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Full Paper

Measurement of Trace Amounts of Anesthetic Thiopental using Imprinted Sol-Gel on Pencil Graphite Electrode Modified with Carbon Nanotubes as an Electrochemical Sensor

Alireza Nouri,[1] Nazila Gholipour,[1] Ali Sobhani-Nasab,[2] Mohammad Javad Taghizadeh,[3] and Mehdi Rahimi-Nasrabadi[1,*]

[1]*Faculty of Pharmacy, Baqiyatallah University of Medical Sciences, Tehran, Iran*
[2]*Physiology Research Center, Institute for Basic Sciences, Kashan University of Medical Sciences, Kashan, Iran*
[3]*Department of Chemistry, Imam Hossein University, Tehran, 1955735345, Iran*

*Corresponding Author, Tel.: +98-2182483409
E-Mail: rahiminasrabadi@gmail.com

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Abstract- In this study, a sensitive electrochemical sensor was developed for the trace determination of thiopental using a sol–gel molecularly imprinted polymer on a pencil graphite electrode modified with functionalized carbon nanotubes. The carbon nanotubes were pretreated using a mixture of nitric and sulfuric acids to enhance surface reactivity. Electrochemical characterization was performed using cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS). The sensor's performance was evaluated based on the oxidation and reduction currents of a redox probe, demonstrating superior recognition ability of the imprinted sol–gel electrode compared to its non-imprinted counterpart. Several experimental parameters, including pH and target molecule concentration, were optimized to achieve maximum response. Under optimal conditions, the sensor exhibited a linear response to thiopental in the concentration range of 0.05–75 µM, with a detection limit as low as 10 nM. The applicability of the sensor was successfully demonstrated through the analysis of thiopental in real biological samples.

Keywords- Thiopental; Sol–gel; Molecularly imprinted polymer; Pencil graphite electrode; Carbon nanotubes

1. INTRODUCTION

Thiopental (also known as Pentothal), chemically designated as 5-ethyl-6-oxo-5-(pentan-2-ylsulfanyl)pyrimidin-4-olate, is a barbiturate characterized by its ultra-short duration of action. It is widely used as an induction agent for anesthesia in both human and veterinary surgeries of brief duration. Beyond its anesthetic application, thiopental has also proven beneficial in managing elevated intracranial pressure and in the prophylaxis and treatment of cerebral ischemia [1-5]. However, due to its prolonged recovery profile, thiopental is not recommended for sustained maintenance of anesthesia or for inducing consciousness over extended periods [6]. Consequently, accurate monitoring of thiopental concentrations in biological samples is crucial in clinical contexts [7]. To address this need, various analytical techniques have been employed to quantify thiopental in biological matrices, including high-performance liquid chromatography (HPLC) [8,9], UV–vis spectrophotometry [10], spectrofluorimetry [11], coulometry [12], gas chromatography (GC) [13], gas chromatography-mass spectrometry (GC-MS) [14], capillary electrophoresis [15], potentiometry [16], and stripping voltammetry [17]. The present study is aimed at the development of a novel electrochemical sensor capable of detecting thiopental with enhanced sensitivity and selectivity.

Chemical modification of electrode surfaces is a key approach to enhance electrochemical sensor performance [18-25]. This study aimed to develop a rapid, sensitive, and reliable voltammetric method for thiopental detection using a pencil graphite electrode (PGE) modified with a molecularly imprinted polymer (MIP)-based nanocomposite. The strategy involves adsorption and pre-concentration of thiopental on the modified surface, followed by voltammetric detection [26-30]. In recent years, MIP-based electrochemical sensors have gained prominence due to their high selectivity, stability, low cost, and capability for molecular recognition. MIPs are synthesized via polymerization around a template molecule, which upon removal, leaves behind specific binding sites complementary to the target. Despite their advantages, conventional MIPs face challenges such as slow diffusion, low imprint density, and poor sensitivity [31,32]. The measurement of drugs using electrochemical sensors based on various nanocomposites is a very promising idea for research projects [33-38]. To overcome these limitations, a sol-gel method was employed for MIP preparation, offering benefits like mild reaction conditions, environmental compatibility, and formation of a porous, highly cross-linked matrix. However, sol-gel films typically suffer from poor conductivity [39-46]. To address this, carbon nanotubes (CNTs)) was incorporated into the MIP-sol-gel matrix to enhance electron transfer and improve sensitivity. CNTs contribute exceptional electrochemical and mechanical properties, Functionalization of CNTs was necessary to ensure dispersion and stability [47-49]. The resulting MIP-sol-gel@CNT composite was applied to PGE for selective and efficient electrochemical detection of thiopental, addressing the scarcity of existing analytical methods for this compound.

