

Developing a sensitive DNA biosensor for the detection of flutamide using electrochemical method

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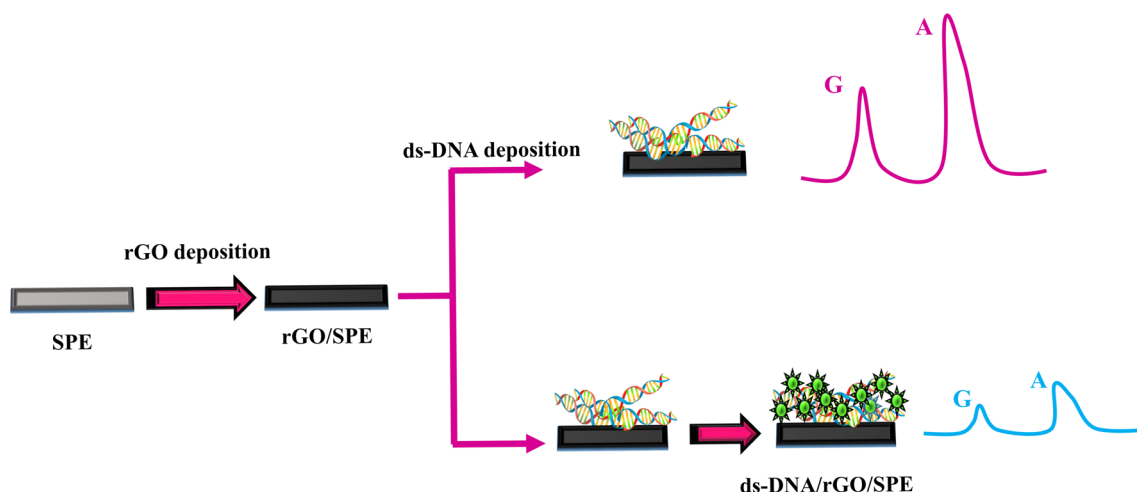
Abstract An uncomplicated method was applied to fabricate a novel electrochemical DNA biosensor for the determination of flutamide. This biosensor was composed of pretreated screen-printed electrode (SPE) that was modified with reduced graphene oxide (rGO) and decorated with ds-DNA. This biosensor was applied via the interaction of small molecules such as flutamide with the DNA. The interaction of salmon sperm ds-DNA with flutamide on the surface of ds-DNA-modified rGO–SPE (ds-DNA/rGO/SPE) was examined. The oxidation currents of guanine and adenine were used as probes in order to peruse the resulting interaction. Through the interaction of flutamide with

the DNA, the oxidation currents of guanine and adenine declined at the ds-DNA/rGO/SPE, using differential pulse voltammetry (DPV). There was a linear dependence of the adenine oxidation currents and the flutamide concentration in the range of 0.0025–0.075 and 0.075–7.50 nmol L⁻¹, with a limit of detection 1.5 pmol L⁻¹. The relative standard deviations (RSD) for five replicate measurements of 0.05 and 5.0 nmol L⁻¹ flutamide were obtained as 4.5 and 3.5%, using the adenine signal. This method was successfully applied to determine the trace amount of flutamide in real samples.

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Graphical abstract



Keywords DNA biosensor · Flutamide · Reduced graphene oxide-modified screen-printed electrode · Differential pulse voltammetry

Introduction

Flutamide (4-nitro-3-trifluoromethylisobutylanilide) is a synthetic, nonsteroidal antiandrogen (NSAA) compound that has considerable therapeutic applications in prostatic cancer [1]. This anticancer drug and its primary hydroxyl metabolite by the cytochrome P₄₅₀ system can reduce the metabolism of C₁₉ steroids at the target cells in the secondary sex organs [2].

Previous studies have shown that treating prostate cancer often need to androgen suppression therapy and utilize of androgen receptor antagonists [3]. The mentioned compounds vie with endogenous hormone agonists testosterone and dihydrotestosterone (DHT) to bind to the androgen receptor, but it must be noted that they do not support the formation of productive androgen receptor transcription complexes [4]. Flutamide is employed as an antineoplastic and antiandrogen drug. It has been shown to be a powerful nonsteroidal androgen antagonist [5] that can block androgen receptor sites and therefore treat the prostate cancer.

Various techniques have been utilized to determine the flutamide including UV–Vis spectrophotometry [6], high-performance liquid chromatography [7], gas–liquid chromatography [8], and polarography [9]. The expensive instrumentation, sophisticated and time-consuming procedures, and low sensitivity are some disadvantages of the reported papers for flutamide detection. Electrochemical methods compared with other methods are better alternative as they are affordable, highly sensitive and have acceptable reliability and reproducibility [10].

The study of the DNA–drug interactions [11, 12] is very interesting and remarkable; it can not only help to understand the mechanism of action of the drug but also contributes to the design of new drugs [13]. Electrochemical inspection of interaction between DNA and different drugs can provide a quick and inexpensive method to determine the drugs. In order to manufacture of DNA-based biosensors, single- or double-strand DNA is immobilized on an electrochemical transducer. Changes in the DNA structure within the interaction of targets with DNA are detected by different electrochemical methods [14–16].

