Anal. Bioanal. Electrochem., Vol. 16, No. 7, 2024, 659-670
https://www.doi.org/10.22034/abec.2024.714690

Analytical & Bioanalytical Electrochemistry

Full Paper

Voltammetric Determination of Mercury (II) Ions in 5-Methoxy-2-({[4-(3-methyl-3-phenyl-cyclobutyl)-thiazol-2-yl]-Hydrazone}-Phenyl-Methyl)-Phenol Modified Glassy Carbon Electrode by Differential Pulse Voltammetry

Hafizullah Sharifi,[1] Şeyma Korkmaz,[1] İbrahim Ender Mülazımoğlu,[2,*] İbrahim Yılmaz,[3,4] and Ecir Yılmaz[2]

[1]*Necmettin Erbakan University, Institute of Science, Chemistry Department, Konya, Turkey*
[2]*Necmettin Erbakan University, Ahmet Keleşoğlu Education Faculty, Chemistry Department, Konya, Turkey*
[3]*Karamanoğlu Mehmetbey University, Kamil Özdağ Science Faculty, Chemistry Department, Karaman, Turkey*
[4]*Bolu Abant İzzet Baysal University, Department of Mathematics and Science Education, Bolu, Turkey*

*Corresponding Author, Tel.: +905061064651
E-Mail: iemulazimoglu@erbakan.edu.tr

Received: 10 May 2024 / Received in revised form: 8 July 2024 / Accepted: 13 July 2024 / Published online: 31 July 2024

Abstract- The aim of this research is to design an electrochemical sensor for the determination of mercury (II) (Hg^{2+}) ions. A new compound 5-methoxy-2-({[4-(3-methyl-phenyl-cyclobutyl)-thiazol-2-yl]-hydrazone}-phenyl-methylene) (MTP) phenol as a substituent on glassy carbon (GC) electrode is reported. The electrochemical behaviors of the new compound were examined by cyclic voltammetry (CV) before characterization of the modified electrode by CV, electrochemical impedance spectroscopy (EIS), linear sweep voltammetry (LSV), and scanning electron microscopy (SEM) techniques. Determination of trace amounts of Hg^{2+} ions using a modified electrode was carried out using differential pulse voltammetry (DPV). To obtain optimized results, the effect of medium acidity and incubation time were investigated. The best results are pH 10.0 and incubation time (90 min), using Britton-Robinson (BR) buffer solution as the supporting electrolyte in all measurements. Under these optimal conditions, the oxidation peak current of Hg^{2+} ions exhibited a linear increase corresponding to concentrations ranging from 5.0 to 20.0 µM and 50.0 to 200.0 mM. Limit of detection (LOD) and limit of quantification (LOQ) are equal to 1.12 and 3.39 µM, respectively.

Keywords- Mercury (II) ions; Modified Sensor Electrode; Differential Pulse Voltammetry; Cyclic Voltammetry; Scanning Electron Microscopy

1. INTRODUCTION

Mercury and its derivatives exert a considerable influence on both environmental integrity and human health [1]. Ingesting even small amounts of mercury can lead to acute or chronic harm to the human body. The toxic effects it triggers include damage to the brain, kidneys, and lungs [2]. In addition, because mercury exhibits high reactivity with selenium, an essential nutrient needed by roughly 25% of genetically distinct enzymes, it can lead to various diseases such as mercury acrodynia, Hunter-Russell syndrome, and Minamata disease [3]. Developing new methods has emerged as one of the most intriguing subjects due to its practical applications. The development of these methods makes it possible to detect harmless levels of mercury.

Typically, mercury ions are detected using various methods including atomic absorption spectroscopy [4], atomic fluorescence spectroscopy [5], inductively coupled plasma atomic emission spectroscopy [6], and coupled plasma mass spectrometry [7] have been studied. Unfortunately, these methods are time-consuming and taxing due to unusually expensive equipment, laboratory setup, and high operational and indirect costs. For proper analysis of mercury decomposition in this field, there is an increasing need to develop an effective detection system that should be simple, sensitive, low equipment, reliable, or economical [8].

Among various techniques for mercury detection, simple and quick methods that have been of special value as a screening test for the existence of different types of this species in the water of industrial processes or food, and it is well known that heavy metals prevent the activity of enzymes.

The utilization of this phenomenon for identifying hazardous toxic elements offers numerous benefits. Initially, detectors employed in enzyme inhibitor assays can exhibit high sensitivity, as the reduction of enzyme activity caused by an inhibitor molecule can be substantial due to the amplification effect. Moreover, enzymes frequently act as specific inhibitors, and in numerous instances, the inhibitory impact of the scrutinized pollutant correlates with its biological toxicity. Consequently, utilizing mercury to inhibit enzyme activity may serve as an effective and straightforward screening method that is both simple and sensitive [9].

