

A Dicysteinyl Tripeptide-Based Electrochemical Sensor for Highly Selective and Stable Detection of Mercury(II) Ions

(Sensor Elektrokimia Berasaskan Dicysteinyl Tripeptide untuk Pengesanan Ion Merkuri(II) yang Sangat Selektif dan Stabil)

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ABSTRACT

Global industrialization has made it necessary to create cheap, quick ways to measure mercury(II) ions (Hg^{2+}) in water systems. This study presents a dicysteinyl tripeptide of NH_2 -Cysteine-Alanine-Cysteine-COOH (CAC)- based electrochemical sensor designed for the highly selective and stable detection of Hg^{2+} ions. The modified electrode was fabricated by the immobilization of the CAC-tripeptide and multi-walled carbon nanotubes (MWNTs) within a Nafion polymer matrix onto a platinum electrode (Pt). Molecular dynamics (MD) simulations confirmed that Hg^{2+} chelation via dicysteinyl thiol groups rigidifies the CAC tripeptide matrix into a structurally stable, low-flexibility conformation. This CAC/MWNTs/Nafion/Pt electrode modification provided a high surface area and superior charge transport capacity, resulting in good sensitivity. Analytical validation demonstrated a linear response range of 10 to 100 nM, with a limit of detection (LOD) of 7.9 nM, effectively exceeding the World Health Organization (WHO) safety criterion of 10 nM. With a relative standard deviation (RSD) of 3.1% ($n = 7$) at 100 nM Hg^{2+} , the modified electrode demonstrated great precision. Moreover, the biosensor exhibited excellent reproducibility and substantial long-term stability, maintaining 85% of its initial electrochemical response after a three-week duration. Selectivity studies showed that most species were quite resistant to competing metal ions, with only Cu^{2+} and Ag^+ causing modest problems. Based on this result, the immobilization of the CAC-tripeptide and multi-walled carbon nanotubes (MWNTs) within a Nafion polymer matrix onto a platinum electrode development seems like a promising early, easily accessible option for environmental water quality monitoring in real time.

Keywords: Dicysteinyl tripeptide; electrochemical sensor; mercury(II) ion; MWNTs

ABSTRAK

Perindustrian global telah menjadikan pembangunan kaedah pengukuran ion merkuri(II) (Hg^{2+}) yang murah dan pantas di dalam sistem air sebagai satu keperluan. Kajian ini membentangkan penderia elektrokimia berasaskan tripeptida disisteinil NH_2 -Sisteina-Alanina-Sisteina-COOH (CAC) yang direka untuk pengesanan ion Hg^{2+} yang sangat selektif dan stabil. Elektrod terubah suai telah difabrikasi melalui imobilisasi tripeptida CAC dan tiub nano karbon berdinding pelbagai (MWNTs) di dalam matriks polimer Nafion ke atas elektrod platinum (Pt). Simulasi dinamik molekul (MD) mengesahkan bahawa pengkelatan Hg^{2+} melalui kumpulan tiol disisteinil menegarkan matriks tripeptida CAC menjadi konformasi fleksibel rendah yang stabil dari segi struktur. Pengubahsuaian CAC/MWNTs/Nafion/Pt elektrod ini menyediakan luas permukaan yang tinggi dan kapasiti pengangkutan cas yang unggul, yang menghasilkan kepekaan yang baik. Pengesanan analitikal menunjukkan julat tindak balas linear antara 10 hingga 100 nM dengan had pengesanan (LOD) sebanyak 7.9 nM, sekali gus berjaya melepasi kriteria keselamatan Pertubuhan Kesihatan Sedunia (WHO) iaitu 10 nM. Dengan sisihan piawai relatif (RSD) sebanyak 3.1% ($n = 7$) pada 100 nM Hg^{2+} , elektrod terubah suai tersebut menunjukkan kejutuan yang tinggi. Selain itu, penderia kimia ini menunjukkan kebolehlulangan yang cemerlang dan kestabilan jangka panjang yang teguh, dengan mengekalkan 85% daripada tindak balas elektrokimia asalnya selepas tempoh tiga minggu. Kajian selektiviti menunjukkan bahawa kebanyakan spesies adalah agak rintang terhadap gangguan ion logam pesaing dengan hanya Cu^{2+} dan Ag^+ yang menyebabkan gangguan sederhana. Berdasarkan keputusan ini, pembangunan elektrod terubah suai melalui imobilisasi tripeptida CAC dan tiub nano karbon berbilang

dinding (MWNTs) di dalam matriks polimer Nafion ke atas elektrod platinum dilihat sebagai satu pilihan awal yang berpotensi dan mudah dicapai bagi pemantauan kualiti air alam sekitar secara masa nyata.

Kata kunci: Ion merkuri (II); MWNTs; penderia elektrokimia; tripeptida disisteinil

INTRODUCTION

Mercury is one of the top ten chemicals of major health concern and World Health Organization (WHO) has established a maximum contaminant level for mercury of 2 µg/L or 10 nM in drinking water (Saturday 2018). Mercury (II) ions (Hg^{2+}) are one of the most usual and stable forms of mercury pollution (Król, Kukulska-Zajac & Gajec 2024). Monitoring Hg^{2+} in an aqueous environment has received increasing interest in the development of various mercury ion sensors (Ali et al. 2022; Radha, Valliyengal & Al-Sayah 2025). For the measurement of Hg^{2+} levels in water samples, several standard analytical methods have been extensively utilized, such as inductively coupled plasma mass spectrometry (Shi et al. 2020) or atomic absorption spectroscopy (Gu et al. 2017). Due to their intricacy, these methods usually require costly equipment, lengthy processes and highly skilled people. Because of these drawbacks, less complex approaches and measuring techniques are sought. Electrochemical sensors are promising analytical tools for providing real-time data with fast procedures, low cost, high selectivity, and sensitivity due to their selectivity and sensitivity.

Voltammetry is one of the most popular and commonly used methods for measuring current as a function of applied voltage (Meri Dayanti et al. 2025). By altering the surface of normal electrodes, one must build a stable, highly targeted, unique interface to increase the current response (Miller et al. 2024). Currently, numerous chemical sensors utilizing the interaction between Hg^{2+} and nitrogen, oxygen, or sulfur atoms in molecules that coordinate heavy metal ions (Meri Dayanti et al. 2025; Pramanik 2026) require enhancements in stability.

The degree of selectivity and sensitivity of a sensor can be improved (Li et al. 2021; Pramanik 2026) by combining a suitable recognition element with an electrochemical transducer, such as the development of gold disk electrodes modified with single-stranded PNA (peptide nucleic acid) for the determination of Hg^{2+} (Pramanik 2026; Xiong et al. 2023). Electrochemical impedance spectroscopy (EIS) and spectrophotometry were used to investigate the interactions between mercury ions and Peptide Nucleic Acid (PNA) strands (UV-VIS). It is advantageous to use DNA-modified electrodes that rely on generating T-T mismatches in mercury preconcentrated, but these Deoxyribonucleic acid (DNA) hybridization processes are relatively sluggish (Hermanová et al. 2025). Carbon nanomaterials have been widely utilized because of their high conductivity and catalytic activity, which help signal

amplification in Hg^{2+} detection (Hermanto et al. 2026; Romaguera-Barcelay et al. 2024).

