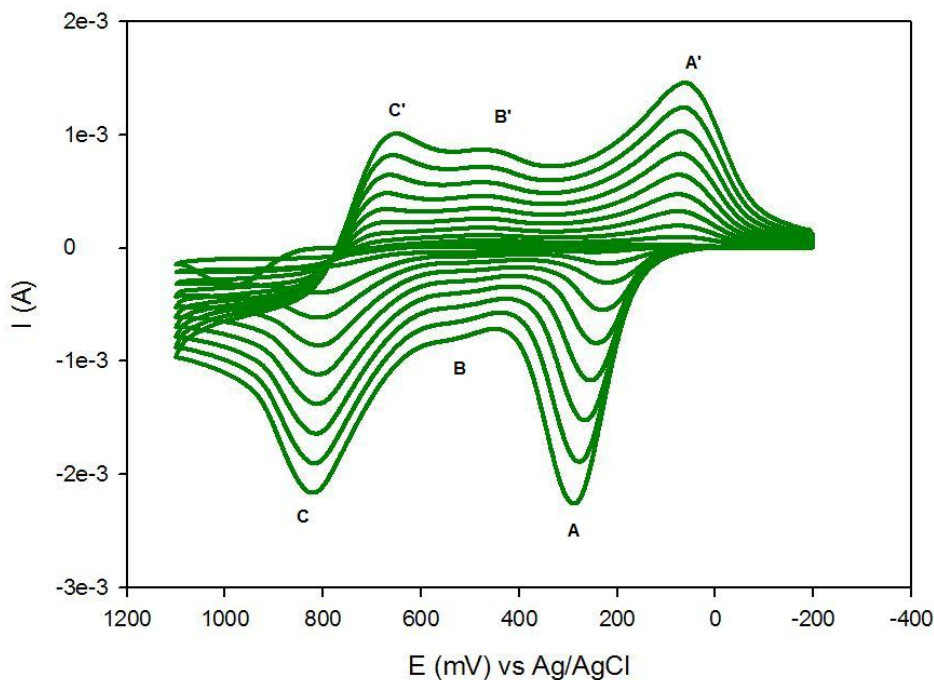
Figure 1. Results for the electropolymerisation of PANI in a 1 M HCl solution on a Pt electrode with the potential scanned from – 200 to +1100 mV at a scan rate of 40 mV/s.

The results obtained for the electropolymerisation of PANI in Figure 1, shows that PANI displays very good redox activity at acidic pH. The CV obtained in Figure 1 further shows two main anodic peaks in A and C, which corresponds to the transformation of leucoemeraldine base to emeraldine salt and the emeraldine salt to pernigraniline salt forms. The reverse scan in the cathodic direction shows the main peaks C′ and A′ that corresponds to the conversion of pernigraniline salt to emeraldine salt and emeraldine salt to leucoemeraldine base. The small redox couple of (B/B′) in the centre of the centre of the CV can be attributed to impurities such as benzoquinone and hydroquinone. With repetitive cycling of the potential an increase in the redox peaks was observed, which indicated the formation of a conducting polymer on the electrode surface (Mathebe et al., 2004; Morrin et al., 2005; Somerset et al., 2007; Somerset et al., 2010a).

Further characterisation of the electrosynthesised PANI polymer was done to compare the results obtained to that of other researchers. To determine the surface concentration of the PANI film, Γ_{PANI}, Brown-Anson analysis (Bard and Faulkner, 2001) was performed. Similarly, Randles-Sevcik analysis (Bard and Faulkner, 2001) of peak current (Ip) versus square root of scan rate ($v^{1/2}$) was performed, to estimate the electron transport diffusion coefficient, D_e, for electrons within the polymer backbone. A summary of the results are shown in Table 1.

Polymer parameter	Mathebe et al., 2004	Nomngongo et al., 2011	This study
Γ_{PANI}	1.85×10^{-7} mol/cm^2	7.8×10^{-7} mol/cm^2	6.19×10^{-8} mol/cm^2
D_e	8.68×10^{-9} cm^2/s	4.07×10^{-8} cm^2/s	4.94×10^{-10} cm^2/s

Table 1. Comparison of the results obtained for the surface concentration and electron transport diffusion coefficient of the electrosynthesised polymers.

