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A novel morphine electrochemical biosensor based on intercalative and electrostatic interaction of morphine with double strand DNA immobilized onto a modified Au electrode

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Abstract

The intercalative and electrostatic interaction of morphine with double-stranded DNA (ds-DNA), which was immobilized onto mercapto-benzaldehyde modified Au electrode, was employed for designing a sensitive biosensor. The interaction of morphine with the immobilized ds-DNA onto the electrode surface has been studied by differential pulse voltammetry (DPV). Under the optimum conditions, a linear dependence for the morphine concentration in the range of 0.05-500 μmol L^{-1} and its oxidation signal was observed and a detection limit of 0.01 μmol L^{-1} for the morphine was obtained. The reproducibility and applicability of the analysis to real samples were also investigated and results demonstrated that this DNA biosensor could be utilized for the sensitive, rapid, simple, and cost effective determination of morphine in urine and blood plasma samples.

Keywords: Morphine; DNA biosensor; Au electrode; Differential Pulse Voltammetry; Mercaptobenzaldehyde

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1. Introduction

Intercalation and groove binding are the two most common modes by which small molecules bind directly and selectively to DNA [1]. Intercalation, which is an enthalpically driven process, results from the insertion of a planar aromatic ring system between DNA base pairs with concomitant unwinding and lengthening of the DNA helix [2]. In contrast, groove binding, which is predominantly entropically driven, involves covalent or non-covalent (electrostatic) interactions that do not perturb the duplex structure to any great extent [3]. Groove-binders are typically crescent-shaped, and fit snugly into the minor groove with little distortion of the DNA structure. It has been suggested that some DNA-binding drugs, especially those classified as minor groove-binders, such as morphine, may exhibit mixed binding modes [4-7].

Morphine is the most abundant alkaloid found in opium. Morphine is used to treat moderate to serious pain. Short-acting formulations are taken as needed for pain. To date, many analytical methods have been developed to determine of morphine concentrations, including high performance liquid chromatography [8–10], gas chromatography–mass spectroscopy [11, 12], fluorimetry [13, 14], chemiluminescence [15–17], surface plasma resonance (SPR) [18], and electrochemical methods [19–25].

Electrochemical methods are generally fast and economical in trace analysis. Compared with other analytical techniques, electrochemical analysis has the advantages of simplicity and high sensitivity, and simultaneously and can give out some mechanism information of medical and biological molecules.

Recently, various modified electrodes have been reported for the electrochemical detection of morphine as cobalt hexacyanoferrate modified glassy carbon electrode (GCE) [19], Prussian blue-modified indium tin oxide (ITO) electrode [26], molecularly imprinted polymer films to

fabricate a microfluidic system for amperometric detection of morphine [27], chemically modified-palladized aluminum electrode [28], Au microelectrode [29,30] in a flow injection system and multiwalled carbon nanotubes modified preheated glassy carbon electrode have also been used for the morphine detection [31], PEDOT modified Pt-electrode [32], and gold nanoparticles modified carbon paste electrode [33].

Electrochemical investigation of DNA-drug interactions can provide a rapid, sensitive, selective and cheaper method for the determination of drugs. The electrochemical DNA biosensors are assayed more easily and rapidly compared to the conventional DNA biosensors.

In recent years we used some nano-compounds modified electrodes as sensors or biosensors for determination of some metal ions [34-38]. In this work, we presented a novel sensitive morphine biosensor based on intercalative and electrostatic interaction between morphine and ds-DNA modified Au electrode. For this purpose, firstly Au electrode was self-assembled with mercapto-benzaldehyde as an arm linker through well-known Au-S bond. Then the residual aldehyde group on the other end of MB was reacted with the modified amino on the ss-DNA and ss-DNA was covalently immobilized to the sensing surface. The ds-DNA was prepared with simple hybridization of ss-DNA with complementary target DNA. Finally, ds-DNA-modified Au electrode was used as an analytical tool to evaluate the interaction of morphine with the ds-DNA. The new biosensor was used for the voltammetric determination of morphine in urine and blood plasma samples.

2. Experimental

2.1. Materials

Morphine was prepared from the Pharma Shimi Pharmaceutical Company (Tehran, Iran). Potassium monohydrogen phosphate, potassium dihydrogen phosphate, $K_3Fe(CN)_6$, and $K_4Fe(CN)_6$ were obtained from the Merck Company. Mercaptohexanol (MCH) and Mercaptobenzaldehyde (MB) were obtained from the Sigma-Aldrich Company. Probe and complementary DNA used in this study, were acquired from the Aminsan Company (Iran) with the following sequence:

Probe ss-DNA (S_1): 5'-NH_2- GAGGAGTTGGGGGAGCACATT-3'

Complementary ss-DNA (S_2): 5'-AATGTGCTCCCCCAACTCCTC -3'

All oligonucleotides were dissolved in 0.1 mol L^{-1} phosphate buffer solutions (prepared using KH_2PO_4 and K_2HPO_4, PBS, pH 5.0) and were kept in refrigerator at 4°C. All other chemicals were of analytical reagent grade and double distilled water (DDW) was used throughout.