2. EXPERIMENTAL SECTION

2.1. Material and Equipment

Thiopental, fentanyl, and azaperone were obtained from the Tofigh Daru, Tehran, Iran. Other chemicals such as ethanol, carbon nanotubes, potassium ferrocyanide, potassium ferricyanide, sodium hydroxide, hydrochloric acid, uric acid, nitric acid, etc., were purchased from Sigma-Aldrich representatives in Iran. Electrochemical experiments were performed using a computer-controlled MicroAutolab system (Metrohm, Germany). pH adjustments and monitoring were conducted using a Metrohm 691 pH meter equipped with a standard glass electrode. An analytical digital balance (Sartorius) was employed for accurate weighing of pharmaceutical compounds. Structural analysis of the synthesized materials was performed via powder X-ray diffraction (XRD) using a Bruker D8 Advance diffractometer with Cu-Kα radiation (λ = 1.5406 Å), operated at 40 kV and 35 mA, across a 2θ range of 2° to 70°. Morphological features were investigated through field-emission scanning electron microscopy (FE-SEM) using a MIRA3 TESCAN microscope. All electrochemical measurements utilized a standard three-electrode configuration comprising a pencil graphite electrode as the working electrode, a platinum wire as the counter electrode, and an Ag/AgCl electrode as the reference.

2.2. Preparation of Carboxyl-Functionalized Carbon Nanotubes (COOH-CNTs):

To introduce carboxylic acid groups onto the surface of carbon nanotubes, 300 mg of pristine CNTs was dispersed in 40 mL of a 3:1 (v/v) mixture of concentrated sulfuric acid and nitric acid. The mixture was subjected to reflux at 80 °C for 12 hours to achieve oxidative functionalization. After cooling, the resulting black dispersion was centrifuged and neutralized to pH 7 using a sodium bicarbonate solution. The solid residue was thoroughly washed with deionized water and ethanol to remove residual acids and then dried in a vacuum oven at 60 °C for 12 h.

2.3. Fabrication and Electrochemical Sensing Strategy

Since thiopental exhibits no significant electroactivity over the accessible potential range on a bare pencil graphite electrode (PGE), an indirect detection method was adopted. In this approach, the redox probe ferricyanide ($[Fe(CN)_6]^{3-/4-}$) was used to monitor the changes on the electrode surface. The electrode was immersed in the probe solution before and after each surface modification step and after exposure to thiopental. Due to its small size and fast, reversible redox behavior, the ferricyanide ion readily accesses the electrode surface, enabling reliable voltammetric responses. The difference in peak current recorded before and after each step was considered as the analytical signal. This strategy enabled indirect electrochemical quantification of the electroinactive thiopental molecule.

A sol-gel labeled coating deposited on a multi-walled carbon nanotube (MWCNT)-modified pencil graphite electrode (PGE) will be used as a sensitive electrochemical sensor for the detection of trace amounts of thiopental. Briefly, 5 mg of MWCNT was added to 2.5 mL of DMF and dispersed by ultrasonication for 6 minutes. Then, 3 µL of this suspension was dropped onto the surface of the pencil graphite electrode (PGE) and allowed to dry for solvent evaporation. A mixture was prepared by combining 3 mL of methyltrimethoxysilane as the monomer with 3 mL of tetraethyl orthosilicate as the cross-linker, 1 mL of 0.1 M HCl as the catalyst, 3 mL of methanol as the homogenizing agent, and 1 mL of water. This mixture was stirred at room temperature for 1 hour to obtain a homogeneous sol. Then, 10 mL of the sol was mixed with 1 mL of an aqueous solution of thiopental (1 mM) and stirred for 1 hour. This solution was used as the thiopental-imprinted sol-gel film (MIP-sol-gel/MWCNT/PGE), while the original sol (without thiopental) was used to prepare the non-imprinted reference sol-gel film. Cyclic voltammetry (CV) was used to form the imprinted sol-gel film on the surface of the MWCNT/PGE in the potential range from –0.4 V to +1.0 V for 5 cycles at a scan rate of 50 mV/s. The prepared MIP-sol-gel/MWCNT/PGE was then dried overnight at room temperature and used for subsequent measurements [49].