Many studies were carried out for the determination of different species, using different electrochemical techniques such as DPV [10], square wave voltammetry [11], and stripping square wave voltammetry [12], with wide working ranges and low detection limits. Electrochemical methods are highly suitable for detecting heavy metal ions because of their high sensitivity, simplicity, selectivity, cost-effectiveness, and ease of construction and operation [13,14]. In this paper, the determination of mercury with an MTP-modified GC electrode is carried out using a simple electrochemical method.

2. MATERIALS AND METHODS

2.1. Chemicals, Reagents and Apparatus

All chemicals, standard solutions, and reagents were of analytical purity and were purchased from Sigma-Aldrich, Acros, and Merck. The Britton-Robinson (BR) buffer solution was created by mixing phosphoric acid, acetic acid, and boric acid in specific proportions relative to 1 M NaOH while maintaining the desired pH level [15]. Ultra-pure water (obtained through the USA MP MINIPURE Purification System, DEST UP, ABD) with a resistance of 18.2 MΩ cm was utilized in all stages of the experiments.

In this study, all electrochemical measurements were utilized in a three-electrode electrochemical cell equipped with GC electrode from BASI (Bioanalytical Systems, USA) model MF-2012 as the working electrode and Pt wire (BAS Model MW-1032) as the counter electrode, in aqueous media Ag/AgCl/3M KCl (BAS Model MF-2063) as reference electrode and in non-aqueous media Ag/Ag^+ (10 mM $AgNO_3$) (BAS Model MF-2042). For all measurements, GAMRY Reference 600+ Potentiostat/Galvanostat/ZRA from Gamry Instruments (USA) was applied. A pH meter (VWR phenomenal, PH 1100 L, UK) was used to check the pH of the medium. Ultrasonic water bath (Kudos, SK2210LHC, China) was used to clean the working electrode after polishing.

2.2. Polishing the working electrode

To obtain repeatable and accurate results, a new, mirror-like surface of the working electrode must be obtained before each experiment. The polishing procedure was routinely performed using a micro cloth pad (Buehler, USA) and 0.05-micron alumina slurry suspension (Baikowski, France) before sonication for 10 min in two stages in deionized water and acetonitrile, respectively [16].

2.3. Synthesis of 5-methoxy-2-({[4-(3-methyl-3-phenyl-cyclobutyl)-thiazol-2-yl]-hydrazone}-phenyl-methyl-phenol

The synthesis reaction of the substance is given in Figure 1. A solution of (2-hydroxy-4-methoxy-phenyl)-phenyl-methanone (eusolex) (2.2824 g, 10 mmol) was prepared in 50 mL of ethanol, onto which a solution of thiosemicarbazide (0.9113 g, 10 mmol) in 20 mL of absolute ethanol was added dropwise. The reaction process was monitored by IR spectroscopy. After adding thiosemicarbazide, the temperature was raised to 323–328 °C and stirred for 2 hours at this temperature. Then the solution was cooled to room temperature and made basic with a 5% NH_3 solution. The resulting precipitate was separated, washed several times with a 5% NH_3 solution, and dried. Finally, single crystals were obtained by evaporating the NH_3 present in the environment. The yield of the reaction was 79%, and the melting point temperature was 406 °C. Characteristic IR bands: 3448 and 3280 cm-1 (n(-NH-)), 3089-3024 (aromatics), 2967-

2858 (n(aliphatics)), 1138 (n(C=O)). Characteristic 1H NMR shifts ($CDCl_3$ d, ppm): 1.39 (s, 3H, -CH_3 in cyclobutane), 3.48 (quint, j = 8.8 Hz, 1H, >CH– in cyclobutane ring), 3.67 (s, 3H, -CH_3 methoxy), 6.17 (s, 1H =CH-S), 6.41 (d, j = 2.2 Hz, 1H, aromatic), 6.45 (q, j = 2.2 Hz, 1H, aromatic), 6.92 (d, j = 8.5 Hz, 1H, aromatic), 7.03-7.12 (m, 3H, aromatics), 7.22-7.26 (m, 2H, aromatics), 7.36 (t, j = 3.8 Hz, 3H, aromatics), 7.66 (q, j = 3.6 Hz, 2H, aromatics), 10.71 (s, 1H, -NH-), 12.43 (s, 1H, -OH). ^{13}C NMR ($CDCI_3$, TMS, 8 ppm): 169.93, 162.07, 155.87, 155.27, 152.15, 148.81, 137.74, 131.25, 129.09, 128.25, 128.16, 127.73, 125. 27, 124.63, 111.21, 106.99, 101.62, 100.90, 58.46, 41.89, 39.85, 38.85, 30.08.