Analysis of the results in Table 1 shows that both the results for the surface concentration of the PANI polymer and the electron transport diffusion coefficient obtained in this study, compares well to that of similar studies performed previously.

3.2. Optimisation of solution pH for Pt/PANI/HRP biosensor

After construction of the Pt/PANI/HRP biosensor, evaluation of the biosensor was performed over the pH range from 4.5 to 7.2, to confirm the optimum current response for the constructed biosensor. A fresh biosensor was constructed and evaluated at each of the pH values evaluated from 4.5 to 7.2. The results obtained are displayed in Figure 2.
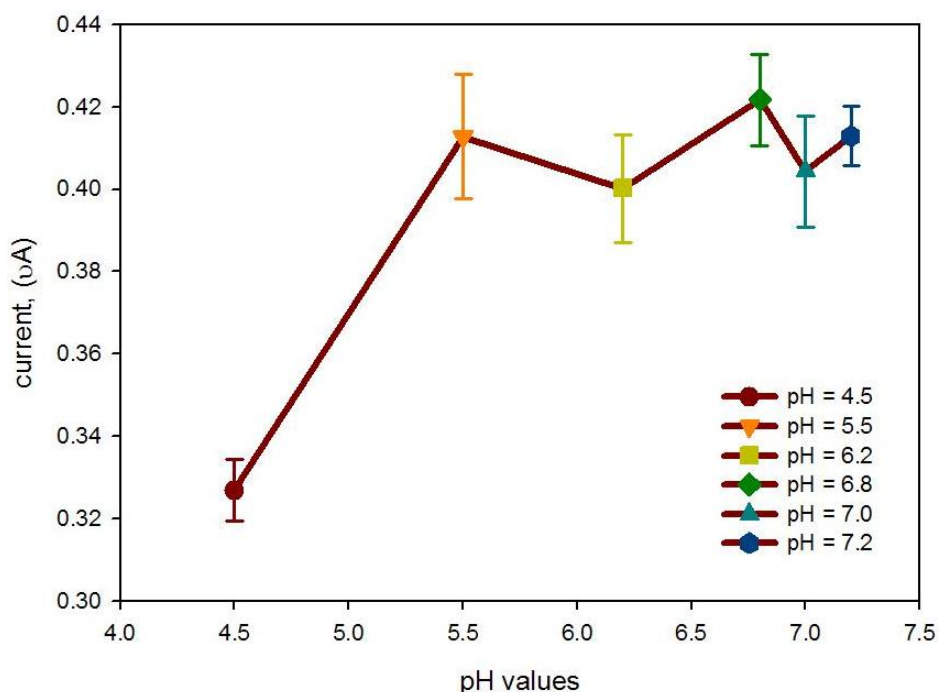

Figure 2. Results obtained for the optimisation of pH for the PtPANI/HRP enzyme electrode in 0.1 M phosphate buffer solution.

Analysis of the results in Figure 2 indicates indicate that a maximum amperometric response and sensitivity was obtained for the biosensor at a pH of 6.8. Similar studies performed on a similar biosensor construction have shown it to operate at an optimum pH range from 6.8 to 7.2 in buffered electrolyte solution. All further biosensor studies was performed at a pH = 6.8 (Mathebe et al., 2004; Nomngongo et al., 2011).

3.3. Differential pulse voltammetric characterisation of Pt/PANI/HRP biosensor

After construction, the amperometric behaviour of the Pt/PANI/HRP biosensor was evaluated in the presence and absence of H_2O_2 as substrate. Both the cyclic and differential pulse behaviour of the biosensor in the presence and absence of the substrate were evaluated, although only the differential pulse voltammetric (DPV) results (Figure 3) will be discussed in this section.

The results obtained for the cyclic voltammetric (CV) behaviour of the Pt/PANI/HRP biosensor (not shown here), have shown that with sequential addition of H_2O_2 to the phosphate buffer (pH = 6.8) solution, the reduction peak current shifted and increased with addition of the substrate (Mathebe et al., 2004; Nomngongo et al., 2011).