3. RESULTS AND DISCUSSION

3.1. FT-IR Spectral Characterization of Functionalized CNT

Infrared spectroscopy serves as a valuable tool for the identification of functional groups due to its ability to generate compound-specific spectral patterns, often regarded as molecular fingerprints. Although this technique does not offer complete structural elucidation, it effectively distinguishes between various bond types, such as single, double, and triple bonds. A significant advantage of IR analysis is its non-destructive nature, allowing samples to remain intact and reusable after measurement—unlike destructive techniques such as mass spectrometry. The method operates by detecting molecular vibrations that arise from the absorption of infrared radiation, making it particularly useful for analyzing polyatomic molecules and identifying organic species with complex spectral profiles. In this work, FT-IR spectroscopy was employed to confirm the presence of functional groups on the surface of the synthesized nanomaterials. As shown in Figure 1, the spectrum of the carboxyl-functionalized multi-walled carbon nanotubes (MWCNT-COOH) displays broad absorption bands in the region of 3600–300 cm^{-1} and a distinct peak at 1636 cm^{-1}, both indicative of O–H stretching from water molecules and hydrogen bonding in carboxylic acid groups. Additionally, the peak at 1557 cm^{-1} is attributed to the structural vibrations of the carbon nanotube backbone. A sharp band at 1735 cm^{-1} corresponds to the stretching modes of carbonyl (C=O) and C–O functional groups, confirming successful functionalization.

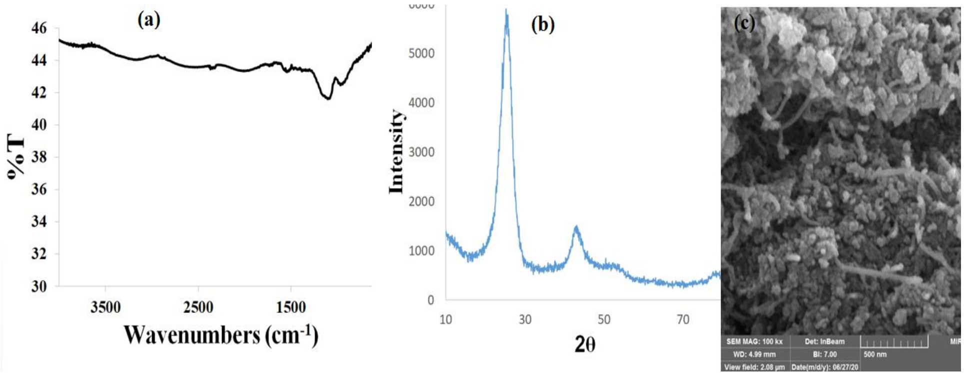

Figure 1. (a) FTIR , (b) XRD and (c) SEM image of Functionalized CNT

3.2. XRD Analysis of Functionalized CNT

X-ray diffraction (XRD) is a well-established method for examining the atomic arrangement in crystalline materials. It exploits the interaction of incident X-rays with the regular lattice of atoms in a crystal, leading to constructive interference and the formation of diffraction patterns. These patterns serve as unique identifiers for specific materials and are widely used for phase identification through database comparison. Unlike amorphous substances that produce diffuse scattering, crystalline compounds yield sharp and distinct diffraction peaks. In this work, XRD was employed to confirm the structural characteristics of multi-walled carbon nanotubes functionalized with carboxylic acid groups. As illustrated in Figure 2, two prominent reflections were observed at 2θ values near 11.25° and 43°, which are attributed to the (002) and (110) lattice planes, respectively. The presence of these peaks supports the successful surface modification of CNTs with carboxylic functionalities.

3.3. Morphological Characterization via SEM

Figure 3 displays the scanning electron microscopy (SEM) images of multi-walled carbon nanotubes after functionalization with carboxylic acid groups. The micrographs exhibit a dense network of entangled nanotubes, indicating a high degree of aggregation. The nanotubes appear to possess a relatively uniform diameter, suggesting consistent morphology throughout the sample.