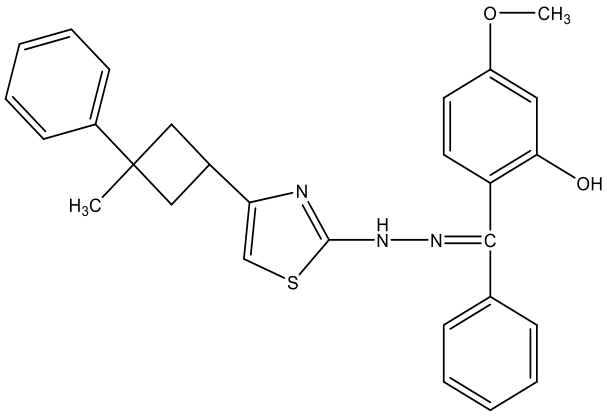

Figure 1. The synthesis reaction of 5-Methoxy-2-({[4-(3-methyl-3-phenyl-cyclobutyl)-thiazol-2-yl]-hydrazone}-phenyl-methyl)-phenol (MTP)

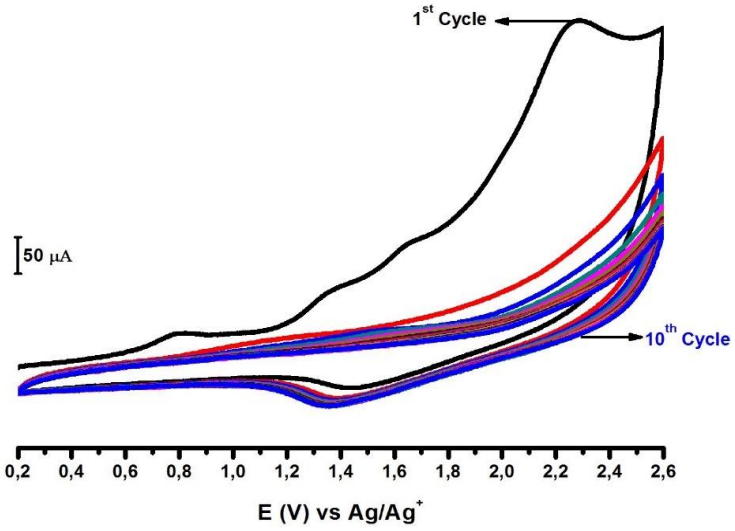

Figure 2. CV voltammogram of the modification of 1 mM MTP prepared in 100 mM NBu_4BF_4 (in CH_3CN) on bare GC electrode surface over the potential range (+0.2 V to +2.6 V) using 0.1 V s^{-1} cyclic voltammogram of scan rate for 10 cycles

3. RESULTS AND DISCUSSION

3.1. Modification of GC Electrode with MTP

Electrode modification can be extensively carried out in both aqueous and non-aqueous media [17, 18]. In this study, the modification process of GC electrode with MTP compound was carried out under the following experimental conditions, potential range +0.2 V to +2.6 V, scanning rate of 0.1 V s^{-1} using CV technique in non-aqueous media and repeated for 10 cycles. As illustrated in Figure 2, the MTP molecule bound to the electrode surface at the start of the first cycle and began modifying the GC electrode surface from the second cycle onwards. The large surface area of GC electrode and optimum coverage of the electrode surface by the molecule was aimed, and the modification was performed for 10 cycles to prevent pinholes on the surface.

3.2. Characterization of MTP/GC Electrode

The electrochemical performance of the modified electrode was characterized by CV technique in both non-aqueous media (1 mM ferrocene in CH_3CN including 100 mM NBu_4BF_4) and aqueous media (1 mM potassium ferricyanide in 100 mM H_2SO_4). The same measurements were made using a bare GC electrode.

The significant change in the electrochemical response of the modified electrode contrasted to the response of the bare GC electrode with CV technique is shown in Figure 3. The redox peaks observed when using bare electrodes in both non-aqueous and aqueous media disappeared. Application of modified electrode indicates electron transfer is inhibited due to the modification layer attached to the modified electrode surface.