Mercury electrode and mercury-film electrodes are most valuable tools employed for adsorptive stripping voltammetry; according to the extreme toxicity of mercury and the mercury salts, electrode materials that have the potential to replace mercury are being inspected. The screen-printed electrode (SPE) is one of these electrodes with the good advantages such as affordability, commercial accessibility, and disposability. However, the rate of electron transfer on these electrodes depends on the types of pretreatment before it is employed in the analysis [17].

In this study, a DNA-based biosensor was fabricated and used to determine the trace amounts of flutamide. In order to construct the DNA-modified screen-printed electrode (SPE), the reduced graphene oxide (rGO) was placed on the surface of pretreated SPE to enhance the ds-DNA immobilization. After that, the ds-DNA was immobilized on the rGO-modified SPE (rGO/SPE) by applying the potential under the optimum condition to construct the ds-DNA/rGO/SPE. The interaction of ds-DNA and flutamide leads to more preconcentration of flutamide in ds-DNA/rGO/SPE and improve the detection limit of proposed method.

Materials and methods

Chemicals

All solutions used during the procedure were prepared by reagent-grade chemicals and deionized water. Double-strand salmon sperm DNA was bought from Sigma. Flutamide was obtained from Aldrich Chemicals. Tris-HCl, CH₃COOH, CH₃COONa, NaH₂PO₄, NaCl, and NaOH were purchased from Aldrich Chemicals. Graphite, nitric acid, and sulfuric acid were purchased from Merck.

A stock solution of salmon sperm ds-DNA (1000 mg L⁻¹) was prepared in a Tris-HCl buffer and stored frozen. More diluted solutions of the ds-DNA were made by an acetate buffer solution (pH 4.8) containing 0.02 mol L⁻¹ NaCl. A stock solution of flutamide (2.0 mmol L⁻¹) was made by dissolving certain amounts of flutamide in ethanol and was kept at 4 °C.

Apparatus

Electrochemical measuring was conducted using an Autolab PGSTAT 12, potentiostat/galvanostat, which is equipped with a Metrohm cell Model 663 VA stand. The platinum wire and Ag/AgCl (3 mol L⁻¹ KCl) were chosen as counter and reference electrodes, respectively.

The spectroscopic studies were performed using a double beam spectrophotometer, Jasco model V-750 which is equipped to 1.0 cm quartz cells.

In order to adjust the pH of required solutions, a Metrohm pH meter (Model 827) with a glass electrode (Corning) was used.

Preparation of the DNA-modified SPE (ds-DNA/rGO/SPE)

Preparation of the modified SPE was carried out in three stages. In the first stage, the surface of SPE was cleaned and treated to immobilize the ds-DNA. So, SPE was transferred to an electrochemical cell containing 0.1 mol L⁻¹ NaOH and the potential were cycled between -0.50 and +1.50 V at a scan rate of 100 mV s⁻¹ for 15 cycles. In the second stage, the SPE was transferred to an electrochemical cell containing 25 mL suspensions of graphene oxide (GO), and to bring out the oxygen, a gentle stream of N₂ gas was blown into the solution for 5 min. Then, the potential was cycled between +0.60 and -1.60 V at a scan rate of 25 mV s⁻¹ for 10 times. The emergence of a peak at -1.40 V is related to the irreversible electrochemical reduction of GO, and continuous

increase in the peak current is evidence for successful reduction and immobilization of GO on the surface of SPE.

In order to further reduction of the immobilized GO, 25 mL of borate buffer (pH 9.0) was added to the electrochemical cell and deoxygenation process was conducted by blowing nitrogen gas for 5 min. Similar to the previous step, the potential was cycled between +0.60 and -1.60 V at a scan rate of 25 mV s⁻¹ for 10 times. The color change from brown to black on the electrode surface represents a further reduction of GO. The modified electrode in this section was named rGO/SPE.

In the third stage, the modified SPE was immersed in stirred 15.0 mg L⁻¹ ds-DNA, and the potential of +0.10 V was applied for 120 s. Then, the DNA-modified SPE (ds-DNA/rGO/SPE) was rinsed with acetate buffer solution (pH 4.8) to remove the unbonded ds-DNA. After this, the ds-DNA/rGO/SPE was immersed in the 0.5 mol L⁻¹ acetate buffer solution (pH 4.8) free of the ds-DNA and the potential was scanned between +0.40 and +1.40 V to record the oxidation currents of the adenine and guanine.

Operational conditions

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) measurements were performed in 5.0 mmol L⁻¹ [Fe(CN)₆]^{3-/4-}, as a redox probe, in 0.1 mol L⁻¹ KCl. The impedance spectra were recorded within the frequency range of 0.005 to 10⁵ Hz at polarization potential of 0.15 V. The cyclic voltammograms were recorded using a scan rate of 100 mV s⁻¹.

The DPV measurements were carried out using a pulse amplitude of 60 mV, a modulation time of 0.045 s, and a scan rate of 100 mV s⁻¹ in the background electrolyte [the acetate buffer (pH 4.8) plus 0.02 mol L⁻¹ NaCl] free of ds-DNA.