3.4. Optimization of Electrode Activation and CNT Modification Time

The performance of the working electrode is a key factor in electrochemical analysis, directly influencing the sensitivity and signal reproducibility. In this study, the pencil graphite electrode (PGE) was pretreated in 1 M sodium hydroxide solution, followed by cyclic voltammetric activation within a potential window of –0.4 V to +1.0 V. Post-activation, the electrode response was evaluated using a redox probe solution consisting of 1 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ and 0.1 M KCl. As illustrated in Figure 4a, the highest anodic peak current was recorded after five activation cycles, with a stable and enhanced response achieved after ten cycles, suggesting optimal surface activation. Subsequently, the activated PGE was modified by immersing it in a carbon nanotube dispersion prepared in dimethylformamide (DMF) for various time intervals (5 to 40 minutes). After drying, cyclic voltammetric measurements in the redox probe were conducted (Figure 2a). The results demonstrated that the probe current plateaued after 30 minutes of modification, which was therefore established as the optimal CNT immobilization time. After activation of the pencil graphite electrode (PGE) with 1 M sodium hydroxide, the electrode was modified with carbon nanotubes. For this purpose, the electrode was immersed in a container containing carbon nanotubes dispersed in dimethylformamide (DMF) for different time intervals: 5, 10, 15, 20, 25, 30, 35, and 40 minutes. After each immersion time, the electrode was removed, dried, and then tested using cyclic voltammetry in a solution of 1 mM potassium hexacyanoferrate(II)/ hexacyanoferrate (III) containing 0.1 M potassium chloride (electrochemical probe). The results are shown in Figure 2b. As the results indicate, after 30 minutes, the probe current became stable. Therefore, 30 minutes was selected as the optimal modification time.

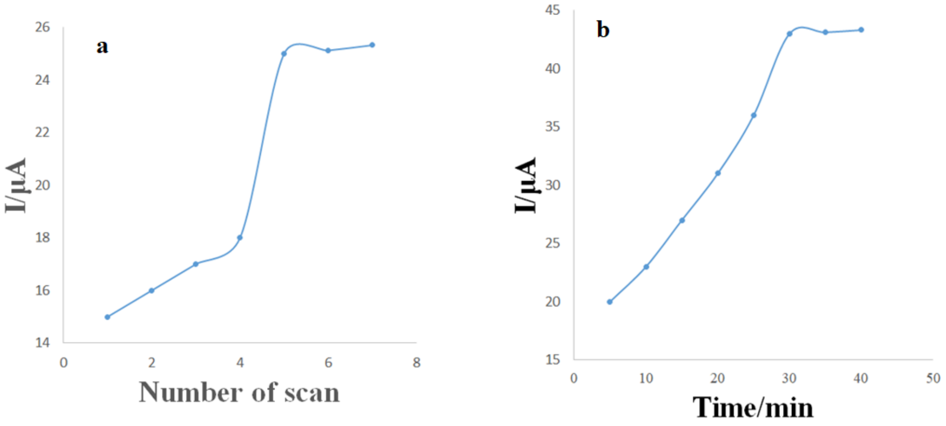

Figure 2. (a) The number of scans of the pencil graphite electrode in 1 M sodium hydroxide solution and (b) the electrochemical probe current at different modification times with carbon nanotubes

3.5. Performance of the Prepared Electrodes Using Cyclic Voltammetry

This section presents the evaluation of various pencil graphite electrodes (PGE): unmodified PGE, PGE modified with carbon nanotubes (CNTs), PGE modified with CNTs and polymer formation, PGE modified with CNTs and thiopental extraction from the polymer network, and PGE modified with CNTs with drug incorporation into the polymer network after its extraction in a 1 mM potassium hexacyanoferrate solution, containing 0.1 M potassium chloride (electrochemical probe). The cyclic voltammetry measurements were carried out at a scan rate of 50 mV/s within a potential range of 0.65 V to -1.5 V. The results are presented in

