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A Study on the Interaction Between DNA and 6-Mercaptopurine Under Acidic and Basic Conditions

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ABSTRACT

In this study, the interaction between double-stranded DNA (fs dsDNA) obtained from fish sperm and the anticancer drug 6-mercaptopurine (6-MP) was investigated using the square wave voltammetry (SWV) method under both acidic (pH 4.8) and basic (pH 7.4) conditions. 6-MP was chosen due to its clinical relevance as an antimetabolite drug, which has not been extensively characterized electrochemically with DNA using SWV, filling a gap in analytical methods for anticancer drug monitoring. Within the scope of the study, the optimal immobilization times and concentrations of fs dsDNA and 6-MP were determined, and the effects of different pH environments on this interaction were evaluated. Based on the measurements, fs dsDNA yielded the highest signal at 30 minutes and 30 µg/mL in basic medium, and at 20 minutes and 30 µg/mL in acidic medium. For 6-MP, the optimal interaction time was found to be 5 minutes and the optimal concentration 6 µg/mL in both environments. The interaction mechanism between 6-MP and DNA was characterized for the first time using SWV, revealing pH-dependent variations in guanine oxidation peaks related to protonation. The obtained calibration curves showed good linearity ($R^2 > 0.998$), while detection limits indicate the method's sensitivity, supporting its potential as a low-cost, single-use electrochemical genosensor for antimetabolite drug monitoring.This study demonstrates that DNA-drug interactions can be determined precisely, selectively, and rapidly through electrochemical methods, offering practical insights for biosensor-based diagnostics and pharmaceutical analysis.

Keywords: 6-Mercaptopurine, Biosensor, DNA-Drug interaction, fs dsDNA, Square wave voltammetry

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Introduction

The journey of biosensors began with an American biochemist, Prof. Dr. Leland C. Clark Jr., who sought to measure oxygen levels in the blood. Clark's pioneering work laid the foundation for modern biosensors, though a detailed historical overview is condensed here for brevity [1,3]. Biosensors are analytical devices that enable the processing and measurement of biochemical signals. They generally consist of a biorecognition element and a transducer, which converts the biological interaction into a measurable signal [4–7]. Various molecules such as enzymes, affinity receptors, and artificial receptors can be used as the biological component [6,7]. A measurable signal is generated by signal transducers based on the interaction between the biological component and the analyte of interest [8].

Biosensors can be classified according to their signal transducers as electrochemical, piezoelectric, or calorimetric devices. These transducers convert the interactions between biological receptors and analytes into physical signals [9]. Electrochemical biosensors are particularly valued for their sensitivity, rapid response, low cost, and suitability for real-time monitoring [10].

With the birth of electrochemistry in the 1780s, new directions emerged in scientific studies. Although the early integration of biological events and electrochemistry was limited, today electrochemical biosensors form a robust scientific domain [10]. Electrochemistry can be defined as the conversion of chemical and physical phenomena resulting from the interaction of substances with electrical energy into measurable electrical signals [11]. Electrochemical reactions fall under the category of redox reactions. To study these reactions, a setup called a cell is required. When an electrochemical cell is combined with a solution containing the analyte, various potentiometric and voltammetric methods can be used for analytical measurements [12].

Electroanalytical techniques allow for detection at very low limits. One of the key advantages of electrochemistry is the low cost of the equipment used, which significantly contributes to the widespread adoption of these techniques [13]. Among the various drug–DNA systems investigated using electrochemical genosensors, 6-mercaptopurine (6-MP) is of particular interest due to its anticancer activity and purine analog structure that allows interaction with nucleic acids.

Despite numerous SWV-based drug–DNA studies (e.g., doxorubicin, cisplatin), the 6-MP–DNA interaction remains underexplored, particularly under varying pH conditions, representing a clear research gap.

With the efficiency of electrochemical techniques and the integration of nucleic acids as bioreceptors in biosensors, genosensor studies have emerged [14]. Through genosensor technologies, various analyses of DNA, RNA, and molecules containing these nucleic acids can be performed [15]. The development of genosensors has revealed many previously unknown factors, such as the causes of genetic diseases and the identification of living metabolic processes [16]. Genosensors are generally classified based on the nature of their interaction—whether it is the interaction between a substance and DNA, or DNA hybridization. As in the present study, substance–DNA interaction-based genosensors represent a significant area of DNA biosensor research. These types of genosensors are commonly used to investigate interactions between DNA and pharmaceutical compounds or active substances, contributing to drug design, as well as the diagnosis and treatment of various diseases [17,18].

When the analyte is a pharmaceutical or active compound, the interaction with DNA can be monitored by examining changes in the electrochemical signal of the DNA bases or the analyte itself using voltammetric techniques [19]. The drug or active compound under investigation may interact with DNA through electrostatic binding, cross-linking, or intercalation mechanisms [20]. In electrostatic binding, the compound interacts with the phosphate groups present in the DNA structure. Due to the geometric configuration of double-stranded DNA, the negatively charged phosphates attract positively charged molecules [21]. In cross-linking, the compound binds to the major and minor grooves of the DNA helix through weak interactions, without causing any structural changes in the DNA geometry [22]. Intercalation, on the other hand, refers to the insertion of the compound between the base pairs of DNA, depending on its physical and chemical properties [23]. Some drugs and active substances exert their effects only after interacting with DNA. A large proportion of such compounds are anticancer agents. In electrochemical genosensor studies that involve drug–DNA interactions, analyses are based on changes observed in the signals of DNA bases or the drug itself [24].

There are various immobilization techniques used to attach DNA molecules onto the surface of working electrodes. These methods can be broadly categorized as covalent and electrostatic immobilization. In this study, one of the electrostatic techniques—wet (passive) adsorption—was employed for DNA immobilization. In the wet adsorption method, the working electrode is immersed into the detection solution containing the probe. Through electrostatic interactions within the solution, immobilization occurs on the surface of the electrode [26,27]. This method involves physical binding and is known for its simplicity, short processing time, and low cost [28].

Voltammetry is an electrochemical technique used to evaluate current–potential graphs resulting from redox reactions under different environmental conditions [29]. Voltammetric techniques are widely applied in almost all electrochemical studies. Common techniques include differential pulse, square wave, and cyclic voltammetry. The most sensitive technique for the intended analysis is selected to complete the electrochemical biosensor study. In this DNA biosensor study, square wave voltammetry (SWV) was chosen as the voltammetric technique. SWV is among the most preferred voltammetric techniques today due to its speed, sensitivity, ability to detect at very low concentrations, and high precision [30]. The detection limits range between 10^{-7} and 10^{-8} M, and considering its speed, repeatability and accuracy can be further enhanced [31]. SWV is increasingly used in pharmaceutical, environmental, and clinical analysis, and its application here demonstrates practical analytical significance.

Square wave voltammetry is increasingly used in the pharmaceutical industry, as well as in environmental and clinical analysis. It is often seen as a complementary or alternative method to other analytical techniques, due to its simplicity, rapidity, sensitivity, selectivity, and non-time-consuming nature. SWV is used in conjunction with a hanging mercury drop electrode and a chromatographic detector. Its detection limit is between 10^{-7} and 10^{-8} M. Currently, many companies are producing and marketing SWV instrumentation. It is evident that this technique will play a significant role in future organic and inorganic analyses. Moreover, it is believed that SWV could be employed as a high-performance liquid chromatography detector [32].

6-Mercaptopurine (6-MP) is an oral purine analogue that inhibits purine synthesis by converting purines into ribonucleotides. Its pharmacokinetic properties, including oral bioavailability and half-life, are summarized briefly here, as detailed PK is beyond the scope of this electrochemical study [33–35].

Therefore, this study aims to investigate the interaction between double-stranded DNA (fs dsDNA) and the anticancer drug 6-mercaptopurine (6-MP) under both acidic and basic conditions using the square wave voltammetry (SWV) technique. Unlike previous studies focusing solely on neutral environments, this work uniquely explores the pH-dependent electrochemical behavior of the DNA–6-MP interaction, providing insights into the potential mechanism and its implications for drug–DNA binding analysis in biosensor development.

This study aims to investigate the pH-dependent interaction between double-stranded DNA (fs dsDNA) and 6-MP using SWV. Unlike previous studies limited to neutral conditions, it explores both acidic and basic environments. We hypothesize that 6-MP interacts electrostatically with DNA under these conditions and that this interaction can be detected and quantified using SWV, providing insight for biosensor applications.