Procedure

In order to investigate the ds-DNA-flutamide interaction, DPV method and UV-Vis spectroscopy were used. In the first stage, the ds-DNA/rGO/SPE was prepared according to "Preparation of the DNA-modified SPE (ds-DNA/rGO/SPE)" section. The ds-DNA/rGO/SPEs was immersed into a well-stirred solution containing 10 mL phosphate buffer solution (PBS, pH 4.0) as a blank solution and a potential of -0.10 V was applied for 120 s. Then, the ds-DNA/rGO/SPE was rinsed and immersed in the background electrolyte, and the potential was scanned between 0.40 and 1.40 V, to record the oxidation currents of guanine and adenine. In the next step, the ds-DNA/rGO/SPEs were immersed in a well-stirred solution containing different concentrations of flutamide

in 10 mL phosphate buffer solution (PBS, pH 4.0) and a potential of -0.10 V was applied for 120 s. Same as the previous step, the DPV measurements was performed to record the oxidation signals of the adenine and guanine. Then, the ds-DNA/rGO/SPE was rinsed with the acetate buffer solution (pH 4.8) to remove any unbonded flutamide and transferred to the electrochemical cell to record the DPV voltammograms under the operational conditions in “Operational conditions” section. The differences between the oxidation currents of adenine before and after interaction with flutamide were considered as an analytical signal.

In the section related to UV–Vis spectroscopy studies, the UV–Vis spectra of flutamide and ds-DNA alone and after interacting with each other were recorded and evaluated to prove their interaction.

Preparation of real samples

In order to prepare plasma sample, three centrifuge tubes were selected. 5 mL tri chloroacetic acid, 5 mL plasma sample, and three different concentrations of flutamide were added to each of the tubes. Then, the contents of the tubes were centrifuged to precipitate the plasma proteins, and 1.0 mL of the supernatant solutions was diluted with 9.0 mL of 0.1 mol L^{-1} PBS (pH 7.0).

To prepare urine sample, different concentrations of flutamide were added to three centrifuge tubes containing 2.0 mL urine sample. Then, the content of each tube was centrifuged and filtered using filter paper. In order to the analysis of the flutamide contents, 1.0 mL of the supernatant solutions was diluted with 9.0 mL of 0.1 mol L^{-1} PBS (pH 7.0).

For samples analysis, a standard addition method (with DPV technique) was used under the operational conditions (as described in “Operational conditions” section).

Results and discussion

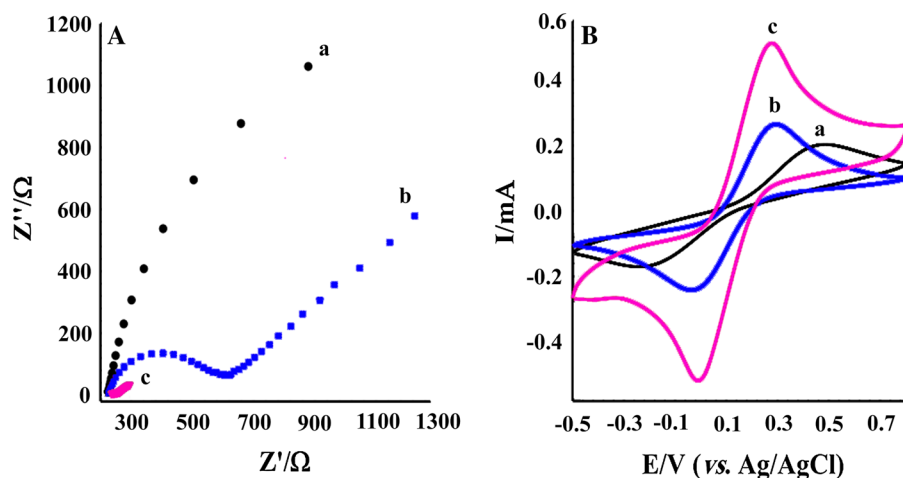
Characterization of ds-DNA/rGO/SPE

DNA is an important biological polymer, which is classified as natural, negatively charged polyelectrolyte due to the present phosphate groups. So, it can be immobilize on the positive surface electrostatically. Applying a positive potential on the surface of SPE leads to better absorption of negatively charged ds-DNA on the SPE electrostatically. Moreover, π – π interaction is also the other reason for the interaction of ds-DNA with SPE surface. Characterization of the ds-DNA/rGO/SPE was performed using SEM, CV, EIS, IR and Raman spectroscopy.

Electrochemical impedance spectroscopy (EIS) is a beneficial method to investigate changes in the electrode surface and has been considered as a label-free technique for the fast and reliable bioanalysis [18]. Figure 1A shows the estimated charge transfer resistance (R_{ct}) from Nyquist plots of (1) untreated SPE, (2) treated SPE and (3) rGO/SPE. As shown in this figure, the R_{ct} related to rGO/SPE is very low compared to treated and untreated SPE due to a decrease in the charge resistance and rich electro-conductivity of SPE after successful immobilization of rGO.