Having the same building blocks as protein, it is possible for a peptide with a specific sequence to substitute for protein in biological analysis could provide high affinity, sensitive, fast, and convenient chemical sensors to analytes such as mercury (Li et al. 2021). Cysteine (Cys) is known as a Hg^{2+} ligand (Yang, Wang & Lv 2025) and has a thiol group that functions as a donor atom for metal ion coordination. According to Pearson's principles (Pearson 1967), mercury (II) ion shows a strong affinity for ligands with 'soft base' donor atoms such as sulfur in cysteine. A steric limitation on the complex formed depends on peptide side chain functionalities. However, there are no clear criteria for the design of highly permeable tripeptide due to various uptake mechanisms involved in the absorption process, including passive and active transport (Hsieh & Liaw 2019). The thiol-metal binding ideally can be improved by increasing the number of thiol S-donors in the tripeptide (Yang, Wang & Lv 2025) as showed by the dithiol peptide Cys-Ala-Cys (CAC) as in Figure 1. A non-reactive alanine (Ala) was chosen as an anchor for the dithiol peptide as it is the simplest α -amino acid after glycine and hardly ever directly involved in protein function.

The electrochemical investigation of tripeptide Cys-Ala-Cys was done to investigate the sensitivity and selectivity response of dicysteiny tripeptides with mercury (II) ions in the solution. The modified electrode was fabricated by the immobilization of the CAC-tripeptide and multi-walled carbon nanotubes (MWNTs) within a Nafion polymer matrix onto a platinum electrode was developed to detect Hg^{2+} ion in water solution. The tripeptide consists of an amino acids sequence of NH_2 -Cysteine-Alanine-Cysteine-COOH (CAC), a recognition element for capturing Hg^{2+} in solution. The bulk nanocomposite can be dropped onto the surface of an electrode surface and used after drying at room temperature (Folorunso et al. 2022). The two cysteiny peptides may bind the mercury (II) ion to create a three- or four-coordinate complex (Kim, Gwon & Kim 2022), resulting in a current response in cyclic voltammetry.

Basic computational modeling was conducted to assess the energetic properties and structural stability of the CAC tripeptide- Hg^{2+} complexes to complement the electrochemical investigations. The study evaluated the conformational integrity and binding strength of that dicysteiny tripeptide when coordinated with Hg^{2+} metal ions by employing molecular mechanics principles and

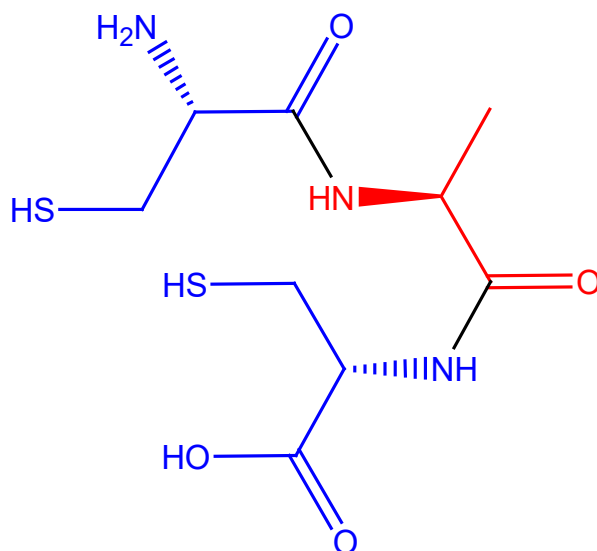


FIGURE 1. Tripeptide Cysteine-Alanine-Cysteine (CAC)

basic molecular dynamics simulation. The electrochemical investigations and computational modeling findings have significantly contributed to establishing a strong foundation for further exploration and utilization of the CAC/MWNTs/Nafion/Pt modified electrode in real-world environmental monitoring and analytical chemistry applications.

MATERIALS AND METHODS

REAGENTS AND MATERIALS

Nafion, a perfluorinated ion-exchange resin (117 solutions, ~5 wt% solutions in a mixture of lower aliphatic alcohols and water)(Chen et al. 2007), was purchased from Fluka (Switzerland). Multi-walled carbon nanotubes (90%) and potassium phosphate monobasic (SigmaUltra, 99.0%) were purchased from Sigma-Aldrich (Sydney, Australia) (Nor Azah et al. 2011). Tripeptide CAC was purchased from Bachem (Germany) and Hg(II) nitrate was purchased from Aldrich (U.S.A., Germany). Merck supplied the sodium acetate and sodium hydroxide (Germany). Stock solution (1.00 g/L) of Hg^{2+} ions is prepared from $\text{Hg}(\text{NO}_3)_2$ in 0.1 M HNO_3 . The supporting electrolyte used to prepare different analyte concentrations is 0.1 M acetate buffer (pH 5.5). All solutions will be prepared using deionized water and without further purification (Helen Rani et al. 2024). Solutions of lower concentrations will be prepared by appropriate dilutions immediately before their use.

The electrode was polished before immobilizing 0.3 and 0.05 μm alumina slurry. Cleansing the surfaces with ultrasonication in distilled water and 100% ethanol between each polishing stage eliminates impurities. In preparation of CAC/MWNTs/Nafion/Pt modified

electrode, two milligrams of pure multi-walled nanotubes (MWNTs) are dispersed in 2.0 mg of CAC peptide and 1.0 mL Nafion using ultrasonic agitation. The Pt electrode surface was coated with 20 μL of this black dispersion, which evaporated and dried at room temperature. This method improves the electrode surface's sensitivity and stability significantly by enabling the development of a thin, homogeneous coating. All experiments were performed at room temperature ($25 \pm 1^\circ\text{C}$) (Morsy et al. 2022).

For current performance purposes in cyclic voltammetry, electrodes were modified with counterpart free tripeptide (MWNTs/Nafion/Pt modified electrode). The possibility of interference effects will be ascertained by electrochemically studying the Hg^{2+} solution in the presence of an excess of foreign ions. Rivers in Sungai Kajang, Selangor, were used to gather water samples. A 0.45 m membrane filter removed suspended particles to filter the water samples. Before usage, the filtered water samples were kept in pristine, acid-washed glass bottles at 4°C . Several standard solutions of metal ions, including Cu^{2+} , Fe^{3+} , Zn^{2+} , Ni^{2+} , Pb^{2+} , Cd^{2+} , and Ag^+ , had been generated at 100 nM concentrations, similar to the values in the calibration study to explore the interference of other metal ions on the performance of the chemical sensor (Liu et al. 2010). The selectivity of the modified sensor was evaluated by exposing it to a standard concentration of 100 nM Hg^{2+} . Its voltammetric response was subsequently compared against mixtures containing 100 nM Hg^{2+} in the concurrent presence of co-existing metal ions or organic interference (Govindaraju et al. 2018). This comparison allowed for calculating the chemical sensor's relative sensitivity (%) for each metal ion and an organic compound. A relative sensitivity of less than 90% suggested there was significant interference.