2.2. Apparatus and instrumentations

CVs and DPVs were performed with an EN50081-2 electrochemical workstation (Declaration of the company, Netherlands). A three-electrode system was employed with Pt wire as auxiliary electrode, Ag/AgCl (saturated KCl) as reference electrode, and Au or modified Au electrode as the working electrodes (Azar electrode-Iran). Electrochemical impedance spectroscopy (EIS) measurements were performed on an Autolab 302 electrochemical workstation.

2.3. Biosensor fabrication

Initially, the Au electrode was polished to a mirror-like surface with 10-50 μm alumina slurry and was washed with ethanol and water. Then, Au electrode thoroughly was rinsed with Piranha solution (7:3 mixtures of concentrated sulfuric acid and 30% hydrogen peroxide). Then, the Au electrode was electrochemically cleaned using several potential cycling between -0.2 - 1.6 V versus Ag/AgCl in 0.5 mol L^{-1} H_2SO_4. For assembly of MB, the cleaned Au electrode was immersed into 1 mmol L^{-1} MB solution for 12 h at room temperature, where the assembly of MB monolayer was performed via sulfur–Au reaction. Then, the obtained electrode was completely cleaned with DDW to remove unassembled thiol component and was denoted as (Au/MB). Following MB self-assembling, the sensor surface was washed with DDW and was subsequently back-filled by spotting 50 μL of a 0.01 mol L^{-1} aqueous solution of MCH onto the electrode surface and let to incubate for 30 min. This step was performed in order to improve the organization of the sensing surface by reorienting the MB for optimal and efficient operation by removing those non-specifically physisorbed onto the electrode surface. The sensor was then thoroughly washed by DDW, leaving an organized mixed SAM of chemisorbed MB and MCH. Then the Au/MB electrode was incubated at 10^{-7} mol L^{-1} S_1 for another 2 h to covalently immobilize the 5'-NH_2 modified ss-DNA (S_1), and thus the ss-DNA modified electrode was obtained after washing with PBS, was denoted as Au/MB/S_1. For constructing of the Au/MB/ds-DNA, Au/MB/S_1 was immersed in 0.1 mol L^{-1} PBS (pH 5.0) containing 10^{-7} mol L^{-1} of target S_2 with shaking for 30 min at 37 °C. After hybridization, the obtained electrode was washed with the same PBS solution and DDW to remove nonspecifically bounded DNA and the resulted

biosensor was denoted as Au/MB/ds-DNA. For comparative purpose, Au/ds-DNA was prepared with immersing the cleaned Au electrode in 10^{-7} mol L^{-1} S_1 followed by hybridization with 10^{-7} mol L^{-1} S_2.

2.4. Preparation of real samples

Urine samples were stored in refrigerator immediately after collection. Ten milliliters of the sample was centrifuged for 30 min at 2000 rpm. The supernatant was filtered out using a 0.45 μm filter and then was diluted 5-times with the PBS (pH 5.0). The solution was transferred into the voltammetric cell to be analyzed without any further pretreatment. The standard addition method was used for the determination of morphine in urine real sample.

In order to precipitate proteins in the plasma samples, 1.0 mL of the sample was treated with 20 mL perchloric acid ($HClO_4$, 20% v/v). Then, the mixture was vortexed for a further 30 s and then was centrifuged at 1000 rpm for 20 min. The solution was diluted five times with the PBS (pH 5.0) and was transferred into a voltammetric cell. Once more, the standard addition method was applied for the determination of morphine contents.

2.5. Electrochemical measurements

Twenty milliliters of 0.1 mol L^{-1} PBS buffer solution (pH 5.0) containing a specific amount of standard solution of morphine was added to an electrochemical cell. CV measurements were recorded in the potential range of 0.0 - 0.8 V at a scan rate of 100 mV/S. DPV was employed with the following parameters: $E_{initial}$ = 0.2 V, E_{final} = 0.7 V, scan rate = 50 mV/S, pulse amplitude = 50 mV, and pulse width 50 mS. The EIS measurements were performed in 5.0 mmol

L^{-1} $[Fe(CN)_6]^{3-/4-}$ containing 0.10 mol L^{-1} KNO_3 with the frequency range from 10^5 to 0.1 Hz at the formal potential of the system, $E^o = 0.182$ V.

3. Result and discussion

3.1. Optimization of DNA biosensor conditions

The effect of ds-DNA concentration based on morphine oxidation signal was explored in different ds-DNA concentrations from 10^{-9} to 10^{-5} mol L^{-1} (Fig. 1A) by DPV in 0.1 mol L^{-1} phosphate buffer solution (pH 5.0) containing 50 μmol L^{-1} of morphine at the scan rate of 50 mV/S. The morphine oxidation signal increased up with increasing in the DNA concentration to 10^{-7} mol L^{-1}. After this concentration value, the magnitude of the morphine signal leveled off. Thus, the optimum immobilization concentration for ds-DNA was chosen as 10^{-7} mol L^{-1} in order to obtain the full surface coverage of MB modified Au electrode.