In Figure 3 the DPV results obtained for the evaluation of the Pt/PANI/HRP biosensor are shown.
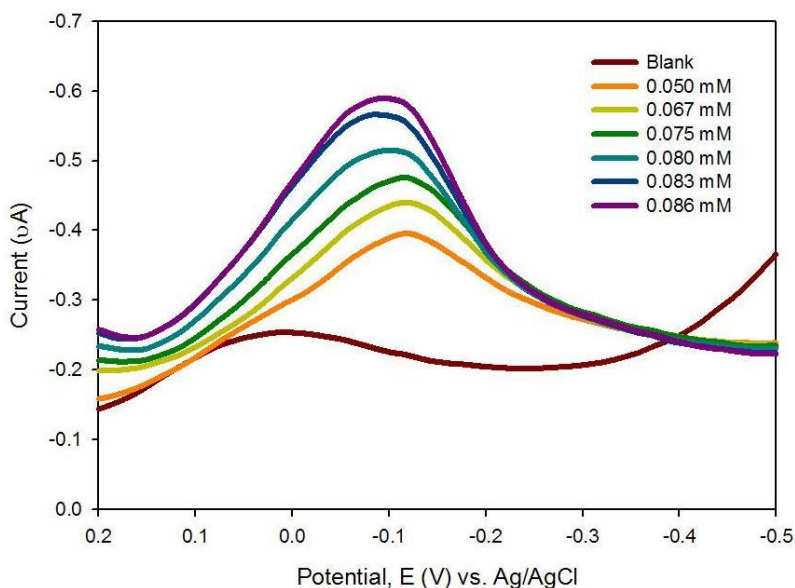

Figure 3. Net cathodic differential pulse voltammograms (DPVs) of the Pt/PANI/HRP biosensor in the presence of increasing concentrations of H_2O_2 as substrate. The experimental conditions were: frequency, 20 Hz; amplitude, 20 mV; and potential step, 20 mV.

The evaluation of the DPV results was done by cycling the potential in the cathodic direction from $+ 0.4$ to $- 1.2$ V (vs. Ag/AgCl). The results obtained in Figure 3 have shown that the in the absence of the substrate, no electrocatalytic reduction of H_2O_2 was observed. However, as soon as H_2O_2 was added, an increase in the current of the peak observed at approximately $- 0.2$ V (vs. Ag/AgCl) occurred, thereby demonstrating effective electrocatalytic reduction of H_2O_2. With subsequent additions of substrate to the 0.1 M PBS, both a positive shift in the reduction peak and reduction peak current was evident, proportional to increased H_2O_2 concentration. Similar observations for the same biosensor were made by Mathebe et al. (2004) and Nomngongo et al. (2011).

In order to explain the DPV results obtained, the mechanism involved for the Pt/PANI/HRP biosensor is shown in Figure 4. This scheme shows that when the Pt/PANI/HRP bioelectrode is charged at a constant potential of $- 0.2$ V (vs. Ag/AgCl), the H_2O_2 substrate reduction charge is propagated along the PANI polymer chain to the Pt electrode surface, by fast electron transfer reactions. It is further shown that the PANI redox species ($PANI^0 \leftrightarrow PANI^+$) is involved in the reduction charge propagation.
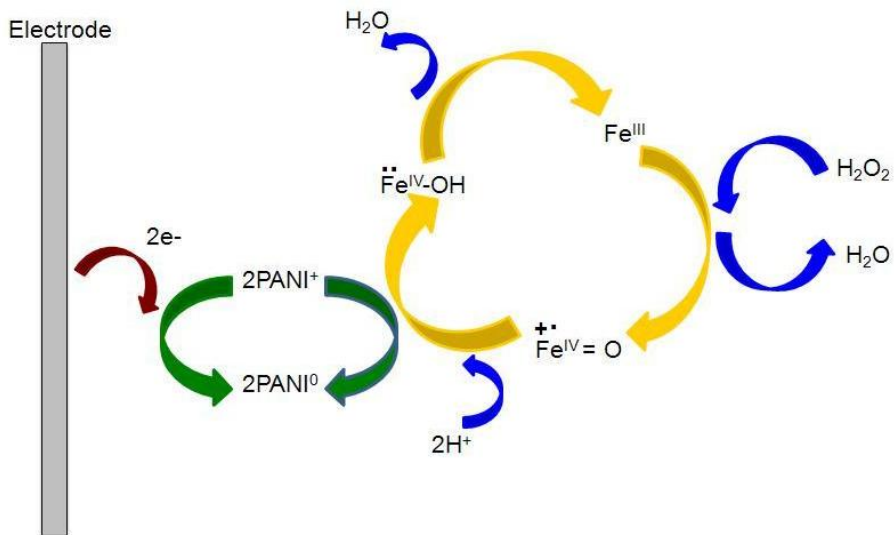
Figure 4. The Pt/PANI/HRP biosensor mechanism indicating the redox species that are either electron donors or hydrogen donors in the reaction mechanism (Iwuoha et al., 1997).