INSTRUMENTATION

Electrochemical evaluation with cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were carried out using an Autolab (Type III) (Microchemie) interfaced to a computer running the G.P.E.S. (version 4.9) software in a conventional three-electrode cell consisting of a working electrode of platinum (Pt), a pseudo reference electrode of silver/silver chloride (Ag/AgCl) and an auxiliary electrode of platinum wire. The signal of the accumulated modified and unmodified Pt electrode in the supporting electrolyte was measured using CV by scanning the potential from +0.5 to -0.6 V with a scan rate of 100 mVs⁻¹ performed in each solution unless specified otherwise. All transmission electron microscopy (TEM) images were taken using a J.E.O.L. 2000FX microscope with a tungsten filament as an electron source; an acceleration voltage of 200 kV was applied (Nor Azah et al. 2011).

A classical molecular dynamic in computational modeling was performed to investigate the stability of the tripeptide-mercury (II) complex using G.R.O.M.A.C.S. software version 5.0.4 (Leap Frog Algorithms) (Xia & Zhang 2014). Applying an electrostatic and Van der Waals cut-off at 0.8 nm with atomic positional restraints set at 1×106 kJ mol⁻¹ nm⁻¹ enhances the overall system stability.

RESULTS AND DISCUSSION

STABILITY BY MOLECULAR MODELING

Basic computational modeling was performed to investigate the stability of CAC tripeptide-Hg²⁺ complexes. Computational modeling examined the stability of two tripeptide-mercury (II) complexes (Marinova & Tamahkyarova 2024). The CAC dicysteiny l tripeptides were designed as a detection tool for Hg²⁺ metal ions. Applying the principles of molecular mechanics, simple molecular dynamics simulations were conducted on the tripeptide and its complexes with Hg²⁺ in an aqueous environment. Energetic properties were evaluated to indicate system stability, while atomic displacements and fluctuations analysis were performed to determine the structural stability of the dicysteiny l tripeptides when bound to Hg²⁺ metal ions.

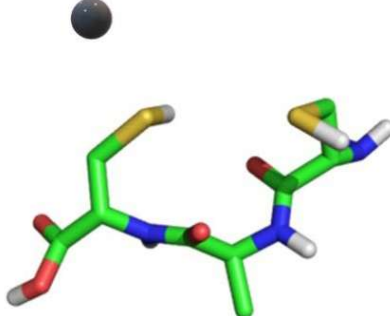
As in Table 1, the average potential energy from the last 2ns of MD simulations indicated CAC- Hg²⁺ had an increase of potential energy during the presence of mercury. The increment in potential energy (252.6 kJ/mol) from CAC to CAC- Hg²⁺ showed that the tripeptide complex was slightly destabilized when mercury was present. The possible energy plot exhibited a consistent energy profile for the systems studied. The structural configuration of the systems during the final molecular dynamics (MD) sampling window is detailed in Table 1. The average potential energy shifts from -40126.5 ± 4.1 kJ mol⁻¹ (free CAC) to slightly less

negative value of -39874 ± 6.5 kJ mol⁻¹ upon binding with Hg²⁺. While this slight energy difference reflects the localized conformational strain and steric adjustment required to accommodate the heavy metal ion within the classical force field parameters, the systems successfully transition into a highly rigidized design, low flexibility state. This structural stabilization is directly confirmed by the sharp drop in the Root Mean Squared Deviation (RMSD) from. 0.16 ± 0.05 nm to 0.12 ± 0.02 nm. A drastic reduction in the total C_α B-factor from 38.10 to 11.60 for a highly rigidified, conformationally stable coordination complex driven by the dicysteiny l thiol group. Furthermore, the atomic displacements and fluctuations analysis indicated that the tripeptide remains structurally stable when bound to Hg²⁺ metal ions.

In addition to the potential energy findings, it is important to delve deeper into the atomic displacements and fluctuations to understand the structural stability of the CAC tripeptide-Hg²⁺ complexes. By analyzing the atomic displacements and fluctuations, it is possible to elucidate the structural dynamics of the tripeptide in the presence of mercury ions. This analysis will provide valuable insights into the interactions between the tripeptide and the Hg²⁺ ions, shedding light on the stability and conformational changes within the complex. Furthermore, an in-depth investigation into the molecular dynamic's simulations beyond the last 2ns will offer a more comprehensive view of the energetic favorability and stability of the CAC tripeptide-Hg²⁺ complexes. Extending the analysis to encompass a longer duration makes it possible to capture additional nuances in the energy profile and better understand the dynamic behavior of the systems studied. RMSD profiles showed the inconsistent movement of unbound dicysteiny l tripeptides in an aqueous solution. In turn, the presence of Hg²⁺ produced more consistent profiles for CAC- Hg²⁺. CAC- Hg²⁺ had a more stable RMSD profile with an average reduction of 0.12 nm from CAC in aqueous.

The effect of Hg²⁺ on the flexibility of the tripeptide was measured using the atomic fluctuation analysis from the last 2 ns of MD simulation (Pinak 2002). The theoretical B-factor was calculated and mapped onto the peptide structure in Figure 2. It was found that the presence of Hg²⁺ greatly reduced the local flexibility of the side chains at both ends, CAC/CAC-Hg²⁺. These preliminary results showed that a Cys-Cys combination could detect Hg²⁺ with alanine rendered as a middle amino acid in combination with tripeptide. Unfortunately, the current force field employed here cannot produce precise interaction energy between the peptide and the metal ion. The structural stability of the dicysteiny l tripeptides when bound to Hg²⁺ metal ions, as evaluated through atomic displacements and fluctuations analysis, may not fully represent the dynamic nature of molecular interactions in a biological environment. In biological systems, factors such as protein folding, conformational

TABLE 1. Average potential energy from the last 2ns of MD simulations indicated CAC- Hg²⁺

System	CAC	CAC- Hg ²⁺
 Carbon	 Sulfur	 Hydrogen
 Oxygen	 Nitrogen	 Mercury
Model		
Potential energy (kJ mol ⁻¹)	-40126.7 ± 4.1	-39874.1 ± 6.5
Temperature (K)	300.20 ± 0.06	298.12 ± 0.11
Pressure (bar)	1.49 ± 0.02	1.40 ± 0.42
Density (kg m ⁻³)	960.28 ± 8.66	973.78 ± 0.25
Volume (nm ³)	31.07 ± 0.01	27.64 ± 0.01
Average Root Mean Square Deviation (R.M.S.D.)	0.16 ± 0.05	0.12 ± 0.02
Total B-factor of Cα	38.10	11.60

