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Research Article

Investigation of Electrochemical Properties and Behaviors of NBITEP Molecule; Availability of Sensor Electrode in Determination of Hg^{2+} with SWAdSV Technique

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ABSTRACT

This Master's Thesis focuses on the synthesis and structural elucidation of 4-(2-((6-nitro-1h-benzo[d]imidazol-2-yl)thio)ethyl)phenol (NBITEP) molecule, along with its electrochemical behaviors. Electrochemical studies were carried out employing a glassy carbon (GC) electrode as the working electrode. The cyclic voltammetry (CV) technique was utilized for modification and characterization processes. Furthermore, the potential of the modified surface as a sensor electrode for Hg^{2+} ion detection was investigated using square wave adsorptive stripping voltammetry (SWAdSV). Detailed examinations were conducted in a phosphate buffer solution (PBS) medium specifically for Hg^{2+} ion detection, demonstrating the suitability of the reduced NBITEP modified GC electrode surface as a sensor electrode.

Araştırma Makalesi

NBITEP Molekülünün Elektrokimyasal Özelliklerinin ve Davranışlarının İncelenmesi; SWAdSV Tekniği ile Hg^{2+}'nın Tayininde Sensör Elektrotun Kullanılabilirliği

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Anahtar Kelimeler
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Hg^{2+}'nın Tayini

ÖZ

Bu çalışmada, 4-(2-((6-nitro-1h-benzo[d]imidazol-2-il)tiyo)etil)fenol (NBITEP) molekülü sentezlenmiş, karakterize edilmiş ve çalışma bir Yüksek Lisans Tezi olarak sunulmuştur. Elektrokimyasal incelemelerde camsı karbon (GC) elektrot, çalışma elektrodu olarak kullanılmıştır. Modifikasyon ve karakterizasyon işlemlerinde dönüşümlü voltametri (CV) tekniğinin kullanıldığı çalışmada, modifiye edilmiş yüzeyin Hg^{2+} iyonu için bir sensör elektrot olarak kullanılabilirliği, kare dalga adsorptif sıyırma voltametri (SWAdSV) tekniği ile araştırılmıştır. Fosfat tampon çözeltisi (PBS) ortamında gerçekleştirilen çalışmalar, Hg^{2+} iyonu için indirgenmiş NBITEP modifiye GC elektrotun bir sensör elektrot olarak kullanılabileceğini ortaya koymuştur.

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Bu çalışmanın hazırlanma sürecinde bilimsel ve etik ilkelere uyulduğu ve yararlanılan tüm çalışmaların kaynakçada belirtildiği beyan olunur (İ.E. Mülazımoğlu). It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (İ.E. Mülazımoğlu).
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Yazarlar, bu makalede bildirilen çalışmayı etkiliyor gibi görünebilecek bilinen hiçbir rakip mali çıkarları veya kişisel ilişkileri olmadığını beyan ederler. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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1. Introduction

The investigation of molecules with desirable electrochemical properties is crucial for the development of advanced sensing platforms. In this context, the NBITEP molecule has garnered attention due to its intriguing structural features and potential applications in electrochemical sensing. This article aims to elucidate the electrochemical behavior of NBITEP and explore its utility as a sensor electrode for the detection of Hg^{2+} ions, which pose significant environmental and health risks.

Electrochemical methods offer distinct advantages over traditional spectrophotometric techniques, including cost-effectiveness, in situ and continuous monitoring, portability, and straightforward instrumentation, often requiring minimal sample pretreatment.

Multiwall carbon nanotubes, categorized as innovative carbon materials, find widespread use across various domains, particularly in electrochemical sensing. This popularity is attributed to their high porosity, hollow structure, exceptional electrical conductivity, substantial specific surface area, and favorable biological activity (Hu et al., 2011; Islamoğlu et al., 2023; Korkmaz et al., 2023). Despite the many superior characteristics of multiwall carbon nanotubes, there is ongoing interest in enhancing their unique performance further. The goal is to fully exploit their extensive specific surface area and high loading capacity to support other nanoparticles. Combining multiwall carbon nanotubes with nanomaterials, such as metal nanomaterials, has been shown to provide a substantial specific surface area, excellent electrical conductivity, and biological compatibility (Leventis et al., 2005; Zheng et al., 2008), facilitating accelerated electron transfer between biological molecules and the electrode surface.