Figure 4 also shows that the substrate H_2O_2 is reduced by HRP (in the ferric (Fe^{III}) resting state) to form water, which results in the oxidation of HRP to form the oxyferryl HRP-I ($Fe^{IV} = 0$) compound. In turn this compound undergoes a two-electron reduction step to form an intermediate compound called hydroxyferryl HRP-II (Fe^{IV}-OH). With continued charge propagation taking place, the hydroxyferryl HRP-II compound goes back to the ferric HRP resting state and the process is repeated (Iwuoha et al., 1997; Nomngongo et al., 2011).

3.4. Cadmium (II), lead(II) and mercury (II) inhibition studies

The results obtained for the inhibition studies for each of the Cd^{2+}, Pb^{2+} and Hg^{2+} metal ions determined with the Pt/PANI/HRP biosensor are discussed in this section. Other studies (Zhao et al., 1996; Shyuan et al., 2008; Nomngongo et al., 2011) have shown that enzymes (e.g. HRP, alkaline phosphatase) are known to be inhibited by metals such as Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+} and Hg^{2+}. Three different heavy metal concentrations were evaluated, ranging from a relatively low, intermediary to higher concentrations. The inhibition results obtained for each of these concentrations (added sequentially) are evaluated and discussed in the following paragraphs.

3.4.1. Inhibition results for lowest metal concentration investigated

The percentage inhibition plots obtained for the inhibition of HRP when aliquots of 0.001 ppb of Cd^{2+}, Pb^{2+} and Hg^{2+} was sequentially added to the 0.1 M PB (pH = 6.8) solution, are shown in Figure 5.
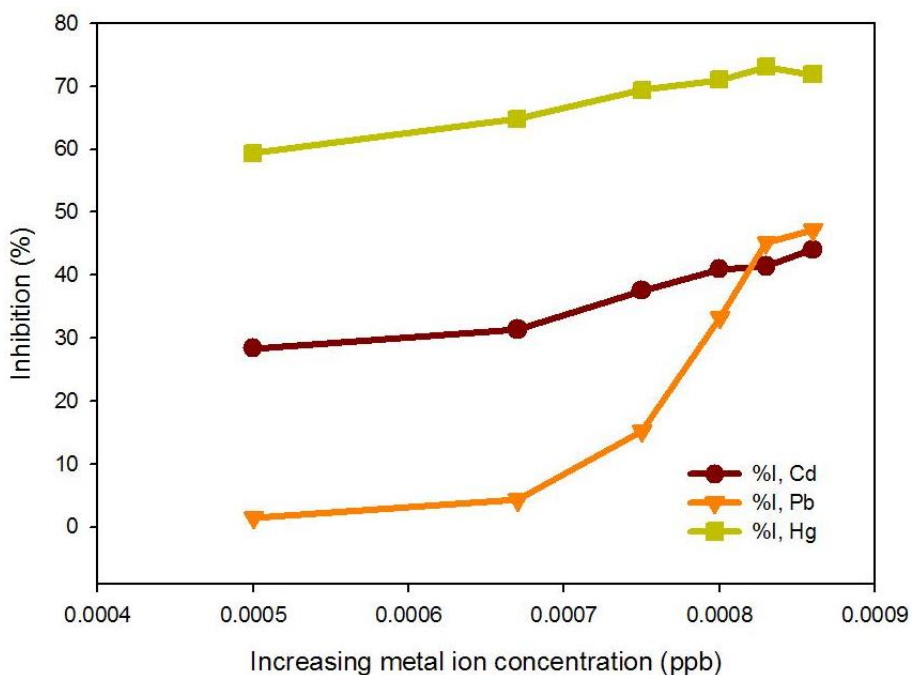

Figure 5. Results obtained for inhibition of the Pt/PANI/HRP biosensor in the presence of 0.001 ppb of Cd^{2+}, Pb^{2+} and Hg^{2+}, respectively.