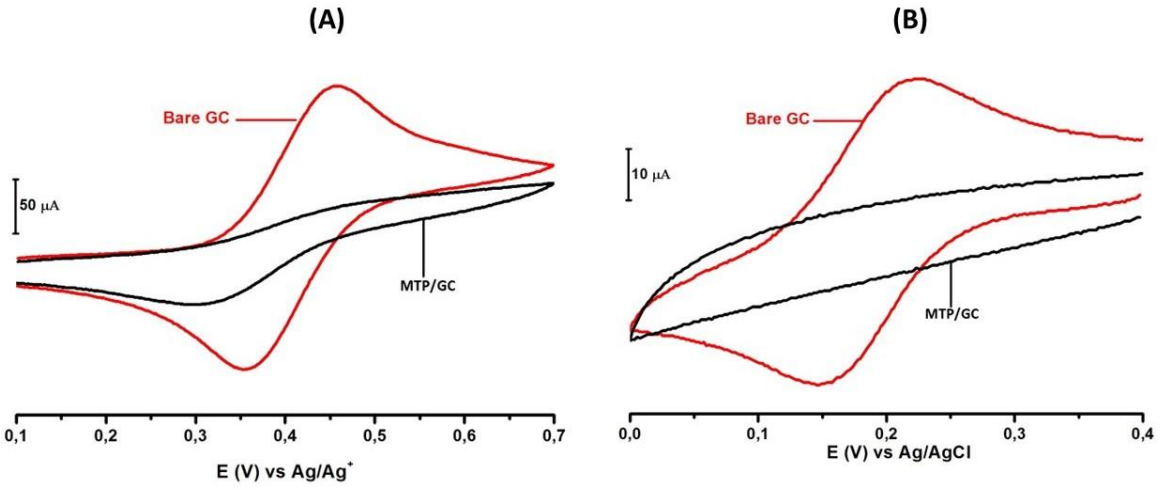

Figure 3. Superimposing of cyclic voltammograms of MTP/GC electrode (A) In ferrocene redox probe vs. Ag/Ag^+, (B) In Fe $(CN)_6^{3-}$ redox probe vs. Ag/AgCl/ 3M KCl reference electrode, 0.1 V s^{-1} scan rate

Characterization of the modified electrode by EIS technique used to examine the electrochemical behavior of MTP was performed in a ferricyanide/ferrocyanide ($Fe(CN)_6^{3-/4-}$) mixture (1:1) in 100 mM KCl solution, in the frequency range from 100,000 Hz to 0.05 Hz. The Nyquist plots were recorded using MTP/GC and bare GC electrodes.

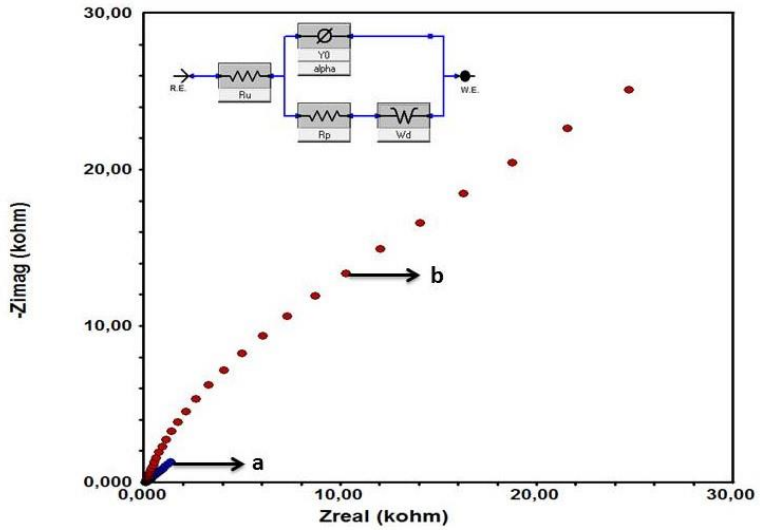

Figure 4. Nyquist plots for electrochemical impedance spectra of (a) bare GC and (b) MTP/GC electrode surface

The obtained Nyquist plots were compared between the modified electrode and the bare GC electrode. The graph of this comparison is shown in Figure 4. When the Nyquist curves were examined, it was understood that while the bare GC electrode surface consented to electron transfer, MTP/GC electrode surface didn't consent to electron transfer, supporting the characterization studies executed out with CV. By examining the size of the semicircle in Nyquist plots obtained with EIS technique, it can be decided to what extent the surface is electroactive or electroactive. In Figure 4, the bare GC electrode surface shows a resistance of approximately 1.5 kOhm, while the resistance on the MTP/GC electrode surface is much larger.

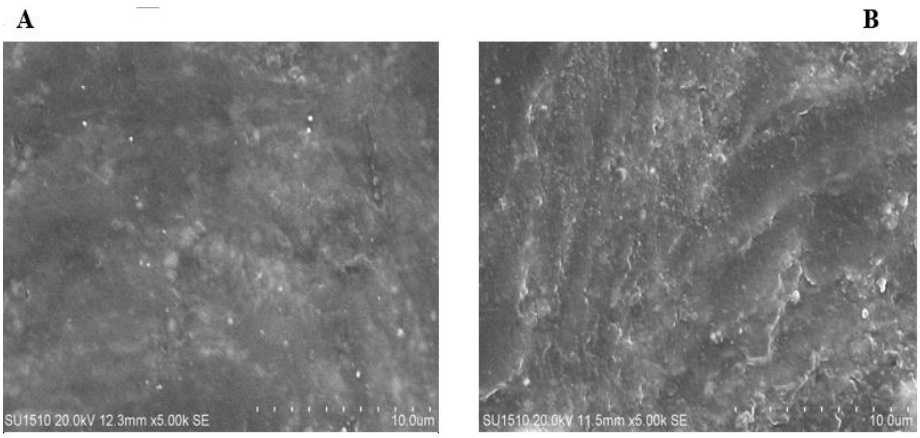

Figure 5. SEM images of (A) bare GC and (B) MTP/GC electrode

Additionally, alongside the CV and EIS characterization processes, the surface morphology of bare GC and MTP/GC electrode surfaces was examined using SEM. SEM images depicting the bare GC and MTP/GC electrode surfaces are provided in Figures 5A and 5B. When the images obtained with the SEM technique were compared, the obvious morphological difference between the two images showed the modification processes had been carried out successfully.

