

A comparative study of Linear range, Sensitivity (S) and Limit of Detection (LOD) for two metal oxide nanocomposite enzyme based biosensors PANI/ZnO/Urease and PANI/MnO₂/Urease for the detection of heavy metal ion Hg (ii) in water

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Manuscript Details

Received :24.08.2023

Accepted: 14.11.2023

Published: 25.11.2023

Available online on <https://www.irjse.in>

ISSN: 2322-0015

Cite this article as:

Ashish P. Mahajan and Atul P. Birajdar. A comparative study of Linear range, Sensitivity (S) and Limit of Detection (LOD) for two metal oxide nanocomposite enzymebased biosensors PANI/ZnO/Urease and PANI/MnO₂/Urease for the detection of heavy metal ion Hg (ii) in water, *Int. Res. Journal of Science & Engineering*, 2023, Volume 11(6): 237-243.

<https://doi.org/10.5281/zenodo.10233312>



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Abstract

Out of all the environmental issues, water pollution by heavy metal ion such as Mercury, Lead Arsenic, Cadmium, Chromium etc most important to be solved. Biosensors present an efficient alternative technique which can lead to the low cost heavy metal ion detector along with the features such as no need of special training for an operator, easily disposable, quick detection and less time consumption. In the present work, we have developed and compared two types of electrochemical amperometric biosensor by immobilizing Urease, to Stainless steel transducer. The stainless steel electrode was modified with PANI/ZnO and PANI/MnO₂ nanocomposite by the method of electropolymerization. Different parameter such as Linear Range, Sensitivity (S) and Limit Of Detection (LOD) were determined and compared. Finally the selectivity of the individual biosensor towards Hg (II) ions was studied by mixing another heavy metal ion Pb(II) in the same solution. It was observed that with increasing amount of Hg (II) ions the response current also increased linearly in the range from 2 mg/l to 7 mg/l. The detection limit was calculated to be 5.04 mg/l. The linear regression equation was $I \text{ (mA)} = y = 0.432[\text{Urea Conc.}] + 0.192$ with correlation coefficient (R²) of 0.980. The obtained high sensitivity value 0. 432 mA/(mg/l) indicated that the proposed biosensors are excellent platform for sensitive detection of Hg (II).

Keywords: Biosensors, Nanocomposites, Heavy metal ions, Hg(II),

1. Introduction

Out of all the environmental issues, water pollution by heavy metal ion such as Mercury, Lead Arsenic, Cadmium, Chromium etc is directly related with health of human being individually as well as society health too. In present days growing industrialization and the use of chemicals in agriculture have contributed to the mixing up many toxic compounds in air, soil, and water, which cause environmental pollution [1]. The pollutants can be identified and quantified by classical analytical techniques such as gas chromatography (GC/MS) or high performance liquid chromatography (HPLC/MS). These techniques are time consuming because of sample preparation and need for pre-concentration, expensive, and, in case of water samples, cannot be performed easily outside the laboratory. The development of electrochemical biosensors is probably one of the most promising ways to solve some problems concerning sensitive, fast and cheap analytical techniques. A biosensor converts the modification of the physical or chemical properties of a biomatrix, which occurs as a result of biochemical interactions, into an electric signal whose amplitude depends on the concentration of defined analytes in the solution [2] & [3].

Mercury is stable in ^{199}Hg , ^{200}Hg , and ^{202}Hg isotopic forms. It is liquid in normal conditions and most likely works in 1 and 2 oxidation states. Mercury is heavier than water and considers to be a dielectric. Human MPR dose of mercury in total is $0.9 \mu\text{g/kgbw/day}$ [4]. (WHO Food Additives Series, 1972, No. 4).

In the present work, we have developed and compared two types of electrochemical amperometric biosensor by immobilizing biological element such as enzyme 'Urease', to the transducer surface 'Stainless steel'. In First biosensor stainless steel electrode is modified with PANI/ZnO nanocomposite and in second case modified with PANI/MnO₂ nanocomposites by the method of electropolymerization. The developed biosensors are abbreviated as PANI/ZnO/Urease and PANI/MnO₂/Urease. Different parameter such as Linear Range, Sensitivity (S) and Limit Of Detection (LOD) were determined and compared. Finally the selectivity of the

individual biosensor towards Hg (II) ions was studied by mixing another heavy metal ion Pb(II) in the same solution.