Proton is always involved in the electrochemical reaction of organic compounds and exerts significant impact on the reaction speed. Therefore, the effect of varying pH (3.0-8.0) on the electrochemical responses of Au/MB/ds-DNA to determine morphine was studied by DPV in a solution containing 50 μmol L^{-1} of morphine at the scan rate of 50 mV/s (Fig. 1B). The maximum anodic peak current for morphine was found at pH 5.0 (Fig. 1B). Decrease in Morphine oxidation signal at lower and higher pHs can be related to the devaluation of electrostatic interaction between ds-DNA and morphine, because protonation of phosphate groups of DNA in lower pHs or diminish the positive charge of morphine at higher pHs. Therefore, pH 5.0 was chosen for the determination of morphine in this work.

Figure 1.

Melting temperature is also a characteristic of a DNA duplex and a function of the duplex length [39]. The melting temperature of a DNA duplex can be defined as temperature at which, under a given set of conditions, 50 % of the double-stranded DNA (ds-DNA) is denatured to single-stranded DNA (ss-DNA). The melting temperature for the Au/MB/S_1 that was hybridized with the complementary DNA, was studied using previously reported procedure [40]. The melting temperature (T_m) for the fully complementary duplex was obtained 42 ºC, which indicated good stability of the biosensor at room temperature.

3.2. Electrochemical characterization of modified electrode

The biosensor fabrication process could be monitored based on the electron transfer of ferricyanide through the modified electrodes. Fig. 2A shows the cyclic voltammograms obtained for differently modified electrodes in 1 mmol L^{-1} $Fe(CN)_6^{3-/4-}$ of 0.1 mol L^{-1} PBS solution (pH 5.0, 0.1 mol L^{-1} KNO_3) at the scan rate of 50 mV/S [41]. A pair of well defined redox peaks can be observed at the bare gold electrode (curve a). These peaks could be definitely attributed to the redox behaviors of $[Fe(CN)_6]^{3-/4-}$. On the cyclic voltammogram of the Au/MB (curve b), the redox peak current decreased with the increase of the peak-to-peak separation, indicated a quasi-reversible electrochemical redox reactions on the Au/MB and illustrated the MB film has successfully self-assembled on Au electrode. The immobilization of ds-DNA on electrode surface induced a big decrease of the peak current and an increase of the peak to peak potential separation (curve c), indicated that the ds-DNA has been successfully immobilized on the electrode surface and the peak current decrease could be well assigned to the repellence of $[Fe(CN)_6]^{3-/4-}$ by the negatively charged phosphate backbone of DNA.

Electrochemical impedance spectroscopy (EIS) can reflect the impedance changes of the electrode surface during the modification process. Fig. 2B shows the Nyquist plot of the differently modified electrodes in 5 mmol L^{-1} $[Fe(CN)6]^{3-/4-}$ and 0.1 mol L^{-1} KNO_3 with the frequency was varied from 0.1 Hz to 10^5 Hz. The bare Au electrode exhibited a relatively low impedance value and almost a straight line (curve a). The Ret increased to be about 17820 Ω after Au electrode modification with MB (curve b). Impedance value continued to increase to 30500 Ω after ds-DNA immobilization (curve c). It may be attributed to the limited diffusion of $[Fe(CN)6]^{3-/4-}$ toward electrode surface by the negative charged phosphate backbone of ss and ds-DNA. After incubating with 50 μmol L^{-1} morphine, the Ret value further increased to be about 37840 Ω (curve d), which could be well ascribed to the repellence of redox probe from approaching electrode surface by the specific combination of morphine and ds-DNA.

Figure 2.

3.3. Voltammetric behavior of morphine on the surface of the modified electrode

To elucidate the electrode reaction of morphine, cyclic voltammetric technique was used. The cyclic voltammograms were recorded in 0.1 mol L^{-1} PBS solution with pH 5.0 as supporting electrolyte in the absence and presence of 50 μmol L^{-1} of morphine at modified electrodes and were shown in Fig. 3. In our experiments, no redox peak was observed in the absence of morphine at the surface of both modified and unmodified electrodes (Fig. 3a). A weak and broad anodic peak (Fig. 3b) at Au electrode in the presence of 50 μmol L^{-1} morphine indicated that morphine was electroactive at the Au electrode in this electrolyte solution, but this oxidation peak has less sensitivity for drug monitoring, while the Au/MB/ds DNA electrode, exhibited a

dramatic increase of current (Fig. 3c). This behavior can be attributed to the presence of ds-DNA that interacted greatly with morphine and hence more morphine can be introduced onto modified electrode surface and its oxidation peak can be observed.

Figure 3.

On the other hand, as shown in Fig. 4A, the peak currents (i_p) at the Au/MB/ds-DNA electrode in 50 μmol L^{-1} morphine solution (pH 5.0) varied with change of scan rate (□). In the range of 20-175 mV/s, the relations obey the following equation ($R^2 = 0.996$, where □ is in mV/s and Ip is in μA),

Log i_p =−0.444 + 0.820 log □

These results indicated that the electrode process was controlled simultaneously by both diffusion and adsorption [42].

Figure 4.