Figure 3a. As observed, the unmodified PGE shows minimal oxidative current for the electrochemical probe solution due to its low conductivity (Figure 5). However, when modified with CNTs, the PGE exhibits significantly increased oxidative and reductive currents, with a notable peak intensity in its voltammogram (Figure 3). The potential difference between the oxidation and reduction peaks is minimal, indicating a reversible process, which is attributed to the high conductivity of the CNTs. CNTs, with their large surface area, enhance electrode conductivity, facilitating electron transfer and improving electrochemical performance. Thus, the PGE modified with CNTs offers a high-conductivity substrate, making it ideal for polymer network deposition. When the PGE modified with CNTs was coated with the polymer network, a significant decrease in the oxidative and reductive currents was observed (Figure 3). This reduction occurs due to the insulating and non-conductive nature of the polymer network, which prevents the entry and exit of iron species to the electrode's surface, thus reducing the current of the electrochemical probe. This result confirms the successful deposition of the polymer network on the CNT-modified PGE. Upon extraction of thiopental from the polymer network, the oxidative and reductive currents of the electrochemical probe solution increased slightly (Figure 3). The removal of thiopental from the polymer network creates channels that allow iron species to access the electrode surface, thereby increasing the current. When the electrode is re-immersed in a thiopental drug solution (pH 7) for 60 seconds, followed by re-exposure to the electrochemical probe solution, the current decreases again (Figure 3). This decrease is due to the binding of thiopental to the polymer cavities, which results in the closure of some cavities by the analyte, thus reducing the current once more.

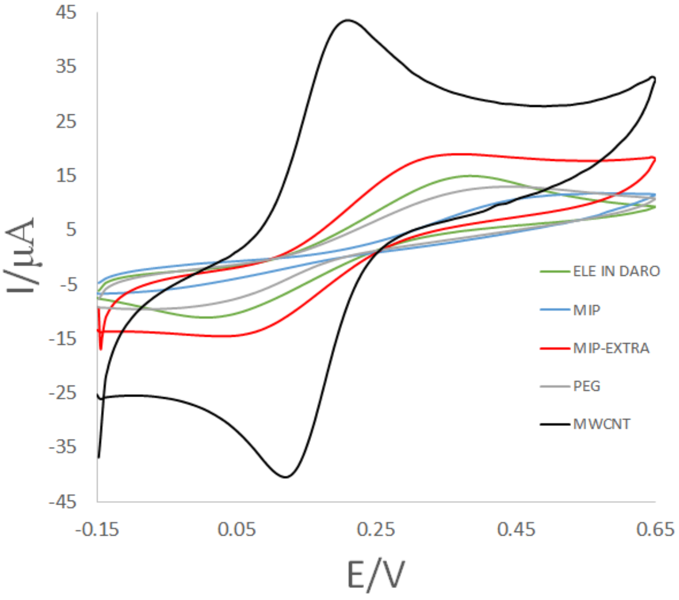

Figure 3. The voltammograms of the prepared electrodes in a 1 mM potassium hexacyanoferrate/potassium hexacyanoferrate solution with the presence of 0.1 M potassium chloride (electrochemical probe)

3.6. Performance Evaluation of the Prepared Electrodes Using Electrochemical Impedance Spectroscopy

This section presents the evaluation of various pencil graphite electrodes (PGE): unmodified PGE, PGE modified with carbon nanotubes (CNTs), PGE modified with CNTs and polymer formation, PGE modified with CNTs and thiopental extraction from the polymer network, and PGE modified with CNTs and drug incorporation into the polymer network after its extraction in a 1 mM potassium hexacyanoferrate solution with 0.1 M potassium chloride (electrochemical probe) using electrochemical impedance spectroscopy within the frequency range of 0.1 kHz to 100 kHz. The results are shown in Figure 6. The Nyquist plots of the different electrodes reveal that the charge transfer resistance, represented by the semicircular arc, for the unmodified PGE is 3 μΩ (Figure 4a), which is due to its low conductivity. For the PGE modified with CNTs, the charge transfer resistance is 0.5 kΩ (Figure 4b). This low resistance is attributed to the large surface area and high conductivity of CNTs. The highest charge transfer resistance (6 μΩ) is observed for the PGE modified with CNTs and polymer formation (Figure 4c), due to the insulating nature of the polymer network. When thiopental is extracted from the polymer network, the charge transfer resistance of the PGE modified with CNTs and thiopental extraction decreases to 2 μΩ, and further reduction to 5 μΩ occurs when the drug is incorporated back into the polymer network (Figures 4d, 4e). These changes are linked to the improved accessibility of iron species as the drug is removed or added back into the polymer network.