*Data are averages over the last 2ns of final MD simulations

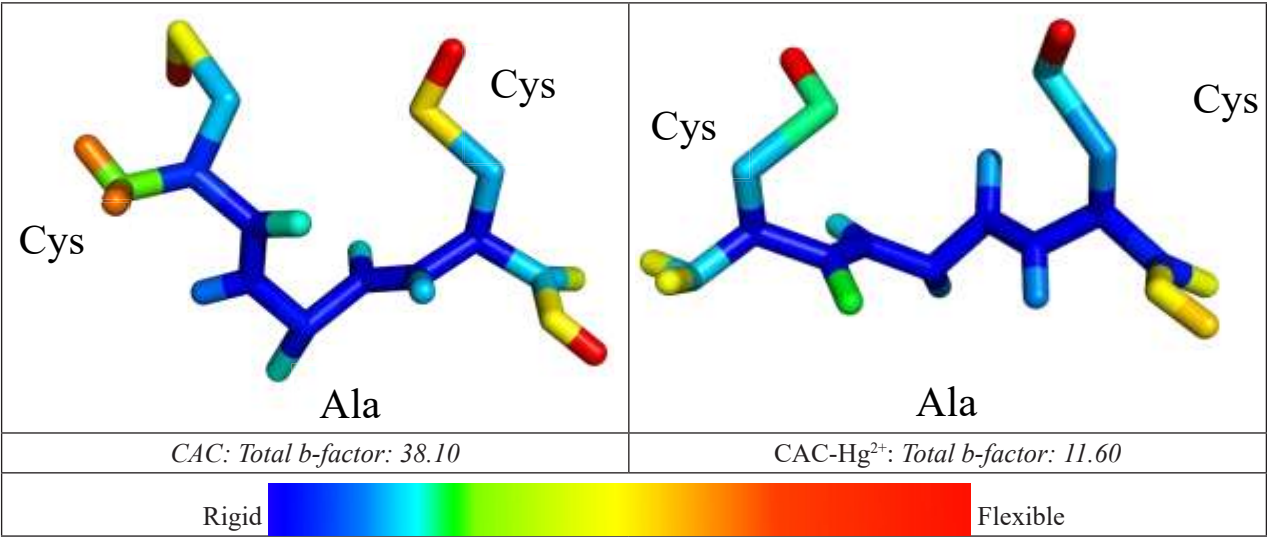


FIGURE 2. Atomic fluctuation analysis from the last two ns of MD simulation

changes, and environmental influences can play significant roles in determining stability and behavior, which may not be fully captured in computational models alone. Therefore, while computational modeling can provide valuable insight, it should be complemented with experimental validation to ensure reliability and accuracy of the findings.

For further investigations, the models here need to be improved either by involving quantum mechanical calculations (QM/MM), a method that calculates the electronic structure of the active site or testing with different types of force field to give us a better understanding on reactions that can be modeled (Kratz et al. 2016). Mehmood and Kulik (2020) and Vasilevskaya et al. (2016) also studied the QM/MM approach for zinc binding sites. Their work confirmed that this method can accurately capture electronic properties and metal coordination (Mehmood & Kulik 2020; Vasilevskaya et al. 2016).

DESIGN OF THE Hg^{2+} DETECTION STRATEGY

The Nafion membrane has a hydrophobic backbone and hydrophilic side chains containing sulfonic acid moieties (Choi et al. 2022), which form proton-conducting clusters when combined with water molecules (Liu et al. 2010). When MWNTs are added to a polymer matrix, their mechanical characteristics can increase dramatically (Li et al. 2021). In this study, we used the Nafion membrane to improve the dispersion ability of MWNTs. The combination of Nafion membrane with MWNTs offers a promising way to improve the dispersion ability of MWNTs and enhance their mechanical characteristics. The hydrophobic backbone of the Nafion membrane, along with its hydrophilic side chains containing sulfonic acid moieties, facilitates the formation of proton conducting clusters when combined with water molecules. This unique property of the Nafion membrane plays a crucial role in promoting the dispersion ability of MWNTs. The voltammetric response of CAC/MWNTs/Nafion/Pt electrode was examined concerning changing the volume of nanocomposite suspension drop-cast into a Nafion solution. A notable increase in peak current was seen as the volume of the drop-cast suspension increased from 10 to 20 μL . However, the electrochemical sensitivity significantly decreased at volumes greater than 30 μL . This drop in performance is attributed to the excessive thickness of the resulting nanocomposite layer, which creates a mass-transport barrier and obstructs the electrode's electrical conducting surface (Zhang et al. 2018). Consequently, an optimal drop-casting volume of 20 μL was selected for the fabrication of the CAC/MWNTs/Nafion onto a Pt electrode. This optimized volume ensures an ideal balance between maximized peak current and optimal sensitivity, allowing for an efficient electron transfer kinetics at the CAC/MWNTs/Nafion onto Pt electrode interface.

Figure 3 shows the MWNTs-Nafion micrographs of TEM figure that provide evidence of the successful incorporation of Nafion polymer into the MWNTs, as seen by the layered bundles and observation of Nafion chains attached to the outer surface of MWNTs.

The presence of a 2.7 nm thick outer layer further confirms the presence of Nafion on the MWNTs. The TEM figure also shows a well-aligned arrangement of individual MWNTs with sizes ranging from 7.5 nm to 9 nm. This suggests that the MWNTs were properly set up and ready for solvent evaporation (Liu et al. 2010). The observed scattering of MWNTs at different angles to the fiber axis in certain locations may be attributed to variations in the synthesis or processing conditions. Overall, the TEM figure provides valuable insights into the structural characteristics of the CAC/MWNTs/Nafion onto Pt electrode, showing the successful integration of Nafion polymer onto the MWNTs and the aligned arrangement and diameter of the MWNTs. Long-term electrochemical stability tests can be conducted to assess the durability and robustness of the modified electrode under continuous Hg^{2+} ion exposure.

ELECTROCHEMICAL CHARACTERIZATION OF THE SENSOR

The electrochemical behavior of Hg^{2+} at CAC/MWNTs/Nafion on Pt electrode was carried out using cyclic voltammetry (CV). To ensure the mechanical integrity and long-term stability of the modified electrode, a thin layer of Nafion was cast onto the CAC/MWNTs/Pt surface. Nafion serves as sturdy polymeric binder that efficiently immobilizes the tripeptide and carbon nanotubes, preventing their leaching during continuous electrochemical tests. Furthermore, its negatively charged sulfonate groups produce a selective environment that enhance the transport of Hg^{2+} cations while limiting interference from anionic species, hence increasing both the sensitivity and selectivity of the sensor. Figure 4 shows the CV responses of a) CAC/MWNTs/Nafion/Pt electrode without the presence of targeting analyte, b) MWNTs/Nafion/Pt electrode with the presence of targeting analyte, and c) CAC/MWNTs/Nafion/Pt electrode with a present of targeting analyte. The CV were performed in potential scanning from -0.5 V to +0.6 V as a possible window (Ferreira et al. 2021). With the addition of CAC tripeptide onto a Pt electrode in 100 nM Hg^{2+} as target analyte concentration, the curve displayed a distinct signal in the form of oxidation (Hg^0 to Hg^{2+}) and reduction (Hg^{2+} to Hg^0) current steps at -0.37 V and +0.26 V, respectively.