Cyclic voltammetry was also carried out to characterize the effects of solution pH on oxidation peak potential of morphine at the Au/MB/ds-DNA. The oxidation peak potential of morphine shifted negatively with the increase of solution pH, indicated that protons take part in the electrode reaction process. The anodic peak potential of morphine was proportional with the solution pH in the range of 4.5 –7.5.

A good linear relationship between the peak potential (E) and the solution pH was also established (data not shown). The linear regression equation was gotten as E (mV) = −62.6 pH + k (n = 4, $R^2 = 0.998$). According to the Nernstian slope (−59.0x/n), where x is the hydrogen ion

participating the electrode reaction and n is the number of electron transferred ≈ −62.6, the loss of electrons was accompanied by the loss of an equal amount of protons and x = n = 1[43].

According to the electron-transfer number n obtained in literature [39], one can conclude that the electrode reaction of morphine on Au/MB/ds-DNA is a two-electron and two-proton process. The proposed reaction mechanism is as scheme 1:

Scheme 1.

3.4. Effect of DNA

DPVs of 100 μmol L^{-1} morphine (Fig. 5) at the bare Au, Au/ds-DNA, Au/MB/ss-DNA, and Au/MB/ds-DNA were displayed. As can be seen, a weak peak was found for morphine at bare Au electrode (Fig. 5a). A dramatically increase in the peak currents with a little positive shift in potential for morphine oxidation signal were observed for Au/MB/ss-DNA (Fig. 5c) and Au/MB/ds-DNA (Fig. 5d), respectively. These results indicated formation of a new association interaction between DNA and morphine. According to the previous research [44] intercalative binding of small molecules with DNA shifted potential to more positive value, while electrostatic binding displaced potential to negative potential value. Ensafi et al [45] reported the intercalative interaction between DNA and morphine with positive shift in DPV current at pH 7.0. In our study, all morphine and DNA solutions were prepared in pH 5.0. In this pH, all morphine molecules were in their cationic form and could interact with negative charge of DNA backbone electrostatically. Therefore, both intercalative and electrostatic interaction could be possible between Morphine and DNA. A little positive shift in peak potential of Morphine oxidation in DPV responses indicated that the intercalative interaction between morphine and ds-DNA, overcame the electrostatic interaction. Furthermore, the oxidation peak current of morphine,

obtained with the Au/MB/ds-DNA (Fig. 5d) was higher than Au/MB/ss-DNA (Fig. 5c) that can be assigned to more intercalative and electrostatic interaction of ds-DNA with morphine.

Compared with the signal obtained with the Au/ds-DNA (Fig. 5b), the Au/MB/ds-DNA (Fig. 5d) produced an obvious increase in signal. This may be due to mercaptobenzaldehyde effect in increasing the immobilization of DNA onto electrode surface because of covalent interaction between MB and ds-DNA that increases the signal of morphine biosensor.

Figure 5.

3.5. Differential pulse voltammetry detection of morphine at Au/MB/ds-DNA

The calibration curve for morphine was characterized by differential pulse voltammetry. The differential pulse voltammograms obtained for a series of morphine solutions with various concentrations were illustrated in Fig. 6A. The calibration graph of the peak current versus concentration was constructed using data from these measurements and the least squares were evaluated using the linear regression method. The results of the measurements were summarized in Fig. 6B. As can be seen from Fig. 6B, there is a linear relationship in the plot of I versus concentration between 0.05 and 500 μmol L^{-1}. That relationship can be described with the following linear regression equation in the mentioned concentration range:

$I\ (\mu A) = 0.059\ C + 7.750,$

where C is concentration of morphine in μmol L^{-1}, with a correlation coefficient of 0.998. The calculated limit of the detection value, LOD = 0.01 μmol L^{-1} with RSD=3.1% was obtained with the S/N = 3. The comparison of proposed method with other electrochemical methods for morphine determination was listed in Table 1 [44, 45, 47-50]. It can be seen that this method provided a broader linear range and lower detection limit for morphine detection than most of

methods listed in Table 1. So the Au/MB/ds-DNA electrode was proved to be an efficient platform for the electrochemical sensor.

Figure 6.

Table 1.

3.6. Selectivity of the biosensor

In order to evaluate the selectivity of the proposed biosensor for the determination of morphine, under optimized experimental conditions described above, the effects of some foreign species on the determination of morphine at 50 μmol L^{-1} were evaluated in detail. Usually there are a number of inorganic ions in urine samples, such as Na^{+}, Mg^{2+}, Ca^{2+}, Cl^{-} and SO_4^{2-}, so these inorganic ions and some other potentially interference compounds were tested to check their levels of interference in the determination of morphine. For this purpose, the tolerance limit was taken as the maximum concentration of the foreign substances, which caused an approximately ±5% relative error in the determination. After the experiments, the results revealed that neither 500-fold of glucose, sucrose, lactose, and fructose, nor 300-fold of Na^{+}, Li^{+}, Ca^{2+}, Mg^{2+}, Al^{3+}, NH_4^{+}, SO_4^{2-}, Cl^{-} and ClO_4^{-}, nor 5-fold of cysteine, codeine, acetaminophen, and diclofenac interfered with the determination of morphine. These results confirmed the suitable selectivity of the proposed biosensor for determination of morphine.