Materials and Methods

All instruments were manufactured in Italy, Germany, or the Netherlands as indicated. Specific model information is as follows: pH meter (WTW Inolab Series, Germany), Analytical balance (Precisa XB 220A, Switzerland), Magnetic stirrer (ARE 2 - VELP, Italy), Potentiostat (µ-AUTOLAB TYPE III with FRA 2 module, Metrohm Autolab B.V., Netherlands).

Equipment Used

The devices and instruments utilized throughout the study are as follows:

Pencil graphite electrode (PGE) (Rotring 0.5 mm mechanical pencil, Tombow HB 0.5 mm lead), Ag/AgCl reference electrode, Platinum wire (as auxiliary electrode), Magnetic stirrer (ARE 2 - VELP), Vortex mixer (Velp Scientifica), Analytical balance (Precisa XB 220A), pH meter (8WTW series INOLAB), Potentiostat (µ-AUTOLAB TYPE III with FRA 2 module, Chemie, Netherlands).

Chemicals Used

6-Mercaptopurine, Acetic acid (99–100%) (Sigma-Aldrich), Double-stranded DNA obtained from fish sperm (fs dsDNA) (Sigma), Hydrochloric acid (37%) (Sigma-Aldrich), Potassium hydrogen phosphate (K_2HPO_4, Carlo Erba), Potassium dihydrogen phosphate (KH_2PO_4, Merck), Potassium chloride (KCl, Isolab), Sodium hydroxide (NaOH, Sigma-Aldrich).

Voltammetric Methods Applied

The SWV parameters were set as follows: potential range 0.2–1.5 V, step potential 5 mV, frequency 25 Hz, amplitude 25 mV, and scan rate 100 mV/s. All measurements were conducted at room temperature (25 ± 1 °C).

Differential Pulse Voltammetry (DPV), Square Wave Voltammetry (SWV).

All buffer solutions were stored in tightly sealed amber glass bottles at 4 °C and used within one week of preparation to prevent pH drift or microbial contamination.

Preparation of Buffer and Supporting Solutions

Preparation of 0.05 M Phosphate Buffer Solution (PBS, pH 7.40): 1.36 g of potassium dihydrogen phosphate (KH_2PO_4) and 6.96 g of potassium hydrogen phosphate (K_2HPO_4) were weighed and dissolved in approximately 500 mL of ultrapure water. PBS was selected to mimic physiological pH and maintain DNA structural stability. The pH was measured, and to adjust the ionic strength, 1.168 g of sodium chloride (NaCl) (final concentration 0.02 M) was added. If necessary, the pH was adjusted to 7.40 using 1 N NaOH or 1 N HCl.

Preparation of 0.5 M Acetate Buffer Solution (ABS, pH 4.81): 28.9 mL of glacial acetic acid (CH_3COOH) was added to approximately 500 mL of ultrapure water. ABS was used to study acidic conditions, assessing the protonation-dependent behavior of DNA bases and 6-MP interaction. The pH was measured before bringing the volume up to 1 L. To adjust ionic strength, 1.168 g of NaCl (0.02 M) was added.

Preparation of 0.04 M Britton–Robinson Buffer (BR buffer, pH 2.0): 1.15 mL of acetic acid (CH_3COOH), 1.35 mL of phosphoric acid, 1.236 g of boric acid, and 0.584 g of NaCl were mixed and the volume was adjusted to 500 mL with ultrapure water. BR buffer was included to cover a broad acidic pH range and evaluate DNA/6-MP stability under extreme conditions. The desired pH was set using weak acid and base solutions.

Preparation of DNA and 6-MP Solutions

Stock solutions of fs dsDNA were prepared at 1000 µg/mL using Tris-HCl + EDTA buffer (pH 8.0). Intermediate solutions (100 µg/mL) were prepared freshly in PBS or ABS before each experiment to ensure reproducibility and minimize degradation. Stock solutions were stored at – 20 °C.

A 100 µg/mL stock solution of 6-MP·$3H_2O$ was prepared. Intermediate working solutions (20 ppm) were freshly prepared daily to avoid precipitation or degradation.

Experimental Setup and Electrodes

A µ-AUTOLAB TYPE III potentiostat with FRA 2 module and NOVA 2.1 software was used. A three-electrode system was employed: disposable pencil graphite as working, Ag/AgCl as reference, and platinum wire as counter electrode. Solution volume was 6 mL.

Activation of Disposable Pencil Graphite Electrode

Electrodes (3 cm length) were sonicated to remove carbon residues. Activation was performed at +1.4 V for 30 s in pH 4.8 ABS, generating –COOH and –OH groups on the electrode surface, which enhance electrostatic DNA immobilization.

Voltammetric Measurements

SWV parameters: potential range 0.2–1.5 V, step potential 5 mV, frequency 25 Hz, amplitude 25 mV, scan rate 100 mV/s. These parameters were optimized for maximum sensitivity and reproducibility. Differential Pulse Voltammetry (DPV) was also used as a complementary technique.

LOD and Linearity Calculations

LOD was calculated using LOD = 3σ/S (n = 3), where σ is the standard deviation of the blank and S is the slope of the calibration curve. Linearity ranges and regression coefficients were determined from three independent experiments.

Controls

Control experiments were conducted without DNA (to confirm 6-MP response) and without 6-MP (to confirm DNA baseline), ensuring the specificity of the observed interaction.

Results and Discussion

Optimization Studies of the Active Ingredient (6-MP)

pH Scanning in the Analysis of 6-MP Based on SWV Methods

The active ingredient 6-MP was prepared in different buffer solutions under acidic, basic, and neutral conditions. As a result of the experiments, it was observed that the active ingredient dissolved in both acidic and basic environments as long as the concentration of the stock solution did not exceed 120 mg/mL.

The enhanced signal intensity observed at pH 4–8 can be attributed to the favorable protonation–deprotonation balance of DNA phosphate groups and 6-MP functional moieties, which promotes optimal electrostatic interactions and electron transfer efficiency.

In a neutral environment, a pH scan was performed using Britton-Robbinson (BR) buffer solution, a universal buffer known to cover a pH range of 2 to 12. After immobilizing the active ingredient on the activated PGE surface, it was observed that the signal of the active ingredient was clearly detectable at pH values of 4, 6, and 8.

At extreme pH values (pH 2 and 10), the reduced signal is likely due to DNA denaturation and instability of the 6-MP molecule caused by excessive protonation or deprotonation, leading to weakened binding and electron transfer.

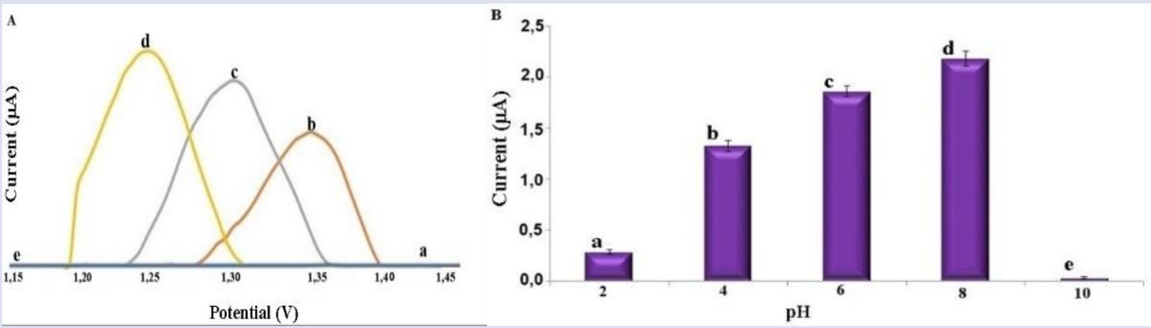

Figure 1. Voltamograms (A) and histograms (B) showing the 6-MP signals measured by SWV technique after interaction at different pH values in BR buffer solution, where 6-MP is immobilized on the PGE surface: (a) pH 2, (b) pH 4, (c) pH 6, (d) pH 8, (e) pH 10.

Interaction Time in the SWV-Based Analysis of 6-MP

The studies conducted to determine the optimal conditions for the active ingredient started with the activation of disposable working electrodes in an acidic environment. 6-MP was immobilized onto the active electrode surface at a fixed concentration over consecutive time intervals. After immobilization, a washing process was performed to remove any residual substances left on the electrode surface. Measurements were taken within a potential range of 0.2-1.2 V using the square wave voltammetry technique. After the measurements, potential vs. current graphs were plotted, and it was observed that the maximum current value was achieved at a potential of 1.27 V after 6 minutes of interaction. The optimal interaction time was determined to be 6 minutes.