3.3. Effect of Scan Rate Using LSV

One of the important steps when examining the electrochemical behavior of a new molecule is to determine whether the molecule reaches the electrode surface in the solution media with electrochemical diffusion control. For this purpose, scanning speed studies were carried out. In these studies, 1 mM MTP solution prepared in 100 mM NBu_4BF_4 support electrolyte media prepared in CH_3CN was used. The study was carried out using LSV and the superimposed voltammograms were given in Figure 6. Voltammograms were taken at scanning rates of 10, 25, 50, 100, 200, and 300 mV s^{-1}, in the potential range of 0.2 V to 1.2 V. According to the Randles-Sevcik equation, the linear relationship seen in the graph plotting peak current versus the square roots of scanning speeds suggests that the molecule binds to the electrode surface in a diffusion-controlled manner.

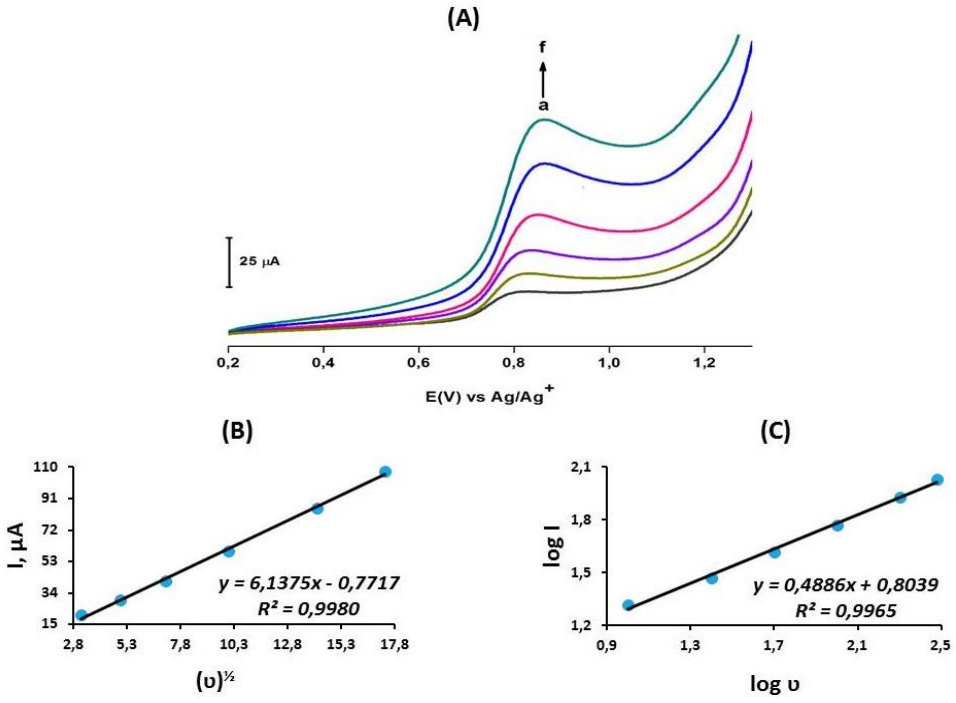

Figure 6. (A) Impact of scan rate on 0.1 mM MTP in BR pH 10.0 at GC electrode from 0.2-1.2 V s^{-1}, (B) Calibration plot of the redox peak currents vs. square root of scan rate, (C) Relation between log scan rate and log peak current

As shown in Figure 6B, the oxidation peak currents (I_{pa}) are plotted against the scan rate (υ) over the studied range.

$$I_{pa}\ (\mu A) = -0.7717 + 6.1375\ \upsilon^{1/2};\ R^2 = 0.9980$$

The linear relationship was acquired (Figure 6C) and was depicted by the equation:

$$\log I_{pa} = 0.4886 \log + 0.8039;\ R^2 = 0.9965$$

The correlation coefficient, $R^2 = 0.9980$, for the obtained line in Figure 6B suggests a strong correlation for the linear fit. Additionally, the slope of the plot of log Ip against log ν in Figure 6C is approximately 0.5, indicating that the molecule was transported to the electrode surface in a diffusion-controlled manner.