CV technique provides qualitative information about electrochemical reaction mechanisms, several quantitative properties of the charge transfer reactions between electrolyte and electrons with respect to the surface of electrode [19]. Figure 1B shows the cyclic voltammograms of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at various stages of the surface modification. Two peaks related to the oxidation and reduction of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ were observed at the untreated SPE with a peak potential separation of 28 mV [Fig. 1B (a)]. After treatment of the SPE, the currents increased and the peak potential separation decreased

Fig. 1 **A** Impedance spectra, and **B** CVs of *a* untreated SPE, *b* treated SPE and *c* rGO/SPE in 0.1 mol L^{-1} PBS (pH 7.0) containing 5.0 mmol L^{-1} $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and 0.1 mol L^{-1} KCl. Condition: polarization potential: 0.15 V, frequency: 0.005–105 Hz with amplitude of 10 mV



clearly [Fig. 1B (b)]. Our results [Fig. 1B (c)] demonstrated that after rGO immobilization, the peak current of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ increased due to the larger electro-active surface area and faster electron transfer of rGO/SPE so that these results were in good agreement with the results obtained from the EIS.

To investigate the changes at the surface of SPE at various stages of the modification with rGO, FE-SEM technique was used too. As shown in Fig. 2A (a), graphite layers and empty spaces between the graphite layers are visible at the surface of SPE. Also, nanosheet structure of graphene related to rGo/SPE is clearly shown in Fig. 2A (b).

FT-IR and Raman spectra were analyzed to assess the rate of GO reduction. FT-IR spectra of exfoliated graphene oxide (EGO) showed the specific absorption bands that correspond to the stretching vibration of O–H, C = O of carboxyl and carbonyl groups, C = C, C–O–C and C–O. As shown in Fig. 2B after the electrochemical reduction of GO, the absorption bands removed and one carbon–carbon double bond in 1618 cm^{-1} and a weak peak of C = O group in 1741 cm^{-1} remained which represent an effective removal of oxygen-containing groups and synthesis of the rGO.

Raman spectra of the EGO include both G and D bands in 1595 and 1355 cm^{-1} (Fig. 2C). The D and G bands are related to Sp^3 hybrid carbon and graphite crystalline state (E_{2g}), respectively. Raman spectra of graphite powder have G band and D band that represent the synthesis of GO.

After the formation of rGO, the bands showed a negative shift, and also, the ratio of bond D to bond G, which represents the synthesis of rGO was reduced.

Calculate the surface area of the electrodes

To calculate the surface area of the modified and unmodified electrodes, the SPE and rGO/SPE were transferred to an electrochemical cell containing $[\text{Fe}(\text{CN})_6]^{3-/4-}$ separately and their voltammograms were recorded in the potential range of -0.40 and $+0.90\text{ V}$.

In order to estimate the surface area of these electrodes, the Randles–Sevcik equation was used. According to the following equations,

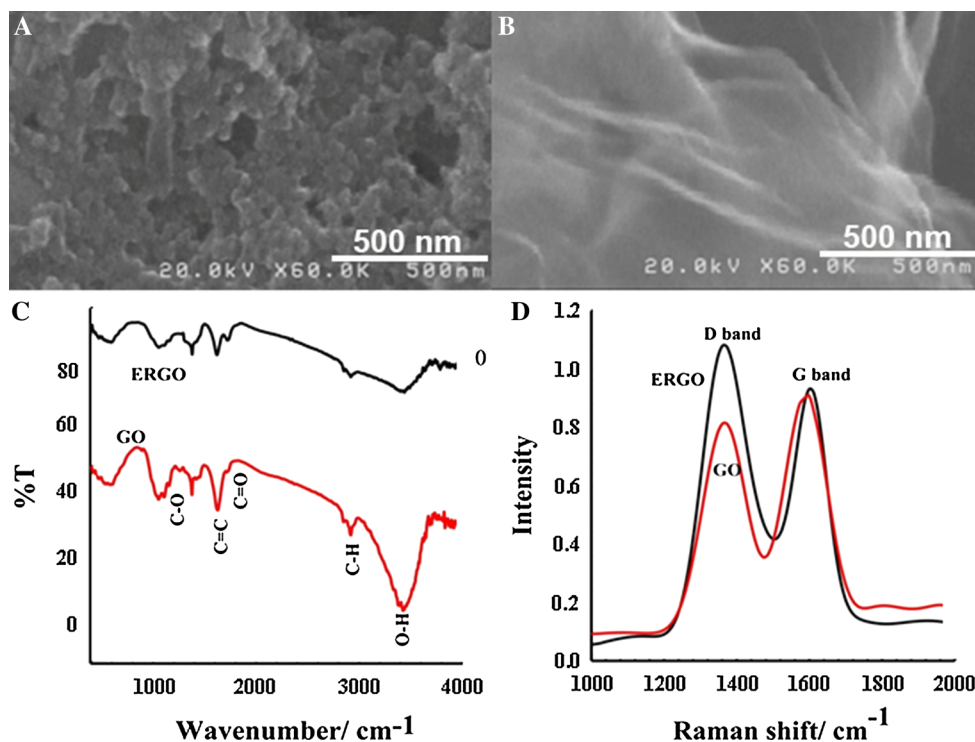
$$I_p = (2.69 \times 10^5) n^{3/2} A D^{1/2} \nu^{1/2} C$$

where I_p is the current, n is the number of electrons, A is the working electrode area, ν is scan rate and other parameters have usual meanings, and the surface area related to SPE and rGO/SPE was obtained as 1.52×10^{-4} and $2.95 \times 10^{-4}\text{ cm}^2$, respectively.