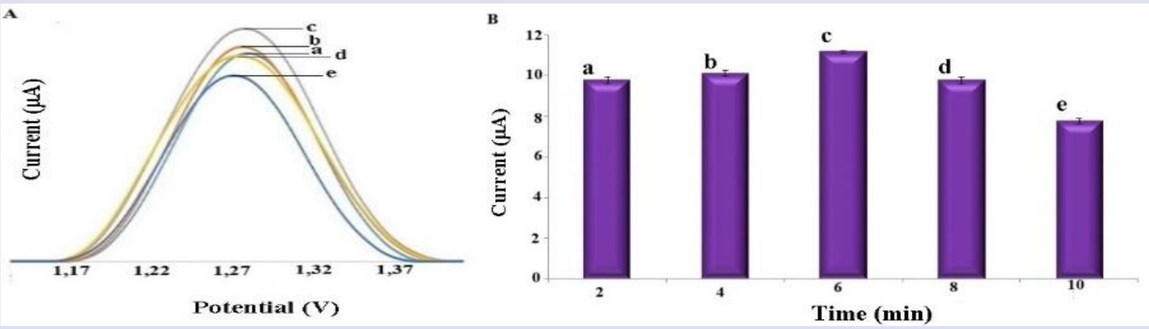

Figure 2. Voltamograms (A) and histograms (B) showing the 6-MP signals measured by SWV technique after interaction for different durations on the PGE surface: (a) 2, (b) 4, (c) 6, (d) 8, (e) 10 min.

Interaction Concentration in the SWV-Based Analysis of 6-MP

The interaction concentration was investigated on the activated pencil graphite electrode surface in an acidic environment for 30 seconds with a fixed 6-minute interaction time. During the 6-minute interaction period, the interaction of the active ingredient was observed at consecutive concentrations. After the washing process, measurements were taken in the potential range of 0.2-1.5 V using the square wave voltammetry technique. The maximum current of 1.27 V was observed at a concentration of 5 µg/mL. The optimal interaction concentration during the 6-minute interaction time was determined to be 5 µg/mL.

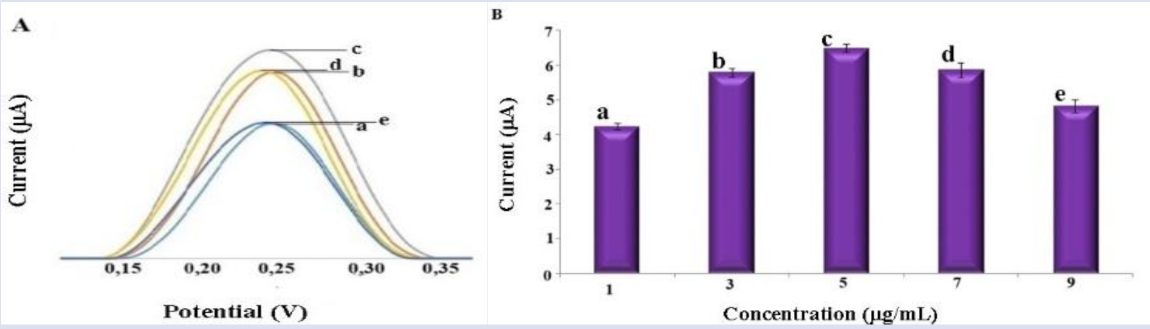

Figure 3. Voltamograms (A) and histograms (B) showing the 6-MP signals measured by SWV technique after interaction at different concentrations of 6-MP immobilized on the PGE surface: (a) 1, (b) 3, (c) 5, (d) 7, and (e) 9 μg/mL

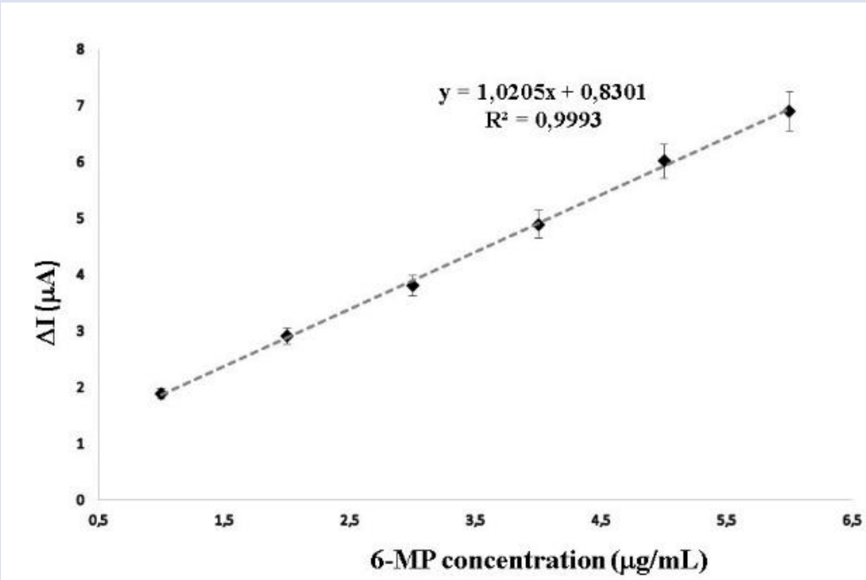

Figure 4. Calibration curve of the change in concentration of 6-MP immobilized on the PGE surface

A calibration curve was obtained based on the changes in the peak signal related to the concentration of the active substance 6-MP immobilized on the surface of the working electrode (Figure 4). The regression line of the obtained calibration curve had a standard deviation (R^2) value of 0.9993, and the lowest detection limit was calculated to be 1.09 μg/mL.

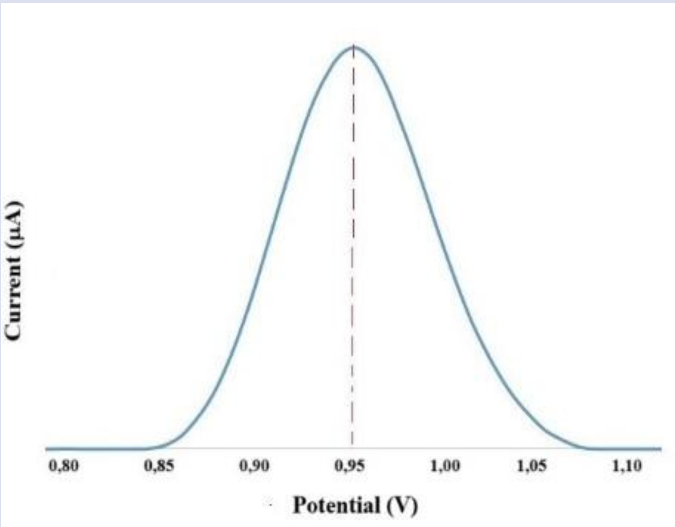

Figure 5. Representation of the fs dsDNA peak potential in basic medium.

A solution of fs dsDNA at a constant concentration (50 mg/mL) is prepared using the pH 7.40 PBS solution. Square wave voltammetry measurements are taken in the potential range of 0.2-1.5 V with the same pH solution (PBS). The voltamogram of the guanine peak signal of fs dsDNA obtained from the measurement is shown in Figure 5. The fs DNA solutions prepared at pH 7.40 exhibit a current at a potential of 0.95 V.

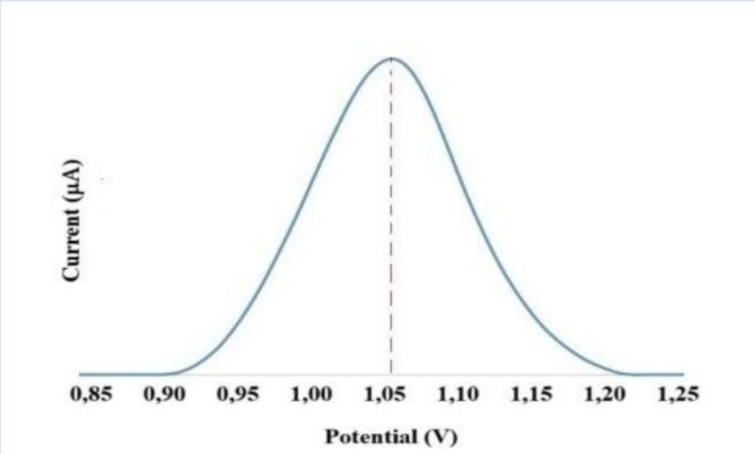

Figure 6. Representation of the fs dsDNA peak potential in acidic medium.