3.4. Effect of pH on Sensor Response

In voltammetric investigations, choice of supporting electrolyte and pH value is essential for elucidating the electrochemical behaviors of an analytical compound. pH value of the electrolyte for the modified electrode can influence the shape of the peak, peak potential, and peak current. Square wave voltammograms were obtained using the SWV technique on the MTP/GC electrode over a potential range of -0.5 V to +0.5 V, with 1.0 mM Hg^{2+} solutions prepared in BR buffer solutions at different pH values ranging from 2.0 to 12.0. The overlayed voltammograms were presented in Figure 7A.

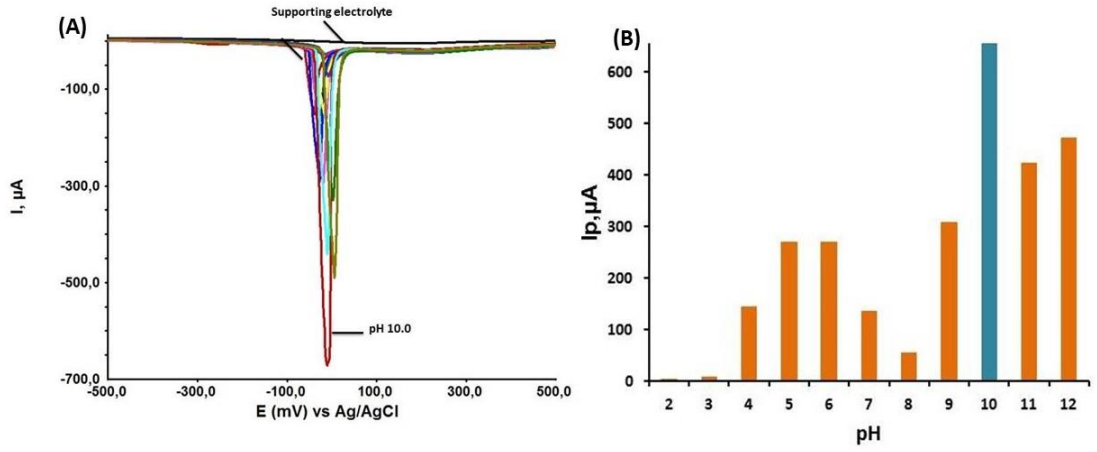

Figure 7. (A) Superimposed voltammograms of 1mM Hg^{2+} from -0.5 to +0.5 V using 0.1 V s^{-1} by DPV with MTP/GC electrode at different pH values (2.0-12.0); (B) Peak current values for Hg^{2+} ions with various pH on MTP/GC electrode

When the peak currents were compared according to different pH values in BR buffer in Figure 7B, the most suitable pH value was determined to be 10.0.

3.5. Effect of Incubation Time

To improve the sensitivity and detection limit of the proposed method, an incubation study was performed using DPV technique. The incubation time of Hg^{2+} ions using DPV technique was investigated in the range of 0, 15, 30, 45, 60, 75, 90, 105, 120 minutes in Figure 8A. Optimum incubation time was determined as 90 minutes to ensure the highest absorption of Hg^{2+} ions. Obtained a graph was drawn between the peak current values and the incubation time in Figure 8B. This value was used in the rest of the study.

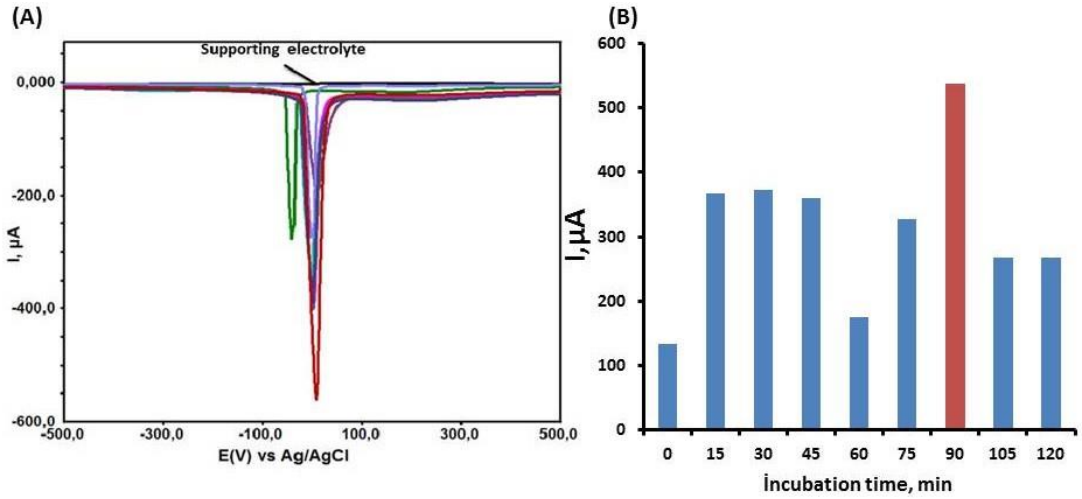

Figure 8. (A) Superimposing DPV voltammograms for different incubation times in the potential range of -0.5 V/ +0.5 V, using MTP/GC electrode, of 1 mM Hg^{2+} solution prepared in 0.1 M BR (pH=10.0); 0, 15, 20, 25, 30, 35, 40, 45, 50 m, 55, 60, 90, 120 minutes); (B) Peak current values for Hg^{2+} ions with various incubation times on MTP/GC electrode