The inspection of flutamide–ds-DNA interaction

In order to prove the interaction between the ds-DNA and flutamide, DPV method and UV–Vis spectroscopy were used separately.

Fig. 2 SEM images of **A** SPE, and **B** rGO/SPE. **C** FT-IR spectra of exfoliated graphene oxide (EGO) and **D** Raman spectra of exfoliated graphene oxide (EGO)



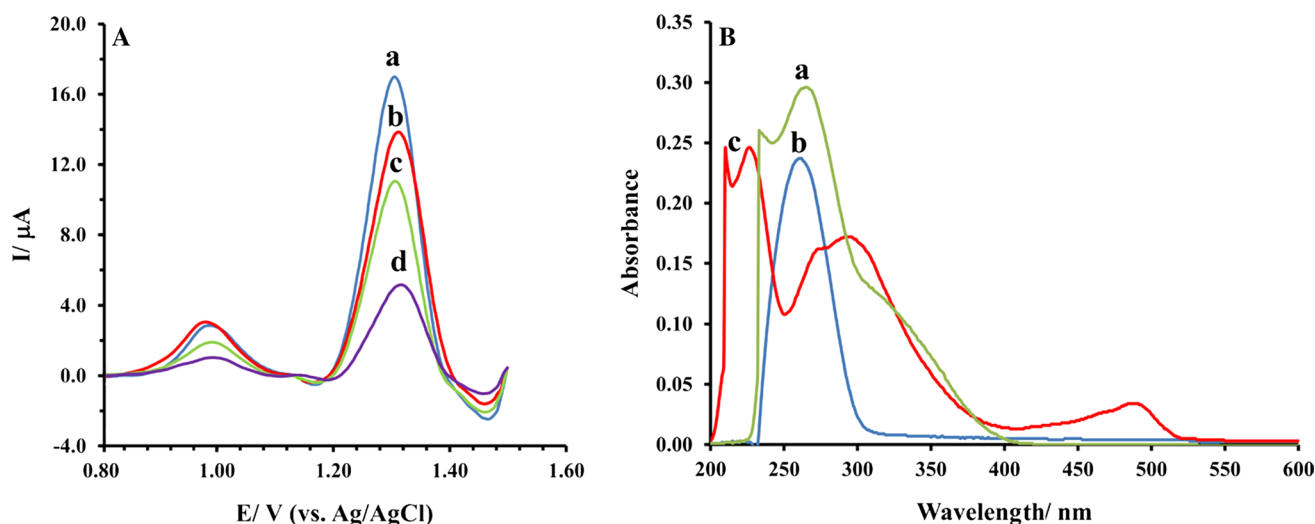


Fig. 3 **A** Differential pulse voltammograms of the interaction between flutamide and ds-DNA/rGO/SPE, oxidation currents of guanine and adenine after interaction with (from up to down) 0.0, 0.0025, 0.05 and 5.0 nmol L⁻¹ flutamide at the surface of ds-DNA/rGO/SPE. Conditions: ds-DNA immobilization (15.0 mg mL⁻¹) on rGO/SPE at

+0.10 V during 120 s in acetate buffer (pH 4.8); flutamide incubation: under the applying the potential -0.1 V during 120 s in PBS buffer (pH 4.0); measurement: scanning between +0.40 and +1.40 V in acetate buffer (pH 4.8). **B** UV-Vis spectra of *a* 4.0 μg mL⁻¹, *b* 10.0 μg mL⁻¹ ds-DNA and *c* flutamide plus ds-DNA (10.0 μg mL⁻¹)

Figure 3A shows the DPV voltammograms of the ds-DNA on the surface of ds-DNA/rGO/SPEs before and after interaction with flutamide. According to what has been demonstrated in the previous articles, the oxidation currents of guanine and adenine are located at around +1.0 and +1.3 V, respectively [20]. Figure 3A (a) shows the DPV voltammograms of ds-DNA before interaction with flutamide. As can be seen in this figure, the peak potential of guanine and adenine at the surface of ds-DNA/rGO/SPEs is located in +0.80 and +1.30 V, respectively. By adding flutamide to the blank solution, the decrease in the oxidation currents of adenine and guanine was observed so that higher concentrations of flutamide were led to a further reduction in the currents [Fig. 3A (b–d)]. This observation confirms the interaction between flutamide and the ds-DNA on the surface of ds-DNA/rGO/SPEs.

The UV-Vis spectra of flutamide and ds-DNA alone and after their interaction are shown in Fig. 3B. Maximum absorbance of flutamide is located at 263 nm [Fig. 3B (a)], and the maximum absorbance of the ds-DNA is observed at 267 nm [Fig. 3B (b)]. The absorption spectra of flutamide plus ds-DNA are shown in Fig. 3B (c). A substantial reduction in absorbance related to flutamide confirmed that there is an interaction between flutamide and the ds-DNA. The results of UV-Vis spectrophotometry and diminishing of adenine and guanine oxidation currents are acceptable evidence to prove the flutamide–ds-DNA interaction.