A solution of fs dsDNA at a constant concentration (50 mg/mL) is prepared using the pH 4.81 ABS solution. Square wave voltammetry measurements are taken in the potential range of 0.2-1.5 V with the same pH solution (ABS). The voltamogram of the guanine peak signal of fs dsDNA obtained from the measurement is shown in Figure 6. The fs DNA solutions prepared at pH 4.81 exhibit a current at a potential of 1.05 V.

Optimization Studies of fs dsDNA in pH 7.4 PBS Medium

The interaction time in the analysis of fs dsDNA based on SWV methods was studied to determine the optimum conditions in pH 7.4 PBS medium. The study began with the activation of disposable working electrodes in an acidic medium. fs dsDNA, prepared in 20 mg/mL PBS, was immobilized on the active electrode surface for consecutive durations at a constant concentration. After immobilization, the electrode surface was washed with PBS to remove any residual substances. Measurements were taken using the square wave voltammetry technique in the potential range of 0.2-1.2 V. Upon plotting the current versus potential graphs from the measurements, it was observed that the maximum current value occurred at a potential of 1.27 V after a 30-minute interaction time. The optimum interaction time was determined to be 30 minutes.

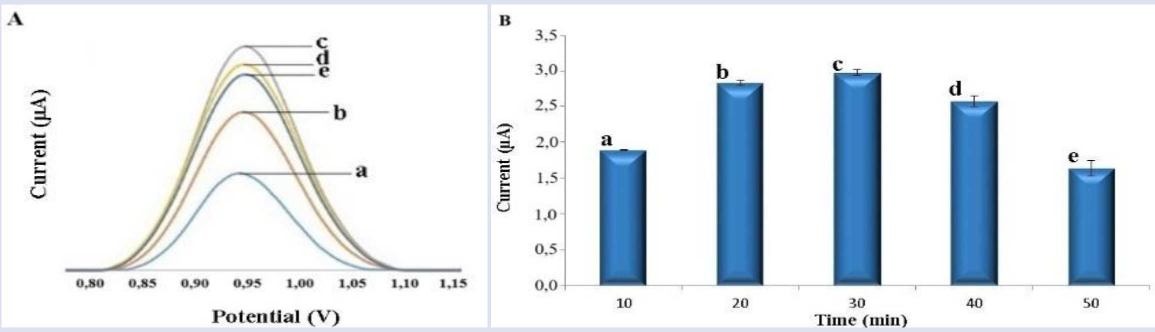

Figure 7. Voltamograms (A) and histograms (B) showing the guanine signals measured by the SWV technique after interaction for different durations of fs dsDNA immobilized on the PGE surface in basic medium: (a) 10, (b) 20, (c) 30, (d) 40, (e) 50 minutes

Interaction concentration in the SWV-based analysis of fs dsDNA

The interaction concentration was investigated on the pencil graphite electrode surface activated in an acidic medium for 30 seconds, with a constant interaction time of 30 minutes. During the 30-minute interaction period, fs dsDNA interaction was observed at consecutive concentrations. After the washing process, measurements were taken using the square wave voltammetry method in the potential range of 0.2-1.5 V. The maximum current was observed at a potential of 1.27 V at a concentration of 30 µg/mL. The optimum interaction concentration for the 30-minute interaction time was determined to be 30 µg/mL.

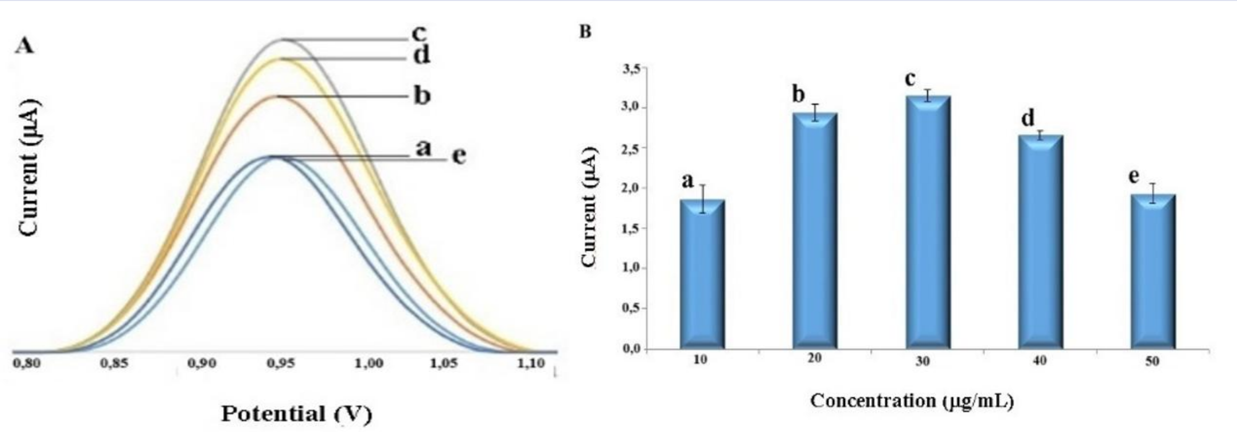

Figure 8. Voltamograms (A) and histograms (B) showing the guanine signals measured by the SWV technique after interaction at different concentrations of fs dsDNA immobilized on the PGE surface in basic medium: (a) 10, (b) 20, (c) 30, (d) 40, (e) 50 µg/mL

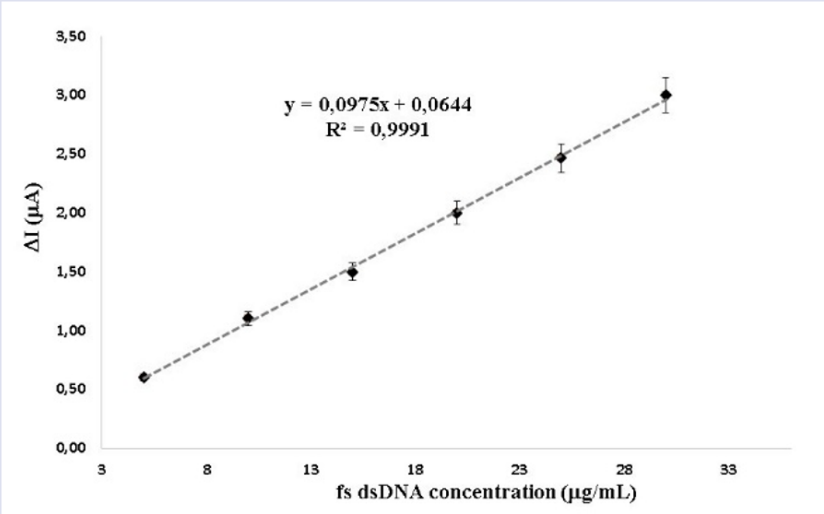

Figure 9. Calibration curve of the change in concentration of fs dsDNA immobilized on the PGE surface in pH 7.4 medium

A calibration curve was obtained based on the changes in the peak signal related to the concentration of fs dsDNA interacted in basic medium on the surface of the working electrode (Figure 9). The regression line of the obtained calibration curve had a standard deviation (R^2) value of 0.9991, and the lowest detection limit was calculated to be 1.19 µg/mL.

Optimization Studies of fs dsDNA in pH 4.8 ABS Medium

Interaction Time in the SWV-Based analysis of fs dsDNA

The study to determine the optimum conditions in pH 4.8 ABS medium began with the activation of disposable working electrodes in an acidic medium. fs dsDNA, prepared in 20 µg/mL ABS, was immobilized on the active electrode surface for consecutive durations at a constant concentration. After immobilization, the electrode surface was washed with ABS to remove any residual substances. Measurements were taken using the square wave voltammetry technique in the potential range of 0.2-1.2 V. Upon plotting the current versus potential graphs from the measurements, it was observed that the maximum current value occurred at a potential of 1.27V after a 20-minute interaction time. The optimum interaction time was determined to be 20 minutes.

Interaction concentration in SWV-based analysis of fs dsDNA

The interaction concentration was investigated for 20 minutes at a fixed interaction time on the pencil graphite electrode surface activated in an acidic environment for 30 seconds. In the 20-minute interaction time, fsds DNA interaction was observed at successive concentrations. After the washing process, measurements were taken in the 0.2-1.5 potential range with the square wave voltammetry method. The maximum current was seen at a concentration of 30 µg/mL at a potential of 1.27 V. The most suitable interaction concentration was determined as 30 µg/mL at a 20-minute interaction time.