2. Materials and Methodology

All chemical used were of analytical grade. Aniline, ammonia solution (min 25%), zinc nitrate (96%), acetone, Sulphuric acid, MnSO₄, KMnO₄ from Merck Ltd, Mumbai, Urease (Jack bean mill) from Loba Chemie, mercury nitrate, lead nitrate, phosphate buffer solution and Urea were procured from Sigma-Aldrich, were used as received without further purification except aniline. Aniline was used after distillation by a known technique. Double distilled water was used throughout this work.

(a) Chemical Synthesis of Nano-sized ZnO and MnO₂

The sample of pure zinc oxide compound was prepared by co-precipitation method. The solution of 0.2M of zinc nitrate $[\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}]$ was prepared in distilled water and to this solution ammonia solution was added drop wise till the pH adjusted to 8. The hydrated zinc hydroxide get thus formed was thoroughly washed with distilled water and transfer to flask fitted with water condenser. The gel was continuously stirred for 6 Hours and temperature was maintained around 85°C . then, the crystalline powder was filtered and oven dried [5].

A mixture of MnSO₄ (1.0M) and KMnO₄ (0.5M) solution was stirred for 4 h at 70°C . The obtained precipitates were washed several times with distilled water followed by ethanol to remove impurities, and then dried in vacuum at 110°C for 5 h. The dried powder was put into muffle and heated at 300°C for additional 3 h. These powders were acidified with 2.0M H₂SO₄ at 90°C for 2 h. Finally, the product was washed with distilled water and vacuum dried [6].

(b) Electropolymerization of PANI/ZnO and PANI/MnO₂ nanocomposite

For electropolymerization of aniline, different weight percentage of nanostructure ZnO (15%) were added in 1 M H₂SO₄ containing of 0.4 M aniline. Then PANI/ZnO

composite were electrochemically polymerized on stainless steel electrode by applied potentials to Pt working electrode at 50 mV/s between the scanning potential -0.2 to 0.8 volt [7].

Similar procedure followed for the PANI/ MnO₂ nanocomposites with 15% weight percentage were deposited on Stainless Steel surface by linear swiping the voltage in range -200 mV to +1100 mV at 50 mVs⁻¹ for 20 cycles. After a 20 cycles the sufficient amount of layer was deposited. The electrodes are washed with distilled water and dried at room temp and used for further studies.

(c) Urease Immobilization

The immobilization of Urease on PANI /ZnO and PANI / MnO₂ matrix on Stainless steel electrode was done using Physical Adsorption method. The electrodes were dipped in a pH 4 acetic acid solution, washed with water and then left overnight at 5^o C in contact with an urease solution containing 2 mg of the enzyme (urease) per ml of pH 5.6 phosphate buffer. The next day, the membrane was washed with a pH 7 phosphate buffer solution. The prepared PANI/ZnO/Urease and PANI/ MnO₂/ Urease electrodes will be always stored dry at 4^o C [8].

(d) Experimental Setup for Calibration curves (Sensitivity, LOD, Linear range of detection)

The calibration of modified biosensor was studied with 10 mM Urea solution in phosphate buffer solution. The stock solution of heavy metal ion Hg(II) is prepared in doubly distilled water. An adequate potential was applied and, once a steady-state current was set, a defined amount of urea stock solution was added to the measuring cell. Then, fixed portions of the heavy metal ion stock solution were added consecutively. The addition of heavy metal ion solution resulted in a change in current to the amount of ions added. Enzyme electrodes were conditioned in a phosphate buffer solution for 5 min between each calibration setting. The inhibition time was taken to be 20 min for Hg (II) as obtained from the optimization curve. While using PANI/ZnO/Urease biosensor the pH of the solution was maintained at optimum value 8.5. Whereas, that for PANI/MnO₂/Urease biosensor pH was 6.0.

3. Results and Discussion

(a) Hg (II) detection

The calibration curve for Hg (II) ions with 10 mM Urea using PANI/ZnO/Urease biosensor is shown in **Figure 1 (a)**. From the graph it can be observed that with increasing amount of Hg (II) ions the response current also increased linearly in the range from 3 mg/l to 9 mg/l. The detection limit was calculated to be 4.22 mg/l. The linear regression equation was $I \text{ (mA)} = y = 0.669[\text{Urea Conc.}] + 1.746$ with correlation coefficient (R^2) of 0.945. The sensitivity is the slope of calibration curve, which is found to be 0.669 mA/(mg/l).