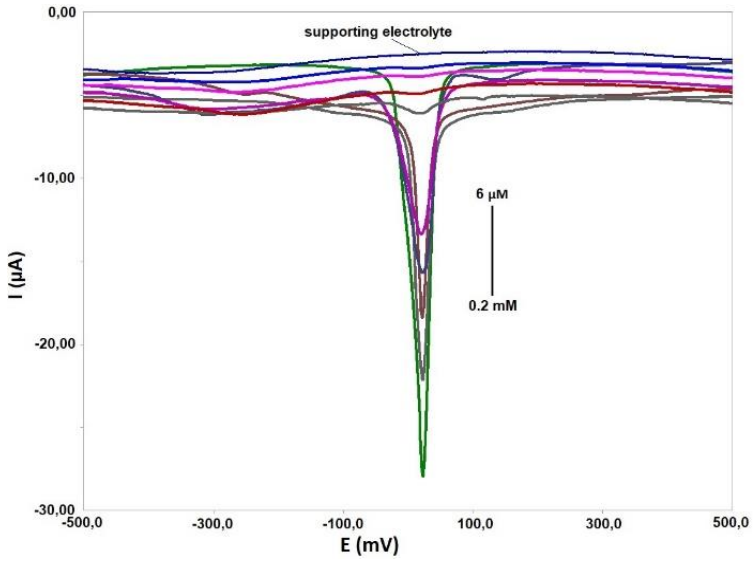

Figure 9. Overlayed voltammograms of MTP/GC electrode obtained from DPV in BR (pH 10.0) in the concentration range from 0.2 mM to 6 µM Hg^{2+}

3.6. Determination of Hg^{2+} Ions on MTP/GC Electrode by DPV

Electrochemical determination of Hg^{2+} ions at different concentrations was examined using DPV due to its narrowest detection potential range. Using the DPV technique of MTP-modified GC electrode in pH= 10.0 BR buffer, DPVs were taken using 0.2 mM and 6 µM Hg^{2+} solutions after incubating for 90 minutes. Superimposed voltammograms are given in Figure 9.

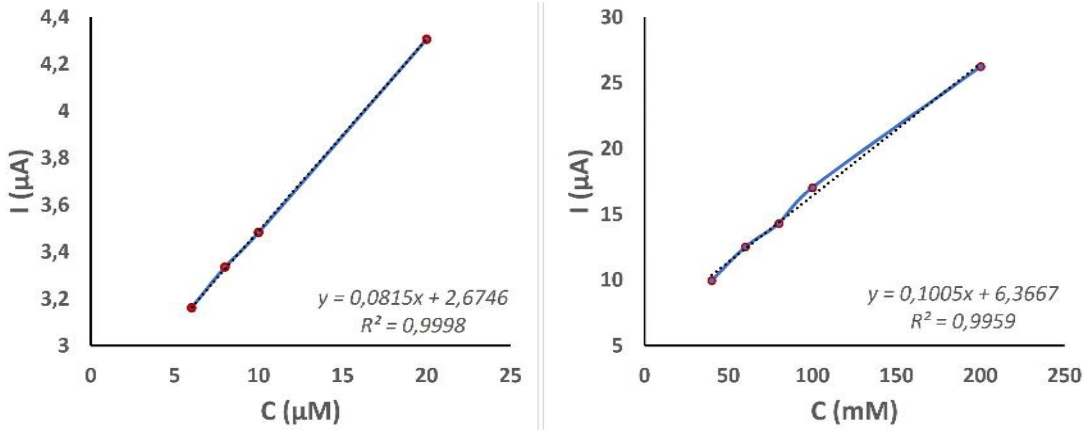

Figure 10. The plots of I_{pa} versus concentration of mercury Hg^{2+} ions

From the calibration curve of linearly increasing anodic peak current (I_{pa}), two linear equations one with $I_{pa} = 0.0815C_{Hg2+} + 2.6746$) and the other with ($I_{pa} = 0.1005C_{Hg2+} + 6.3667$ were created and the corresponding correlation coefficients were calculated as 0.9998 and 0.9959, respectively.