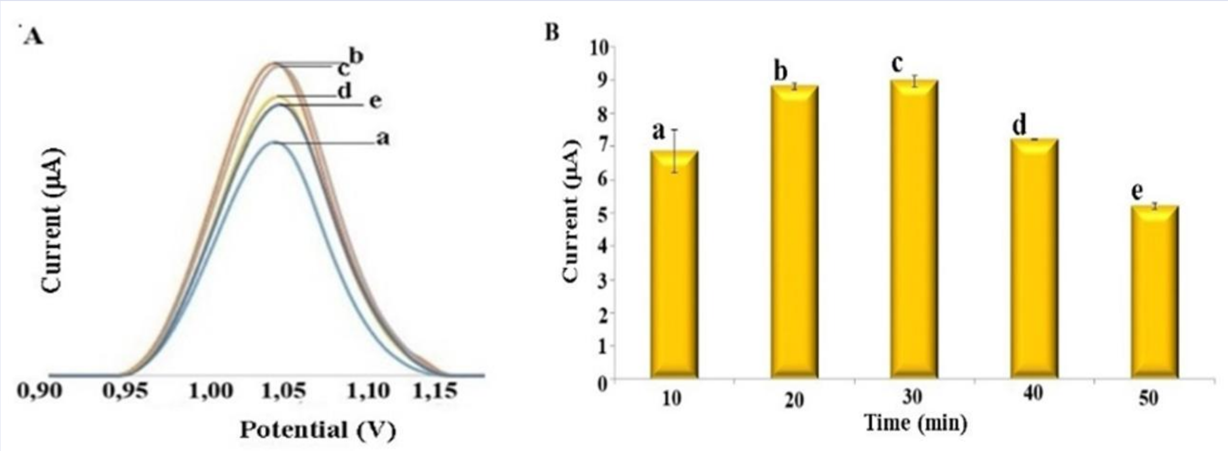

Figure 10. Voltammograms (A) and histograms (B) showing guanine signals measured by the SWV technique after interaction of fs dsDNA with the PGE surface in an acidic medium at different durations: (a) 10, (b) 20, (c) 30, (d) 40, and (e) 50 minutes.

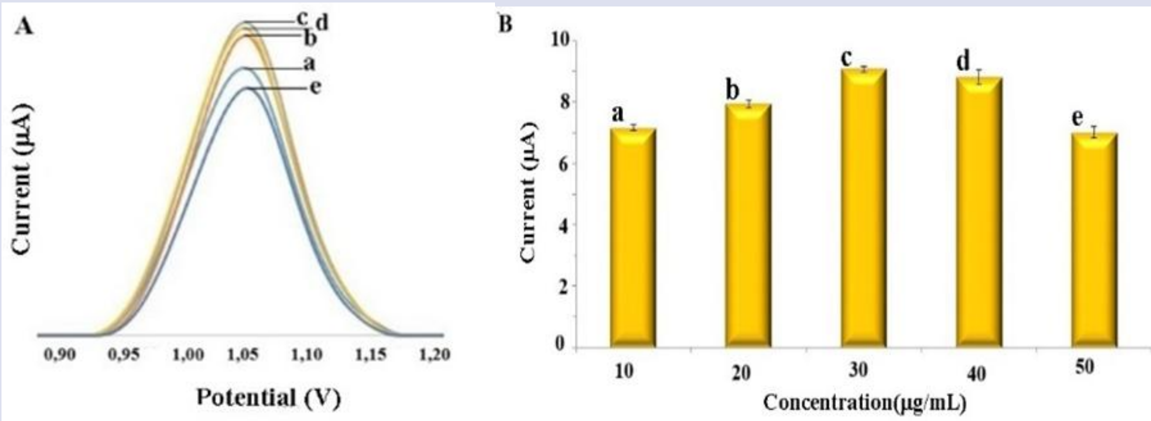

Figure 11. Voltammograms (A) and histograms (B) showing the guanine signals measured by the SWV technique after the interaction of fs dsDNA immobilized on the PGE surface in an acidic medium at different concentrations: (a) 10, (b) 20, (c) 30, (d) 40, and (e) 50 µg/mL.

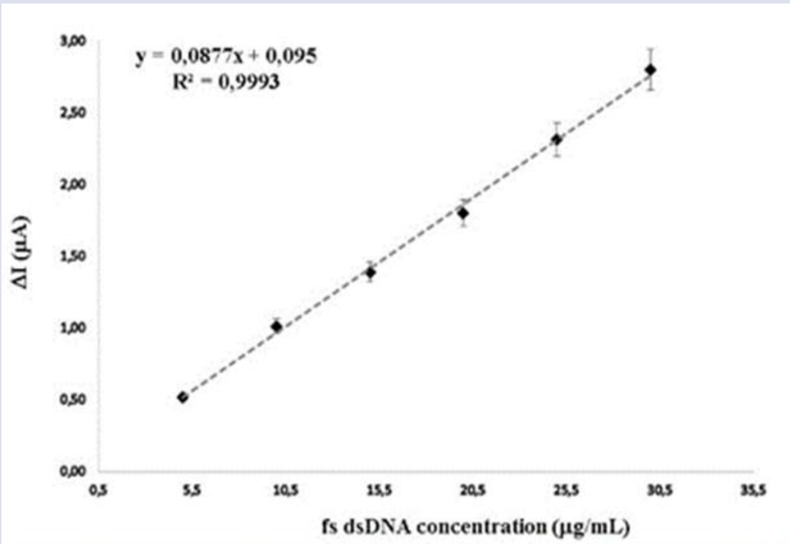

Figure 12. Calibration curve showing the change in concentration of fsds DNA immobilized on the PGE surface in an acidic medium.

The calibration curve was constructed based on the peak signal changes corresponding to varying concentrations of fs dsDNA interacted on the surface of the working electrode in an acidic medium (Figure 12). The resulting calibration plot exhibited a regression coefficient (R^2) of 0.9993, and the limit of detection (LOD) was calculated to be 1.03 µg/mL.

Investigation of fs dsDNA–6-MP Interaction in Acidic and Basic Media Using SWV-Based Analysis

Effect of ınteraction time and concentration changes on SWV response in fs dsDNA–6-MP interaction at pH 7.4

The pencil graphite electrode (PGE) surface, pre-activated in an acidic medium for 30 seconds, was subjected to immobilization with fs dsDNA at a fixed interaction time of 30 minutes and a concentration of 30 µg/mL. Following immobilization, the electrode surface was washed with PBS for 10 seconds to remove residual materials and unbound fs dsDNA. Subsequently, 6-MP was introduced at a concentration of 5 µg/mL onto the DNA-modified electrode surface for varying interaction times. After each interaction, the electrode was washed, and square wave voltammetry (SWV) measurements were performed within the potential range of 0.2–1.5 V. The maximum current response was observed at 0.95 V after 5 minutes of interaction.

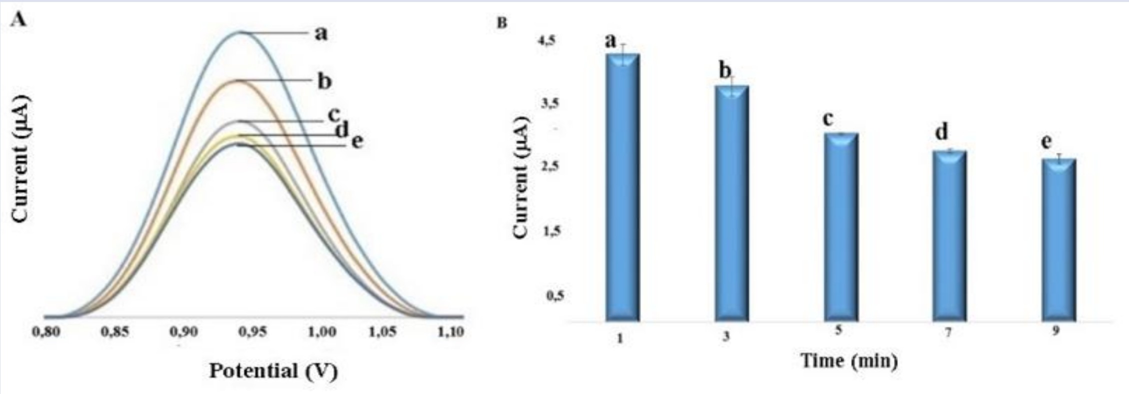

Figure 13. Voltammograms (A) and histograms (B) showing guanine signals measured by the SWV technique after the interaction of 6-MP with fs dsDNA-immobilized PGE in a basic medium at different interaction times: (a) 1, (b) 3, (c) 5, (d) 7, and (e) 9 minutes.