Figure 1 (b) shows calibration curve for Hg (II) ions with 10 mM Urea using PANI/MnO₂/Urease biosensor. From the graph it can be observed that with increasing amount of Hg (II) ions the response current also increased linearly in the range from 2 mg/l to 7 mg/l. The detection limit was calculated to be 5.04 mg/l. The linear regression equation was $I \text{ (mA)} = y = 0.432[\text{Urea Conc.}] + 0.192$ with correlation coefficient (R^2) of 0.980. The sensitivity is the slope of calibration curve, which is found to be 0.432 mA/(mg/l).

Table 1 shows that the limit of detection of PANI/ZnO/Urease biosensor for Hg (II) detection is lower than that of PANI/MnO₂/Urease biosensor. The sensitivity is high in case of PANI/ZnO/Urease biosensor. Thus with PANI/ZnO/Urease biosensor we obtained lower detection limit with high sensitivity. Hence this biosensor is preferred for the detection of Hg (II) ions.

For comparison, the analytical performances such as the linear range and the limit of detection of the proposed biosensor and other enzyme based biosensors reported in the literatures were all summarized in Table 1. As can be seen, the linear range of the proposed biosensor was located in a relative lower range of Hg (II) concentrations. And the limit of detection for Hg (II) obtained by the proposed biosensor was much lower than by the previous reported biosensors. Also the results of high sensitivity values indicated that the proposed biosensors are excellent platform for sensitive detection of Hg (II).

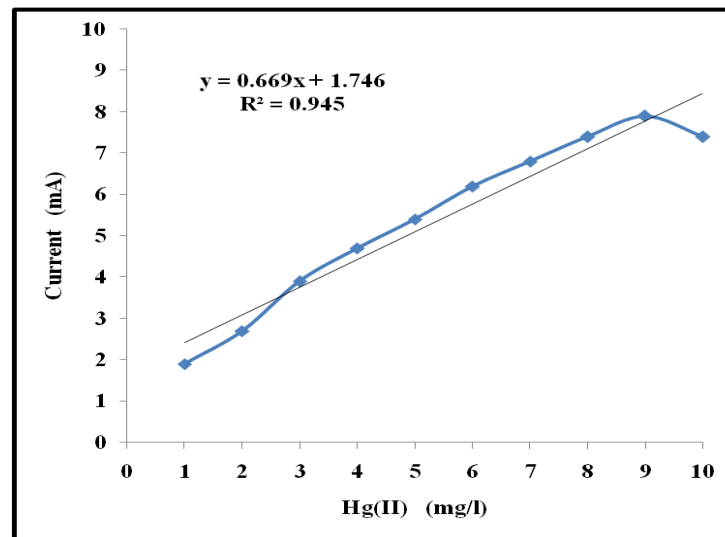


Figure 1 (a): Calibration curve for Hg (II) ions using PANI/ZnO/Urease biosensor.