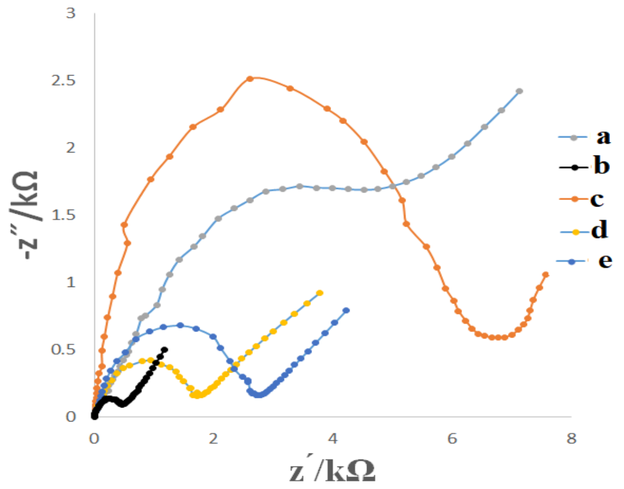

Figure 4. The Nyquist plots of the prepared electrodes.

3.7. Investigation of parameters influencing sensor construction

After the preparation of the sensor with molecularly imprinted polymer using the sol-gel method, various factors affecting its performance were studied and optimized.

3.7.1. Optimization of the Number of Cycles for Electropolymerization

A crucial parameter in forming the molecularly imprinted polymer through the sol-gel method is the number of cycles used to create the polymer network. If the number of cycles is too low, the polymer network may not adhere properly to the electrode surface or may lack stability. Conversely, excessive cycles lead to an increased polymer thickness, which in turn reduces the sensor's sensitivity due to restricted access to the polymer's cavities. In this study, polymerization cycles were tested for 5, 10, 15, 20, and 30 cycles. After 5 and 10 cycles, polymerization did not form adequately, as the oxidation and reduction peaks for the electrochemical probe did not show significant change. After 15 cycles, the oxidation and reduction peaks almost disappeared, indicating proper polymer formation. Therefore, 15 cycles were selected as the optimal value (Figure 5a).

3.7.2. Optimization of the Extraction Time of the Analyte from the Polymer Network

The extraction time of the analyte from the polymer network was optimized to achieve the best sensitivity and response. The polymer-coated sensor, containing thiopental, was placed in a methanol solution and gently stirred. At 10-minute intervals, the sensor was removed after various durations (10, 20, 30, 40, 50, 60, and 70 minutes), allowed to dry, and then tested in the electrochemical probe solution using cyclic voltammetry. The results indicated that the oxidation current of the probe increased with time. After 50 minutes, this current plateaued, suggesting 50 minutes as the optimal extraction time (Figure 5b).

3.7.3. Optimization of Re-extraction Time of Thiopental

After extracting thiopental from the polymer network using methanol, the modified electrode was immersed in a 5 μM thiopental solution. The sensor was stirred for 10, 20, 30, 40, 50, 60, 120, 180, and 240 seconds, then dried and tested again using cyclic voltammetry. The results showed that the oxidation current decreased with time, and after 60 seconds, the current remained stable. Therefore, 60 seconds was chosen as the optimal re-extraction time (Figure 5c).

3.7.4. Effect of pH on electrochemical measurements

pH plays a significant role in electrochemical measurements. In this study, the pH effect was tested for the sensor in a 5 μM solution. The best response was observed at pH 8, which was chosen as the optimal pH for subsequent experiments (Figure 5d).

3.7.5. Monomer-to-Drug ratio in polymer network preparation

The ratio of monomer to drug in preparing the polymer network was also studied. The electrode exhibited varying behaviors with different monomer-to-drug ratios. Ratios of 1:5, 1:10, 1:15, 1:20, 1:25, and 1:30 were tested, with a 1:20 ratio showing the best response.

Therefore, a monomer-to-drug ratio of 1:20 was selected as the optimal ratio for the construction of the electrochemical sensor.