3.7. Stability, reproducibility, and repeatability of Au/MB/ds-DNA

Stability of the Au/MB/ds-DNA was tested by keeping the electrode in phosphate buffer solution (pH 5.0) for 10 days and then recording the DPVs for 100 μmol L^{-1} of morphine and comparing

them with the DPVs obtained in the same solution before immersion. The results indicated that peak current decreased only slightly for the Au/MB/ds-DNA, indicated that the Au/MB/ds-DNA had good stability (data not shown). Five Au/MB/ds-DNA examples fabricated independently were used to determine 100 μmol L^{-1} of morphine, and the RSD was 4.1%, revealed good reproducibility of the electrode. To ascertain the repeatability of the electrode, 10 measurements of morphine were carried out using the Au/MB/ds-DNA at intervals of 1 h. The RSD was found to be 3.6%, indicated that the Au/MB/ds-DNA had also excellent repeatability.

3.8. Analytical application

In order to investigate the accuracy of the method, urine and blood plasma samples were analyzed to determine their morphine contents. In this regard, the samples were prepared as described in the sample preparation section. For blood plasma and urine samples, the concentrations of morphine in the samples were determined. Then, known concentrations of morphine were added into the same sample volume before their being centrifuged. After sample preparation, the Morphine in the spiked samples was measured simultaneously. The data obtained with the proposed biosensor for spiked morphine in urine and blood plasma samples were presented in Table 2. The results showed that the determination of morphine was satisfactorily accurate.

Table 2.

4. Conclusions

CV and DPV techniques studied the electrochemical behavior of morphine at the surface of Au/MB/ds-DNA. The results revealed that the morphine interacted with ds-DNA with both electrostatic and intercalation modes in (pH 5.0). Under the optimized conditions, the anodic peak currents were linear to morphine concentrations in the range of 0.05-500 μmol L^{-1}, and a detection limit about 0.01 μmol L^{-1} was obtained. This electrode presents advantages of easy fabrication, excellent stability and sensitivity. The results obtained are promising and demonstrate the utility of the developed method for the determination of morphine content in urine and blood plasma samples without additional steps of extraction, clean-up, and derivatization.

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Figures legend:

Figure 1. DPVs of 50 μmol L^{-1} morphine oxidation signals (n = 3) measured using (A) various concentration of ds-DNA immobilized onto the surface of MB modified Au electrode. (B) Au/MB/ds-DNA at different pHs (3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0) at a scan rate of 50 mV/S.

Figure 2. (A) Cyclic voltammograms of the bare Au (a), Au/MB (b), and Au/MB/ds-DNA (c) in 1 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol L^{-1} KNO_3 at the scan rate of 50 mV/S. (B) Electrochemical impedance spectrosgrams of the bare Au (a), Au/MB (b), Au/MB/ds-DNA (c) and Au/MB/ds-DNA/morphine (d) in 5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol L^{-1} KNO_3 with the frequency varied from 0.1 Hz to 10^5 Hz.

Figure 3. Electrochemical responses of bare and MB/ds-DNA modified Au electrodes at absence of morphine (a), Au at presence of 50 μmol L^{-1} morphine (b), and Au/MB/ds-DNA at presence of 50 μmol L^{-1} morphine (c) in 0.1 mol L^{-1} PBS solution, scan rate 100 mV/S.

Figure 4. (A) Cyclic voltammograms of 50 μmol L^{-1} Morphine in 0.1 mol L^{-1} PBS (pH 5.0) at Au/MB/ds-DNA at various scan rates: 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, and 175 mV/s from inner to outer, respectively. (B) Plot of log Ip vs. log □.

Figure 5. DPV for the bare Au (a), Au/ds-DNA (b), Au/MB/ss-DNA (c) and Au/MB/ds-DNA (d) in PBS (pH 5.0) with 50 μmol L^{-1} morphine. The concentration of all DNAs immobilized onto electrodes surface was 10^{-7} mol L^{-1}.

Figure 6. (A) Differential pulse voltammograms for morphine at a Au/MB/ds-DNA electrode in the presence of various concentrations of morphine. The numbers 1–12 correspond to 0.05, 0.5, 1, 10, 25, 50, 75, 100, 150, 200, 300, and 500 μmol L^{-1} of the morphine. (B) Calibration curve for morphine at an Au/MB/ds-DNA electrode.