Optimization of effective factors on the sensitivity of ds-DNA/rGO/SPE

In order to find the lowest detection limit and highest sensitivity, the effect of various factors on the response of ds-DNA/rGO/SPE (the oxidation currents of adenine and guanine) was effectively evaluated and optimized. These factors were divided into two different categories which are as follows:

1. Effective factors on the construction of ds-DNA/rGO/SPE.
2. Effective factors on the flutamide determination.

Optimization of the effective factors on ds-DNA/rGO/SPE construction

In order to optimize the effective factors on ds-DNA/rGO/SPE construction, some factors such as absorption potential of ds-DNA, ds-DNA concentration and immobilization time of ds-DNA were inspected.

To achieve the best absorption potential for ds-DNA immobilization, different potentials, in the range of -0.20 and +0.30 V, were selected and applied for 60 s to immobilize 10.0 mg L⁻¹ ds-DNA. As shown in Fig. 4A by applying +0.10 V as an absorption potential, the adenine and guanine currents reach to the maximum and then level off. So this potential was selected for ds-DNA immobilization through the work.

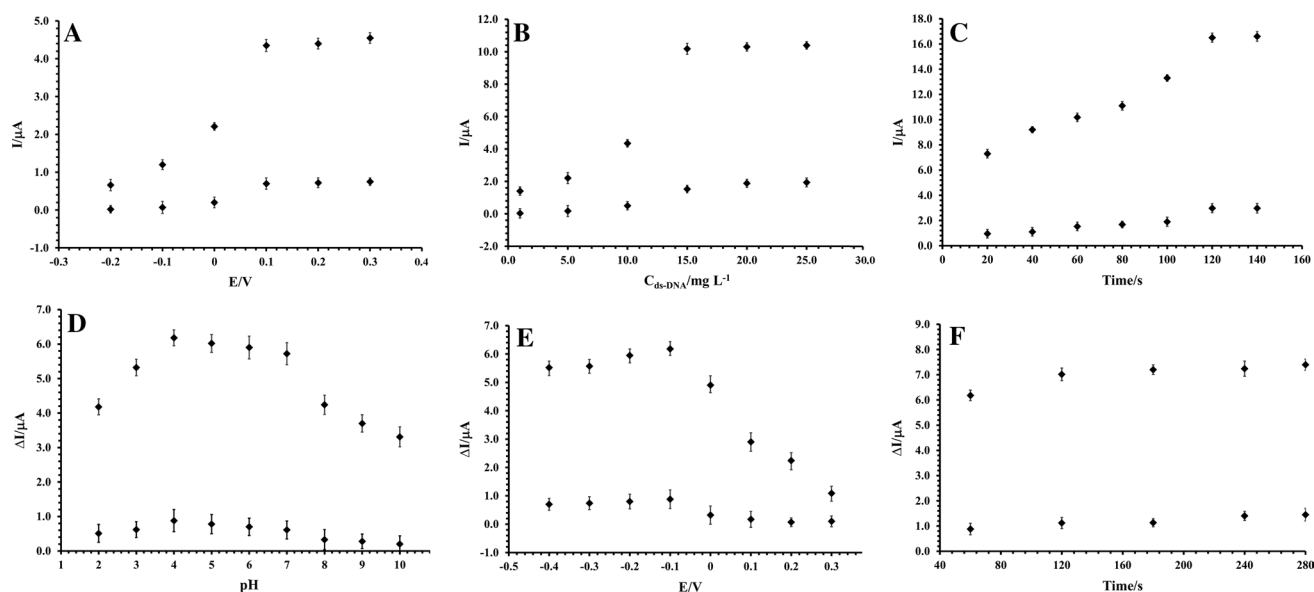


Fig. 4 **A** Influence of deposition potential of ds-DNA on DPV response of adenine and guanine; **B** influence of ds-DNA concentration on DPV response of adenine and guanine to construct the ds-DNA/rGO/SPE; **C** influence of immobilization time of ds-DNA on

the DPV response of adenine and guanine; effect of **D** changing the pH of flutamide solution; **E** deposition potential of flutamide, and **F** deposition time of flutamide on the response of ds-DNA/rGO/SPE

In order to achieve the best concentration of the ds-DNA, several concentrations of the DNA in the range of 1.0 and 25.0 mg L⁻¹ were tested and the results demonstrated that 15.0 mg L⁻¹ of the DNA have the highest adenine and guanine currents compared with other concentrations (Fig. 4B). Therefore, this concentration was selected for ds-DNA/rGO/SPE construction.

In the last stage of the optimization related to effective factors on the ds-DNA/rGO/SPE construction, the immobilization time of the ds-DNA, in the range of 20–140 s, was altered, and the adenine and guanine currents were followed. The obtained currents in this time frame showed that at the time of 120 s, the maximum current and sensitivity are achieved (Fig. 4C).

Optimization of the effective factors on flutamide determination

The factors examined in this section included (1) pH of flutamide solution, (2) deposition potential of flutamide, (3) deposition time of flutamide.

To evaluate the best pH of 0.050 nmol L⁻¹ flutamide solution, the pH of PBS was changed in the range of 2.0–10.0 by applying a potential of -0.10 V for 60 s. Our results (Fig. 4D) showed that the maximum analytical signal was achieved at pH 4.0. Therefore, this pH was selected as the best pH for interaction between flutamide

and ds-DNA. It can be argued that in acidic pH, the functional groups of flutamide are protonated and the interaction between flutamide and the ds-DNA reaches the maximum amounts and in neutral and alkaline pH the interaction reaches to minimum due to repulsion between free electrons of the flutamide and the negative charge of the phosphate groups of ds-DNA.