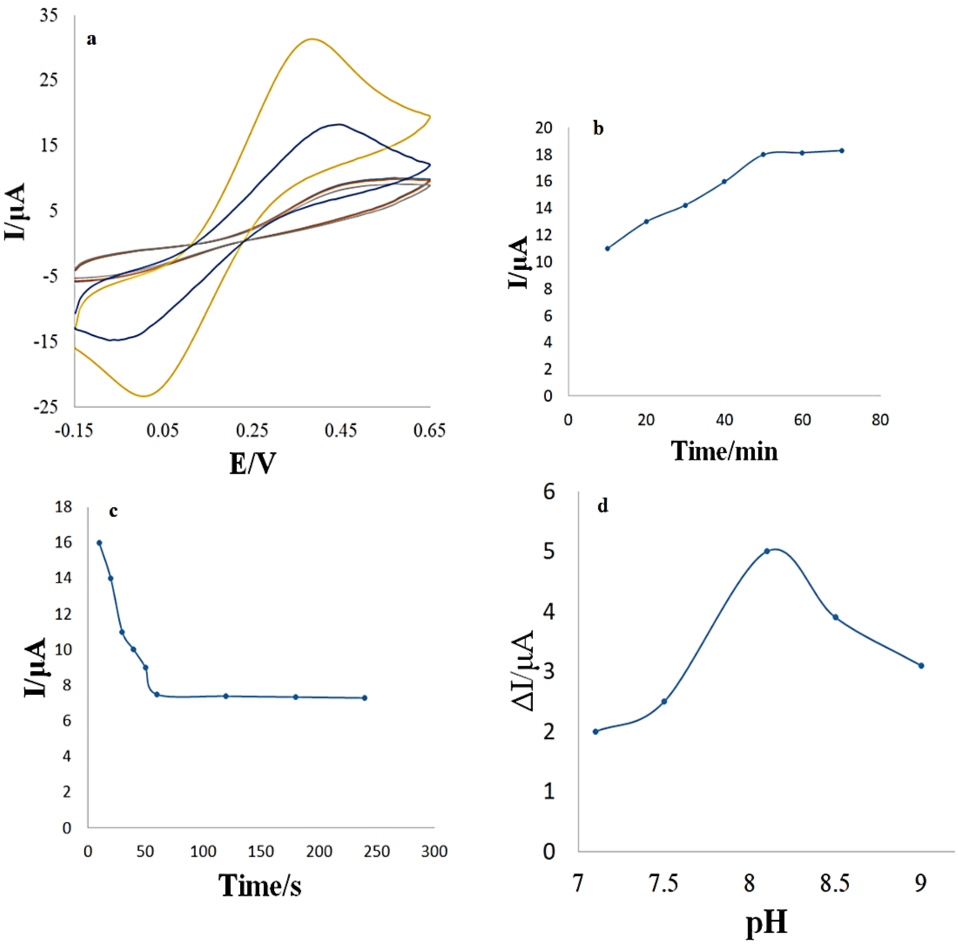

Figure 5. (a) Number of Cycles in Polymerization for Sensor Preparation, (b) Effect of Extraction Time of Analyte from the Polymer Network, (c) Investigation of Thiopental Adsorption by the Prepared Sensor and (d) Effect of pH on Thiopental Measurement

3.8. Analysis of different concentrations of thiopental

Differential Pulse Voltammetry (DPV) was employed to analyze low concentrations of thiopental in the electrochemical probe solution. After the sol-gel imprinting process with thiopental, voltammograms of the probe were recorded under optimized conditions. The voltammograms obtained from this experiment are shown in Figure 6. As observed, with an increase in the thiopental concentration, many sites on the polymer are occupied by the drug, reducing the probe's ability to interact for electrochemical reactions. Consequently, the current decreases. The plot of current versus thiopental concentration is shown in Figure 3-12b. The results indicate that the linear range between current response and thiopental concentration for the modified electrode is 0.05–0.75 µM, with a detection limit of 10 nM.