Recent findings underscore the suitability of carbon nanomaterials, including carbon nanotubes, graphene, carbon nanopowders, or nanofibers, in the preparation of electrochemical sensors due to their large active surface area and superior electrical conductivity (Li et al., 2013). Graphene-based materials, with their distinctive structure, have garnered significant attention in recent years. The conjugated, one-atom-thick, two-dimensional structure imparts excellent electron transport mobility, mechanical strength, and thermal stability to graphene (Pumera et al., 2010; Shao et al., 2010), enhancing its potential applications in electrochemical sensors (Mülazımoğlu & Mülazımoğlu, 2013; Mülazımoğlu & Mülazımoğlu, 2012; Mülazımoğlu et al., 2011).

2. Material and Method

Experimental procedures involved the synthesis and characterization of NBITEP, followed by the modification of electrode surfaces and electrochemical studies using cyclic voltammetry. The SWAdSV technique was employed for the sensitive determination of Hg^{2+} ions, utilizing reduced NBITEP modified GC electrode surfaces. Comprehensive analyses were conducted in PBS media to evaluate the sensor performance and detection capabilities.

2.1. Instruments and chemicals

All the chemicals were used from Fluka, and Sigma-Aldrich, all chemicals were used without any purification. Three electrodes electrochemical cell setup supplied with bare GC as working electrode, Pt wire (BAS Model MW-1032) as counter electrode and Ag/AgCl/ 3M KCl (BAS Model MF-2063) as reference electrode. Reference 600+ Potentiostat/Galvanostat/ZRA from Gamry (USA) was used in all measurements. Bare GC with a geometric area of 0.071 cm^2 or NBITEP modified GC electrode BAS (Bioanalytical Systems, West Lafayette, IN, USA) model MF-2012 were used as a working electrode in all electrochemical experiments such as CV, and SWAdSV.

2.2. Synthesis of 4-(2-((6-nitro-1H-benzo[d]imidazol-2-yl)thio)ethyl)phenol (NBITEP)

A solution containing 2-mercapto-5-nitrobenzimidazole (1.0 mmol), 4-hydroxyphenethyl bromide (1.0 mmol), and K_2CO_3 (1.2 mmol) in acetone was refluxed at 40 °C for 2 hours. Following cooling, the solvent was evaporated to dryness. The resulting residue was then treated with 25 mL of water. The solidified product was filtered, washed with water, and subjected to recrystallization from ethanol, yielding the compounds as reported by Yurttaş et al. in 2014 and 2016 (Yurttaş et al., 2014; Yurttaş et al., 2016). The analysis results from FTIR and NMR (1H and 13C) are as follows: Yield: 76%, melting point: 150.5 °C. FTIR (ATR) cm^{-1}: 3373 (N–H), 3205 (O-H), 1612–1421 (C=C, C=N), 1512-1319 (NO2), 819 (1,4-disubstituted benzene). ^{1}H-NMR (500 MHz, DMSO-d6): δ = 2.95 (2H, t, J=7.50 Hz, -CH2-), 3.52 (2H, t, J=7.50 CH2), 6.71 (2H, d, J=8.50 1,4-phenyl), 7.10 (2H, d, J=8.00 1,4-phenyl), 7.56 (1H, d, J=9.00 Hz, Benzimidazole-H4), 8.03 (1H, dd, J=2.50, J=8.50, Hz, Benzimidazole-H5), 8.29 (1H, d, J=9.00 Hz, Benzimidazole-H7), 9.26 (1H, s, OH), 13.29 (1H, s, Benzimidazole-NH). ^{13}C-NMR (125 MHz, DMSO-d6): δ =33.18, 34.81, 110.59, 113.69, 115.61, 115.67, 117.38, 129.99, 130.45, 139.45, 141.97, 142.01, 156.36. HRMS (m/z): [M+H]+ calculated for C15H13N3O3S: 316.0750; found 316.0750 (Fig. 1).

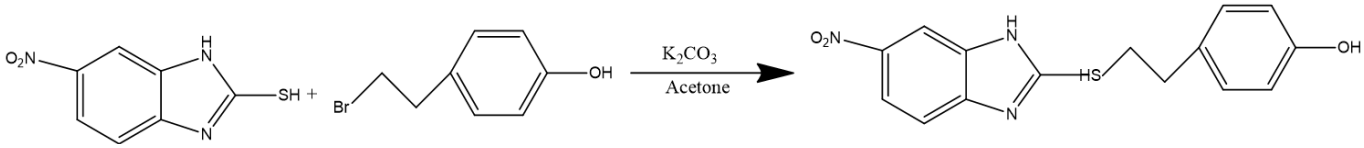

Figure 1. Synthesis mechanism of NBITEP molecule.