The MWNTs/Nafion/Pt electrode shows the anodic and cathodic peaks almost at similar voltage. Still, the observed current response for Hg^{2+} was lower than those at the CAC/MWNTs/Nafion/Pt electrode. The CAC/MWNTs/Pt electrode has remarkably improved Hg^{2+} sensitivity. The absence of a significant peak within

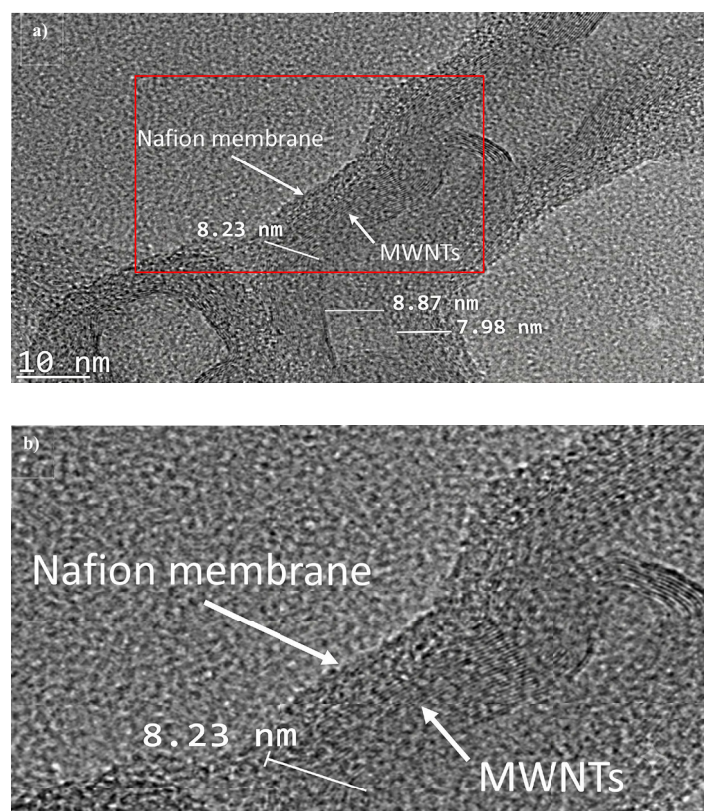


FIGURE 3. (a) TEM micrograph of the MWNTs/Nafion polymer composite at the optimal mass ratio; (b) High magnification view of the boxed area in (a) illustrating the morphological details and the thin Nafion coating on the carbon nanotubes

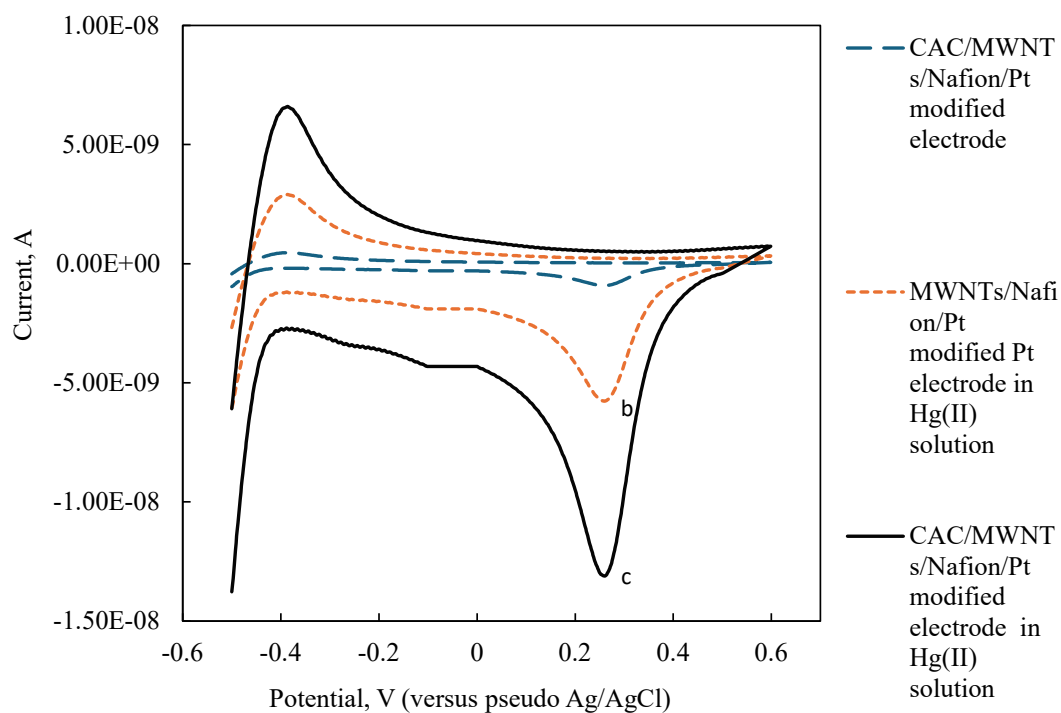


FIGURE 4. Cyclic voltammetry (CV) responses of: (a) the CAC/MWNTs/Nafion/Pt electrode in the absence of Hg^{2+} ; (b) the MWNTs/Nafion/Pt control electrode in the presence of Hg^{2+} and (c) the CAC/MWNTs/Nafion/Pt electrode in the presence of 100 nM Hg^{2+}

the potential window in the solution without targeting Hg^{2+} highlights the electrode's selectivity (Kassa et al. 2022). Notably, a minor peak at +0.25 V in the presence of 0.1 M acetate buffer suggests the electrode's sensitivity to the analyte. The CAC tripeptide, with its specific binding sites for Hg^{2+} ions, forms a strong complex with the target analyte. This interaction greatly enhances the electrode's sensitivity and selectivity towards Hg^{2+} ions. Additionally, the large surface area of MWNTs provides ample sites for adsorption, further amplifying the electrode's performance in detecting Hg^{2+} ions. The synergy between these components has improved sensitivity and opened avenues for advanced detection and quantification of Hg^{2+} ions. Intriguingly, the absence of a significant peak within the potential window in the solution without targeting Hg^{2+} underlines the electrode's remarkable selectivity towards the analyte. The minor peak observed at +0.25 V in the presence of 0.1 M acetate buffer further reinforces the electrode's sensitivity to Hg^{2+} , showcasing its potential for precise and accurate detection in complex sample matrices.

The calibration curve for Hg^{2+} is shown in Figure 5 and is done in differential pulse voltammetry (DPV) at potential -0.37 V and +0.26 V, anodic and cathodic peak current, respectively.

The current increased linearly with the addition of Hg^{2+} in the concentration of 10 – 100 nM, with a linear regression equation of $y = -5 \times 10^{-11}x + 6 \times 10^{-9}$ and

correlation coefficient (R) of 0.9911 for current peak height of anodic (I_{pa}) and linear regression equation of $y = -1 \times 10^{-10}x - 1 \times 10^{-8}$ and correlation coefficient (R) of 0.9976 for current peak height of cathodic (I_{pc}). The limit of detection (LOD) was calculated to be 7.9 nM ($3 \times \text{Sd}$ of the blank response/slope of the calibration plot).

Table 2 compares electrochemical mercury (II) ion performance with the previously reported work. The most sensitive sensors achieve detection limits in the low picomolar to sub-nanomolar range. Alharthi, Fallatah and Al-Saidi (2021) demonstrated in their publication the utilization of a Hg^{2+} selective electrode (Hg^{2+} -SE) for detecting ultra-trace amounts of Hg^{2+} in different real-life samples. They introduced a new Schiff base ionophore called bromo-2-[4-methoxyphenyliminomethyl]phenol as a critical component of the suggested electrode. Labib's group reported the characterization of an electrochemical Hg^{2+} sensor using a thymine-containing linear DNA probe modified with methylene blue for the sensor (Labib, Sargent & Kelley 2016). The linear dynamic range of this sensor was 10 nM to 500 nM, with a 10 nM detection limit. Li et al. (2021) created TP-2 (TPE-Trp-Pro-Gln-His-Glu- NH_2), a fluorescent peptide probe, which harnessed aggregation-induced emission effects and displayed good selectivity for Hg^{2+} . The detection limit for Hg^{2+} in buffer solution was 41 nM, marking a significant discovery. Wannassi et al. (2025) reported 6 nM using nickel porphyrin complexes.

