

A maize Tassel-Multiwalled Carbon Nanotubes Modified Glassy Carbon Electrode for Amperometric Environmental Monitoring of Pb (II) and Cu (II) via Horseradish Peroxidase Inhibition

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Abstract. This paper describes the fabrication and application of an inhibition-based enzyme biosensor proposed for environmental monitoring and assessment of heavy metal ions in aqueous solution. A maize tassel- multiwalled carbon nanotube (MT-MWCNT) composite material was prepared and used to immobilize horseradish peroxidase (HRP) on a glassy carbon electrode (GCE) to produce a biosensor responsive to H₂O₂. The biosensor was incubated in heavy metal solutions and the inhibited cathodic response determined amperometrically. The response was linear ($R^2 > 0.99$) over the concentration ranges 0.092 – 0.55 and 0.068 – 2.0 mg/L for Pb²⁺ and Cu²⁺, respectively, with limits of detection (LOD) values of 2.3 and 4.1 µg/L. Relative error values of 7.93 and 2.55 % were obtained for Pb²⁺ and Cu²⁺, respectively, when the biosensing technique was validated using a standard reference material. Recoveries of 96-104% were obtained when applied to the measurement of Pb²⁺ and Cu²⁺ in tap water. The fabricated biosensor exhibited good stability, repeatability and reproducibility.

Keywords: environmental monitoring, heavy metals, biosensor, maize tassel.

1. Introduction

The toxicity, non-biodegradability and environmental persistence of heavy metals such as lead and copper is a matter of serious public health concern [1]. Over the last decade or so inhibition-based enzyme biosensors have emerged as an important technique for monitoring pollutants such as heavy metals [2]-[4]. The attraction of biosensors compared with sophisticated analytical instrumentation such as an inductively-coupled plasma atomic emission - mass spectrometer (ICP-MS) lies, among other things, in their lower cost, rapid response and on-site applicability. In this study maize tassel (MT), an agro-waste biomaterial, was blended with non-functionalized multi-walled carbon nanotubes (MWCNT) to produce a composite material which was used to modify a glassy carbon electrode (GCE) to fabricate a biosensor for determining trace metal ions *via* their inhibition of the activity of immobilized horseradish peroxidase (HRP) towards H₂O₂.

2. Methods

The composite was prepared by dispersing a blend of MT and MWCNT in DMF. A drop of the dispersion was placed onto the surface of the GCE which was then coated with HRP in phosphate buffer solution (PBS) (pH 7.0) and Nafion as a binder. The preparation of the HRP/MT-MWCNT/GCE and electron transfer is shown in Figure 1.

FTIR and UV-Vis spectroscopy were used to assess the stability and biological activity, respectively, of the HRP before and after immobilization on the MT-MWCNT composite. The response of the biosensor- the

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fabricated HRP/MT-MWCNT/GCE- towards H_2O_2 was studied using linear sweep voltammetry (LSV) and cyclic voltammetry (CV) scanned from +0.6 to -0.6 V, and amperometry at -0.3 V.

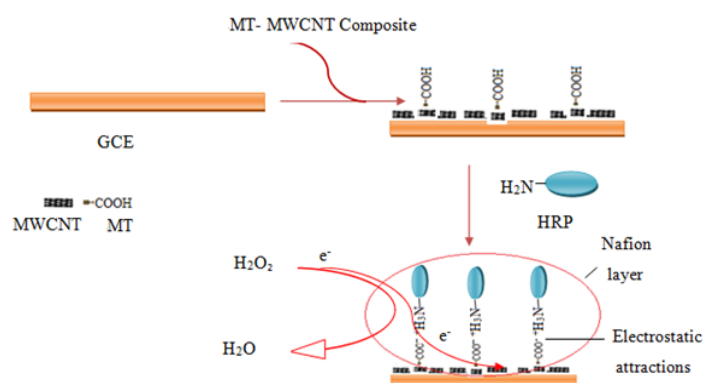


Fig. 1: Sensor fabrication and electron transfer between the modified electrode and HRP.