2.3. Preparation and polishing GC electrode surface

The GC electrode underwent preparation for the experiments through a polishing process on micro cloth pads (Buehler, USA) to achieve a mirror-like appearance. This involved initial polishing with fine wet emery papers (grain size 4000), followed by polishing with 1.0 and 0.3 mm alumina slurry. Subsequently, the GC electrode underwent sonication in both water and a 1:1 (v/v) mixture of isopropyl alcohol (IPA) and CH_3CN (IPA + CH_3CN) for 10 minutes, following a specific sequence (Mülazımoğlu et al., 2012; Mülazımoğlu et al., 2011).

3. Results and Discussion

The electrochemical characterization revealed distinctive features of NBITEP-modified electrode surfaces, indicating favorable adsorption and redox processes. The SWAdSV technique demonstrated high sensitivity and selectivity towards Hg^{2+} ions, facilitated by the unique properties of NBITEP. Moreover, the study elucidated the underlying mechanisms governing the electrochemical behavior of NBITEP and its interaction with Hg^{2+} ions, providing valuable insights into sensor design and optimization.

3.1. Modification and characterization of GC electrode surface

As a step after synthesis processes and structure analysis, modification processes were carried out using the electrochemical CV technique. By using 1 mM NBITEP solution prepared in 100 mM NBu_4BF_4 solution dissolved in acetonitrile in anhydrous medium, the modification was made against the Ag/Ag^+ reference electrode in the range of 0.0 V to +2.3 V, at a scanning speed of 100 mV s^{-1} and for 10 cycles. The voltammogram of the modification is given in Figure 2.

Upon examining the modification voltammogram in Figure 2A, it becomes apparent that the peaks vanish starting from the second cycle. This observation suggests the coverage of the electrode surface by the molecule. Despite initiating the modification process from the first cycle, conducting it through 10 cycles serves the purpose of avoiding the persistence of small openings, referred to as pinholes, on the surface. In this investigation, the characterization processes employed the CV technique in both aqueous and non-aqueous mediums.

The reduction of the nitro group in the molecule was carried out using CV in 100 mM HCl medium with a potential range from +0.2 V to -1.0 V, scanning speed of 100 mV s^{-1} and with 10 cycles (Figure 2B). After modification and reduction, surface characterizations were made with CV using ferrocene in non-aqueous medium (Figure 3A) and ferricyanide redox probes in aqueous medium (Figure 3B).