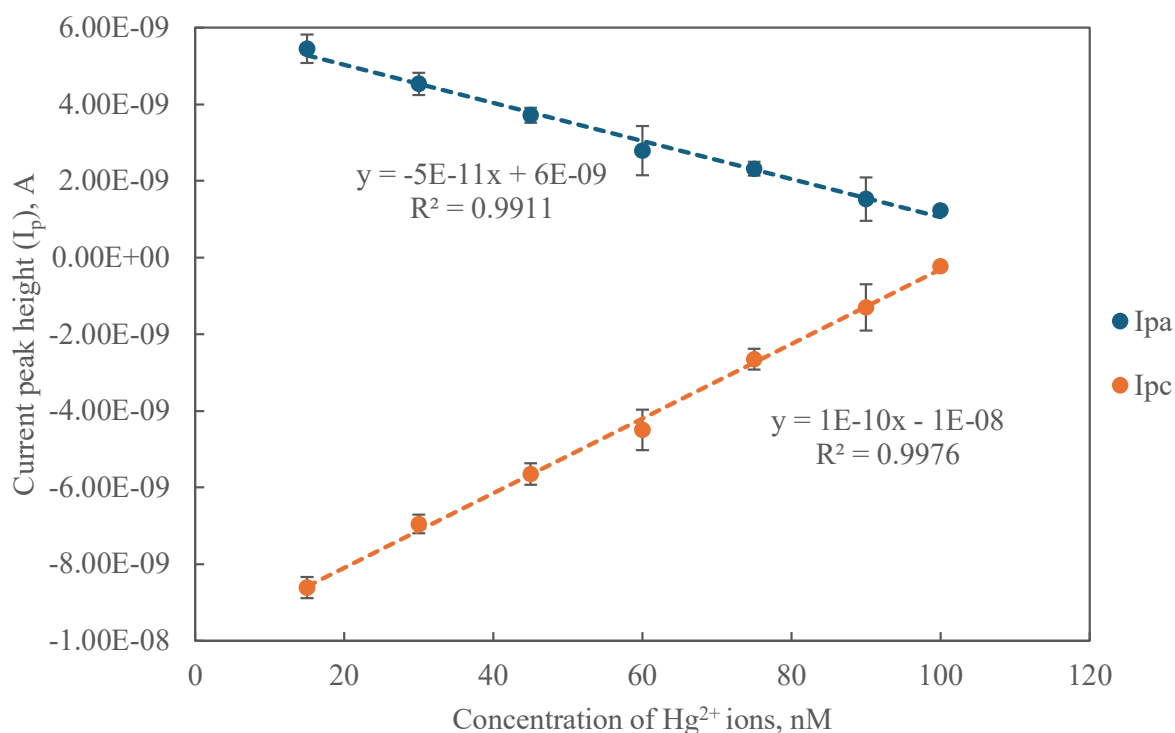


FIGURE 5. Calibration curve differential pulse voltammetry (DPV) at potential -0.37 V and +0.26 V (n=3)

TABLE 2. Comparison of electrochemical Hg²⁺ ion performance with the previously reported

Recognition element	Method of detection	Linear Range	Detection Limit	References
Schiff base, namely 4-bromo-2-[4-methoxyphenylimino) methylphenol	Potentiometric Titration	9 nM and 4000 nM	8 nM	(Alharthi, Fallatah & Al-Saidi 2021)
Methylene blue (M.B.)-modified and thymine-containing linear D.N.A. probe	Square wave voltammetry (SWV))	10 nM and 500 nM	10 nM	(Labib, Sargent & Kelley 2016)
TP-2 (TPE-Trp-Pro-Gln-His-Glu-NH2)	Fluorescence probe	0 – 15 µM	41 nM	(Li et al. 2021)
(5,10,15,20-Tetrakis(p-bromophenyl) porphyrinato] Nickel (II) [Ni (TBrPP)]	Cyclic Voltammetry (CV) and Differential Pulse Voltammetry (DPV)	0.006 – 10 µM	6.0 nM	(Wannassi et al. 2025)
CAC/MWNTs/Pt	Cyclic Voltammetry (CV) and Differential Pulse Voltammetry (DPV)	15 – 100 nM	7.9 nM	Present work

All sensors demonstrated excellent selectivity against competing metal ions, with recovery rates typically 96-103% in real samples.

After comparing the electrochemical mercury ion performance with previously reported work, it is evident that the immobilization of the CAC-tripeptide and multi-walled carbon nanotubes (MWNTs) within a Nafion polymer matrix onto a platinum electrode shows significant promise for sensitive and accurate detection of Hg²⁺. Our investigation determined a lower limit of detection (LOD) for the suggested sensor of 7.9 nM, less than the maximum contamination level for mercury allowed in drinking water by the WHO. This LOD data proves a significant advantage as the CAC/MWNTs/Nafion/Pt electrode sensor can detect samples contaminated with 1.0 µg/L of mercury, a concentration that exceeds permissible limits in numerous countries. By leveraging the unique properties of the immobilization of the CAC-tripeptide and multi-walled carbon nanotubes (MWNTs) within a Nafion polymer matrix onto a platinum electrode, this research contributes to the advancement of mercury ion detection techniques.

SCAN RATE STUDY

Analyzing the dependency of peak current on the scan rate provides critical insights into whether the electrochemical process is controlled by diffusion from the bulk solution, adsorption of the analyte onto the electrode surface, or a complex interaction of both mechanisms (Alarcón et al. 2021). Various scan rates ranging from 20 mV/s to 200 mV/s were used to measure the stripping peak current of Hg²⁺ ions on the modified electrode. The stripping peak current exhibited a proportional increase with higher scan rates, indicating that elevated scan rates can enhance the electrode's sensitivity for Hg²⁺ detection.

Figure 6 presents a plot of the cathodic peak current (I_{pc}) versus the square root of the scan rate ($v^{1/2}$). The observed relationship exhibits near-linearity across the entire studied range. According to the Randles-Sevcik equation, a primary linear correlation between I_p and $v^{1/2}$ is indicative of a diffusion-controlled process, implying that the rate-limiting step is the transport of Hg²⁺ ions from the bulk electrolyte to the electrode interface (Kant & Prerna 2022). The empirical equation describing this relationship was determined as I_{pc} (A) = $1 \times 10^{-0.8} \times -6 \times 10^{-8}$, with a correlation coefficient (R^2) of 0.9631, as in Figure 6.