Table 1. Comparison of mercury determination with other modified electrodes

Electrode	Technique	LOD	Reference
GPE	CV	6.6 µM	[19]
Au-PEDOT	ASV	5.0 µM	[20]
Graphene–MnO_2	LSV	2.0 µM	[21]
CPE	DPASV	1.6 µM	[22]
MTP/GC	DPV	1.12 µM	**This work**

This study has a low detection limit and a wide working range compared to different working electrodes and different electrochemical techniques given in Table 1.

4. CONCLUSION

In this research, the analytical performance of a new sensor MTP/GC electrode for the determination of mercury by the DPV method was investigated. In the first step, the potential

range was optimized and the effect of pH values was investigated using BR buffer solutions from 2.0 to 12.0. Best DPV responses were in BR buffer solution at pH 10 and potential range between +0.2 and +2.6 V with a scan rate of 0.1 V s^{-1}. According to the above conditions, MTP/GC electrode was obtained LOD and LOQ values for Hg^{2+} at micromolar levels. The LOD and LOQ for Hg^{2+} ions were 1.12 and 3.39 µM, respectively. Since the MTP/GC electrode has the highest sensitivity to Hg^{2+} ions, the sensitivity and ability to detect micromolar levels of mercury at very low concentrations at BR pH 10.0 has been carefully studied. According to the obtained results, DPV technique using MTP/GC electrode may be a useful alternative to more expensive chromatography-mass spectrometry methods in the determination of Hg^{2+} ions.

Acknowledgments

This study was conducted as part of Hafizullah Sharıfi's master's thesis.

Declarations of interest

The authors declare no conflict of interest in this reported study.

REFERENCES

[1] P.B. Tchounwou, W.K. Ayensu, N. Ninashvili, and D. Sutton, Environ. Toxicol. Int. J. 18 (2003) 149.
[2] M. Nylander, L. Friberg, and B. Lind, Swed. Dent. J. 11 (1987) 179.
[3] M.L.A. Cuvin-Aralar, and R.W. Furness, Ecotoxicol. Environ. Saf. 21 (1991) 348.
[4] A.F. Danet, M.C. Bratu, M.C. Radulescu, and A. Bratu, Sens Actuators B Chem. 137 (2009) 12.
[5] I. Murkovic, and Otto S. Wolfbeis, Sens Actuators B Chem. 39 (1997) 246.
[6] X. Zhu, and S.D. Alexandratos, Microchem. J. 86 (2007) 37.
[7] D.S. Bushee, Analyst 113 (1988) 1167.
[8] J. Gong, T. Zhou, D. Song, and L. Zhang, Sens. Actuators B Chem. 150 (2010) 491.
[9] X. Niu, Y. Ding, C. Chen, H. Zhao, and M. Lan, Sens. Actuators B Chem. 158 (2011) 383.
[10] N. İslamoğlu, İ. E. Mülazımoğlu, and A. Demir Mülazımoğlu, Anal. Methods 15 (2023) 4149.
[11] Ş. Korkmaz, W. Bosnali, İ. E. Mülazımoğlu, A. Demir Mülazımoğlu, Food Anal. Methods 16 (2023) 1738.
[12] T. Gebregiyorgis, M. Tessema, S. Admassie, G. Birhanu, and A. Ashenef, Anal. Bioanal. Electrochem. 11 (2019) 1070.

[13] N. Durmuş, E. Yılmaz, A. Demir Mülazımoğlu, B. Mercimek, A. Çukurovalı, İ. Yılmaz, and İ. E. Mülazımoğlu, Desalin Water Treat. 112 (2018) 34.
[14] A. Demir Mülazımoğlu, İ. E. Mülazımoğlu, and E. Yılmaz, Rev. Anal. Chem. J. 31 (2012) 131.
[15] İ. E. Mülazımoğlu, A. Demir Mülazımoğlu, and E. Yılmaz, Desalination 268 (2011) 227.
[16] A. Demir Mülazımoğlu, İ.E. Mülazımoğlu, Food Anal. Methods 6 (2013) 141.
[17] H.H. Çelik, S. Özcan, A. Demir Mülazımoğlu, E. Yılmaz, B. Mercimek, A. Çukurovalı, and İ.E. Mülazımoğlu, Inorg. Chem. Commun. 116 (2020) 107893.
[18] N. İslamoğlu, and A. Demir Mülazımoğlu, Anal. Bioanal. Electrochem. 15 (2023) 1086.
[19] C. Raril, and J.G. Manjunatha, JAST 11 (2020) 1.
[20] S. Anandhakumar, J. Mathiyarasu, and K.L.N. Phani, Anal. Methods 4 (2012) 2486.
[21] J. Lu, X. Zhang, N. Liu, H. Li, Z. Yu, and X. Yan, Electrochim. Acta. 174 (2015) 221.
[22] D.S. Rana, R. Kumar, N. Gupta, K.K. Thakur, and D. Singh, Nano-Struct. Nano-Objects. 36 (2023) 101041.