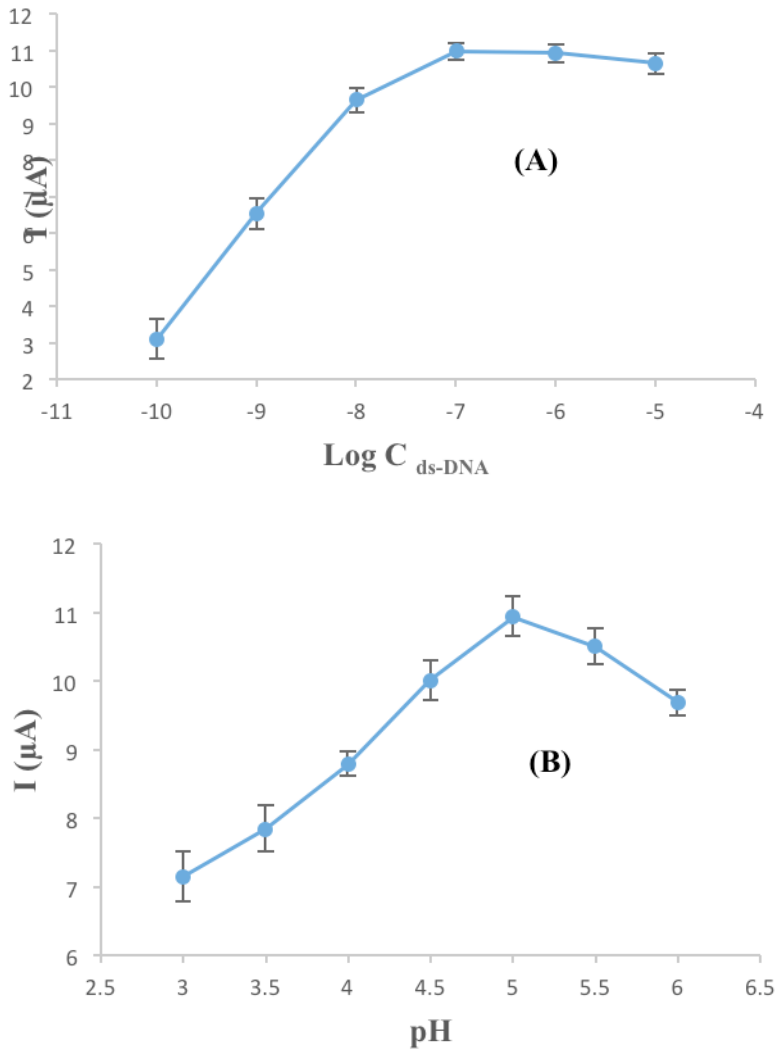


Figure 1.

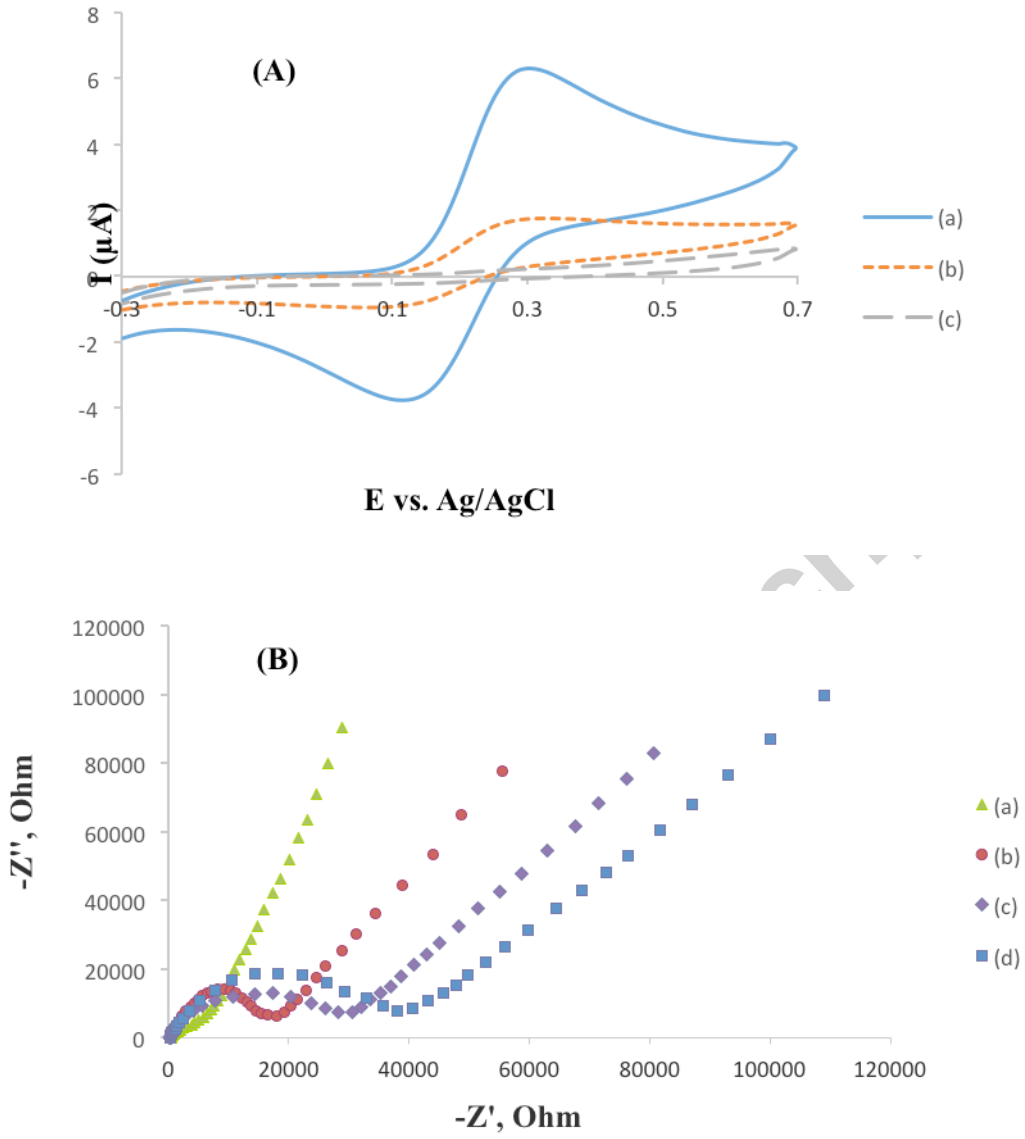


Figure 2.