To select the best deposition potential of flutamide, suitable potential was applied in the range of -0.40 to 0.30 V for 60 s and the responses of the biosensor were followed. According to the results (Fig. 4E) at -0.10 V, the interaction of flutamide and the ds-DNA has the maximum amount, so this potential was used as a deposition potential during the work.

The interaction time between flutamide and the ds-DNA (deposition time of flutamide) is an important factor that must be evaluated. Therefore, in this stage of work, the interaction time was changed between 60 and 280 s. The results showed that 120 s was the best time for the interaction between flutamide and the ds-DNA (Fig. 4F).

Figures of merit

Increasing the concentration of flutamide leads to the corresponding decrease in the guanine and adenine oxidation currents. As shown in Fig. 5A, B, the current of adenine

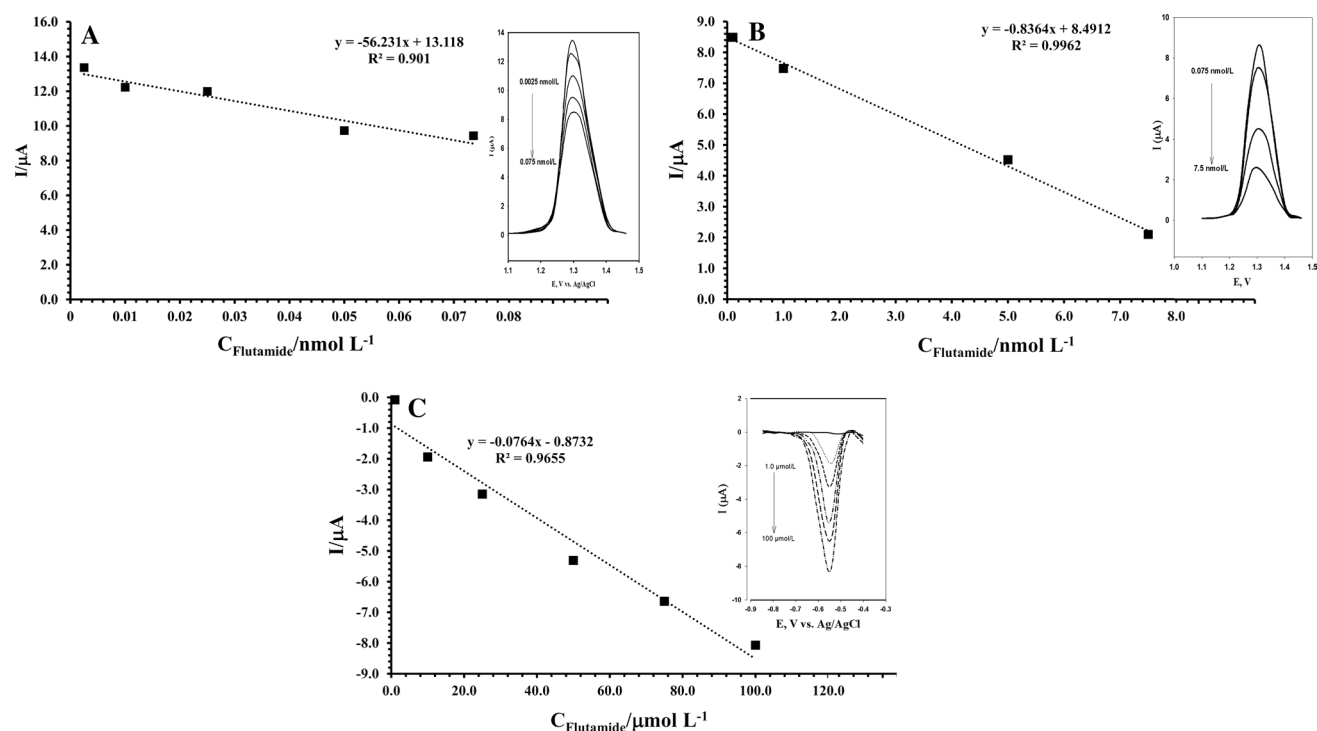


Fig. 5 Calibration curves (**A**, **B**) for determination of flutamide using ds-DNA/rGO/SPE based on the change in guanine and adenine currents after interaction with flutamide under the optimum conditions;

calibration curve **C** for determination of flutamide on the rGO/SPE by following the DPV currents of flutamide. *Insets* show the DPVs

was linear with flutamide concentration over the ranges of 0.0025–0.075 nmol L⁻¹ and 0.075–7.50 nmol L⁻¹. The detection limit (3 s) of the proposed method was estimated 0.0015 nmol L⁻¹ using the proposed procedure. The value of the detection limit for flutamide is better than those obtained for several other reported electrochemical methods as given in Table 1 [21, 22]. As can be seen, the proposed method has a detection limit better than the other reported electrochemical methods.