The biosensor was incubated in 0.1 mM H_2O_2 in the presence of 0.1-2.5 mg/L Pb^{2+} and 0.1-5.0 mg/L Cu^{2+} solutions in 0.1 M PBS (pH 7.0) for inhibition studies. The linear range and LOD of the % inhibition as a function of metal ion concentration were determined. Dixon and Cornish-Bowden plots were used to study the mode of inhibition induced by the trace metal ions. Values for the inhibition constants, K_i , were estimated. The biosensor measurement was validated by applying it to determine Pb^{2+} and Cu^{2+} in a standard reference material. The feasibility of the fabricated enzyme inhibition biosensor for possible environmental applications was assessed by determination of Pb^{2+} and Cu^{2+} in tap water.

3. Results and discussion

3.1. Biosensor characterisation

The FTIR and UV-Vis results (data available but not shown here) indicated that the HRP was not denatured and retained its structure after immobilization in the MT-MWCNT composite film [5], [6]. The enzymatically reduction of H_2O_2 by HRP was evaluated by cyclic voltammetry in 0.1 M PBS at pH 7.0. At these experimental conditions, a cathodic peak around -320 mV versus Ag/AgCl was obtained. Figure 2 shows the cyclic voltammograms of the biosensor in the absence (b) and presence of H_2O_2 (0.01–0.5 mM) (c to d) in PBS (pH 7.0) at the scan rate of 100 mV s^{-1} .

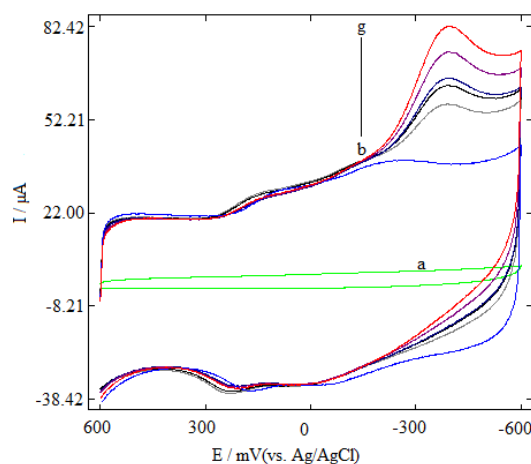


Fig. 2: Cyclic voltammograms of GCE (a), HRP biosensor in 0.1 M PBS, pH 7.0 and 0.1 M KCl (b) without substrate, (c–g) with 0.01–0.5 mM substrate.

An increase in cathodic peak current was observed with increase in substrate concentration. For comparison, the GCE (a) was also scanned in 0.1 M PBS and substrate, no significant response was observed. The linear regression equation is $I_p/\mu\text{A} = 2.095 (\pm 0.1245) + 0.1985 (\pm 0.0052) C/\text{mM}$ ($R = 0.9969$) in the H_2O_2 concentration range from 0.01–0.5 mM. The detection limit of H_2O_2 was 0.85 μM .

3.2. Inhibition studies

The percentage inhibition was found to increase linearly with an increase in the concentration of Pb^{2+} and Cu^{2+} . A typical curve for Cu^{2+} is shown in Figure 3. Inhibition was accompanied by decreased residual enzyme activity, as expected.

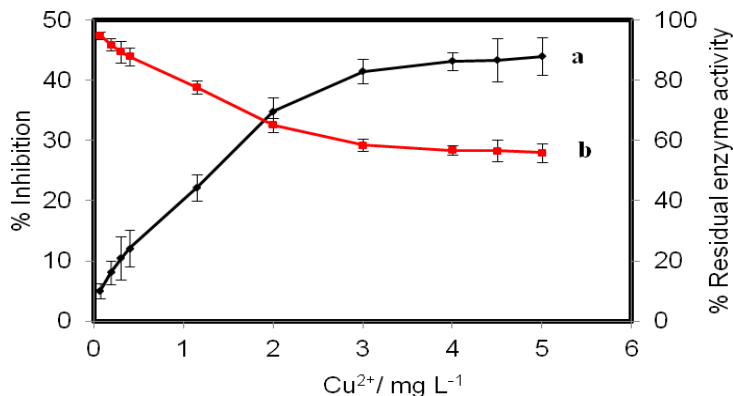


Fig. 3: Dose-dependent enzyme inhibition (a) and residual enzyme activity (b) of Cu^{2+} towards, HRP-catalyzed H_2O_2 , Error bar = \pm S.D. and $n = 3$.