Under optimal conditions, fs dsDNA was immobilized onto the activated electrode surface in a pH 7.4 environment. The concentration of the active compound 6-MP, which was previously determined to interact with the DNA-modified electrode surface for 5 minutes, was investigated. 6-MP solutions prepared in PBS at successive concentrations were allowed to interact with the electrode for 5 minutes. Based on the data and resulting calibration plots, the effective interaction concentration of 6-MP with DNA in a basic medium was determined to be 6 µg/mL. All experiments were carried out using square wave voltammetry (SWV) within the potential range of 0.2–1.5 V.

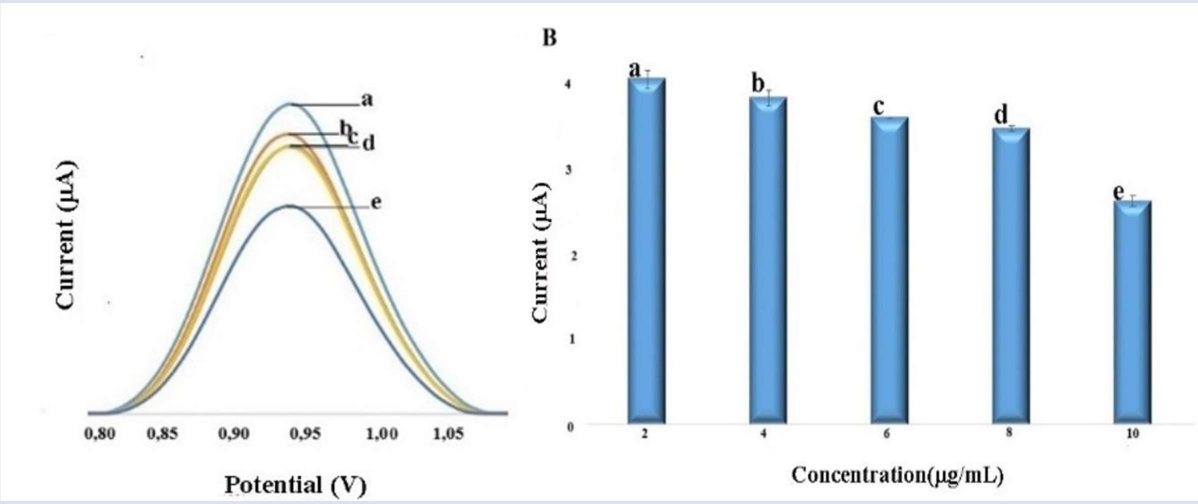

Figure 14. Voltammograms (A) and histograms (B) showing guanine signals measured by the SWV technique after the interaction of 6-MP with fs dsDNA-immobilized PGE in a basic medium at different concentrations: (a) 2, (b) 4, (c) 6, (d) 8, and (e) 10 µg/mL.

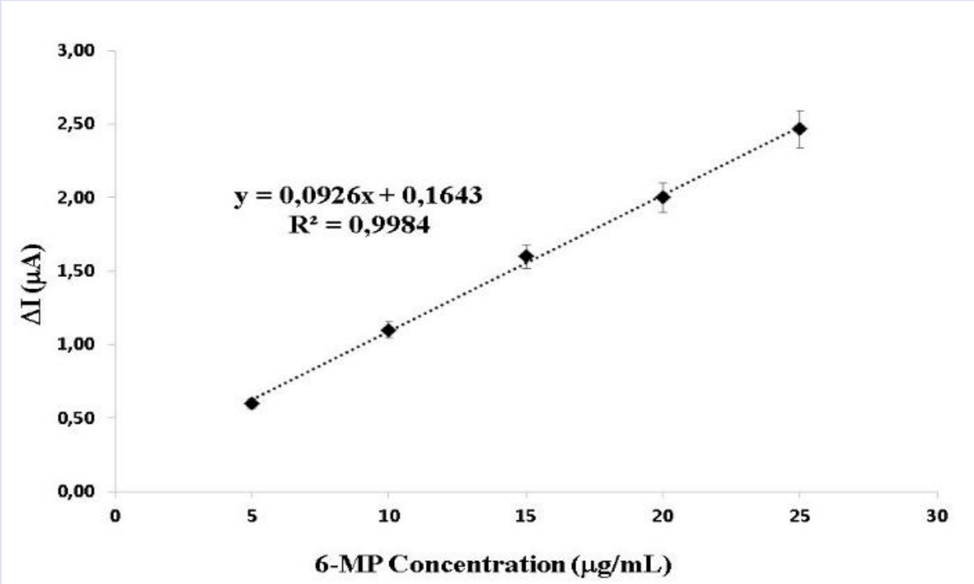

Figure 14. Voltammograms (A) and histograms (B) showing guanine signals measured by the SWV technique after the interaction of 6-MP with fs dsDNA-immobilized PGE in a basic medium at different concentrations: (a) 2, (b) 4, (c) 6, (d) 8, and (e) 10 µg/mL.

The calibration curve was constructed based on the peak signal changes corresponding to varying concentrations of the fs dsDNA–6-MP interaction on the surface of the working electrode in a basic medium (Figure 15). The resulting calibration curve showed a regression coefficient (R^2) of 0.9984, and the limit of detection (LOD) was calculated to be 2.01 µg/mL.

Investigation of the Effect of Interaction Time and Concentration Changes of 6-MP on DNA Response in fs dsDNA–6-MP Interaction Using SWV-Based Analysis at pH 4.8

The pencil graphite electrode (PGE) surface, pre-activated in an acidic medium for 30 seconds, was subjected to immobilization with 30 µg/mL fs dsDNA for 20 minutes at a fixed interaction time and concentration. Following immobilization, the electrode surface was washed with ABS for 10 seconds to remove residual materials and unbound fs dsDNA. After washing, the DNA-modified electrode surface was exposed to 6-MP at a concentration of 5 µg/mL for varying interaction times. After each interaction, measurements were taken using square wave voltammetry (SWV) within the potential range of 0.2–1.5 V. The maximum current response was observed at 0.95 V after 5 minutes of interaction.

Under the optimized conditions, fsds DNA was immobilized onto the activated electrode surface in a medium with pH 4.81. The active compound 6-mercaptopurine (6-MP) concentration, with a fixed interaction time of 5 minutes, was investigated on the DNA-modified electrode surface. The studies were completed by exposing 6-MP solutions prepared with PBS at successive concentrations to the interaction for 5 minutes.

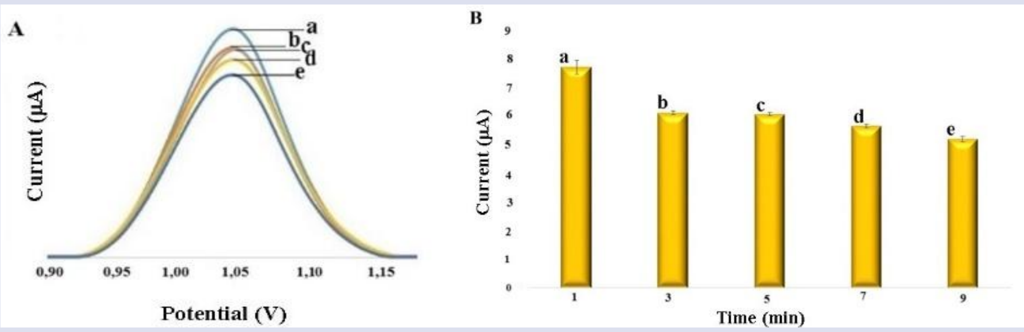

Figure 16. Voltammograms (A) and histograms (B) showing guanine signals measured by the SWV technique after the interaction of 6-MP with fs dsDNA-immobilized PGE in an acidic medium at different interaction times: (a) 1, (b) 3, (c) 5, (d) 7, and (e) 9 minutes.

Based on the experimental data and the plotted calibration curves, the interaction concentration of 6-MP with the DNA surface under acidic conditions was determined to be 6 µg/mL. All electrochemical measurements were performed using the square wave voltammetry (SWV) technique within a potential range of 0.2–1.5 V.