The relative standard deviations for a change in the adenine currents, after interaction with 0.05 and 5.0 nmol L⁻¹ flutamide, were obtained as 4.5% and 3.5% ($n = 4$), respectively.

Comparison between the ds-DNA/rGO/SPE and rGO/SPE performance

To compare the performance of ds-DNA/rGO/SPE and rGO/SPE, the rGO/SPEs were fabricated according to “Preparation of the DNA-modified SPE (ds-DNA/rGO/SPE)” section without ds-DNA immobilization. Then, the rGO/SPEs were transferred to an electrochemical cell containing different amounts of flutamide in PBS (pH 4.0) for 120 s, and the potential was scanned between -0.39 and -0.85 V. As shown in Fig. 5C the reduction current of the rGO/SPEs was increased linearly with flutamide concentration over the range of 1.0–100 μmol L⁻¹. The detection limit (3 s) using rGO/

Table 1 Comparison of the published electrochemical sensor for the determination of flutamide versus the proposed biosensor

Electrode	Method	LOD (nmol L ⁻¹)	Linear dynamic range (nmol L ⁻¹)	References
Carbon paste electrode modified with polymer film	DPV	181.0	7.24×10^4 – 5.79×10^5	[21]
Hanging mercury drop electrode	SWV ^a	4.27×10^2	6.60×10^2 – 4.45×10^3	[22]
Hanging mercury drop electrode	DPP ^b	1.72	2.00 – 1.54×10^4	[23]
Modified SPE with rGO	DPV	0.0015	0.0025–0.0750 and 0.0750–7.5000	The present work

^a Square wave voltammetry

^b Differential pulse polarography

Table 2 Interference study for determination of flutamide at the ds-DNA/rGO/SPE

Species	Tolerant limit (w/w)
Glucose, fructose, sucrose	1000
Sodium citrate, ascorbic acid, urea	500
Magnesium chloride, calcium chloride	400
Hydroxylamine	250
Sodium thiocyanate	100

SPE was estimated as $0.3 \mu\text{mol L}^{-1}$ that compared with a limit of detection achieved with ds-DNA/rGO/SPE is more.

Interference study

After determining the optimum conditions and construction of calibration curve, in order to check the applicability of the sensor for real sample, the effects of chemical and pharmaceutical species that usually found with flutamide in real samples were studied. The obtained data (Table 2) showed that the proposed method has acceptable selectivity for flutamide determination due to the strong interaction of flutamide and ds-DNA compared with other chemical and pharmaceutical species.

Real sample analysis

In order to evaluate the effectiveness of proposed method to measure the flutamide in real samples, the urine and plasma were prepared from medical diagnostic laboratory of Isfahan University of Technology. Sample preparation of urine and plasma was performed according to the method presented in “[Preparation of real samples](#)” section. To determine the flutamide content of real specimens, different concentrations of flutamide were spiked to the test samples and standard addition method was applied. The results of real sample analysis are shown in Table 3. As can be seen in this table, the proposed

method has good accuracy and precision for flutamide determination.

Conclusion

Nucleic acids have developed a new generation of electrochemical sensors so that it can be used to detect and measure the essential drugs. DNA-based biosensors as an efficient, affordable, and high-speed tool can be used in the analysis of drugs. In the study presented, a sensitive modified DNA-based biosensor was designed for the determination of nonsteroidal antiandrogen called flutamide in urine and plasma samples. Graphene and its derivatives are beneficial material because of their efficient and acceptable physical and electronic properties so that can be used for different applications, such as the construction of sensitive sensors, flexible devices, functional nanocomposites, and packaging materials. In this study, to construct the DNA-based biosensor the rGO with small size, high surface to volume and conductivity was used to better immobilization of ds-DNA and improving the signal. In this paper, initially, the interaction of flutamide with the ds-DNA using electrochemical and UV–Vis spectroscopy was investigated. The results indicated that there is an interaction between flutamide and the ds-DNA. The proposed ds-DNA/rGO/SPE showed two dynamic range $0.0025\text{--}0.075$ and $0.075\text{--}7.50 \text{ nmol L}^{-1}$ (at the optimum conditions), with a detection limit of 1.5 pmol L^{-1} . The results showed that proposed method compared with other methods has lower detection limit, and due to the strong interaction of flutamide and ds-DNA, interference of other species reaches to minimum amounts. The wide dynamic range, low detection limit, and low disturbance of other species lead to good performance of ds-DNA/rGO/SPE for flutamide determination. In addition to the mentioned advantages, this method is quick, simple, selective, sensitive, and inexpensive for the recognition and assessment of flutamide.

Table 3 Recovery of flutamide in human serum and plasma using ds-DNA/rGO/SPE

Sample	Sample no.	Added (nmol L^{-1})	Expected (nmol L^{-1})	Found (nmol L^{-1})	Recovery %
Human urine	1	–	–	<LOD	–
	2	1.00	1.00	0.98 ± 0.06	98.6
	3	2.00	2.00	3.03 ± 0.03	101.1
	4	3.00	3.00	4.81 ± 0.26	96.2
Plasma	1	–	–	<LOD	–
	2	1.00	1.00	0.98 ± 0.06	98.6
	3	2.00	2.00	3.03 ± 0.03	101.0
	4	3.00	3.00	5.02 ± 0.18	100.4

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