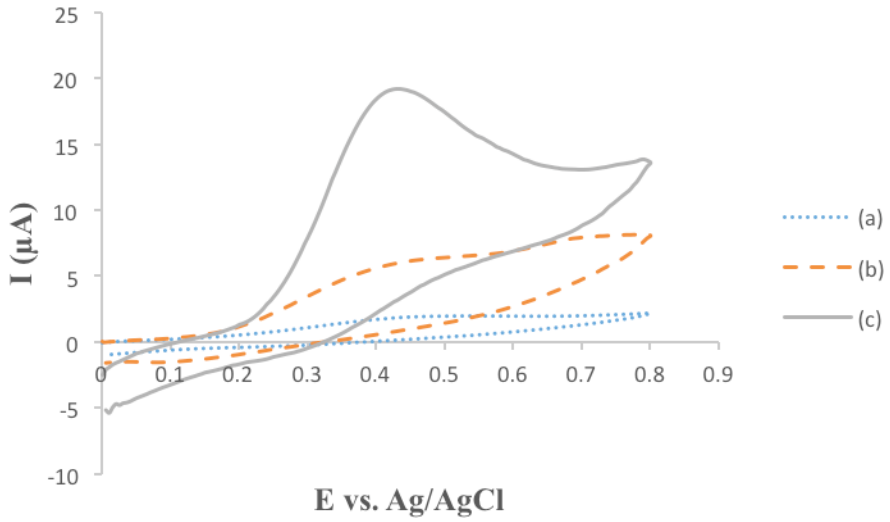


Figure 3.

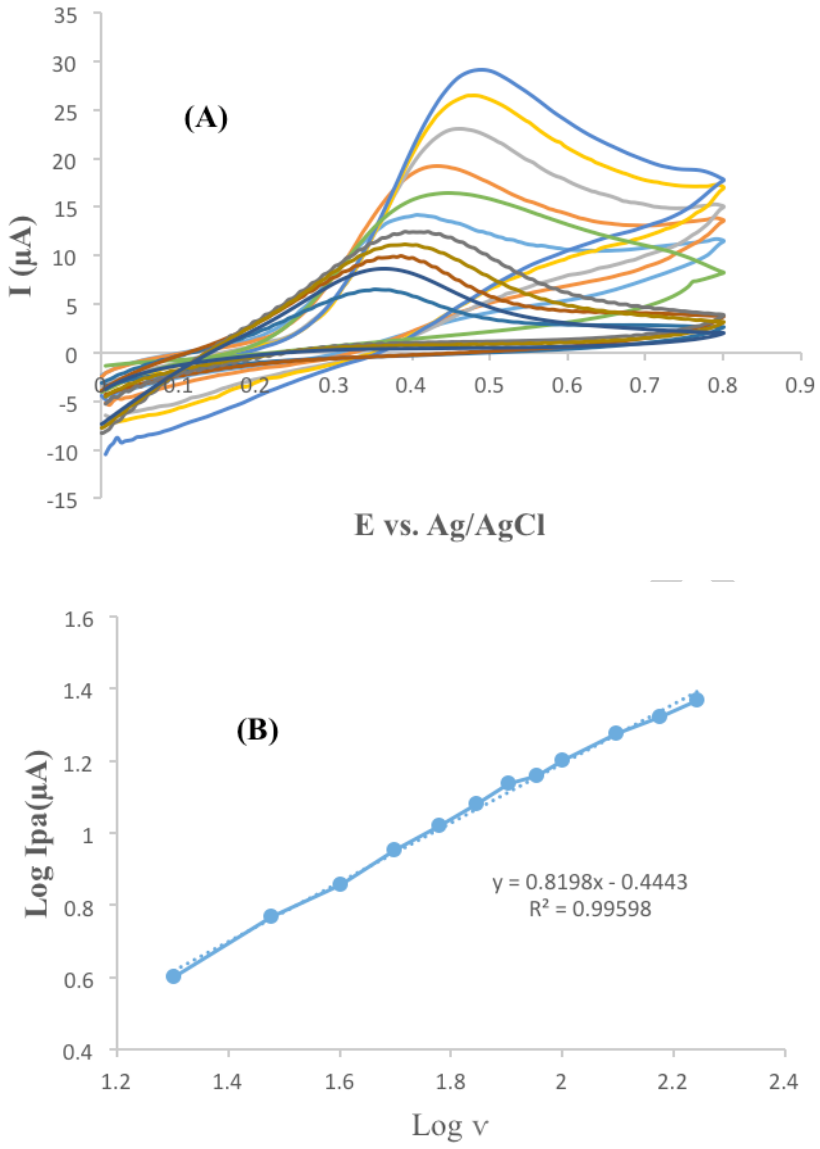


Figure 4.