For the results shown in Figure 5 it was observed that three distinctive patterns of inhibition was obtained for each of the Cd^{2+}, Pb^{2+} and Hg^{2+} metal ions investigated. For metal concen-

trations ranging from 0.001 – 0.004 ppb, it was observed that the decreasing trend of inhibi-tion was $Hg^{2+} > Cd^{2+} > Pb^{2+}$. For higher concentrations the inhibition trend was $Hg^{2+} > Pb^{2+} > Cd^{2+}$. It was further observed that after the initial concentration of 0.001 ppb for each metal was added to the Pt/PANI/HRP biosensor, the first inhibition results were 1.5% (Pb^{2+}), 28.4% (Cd^{2+}) and 59.4% (Hg^{2+}), respectively. This was a clear indication of the initial toxicity of the respective metal ions to HRP as enzyme. The inhibition plots have also shown that for Pb^{2+} a gradual increase in the inhibition was observed as the metal ion concentration was in-creased, with the final percentage inhibition at 47.2%. On the other hand, in the case of Hg^{2+} and Cd^{2+} an initial high percentage inhibition was obtained that gradually increased slightly with increased metal ion concentration. The highest percentage inhibition obtained for Cd^{2+} and Hg^{2+} were 44.1% and 71.9%, respectively.

3.4.2. Inhibition results for intermediary metal concentration investigated

Figure 6 displays the results obtained for the percentage inhibition plots of HRP when aliquots of 0.005 ppb of Cd^{2+}, Pb^{2+} and Hg^{2+} was sequentially added to the 0.1 M PB (pH = 6.8) solution.
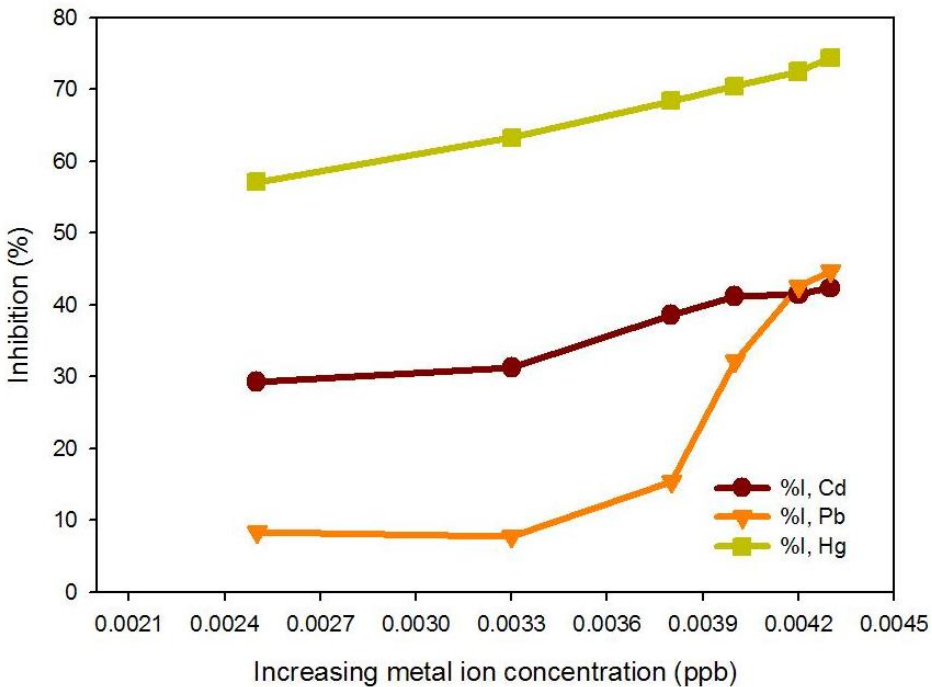

Figure 6. Results obtained for inhibition of the Pt/PANI/HRP biosensor in the presence of 0.005 ppb of Cd^{2+}, Pb^{2+} and Hg^{2+}, respectively.