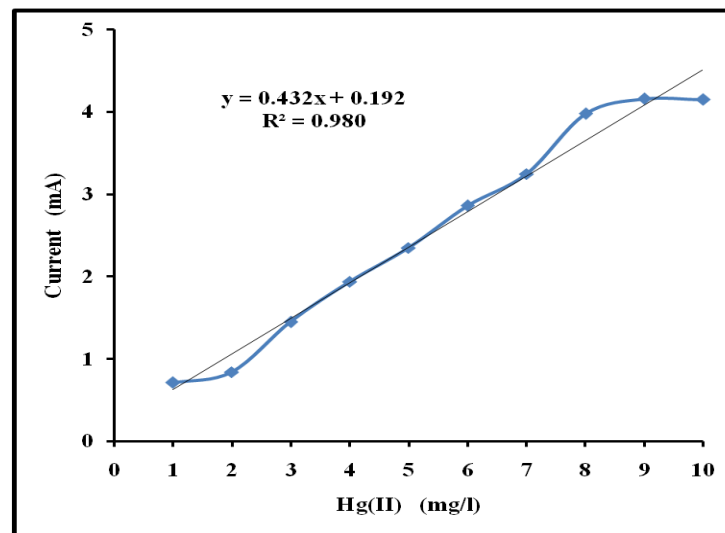


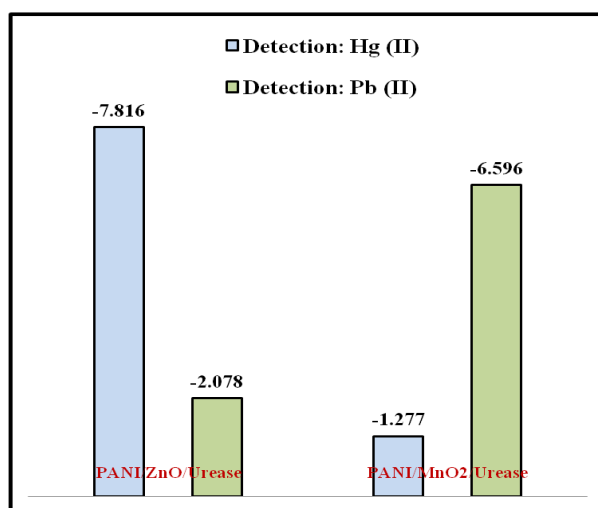
Figure 1 (b): Calibration curve for Hg (II) ions using PANI/MnO₂/Urease biosensor.

Table 1: Parameter of detection of Hg (II) ions.

Biosensor	Linear Range	Sensitivity (S)	Correlation coeff. (R ²)	Limit of Detection (LOD)
PANI/ZnO/Urease	3 -9 mg/l	0.669 mA/ (mg/l)	0.945	4.22 mg/l
PANI/MnO ₂ /Urease	3 -7 mg/l	0.432 mA/ (mg/l)	0.980	5.04 mg/l
Reference [9]	0.1-10 mg/l	0.192 mA/ (mg/l)	0.990	2.00 mg/l
Reference [10]	2.7-13.5	0.33	0.998	5.43 mg/l

Table 2: The Relative Sensitivity of constructed biosensors for detection of Hg (II) and Pb (II) ions.

	PANI/ZnO/Urease	PANI/MnO ₂ /Urease	
Detection: Hg (II) Interferer: Pb (II)	-7.81594	-1.27706	83.66%
Detection: Pb (II) Interferer: Hg (II)	-2.07777	-6.59554	68.50%
	73.42%	80.64%	

**Figure 2:** The Relative selectivity of biosensors to Hg (II) and Pb (II) ions.**(b) Selectivity of biosensors**

The calculated selectivity coefficient results are shown in Table 2. It has been observed that the PANI/ZnO/Urease biosensor is more selective to the Hg (II) as compared to Pb (II). Whereas, the PANI/MnO₂/Urease biosensor is found to be more selective to Pb (II) ions compared to that of Hg (II).

The relative Selectivity of the constructed biosensors was carried out using the obtained values of selectivity coefficient $\log \log K_{A,B}^{amp}$. From the table it is observed that the Selectivity of PANI/ZnO/Urease biosensor to Hg (II) ions is 83.66 % more than that for Pb (II) ions. Similarly, PANI/MnO₂/Urease biosensor is found to be 68.50 % more selective to the Pb (II) ions compared to that for Hg (II) ions.

Table also demonstrates the relative Selectivity of constructed biosensors for the detection of same ion. The Selectivity of PANI/ZnO/Urease biosensor to Hg (II) ions detection is 73.42 % more than that of PANI/MnO₂/Urease biosensor. Also the Selectivity of PANI/MnO₂/Urease biosensor to Pb (II) ion detection is 80.64 % more than that PANI/ZnO/Urease biosensor. These results are graphically shown in Figure 2.

Hence the PANI/ZnO/Urease biosensor can selectively determine Hg (II) ions and PANI/MnO₂/Urease biosensor determines Pb (II) ions with greater selectivity. These results are in good agreement with that obtained earlier during Chronoamperometry study as demonstrated in our earlier research.