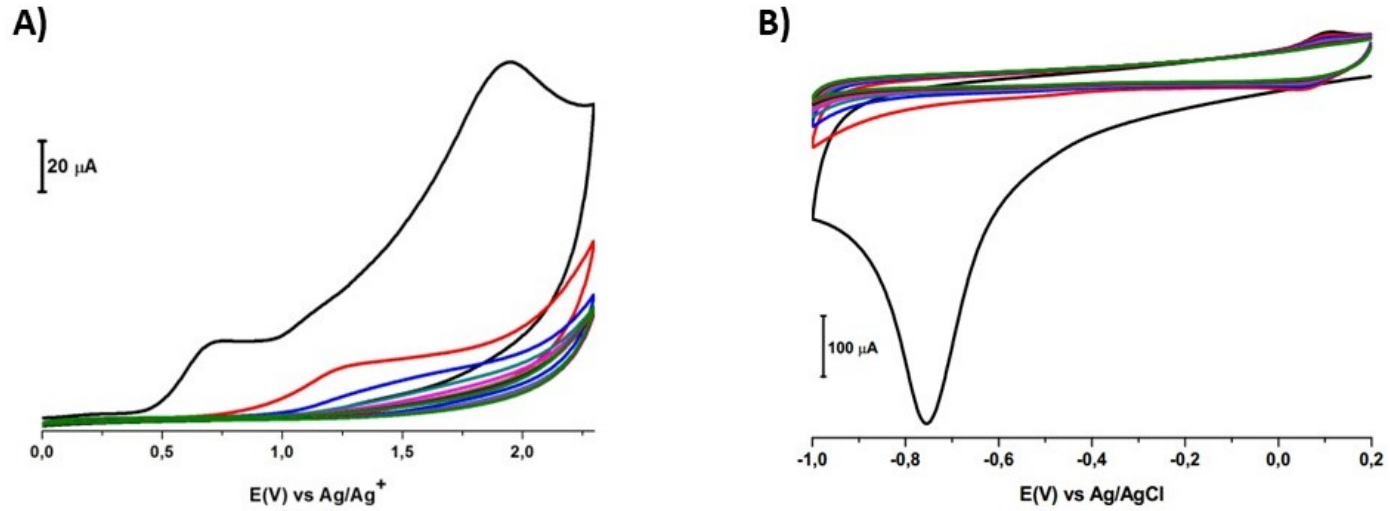

Figure 2. A) The modification voltammogram of NBITEP onto the GC surface was conducted within the potential range of 0.0 V to +2.7 V, employing a scanning rate of 100 mV s-1 with 10 cycles. B) Cyclic voltammogram illustrating the reduction of the nitro group to the amine group on the reduced NBITEP modified GC electrode surface in a 100 mM HCl solution medium.

Figure 2A illustrates the full modification of the NBITEP molecule on the surface of the GC electrode in an non-aqueous medium, while Figure 2B depicts the reduction of -NO_2 and -NH_2 groups on the reduced NBITEP modified GC electrode surface in an acidic medium.

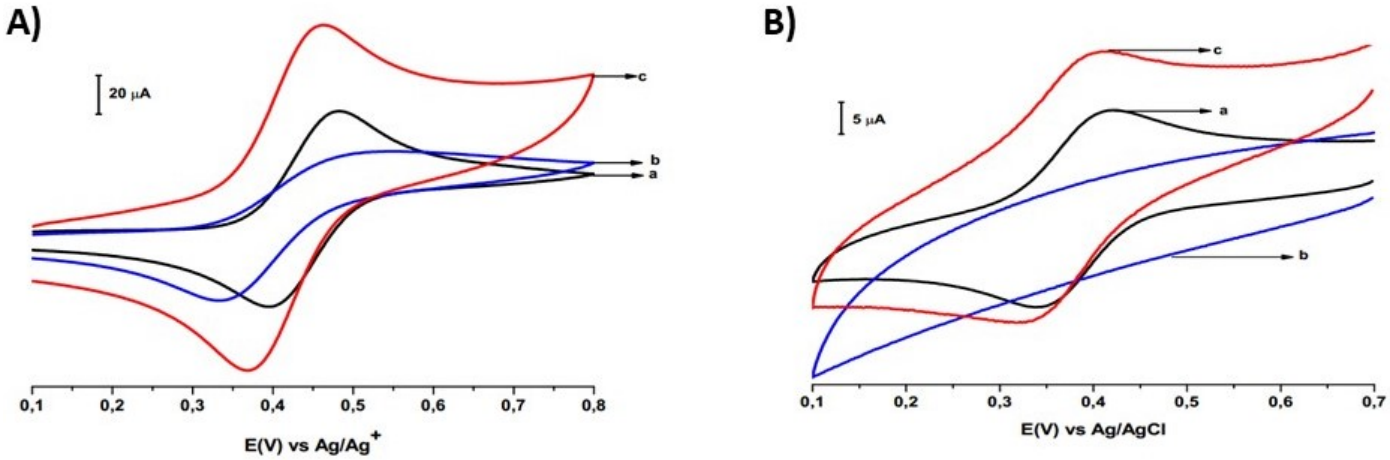

Figure 3. Comparative cyclic voltammograms are presented for: **A)** Ferrocene redox probe solution (1 mM) versus Ag/Ag+ (10 mM) in CH3CN with 100 mM NBu4BF4, employing a scanning rate of 100 mV s-1. **B)** Redox probe solution of Fe(CN)63- (1 mM) versus Ag/AgCl/3 M KCl reference electrode in BR buffer solution at pH 2.0, using a scanning rate of 100 mV s-1. The voltammograms are obtained on **a)** the bare glassy carbon (GC) electrode surface, **b)** the GC electrode surface modified with NBITEP, and **c)** the reduced NBITEP/GC electrode surface.