Figure 6 also displayed the same characteristic trend for the percentage inhibition results, compared to that in Figure 5. For metal concentrations ranging from 0.005 – 0.02 ppb, it was observed that the decreasing trend of inhibition was $Hg^{2+} > Cd^{2+} > Pb^{2+}$. For higher concentrations the inhibition trend was $Hg^{2+} > Pb^{2+} > Cd^{2+}$. The results in Figure 6 are characteristically similar to that obtained in Figure 5 and differences were only observed when the individual percentages were compared. For Pb^{2+} a gradual increase was again observed as the concentration was increased, with the final percentage inhibition obtained at 44.7%. In the case of Hg^{2+} and Cd^{2+} an initial high percentage inhibition was obtained that gradually increased slightly with increased metal ion concentration. The highest percentage inhibition obtained for Cd^{2+} and Hg^{2+} were 42.4% and 74.4%, respectively.

3.4.3. Inhibition results for highest metal concentration investigated

The percentage inhibition plots obtained for the inhibition of HRP when aliquots of 0.01 ppb of Cd^{2+}, Pb^{2+} and Hg^{2+} was sequentially added to the 0.1 M PB (pH = 6.8) solution, are shown in Figure 7.
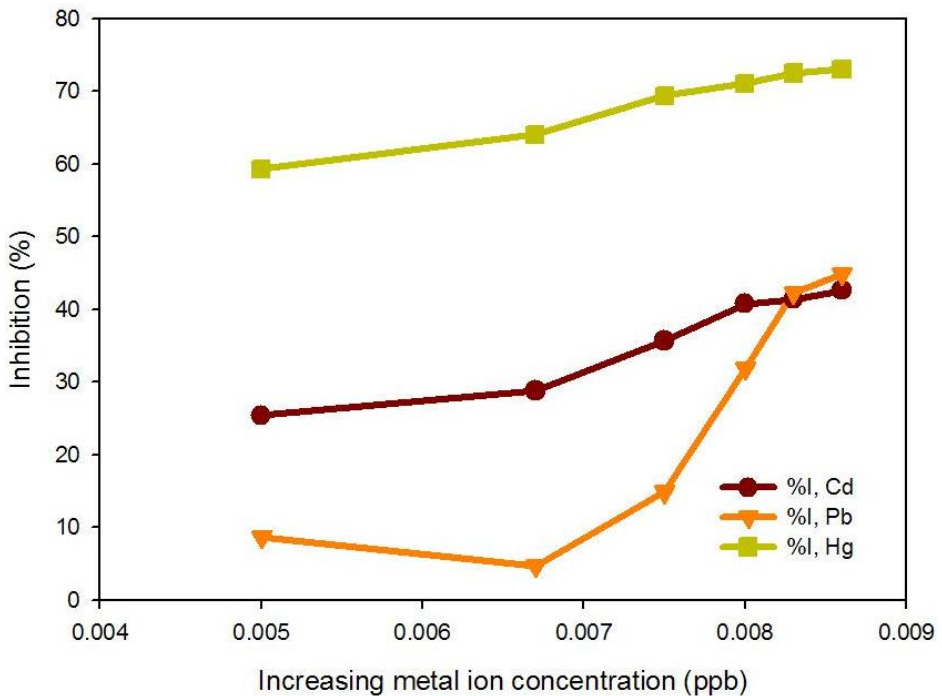

Figure 7. Results obtained for inhibition of the Pt/PANI/HRP biosensor in the presence of 0.01 ppb of Cd^{2+}, Pb^{2+} and Hg^{2+}, respectively.

Similarly, the same percentage inhibition trends as observed for the two previous investigations were observed for the results shown in Figure 7. The decreasing trend of inhibition was $Hg^{2+} > Cd^{2+} > Pb^{2+}$ for the metal ion concentrations ranging from 0.01 to 0.04 ppb, while it changed to $Hg^{2+} > Pb^{2+} > Cd^{2+}$ for the last two concentrations evaluated. The highest percentage inhibition obtained at the highest metal ion concentration of 0.06 ppb evaluated were 42.6% (Cd^{2+}), 44.9% (Pb^{2+}) and 73.1% (Hg^{2+}).