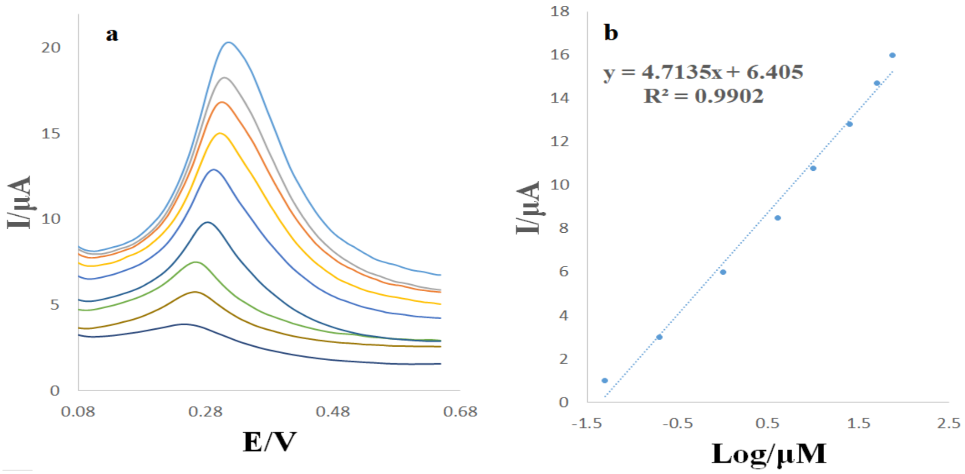

Figure 6. (a) Differential Pulse Voltammetry voltammograms of different thiopental concentrations, b) Calibration curve

3.9. Selectivity of the prepared electrode for thiopental measurement

An important characteristic of an ideal sensor is its selectivity for the target compound among other substances. The selectivity of the sol-gel imprinted electrodes is due to their stability. To evaluate the selectivity of the prepared sensor, the electrochemical response of the sensor was measured in the presence and absence of thiopental along with other compounds such as fentanyl, ketanserin, medetomidine, midazolam, azaperone, uric acid, and ascorbic acid. The results are presented in Figure 7. As can be observed, the presence of other species led to less than 5% variation in the current of thiopental measurement. Therefore, the prepared sensor is capable of accurately detecting thiopental in the presence of a complex matrix without the need for separation.

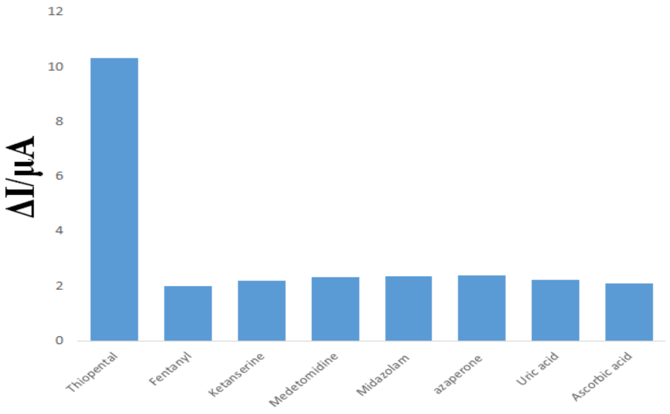

Figure 7. Selectivity of thiopental on the surface of the prepared electrode

3.10. Stability, reproducibility, and real Sample analysis of the prepared sensor

The repeatability of the developed sensor was evaluated by conducting ten successive measurements of 5 µM thiopental, yielding a relative standard deviation (RSD) of 4.73%. To assess reproducibility, five independently fabricated electrodes were tested under identical conditions, resulting in an RSD of 3.62%. The long-term stability of the electrodes was investigated over one month, with weekly measurements showing gradual current decreases of 2.32%, 3.41%, 5.72%, and 7.83%, respectively. These results confirm the satisfactory stability and reliability of the sensor.

3.11. Measurement in real samples

Additionally, the applicability of the sensor for real sample analysis was examined using spiked blood samples and the standard addition method. As presented in Table 1, the recovery rates for thiopental ranged from 97% to 101%, demonstrating the sensor's high accuracy and potential for detecting thiopental in complex biological matrices.

Table 1. Measurement in real samples

Add (μM)	Found (μM)	Recovery %	RSD %
1.00	0.97	97.00	4.30
5.00	4.83	96.60	4.49
1.00	1.05	105.00	4.12

4. CONCLUSION

An electrochemical sensor for thiopental was developed using a pencil graphite electrode modified with carbon nanotubes and a molecularly imprinted polymer prepared via the sol–gel technique. The high surface area and excellent conductivity of the carbon nanotubes facilitated effective polymerization and enhanced electron transfer, resulting in a low detection limit of 10 nM. The sensor demonstrated high sensitivity, selectivity, and reproducibility, and was successfully applied for thiopental determination in real biological samples.

Acknowledgments

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Declarations of interest

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

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