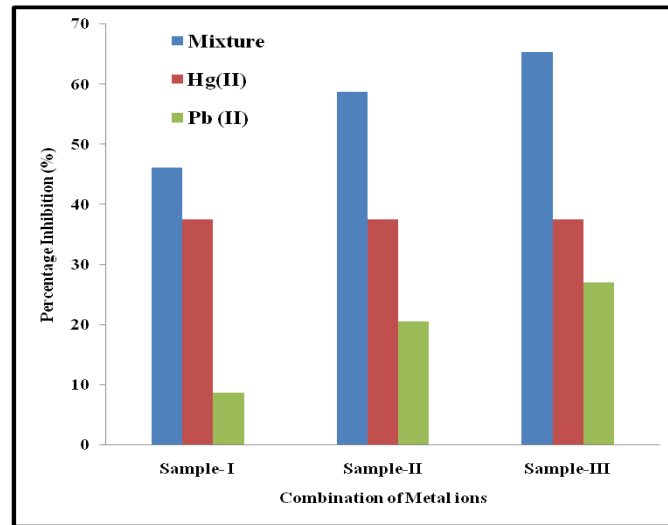


Figure 3 (a): Combined effect of Hg (II),) and Pb (II) concentration on enzyme activity at PANI/ZnO/Urease biosensor. **Sample-I:** Hg(II) (4 mg/l)+Pb (II) (3 mg/l), **Sample-II:** Hg(II) (4 mg/l)+Pb (II) (4 mg/l), **Sample-III:** Hg(II) (4 mg/l)+Pb (II) (5 mg/l).

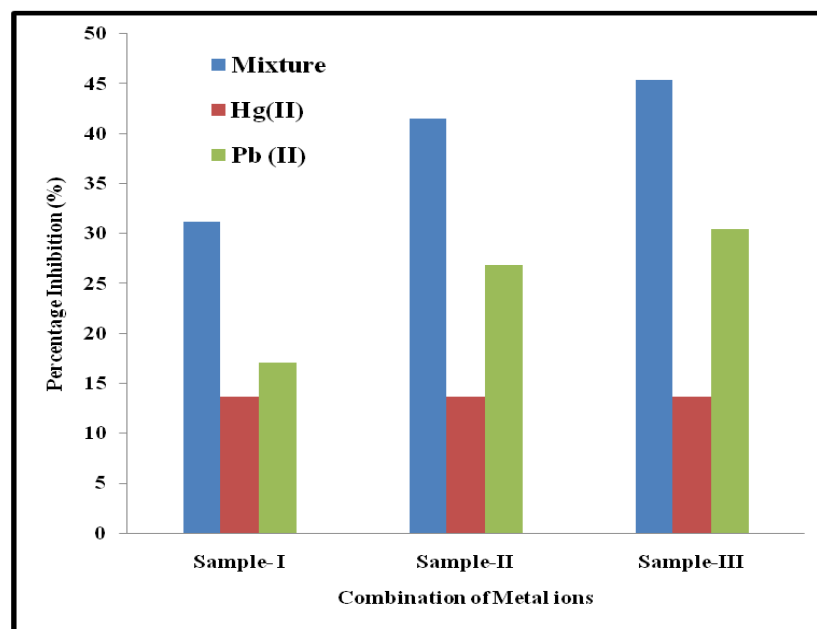


Figure 3 (b): Combined effect of Hg (II),) and Pb (II) concentration on enzyme activity at PANI/MnO₂/Urease biosensor. **Sample-I:** Hg(II) (4 mg/l)+Pb (II) (3 mg/l), **Sample-II:** Hg(II) (4 mg/l)+Pb (II) (5 mg/l), **Sample-III:** Hg(II) (4 mg/l)+Pb (II) (5 mg/l).

(c) Response to Mixture of Hg(II) and Pb(II) ions

Experiments were carried out to determine relative inhibition of Urease by binary combination of Hg (II) and Pb (II) and compared these values with the inhibition of Hg (II) and Pb (II) alone. In this study, Hg (II) concentration at 4mg/l, were mixed with varying

concentration of Pb (II) concentration at 3mg/l, 4 mg/l, and 5 mg/l. The combination of both metal ions exhibit additive effect as depicted in Figure 6. This study on mixture of metal ion can be used as a supporting data for metal induced toxicity by mixture of heavy metal ions using the Urease bioassay. Recent reports on metal

induced toxicological interactions using the Lactase and Aluminium oxidase [11] also support the additive nature of heavy metal ion mixtures, as reported in this work. Thus, the study demonstrates that both the metal ions inhibit Urease significantly higher when they are combined than when they inhibit Urease individually. Various combinations were prepared to study interactive effect.

Conflicts of interest: The author stated that no conflicts of interest.

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