Analysis of the above inhibition results obtained for the three sets of starting metal ion concentrations investigated showed interesting results and similarities. It was observed that the enzyme HRP was inhibited by all three of the metal ions, Cd^{2+}, Pb^{2+} and Hg^{2+}. The inhibition results obtained for Hg^{2+} ions was the highest of the metal ions investigated, clearly indicating the known toxicity of these metal ions. At low to intermediate metal ion concentrations, the results obtained showed a decreasing inhibition trend of $Hg^{2+} > Cd^{2+} > Pb^{2+}$. The highest inhibition results obtained for the Pt/PANI/HRP biosensor was 74.4% for Hg^{2+}, followed by 47.2% for Pb^{2+} and 44.1% for Cd^{2+}.

However, no results for the simultaneous analysis of two or more of either Cd^{2+}, Pb^{2+} or Hg^{2+} were collected. These experiments will be conducted in future with the biosensor system described in this work and published in future papers.

3.4.4. Analytical characteristics of the Pt/PANI/HRP biosensor applied in metal inhibition studies

The amperometric responses of the Pt/PANI/HRP biosensor to various H_2O_2 substrate concentrations were evaluated and compared to that obtained in the presence of selected heavy metal ions of Cd^{2+}, Pb^{2+} and Hg^{2+}. For all three metal ions, decreased biosensor responses were obtained, clearly showing that inhibition was taking place. The analytical characteristics of the Pt/PANI/HRP biosensor were evaluated for each metal ion using various calibration curves to obtain the linear ranges, slopes of the calibration plots, correlation coefficients, limits of detection ($LOD = \frac{3 \times SD}{m}$) and limits of quantification ($LOQ = \frac{10 \times SD}{m}$). For these calculations SD is the standard deviation of the blank signal ($n = 10$) obtained in 0.1 M PB solution, while m is the slope of the calibration curve. The results obtained are listed in Table 2.

Metal ion	Linear range (ppb)	Sensitivity (µA/ ppb)	R^2	LOD (ppb)	LOQ (ppb)
Cd^{2+}	1.5 - 4	3.19×10^{-2}	0.992	0.0579	0.193
Pb^{2+}	1.1 - 5	1.90×10^{-2}	0.991	0.0931	0.310
Hg^{2+}	1.5 - 4	1.20×10^{-2}	0.991	0.0268	0.089

Table 2. Results for the analytical characteristics of the Pt/PANI/HRP biosensor from the various calibration curves for the determination of Cd^{2+}, Pb^{2+} and Hg^{2+} heavy metal ions.

Analysis of the results in Table 2 showed that the Pt/PANI/HRP biosensor was very sensitive for the determination of Hg^{2+} ions, followed by Pb^{2+} and Cd^{2+} ions. This sensitivity was confirmed with the lowest LOD obtained for Hg^{2+} ions. The increasing LOD values obtained for this biosensor was $Pb^{2+} < Cd^{2+} < Hg^{2+}$. These results further compared favourably with that obtained in the study by Nomngongo et al. (2011), which used the same biosensor construction for the determination of Cd^{2+}, Pb^{2+} and Cu^{2+} metal ions.

4. Conclusions

The results obtained in this study have showed that an amperometric Pt/PANI/HRP biosensor can be successfully applied for the inhibition determination of selected heavy metals of Cd^{2+}, Pb^{2+} and Hg^{2+}. The inhibition results obtained for the three sets of starting metal ion concentrations investigated showed interesting results and similarities. It was observed that the enzyme HRP was inhibited by all three of the metal ions, Cd^{2+}, Pb^{2+} and Hg^{2+}. The inhibition results obtained for Hg^{2+} ions was the highest of the metal ions investigated, clearly indicating the known toxicity of these metal ions. At low to intermediate metal ion concentrations, the results obtained showed a decreasing inhibition trend of $Hg^{2+} > Cd^{2+} > Pb^{2+}$. The highest inhibition results obtained for the Pt/PANI/HRP biosensor was 74.4% for Hg^{2+}, followed by 47.2% for Pb^{2+} and 44.1% for Cd^{2+}. The analytical features obtained for the HRP biosensor showed high sensitivity for the determination of Hg^{2+} ions, followed by Pb^{2+} and Cd^{2+} ions. The respective LOD values obtained were 0.027 ppb (Hg^{2+}), 0.058 ppb (Cd^{2+}) and 0.093 ppb (Pb^{2+}).

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