The characterization of the newly obtained surfaces after modification in an non-aqueous medium electrochemically and subsequent reduction in an acidic medium was performed by anodic scanning in the presence of ferrocene redox probe in the non-aqueous medium and cathodic scanning in the presence of ferricyanide redox probe in the aqueous medium. Figures 3A and 3B demonstrate that both in non-aqueous and aqueous media, NBITEP molecule is fully modified and reduction is fully realized. Specifically, while the Bare GC electrode surfaces are electroactive, they become electroinactive due to the presence of the functional group -NO_2 after NBITEP modification, and after reduction, they become electroactive again due to the formation of -NH_2 groups on the surface of the molecule.

3.2. Electrochemical reaction mechanism of oxidation and reduction of NBITEP on GC electrode surface

After the establishment of NBITEP, the nitro groups can undergo electrochemical reduction to form amines, as illustrated in Figure 4. The cathodic sweep of the cyclic voltammogram recorded from a glassy carbon electrode modified with NBITEP exhibits an irreversible reduction peak at approximately −750 mV. The reduction peak area for the conversion of nitro to amine is comparatively smaller on carbon electrodes. In a 100 mM HCl medium, the nitro groups on the modified surface, following characterization procedures, were successfully reduced to amine groups (Mulazimoglu and Demir Mulazimoglu, 2012). Following this process, conducted within the potential range of 0.200 to −1.000 V at a sweep rate of 100 mV s^{-1} and through 10 cycles (Figure 2B), the initially electroinactive surface became electroactive.