Some analytical characteristics of the HRP/MT-MWCNT/GCE modified electrode used as an inhibition biosensor for Pb^{2+} and Cu^{2+} are summarised in Table 1. The Dixon and Cornish-Bowden plots (data available but not shown here) indicated that the inhibition was reversible and mixed for both metal ions studied.

Table 1: Analytical characteristics of the inhibition biosensor.

Analyte	Linear range	LOD	R^2	K_i
Pb^{2+}	0.092 -0.55 mg/L	2.3 $\mu\text{g/L}$	0.9936	2.6
Cu^{2+}	0.068 -2.00 mg/L	4.1 $\mu\text{g/L}$	0.9954	1.8

3.3. Validation

The biosensor measurement was validated by using it to determine Pb^{2+} and Cu^{2+} in a NIST (National Institute of Standards and Technology) standard reference material 1640a for trace metals in natural water. The results are shown in Table 2. In each case the relative error was less than 10%.

Table 2: Validation of the HRP/MT-MWCNT biosensing technique.

Cation ($\mu\text{g/L}$)	Certified value ($\mu\text{g/L}$)	HRP/MT-MWCNT (%)	Relative error ($n = 3$)
Pb^{2+}	12.10 ± 0.050	13.06 ± 0.062	7.93
Cu^{2+}	85.75 ± 0.51	83.56 ± 0.58	2.54

3.4. Stability, repeatability and reproducibility

The stability of the biosensor was first examined in the presence of 0.1 mM H_2O_2 concentration in 0.1 M PBS (pH 7.0). For the same metal concentration, it was observed that after 10 successive series of measurements, the biosensor lost about 30% of the initial sensitivity. In studying the long-term stability, the

HRP/MT-MWCNT biosensor was stored in 0.1 M PBS at 4°C for 18 days and the biosensor response was tested on different days after incubation in the inhibitor. The biosensor did not show a bigger decrease of its initial response for 0.1 mM H₂O₂ after incubation in standard Cu²⁺ ion solution for the different days studied. The repeatability of the HRP/MT-MWCNT biosensor was investigated for fixed Cu²⁺ ion concentrations. Relative standard deviations (*RSD*) of 5.8% were obtained for Cu²⁺. Five modified biosensors were made independently and were investigated for the determination of the same concentrations of Cu²⁺. The modified biosensors showed a relative standard deviation (*RSD*) of Cu²⁺ (6.2%).

3.5. Application

The fabricated biosensor was used for the determination of Pb²⁺ and Cu²⁺ in tap water in order to assess its feasibility for possible application to environmental monitoring. A standard addition method was used (see Table 3). The range of recoveries obtained indicated that the sensor is a good candidate for this type of application.

Table 3: Determination of Pb²⁺ and Cu²⁺ in tap water.

Cation	Added (mg/L)	Found (mg/L)	Recovery (%)
Pb ²⁺	0.50	0.52	104.0
	0.55	0.53	96.4
Cu ²⁺	0.50	0.48	96.0
	1.00	1.01	101

4. Conclusions

The proposed biosensor does not require a complicated process for its construction. The inhibition of horseradish peroxidase immobilized in maize tassel- multi-walled carbon nanotubes composite by Pb²⁺ and Cu²⁺, both metal ions of environmental interest, has been shown. The performance of the electrode makes it a promising candidate for application in the field of environmental monitoring.

5. References

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