However, relying strictly on the $v^{1/2}$ plot can obscure surface confinement effects introduced by advanced nanostructure modification layers. Therefore, to gain deeper insight, a log-log diagnostic plot relating the logarithm of the scan rate ($\log v$) to the logarithm of the peak current ($\log I_{pc}$) was constructed, as shown in Figure 7. The resulting linear relationship had a slope of 0.85, explicitly demonstrating that the electrochemical process operates via a mixed diffusion-adsorption control mechanism on the modified electrode. This intermediate slope value sits between the theoretical limits of 0.5, indicative of purely diffusion-controlled kinetic behavior, and 1.0, which corresponds to an adsorption-controlled process (Kant & Prerna 2022).

The slope of 0.85 in the log-log plot ($\log I_{pc}$ vs. $\log v$) related to Figure 7 explicitly demonstrates a mixed diffusion-absorption control process, in which electroactive molecules migrate from the bulk phase via diffusion but experience localized adsorption limitations at the electrode boundary interface. The linear correlation ($R=0.9918$) between oxidation and reduction peak currents and increasing scan rates is consistent with the fast electron transfer with localized diffusion limitation within this outline framework, supported by data from Figures 6 and 7. The highly interconnected network of

MWNTs embedded within the porous Nafion polymer matrix establishes an expanded electroactive surface area that accelerates long-range mass transport via semi-infinite linear diffusion from the bulk phase. Once the migrating Hg^{2+} ions cross the outer polymer boundary and reach the inner interface, they experience immediate localized trapping and surface confinement. This behavior is driven by the exceptionally strong coordination affinity of the dicysteinyl thiol groups residing on the immobilized CAC-tripeptide macrostructures. The results of the scan rate study on the CAC/MWNTs/Nafion/Pt electrode confirm the efficiency and reliability of the electrode arrangement in detecting Hg^{2+} and suggest its potential for accurate and sensitive Hg^{2+} detection in various environmental samples.

REPEATABILITY AND STABILITY OF THE TRIPEPTIDE SENSOR

Current responses of the same electrode in Hg^{2+} at a concentration of 100 nM were evaluated seven times to determine the repeatability of the CAC/MWNTs/Pt electrode. The relative standard deviation (RSD) for seven repetitions analysis (Zhang, Zhao & Zhang 2023) in 0.1 M acetate buffer containing 100 nM of Hg^{2+} was 3.1% (Data not shown). The low relative standard deviation of 3.1% indicates a high level of repeatability

for the CAC/MWNTs/Pt electrode in detecting Hg^{2+} at a concentration of 100 nM in the acetate buffer solution. This level of consistency is essential for ensuring the reliability of the electrode in practical applications.

The CV peak current in supporting electrolytes containing 100 nM Hg^{2+} tests the long-term stability of the CAC/MWNTs/Pt electrode. Several modified electrodes were built and kept at room temperature to examine the performance of the CAC/MWNTs/Nafion/Pt electrode. The modified electrodes were tested overnight with a solution containing 100 nM Hg^{2+} and kept under 4-5 °C when not used. The long-term stability study shown in Figure 8 maintains 85% performance of the initial signal despite losing some of the analytical signal compared to the production day.

This signal remained stable throughout the study's initial 21 days and depleted in week four at 73%. The long-term stability test results demonstrate the promising performance of the CAC/MWNTs/Nafion/Pt electrode in supporting electrolytes with 100 nM Hg^{2+} . The gradual decline in the signal over the study's initial 21 days, maintaining 85% of the initial signal, indicates robust stability. However, a slight decrease to 73% in week four suggests a gradual decline in performance. Further investigation into the cause of this decline and potential strategies to maintain stability beyond the initial 21 days would be beneficial for enhancing the electrode's long-term performance.

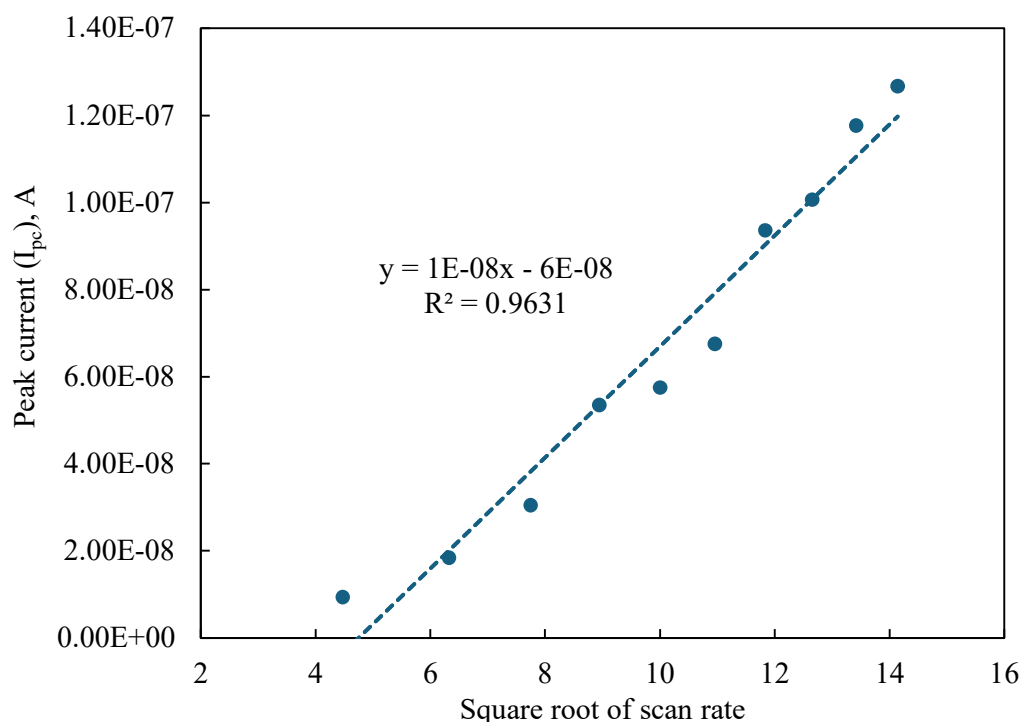


FIGURE 6. The electrochemical behaviour of 100 nM Hg^{2+} was studied under different scan rates (20 to 200 mV/s), plot of peak current vs. square root of scan rate ($v^{1/2}$)