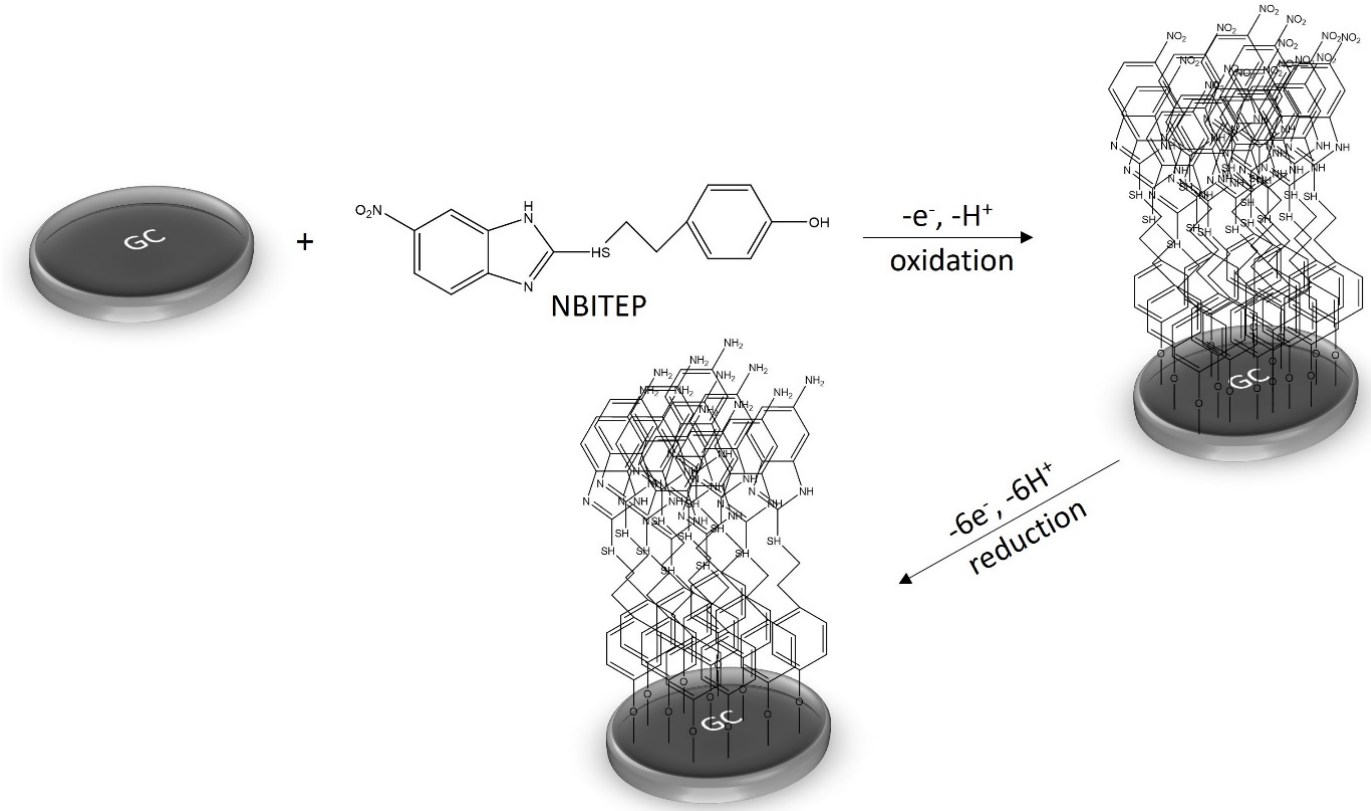

Figure 4. The process involves the modification of NBITEP onto the GC electrode surface, followed by elucidating the mechanism of reduction from the nitro group to the amine group on the surface of the modified electrode.

3.3. Determination of Hg^{2+} onto the NBITEP/GC electrode surface

In this part, first of all, the deposition time study was carried out using the SWAdSV technique (Fig. 5). As a result of this study, it was understood from the superimposed image of the voltammograms that the appropriate time for deposition was 360 seconds.

The overlayed image of the SWAdSV results obtained using solutions of Hg^{2+} ions at different concentrations is given in Figure 6. Accordingly, it seems that Hg^{2+} ion can be determined with the SWAdSV technique using the reduced NBITEP/GC sensor electrode in the concentration range of 1 mM to 1 µM.

For the conclusion and discussion section of the thesis, you can elaborate on the findings and implications of the electrochemical studies conducted on the NBITEP molecule, particularly regarding its potential application as a sensor for detecting Hg^{2+} ions.

Begin by summarizing the key findings of the research. Highlight the successful synthesis and structural elucidation of the NBITEP molecule and its electrochemical behaviors observed through CV technique. Discuss the results obtained from the characterization processes, emphasizing any changes observed in the electrochemical properties of the GC electrode upon modification with NBITEP. This could include shifts in peak potentials, changes in peak currents, and alterations in voltammograms.

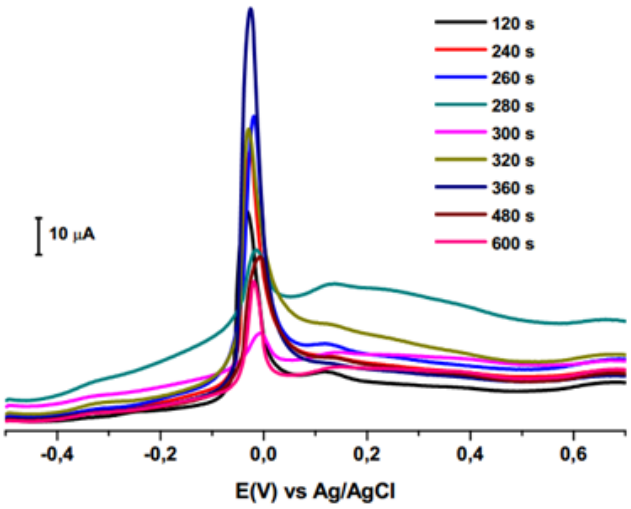

Figure 5. Voltammograms of 1 mM Hg^{2+} using reduced NBITEP/GC electrode surface with applying different accumulation times, under the following conditions: PBS at pH 7.0, wave frequency 25 Hz, pulse size 50 mV and stirring rate 500 rpm, through employing SWAdSV technique.

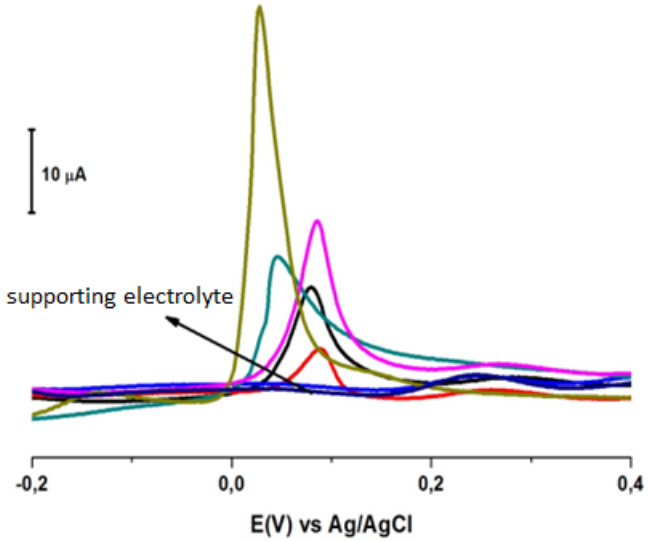

Figure 6. Voltammograms of different Hg^{2+} ion concentrations (1.10^{-3}, 5.10^{-4}, 1.10^{-4}, 5.10^{-5}, 1.10^{-5}, 1.10^{-6} M) using reduced NBITEP/GC electrode surface in PBS at pH 7.0, under the following conditions: -0.2/+0.4 V potential range, accumulation time 360 s, wave frequency 25 Hz, pulse size 50 mV and stirring rate 500 rpm, through employing SWAdSV technique.