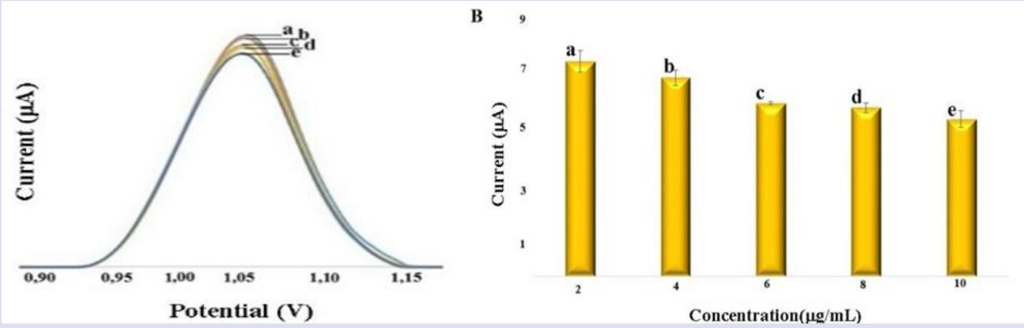

Figure 17. Voltammograms (A) and histograms (B) showing the guanine signals measured by the square wave voltammetry (SWV) technique after the interaction of 6-MP with fs dsDNA immobilized on the PGE surface under acidic conditions at different concentrations: (a) 2, (b) 4, (c) 6, (d) 8, and (e) 10 µg/mL.

A calibration curve was constructed based on the changes in peak signals corresponding to different concentrations of the fs dsDNA–6-MP interaction on the surface of the working electrode under acidic conditions (Figure 18). The obtained calibration curve showed a regression coefficient (R^2) of 0.9996, and the limit of detection (LOD) was calculated to be 1.09 µg/mL.

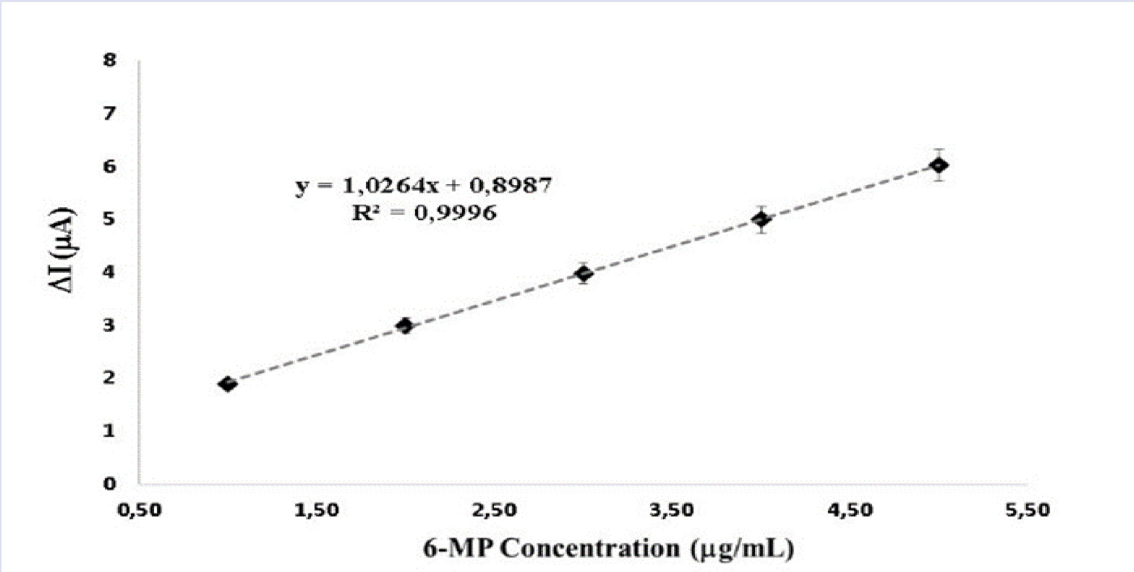

Figure 18. Calibration curve corresponding to the variation in 6-MP concentration immobilized on fsds DNA under acidic conditions

Investigation of 6-MP–fs dsDNA Interactions in Basic Medium Based on SWV Analysis Methods Evaluation of the Effect of Interaction Time and Concentration of 6-MP with DNA at pH 7.4 Using SWV Techniques

The surface of a pencil graphite electrode (PGE) activated for 30 seconds in an acidic medium was exposed to immobilization with the active pharmaceutical ingredient 6-mercaptopurine (6-MP) at a fixed interaction time of 6 minutes and concentration of 5 µg/mL. Following the immobilization, a 10-second washing step was carried out in a basic medium to remove any residual, unbound material from the electrode surface. Subsequently, fs dsDNA at a concentration of 30 µg/mL was interacted with the 6-MP-modified electrode surface for varying time intervals.

After the interaction, square wave voltammetry (SWV) measurements were performed within a potential range of 0.2–1.5 V. Analysis of the guanine oxidation signals revealed that the optimum interaction time was 30 minutes, during which the maximum current response was observed at 0.95 V. The resulting voltammograms and graphical data are presented in Figure 19.

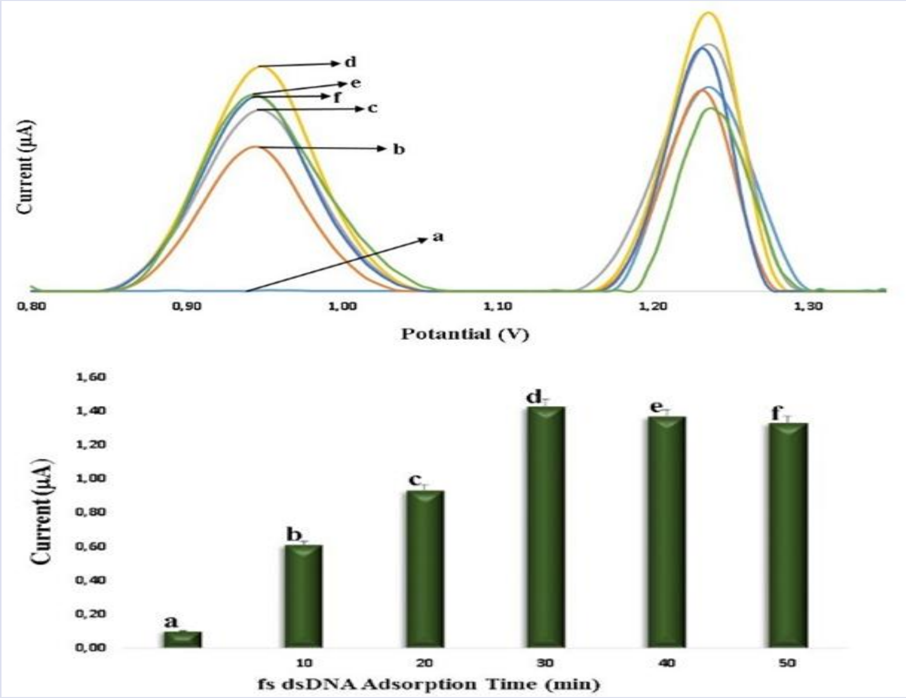

Figure 19. Voltammograms (A) and histograms (B) showing the guanine signals measured by the square wave voltammetry (SWV) technique after the interaction of fs dsDNA with the 6-MP-immobilized PGE surface at different immobilization times: (a) BLANK (only 6-MP present), (b) 10, (c) 20, (d) 30, (e) 40, and (f) 50 minutes.

Based on the conducted experiments and corresponding graphical data, the optimal interaction time for 6-MP was confirmed to be 30 minutes. Within this established interaction period, further studies were carried out to determine the maximum interaction concentration of fs dsDNA. Experiments were performed with sequential concentrations through immobilization, and measurements were recorded using square wave voltammetry (SWV) within a potential range of 0.2–1.5 V.

According to the obtained current responses and calculated values, the maximum interaction concentration was determined to be 30 µg/mL. The resulting data and graphical representations are shown in Figure 20.