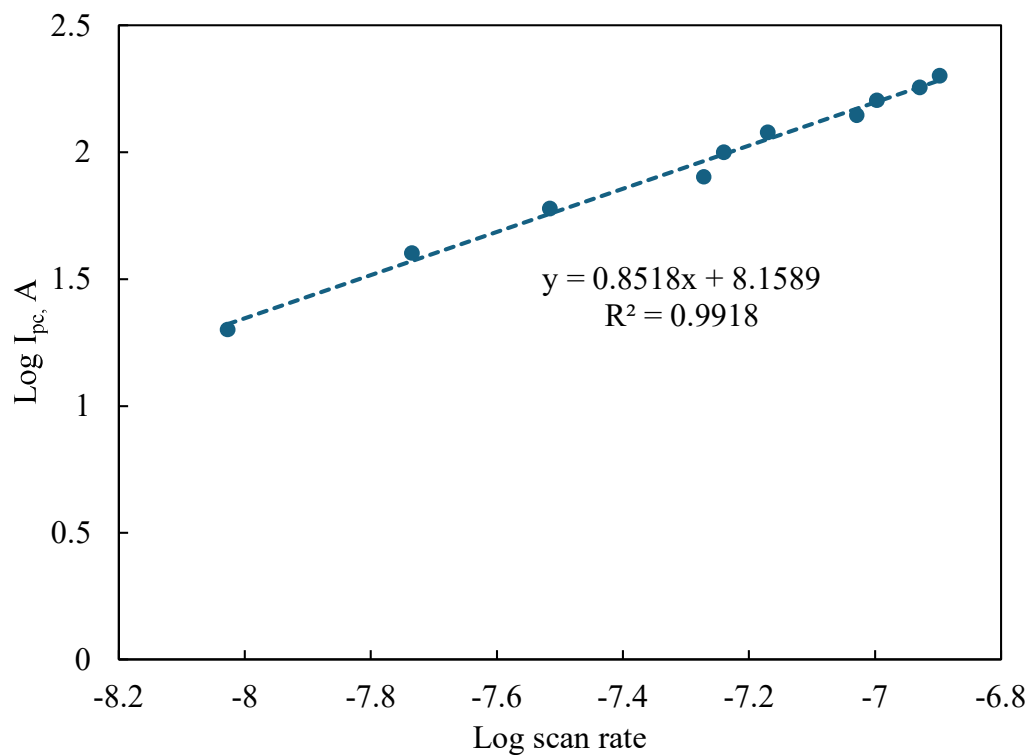


FIGURE 7. Relationship between the logarithms of scan rate ($\log v$) and peak current ($\log (I_{pc})$)

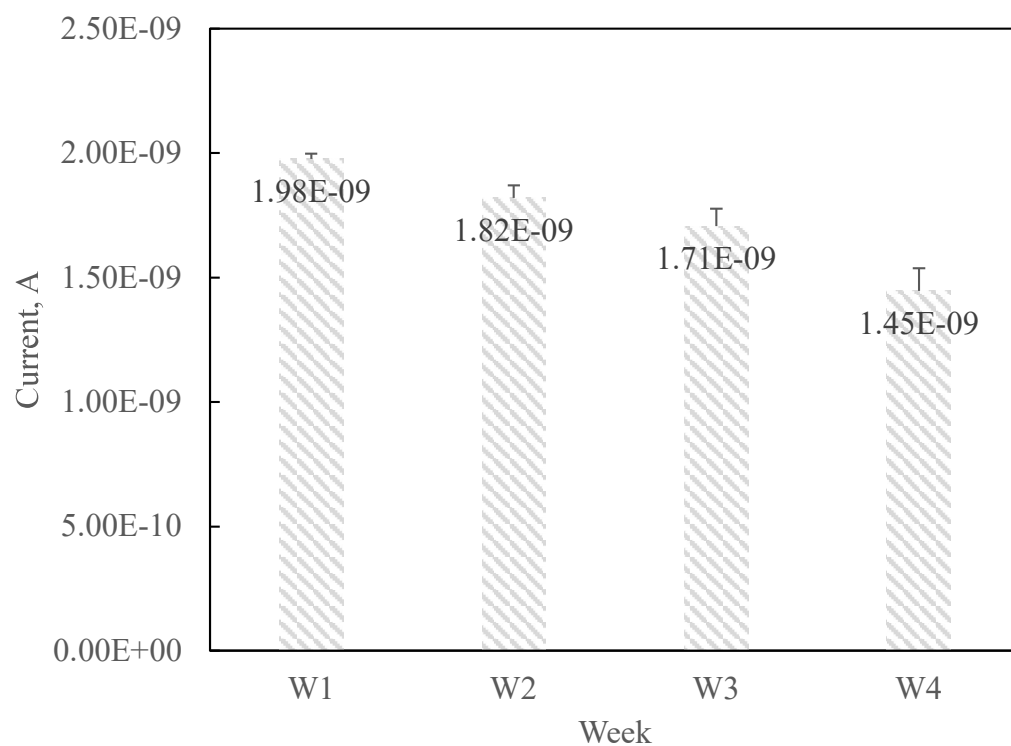


FIGURE 8. Stability study of CAC/MWNTs/Nafion/Pt modified electrode (n=3)

INTERFERENCE AND SELECTIVITY STUDY

The selectivity study of the CAC/MWNTs/Nafion/Pt electrode showed that it is highly selective for Hg^{2+} ions, even in the presence of other metal ions. Metal ions Ag^+ , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cu^{2+} , and Fe^{3+} were studied under optimal conditions, employing 10 nM and 100 nM concentrations for each metal ion. The experiment results have been compared against the control baseline signals recorded for pure 10 nM Hg^{2+} and 100 nM Hg^{2+} solutions (Gong et al. 2016). The experimental results demonstrated that the majority of the tested background metal ions do not interfere with the quantitative detection of mercury (Data not shown). A minor competitive effect was observed for Cu^{2+} and Ag^+ , which showed a relative sensitivity of 80% and 85%, respectively, where minor overlapping stripping peaks were visible within the evaluated potential window. The interference from metal ions such as Cu^{2+} and Ag^+ is due to their higher reduction potential, resulting in their preferential reduction on the surface of the CAC peptide and not interfering with the detection of Hg^{2+} ions. However, the modified electrode of the CAC/MWNTs/Nafion/Pt electrode maintained robust target selectivity, preserving a relative sensitivity for Cu^{2+} and Ag^+ , while maintaining greater than 90% relative sensitivity for all other co-existing ions, due to the specific binding affinity of Hg^{2+} towards the amino acids on the CAC peptide. This unique binding affinity enables the electrode to effectively differentiate Hg^{2+} ions even when other metal ions are present in the sample. Another way to address potential interference from Cu^{2+} and Ag^+ is to consider incorporating additional recognition components or functional groups onto the chemical sensor surface. This approach could help enhance the selectivity of the CAC/MWNTs/Nafion/Pt electrode for Hg^{2+} ions, thereby reducing the potential impact of other metal ions like Cu^{2+} and Ag^+ on its performance.

CONCLUSIONS

This study assesses the impact of immobilization of the CAC-tripeptide and multi-walled carbon nanotubes (MWNTs) within a Nafion polymer matrix onto a platinum electrode for the detection of Hg^{2+} . The integrated MWNTs accelerates electron transfer kinetics and expands the electroactive area, while Nafion serves as a vital polymeric binder that structurally stabilizes the composite matrix on Pt electrode surface. Computational modeling successfully verified the sensor's underlying mechanism, proving that Hg^{2+} coordination by the dicysteinyl thiol groups rigidifies the CAC tripeptide into a highly stable, low-flexibility state, as evidenced by diminished RMSD and C_α B-factor profiles. Analytically, the sensor delivers a good detection limit of 7.9 nM across a linear range of 10 – 100 nM Hg^{2+} , effectively surpassing the safety standards set by the WHO. Moreover, the modified

electrode demonstrates long-term stability and retains good selectivity in complex matrices containing competing metal ions. This tripeptide-based sensor provides a robust and scalable alternative to conventional equipment, offering a disruptive method for real-time environmental monitoring and the protection of global water resources in an increasingly industrialized scenario.

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