Present the results of the investigation into the potential of the reduced NBITEP modified GC electrode as a sensor for detecting Hg^{2+} ions. Discuss the sensitivity, selectivity, and detection limits achieved using SWAdSV in a PBS medium. Compare the performance of the reduced NBITEP modified GC electrode with other existing methods for Hg^{2+} ion detection. Highlight any advantages, such as enhanced sensitivity or lower detection limits, offered by the proposed sensor electrode. Provide insights into the underlying mechanisms governing the electrochemical behavior of NBITEP and its interaction with Hg^{2+} ions at the electrode surface. Discuss any potential redox processes or adsorption phenomena involved in the detection mechanism. Discuss the broader implications of the research findings and potential applications of the reduced NBITEP modified GC electrode in environmental monitoring or analytical chemistry. Suggest future research directions, such as exploring the sensor's performance under different experimental conditions or investigating its applicability in real samples.

Summarize the main conclusions drawn from the study, emphasizing the significance of the findings in advancing the field of electrochemical sensing for mercury detection. By covering these points in the conclusion and discussion section, you can provide a comprehensive overview of the research outcomes and their implications for the scientific community.

4. Conclusions and Recommendations

The Master's Thesis outlined provides a comprehensive exploration of the NBITEP molecule, focusing on its synthesis, structural elucidation, and electrochemical behaviors. Through meticulous experimentation, the study utilized a GC electrode as the working electrode, employing CV for modification and characterization processes. Notably, the research delved into the potential of the modified surface to serve as a sensor electrode for detecting Hg^{2+} ions, utilizing SWAdSV. The detailed examinations conducted in a PBS medium specifically targeted Hg^{2+} ion detection, showcasing the efficacy of the reduced NBITEP modified GC electrode surface as a promising sensor electrode. This work not only advances our understanding of the electrochemical properties of NBITEP but also highlights its potential application in sensitive and selective detection of heavy metal ions, contributing to the field of analytical chemistry and environmental monitoring.

The study employed both alcohol oxidation and alternating voltammetry techniques, common in electrochemical analysis, for modification. In an acidic environment, the nitro group on the surface was effectively reduced to the amine group using alternating voltammetry, resulting in an electroactive surface.

Following the modification process, comprehensive characterization and stability studies were conducted. The reduced NBITEP/GC electrode surface obtained was then investigated for its applicability as a sensor electrode for various metals using square wave stripping voltammetry in a phosphate buffer solution. The findings suggested that the reduced NBITEP/GC electrode surface, derived from these studies, exhibited promising potential as a suitable sensor electrode for detecting Hg^{2+} ions.

A successful and efficient determination of Hg^{2+} ions on a disposable reduced NBITEP/GC electrode surface was achieved in a phosphate buffer solution using the SWAdSV technique. The generated Hg^{2+} standard curve covered a range from 1.0 mM to 1.0 µM, demonstrating the feasibility of a simple, cost-effective, and rapid method for quantifying Hg^{2+} ions.

In conclusion, this research highlights the promising prospects of NBITEP as a sensor electrode for Hg^{2+} detection, leveraging the SWAdSV technique. The comprehensive understanding of its electrochemical properties and behaviors paves the way for the development of efficient and reliable sensing platforms for various environmental and analytical applications. Further exploration and refinement of NBITEP based GC sensors hold significant potential for addressing contemporary challenges in environmental monitoring and public health.

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CRediT author statement

LA: She took part in all experimental procedures for this study, writing and reading the manuscript.
ŞK: She took part in all experimental procedures for this study, writing and reading the manuscript.
YÖ: The synthesis and characterization of the modifier species used in electrode modification have been carried out.
İEM: He carried out the operations of directing, controlling and interpreting the results of the experiments for the study. He also carried out the final reading of the manuscript.

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