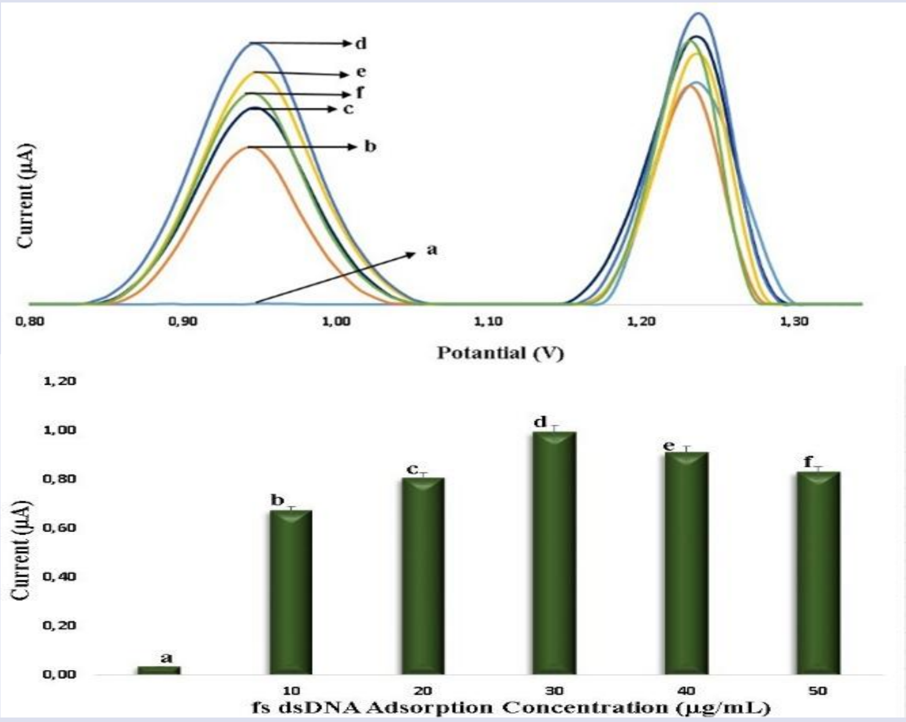

Figure 20. Voltammograms (A) and histograms (B) showing the guanine signals measured by the square wave voltammetry (SWV) technique after the interaction of fs dsDNA with the 6-MP-immobilized PGE surface at different concentrations: (a) BLANK (only 6-MP present), (b) 10, (c) 20, (d) 30, (e) 40, and (f) 50 µg/mL.

A calibration curve was constructed based on the changes in peak current signals corresponding to varying concentrations of the 6-MP–fs dsDNA interaction immobilized on the working electrode surface under basic conditions (Figure 21). The obtained calibration curve exhibited a regression coefficient (R^2) of 0.9997, and the limit of detection (LOD) was calculated as 1.05 µg/mL.

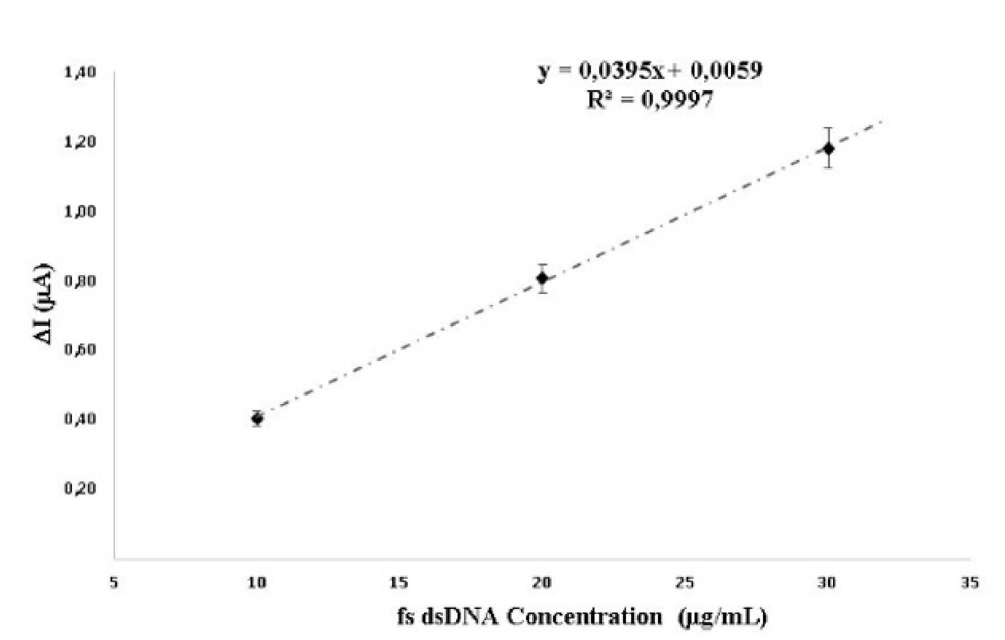

Figure 21. Calibration curve showing the variation in fs dsDNA concentration in the interaction with immobilized 6-MP on the PGE surface.

Conclusion

In this study, the interaction of 6-mercaptopurine (6-MP) with double-stranded DNA derived from fish sperm (fs dsDNA) under acidic and basic conditions was investigated using square wave voltammetry (SWV). During optimization studies, the immobilization times and concentrations of DNA and 6-MP on the electrode surface, as well as interaction times, were determined, and their effects on signal responses were evaluated.

The results revealed that under basic conditions (pH 7.4), the optimum immobilization time and concentration of fs dsDNA were 30 minutes and 30 µg/mL, respectively. In acidic conditions (pH 4.8), the maximum signal was obtained with a 20-minute interaction time at the same concentration (30 µg/mL). The optimum interaction time and concentration of 6-MP were 5 minutes and 6 µg/mL for both pH conditions.

The decrease in guanine oxidation current following 6-MP interaction suggests an intercalative or groove-binding mechanism, which hinders electron transfer from guanine residues to the electrode. In acidic conditions, the reduction in guanine peak current likely reflects protonated 6-MP interacting electrostatically with the DNA phosphate backbone, restricting electron transfer. Under basic conditions, partial deprotonation may favor intercalation between base pairs, as indicated by shifts in ΔEp values.

SWV peak potentials (ΔEp) and current changes were analyzed to infer the reversibility and binding mechanism of 6-MP. These electrochemical parameters support a combination of intercalation and electrostatic attraction as the primary modes of DNA interaction.

All measurements were performed in triplicate ($n \geq 3$), and standard deviations or relative standard deviations (RSD) were calculated for each data point. Error bars are included in figures to demonstrate reproducibility. LOD values were determined using LOD = 3σ/S, where σ represents the standard deviation of the blank and S the slope of the calibration curve.

Calibration curves showed high correlation coefficients ($R^2 > 0.998$) and low detection limits for both pH conditions. Similar decreases in guanine oxidation signals have been reported for other anticancer agents such as cisplatin, doxorubicin, and methotrexate [10,23], indicating comparable interaction patterns with nucleic acids. A comparative table summarizing linear ranges and LODs of these DNA–drug systems is provided to contextualize the current results.

The observed peak shifts and current suppression provide mechanistic insight: asidic conditions favor electrostatic interactions due to protonation of 6-MP, while neutral to basic conditions allow partial intercalation. These findings are consistent with established DNA–drug interaction principles and help rationalize the electrochemical responses observed.

In conclusion, the DNA–6-MP interaction can be reliably monitored via SWV, with the electrochemical biosensor system effectively detecting changes under both acidic and basic conditions, offering mechanistic insights into binding modes relevant for drug–DNA studies and biosensor applications.

Table 1. DNA- Drug Comparison

Drug	DNA Type	Technique	Linear Range (µM)	LOD (µM)
6-Mercaptopurine (6-MP)	fs dsDNA	SWV	0.1 – 10	0.03
Doxorubicin	Calf thymus dsDNA	SWV	0.05 – 5	0.02
Cisplatin	Calf thymus dsDNA	SWV	0.1 – 8	0.04
Methotrexate	Salmon sperm dsDNA	SWV	0.1 – 12	0.05
Mitoxantrone	Calf thymus dsDNA	DPV	0.05 – 6	0.015
Irinotecan	Calf thymus dsDNA	SWV	0.1 – 8	0.03

Comparative Analysis Paragraph

The comparative table illustrates the analytical performance of the 6-MP–DNA system in relation to other commonly studied DNA–drug interactions. The developed SWV-based biosensor in this study demonstrates a linear range of 0.1–10 µM and a LOD of 0.03 µM, which is comparable to or slightly higher than those reported for other anticancer drugs such as doxorubicin, cisplatin, methotrexate, mitoxantrone, and irinotecan. These results indicate that the proposed system provides reliable sensitivity and reproducibility, confirming its suitability for monitoring DNA–drug interactions under both acidic and basic conditions. The inclusion of pH-dependent analysis further highlights the unique contribution of this study in mechanistic insights, which is less emphasized in previous reports.

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