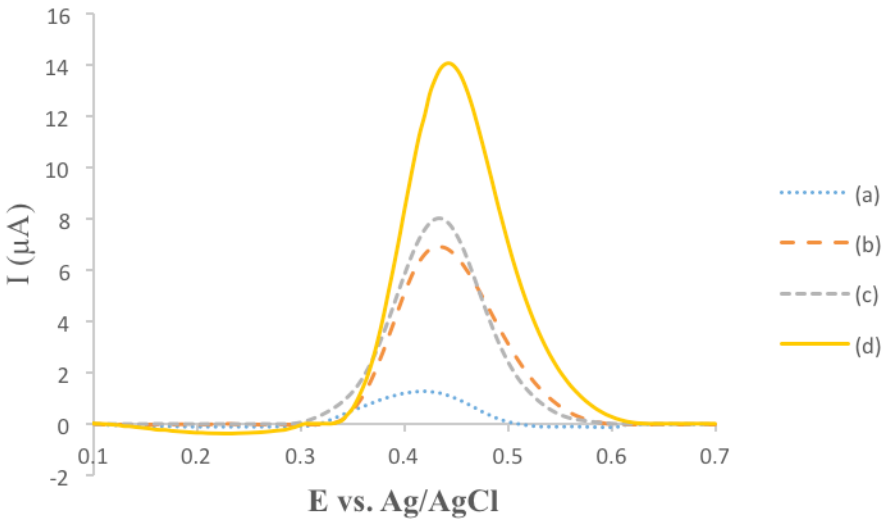


Figure 5.

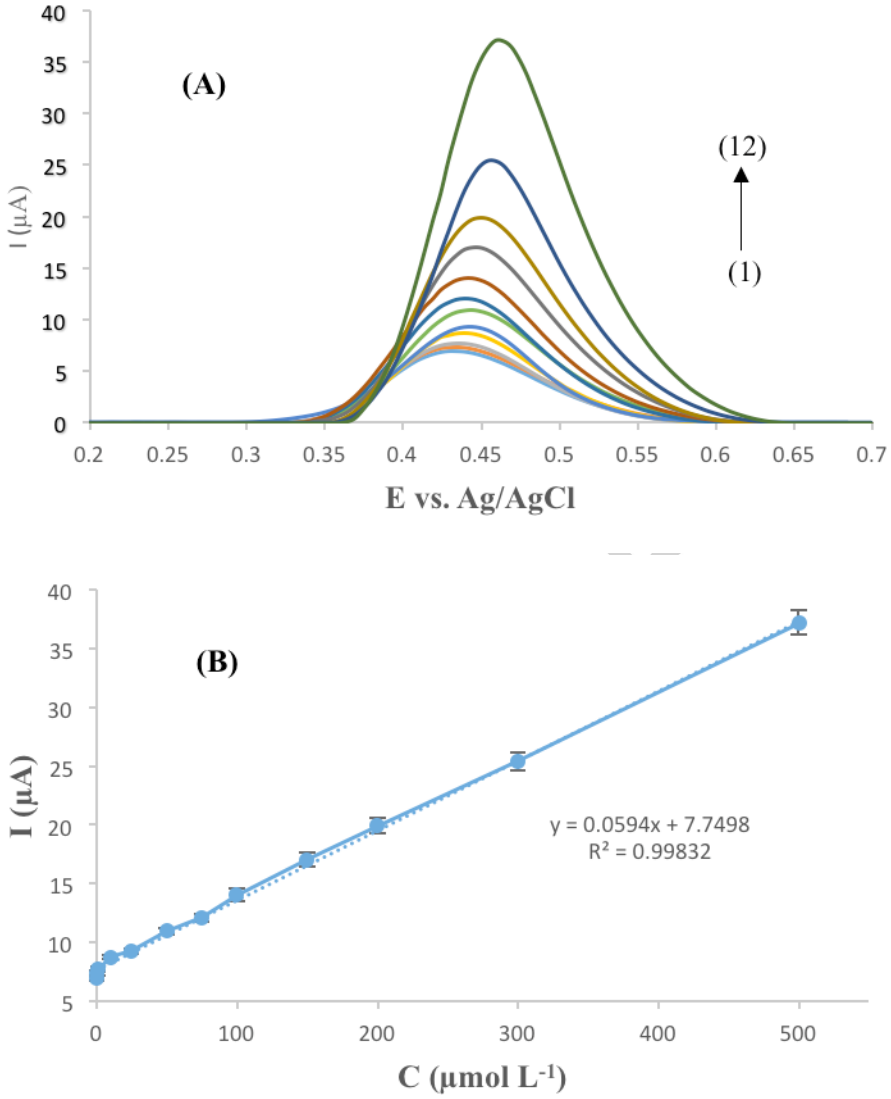
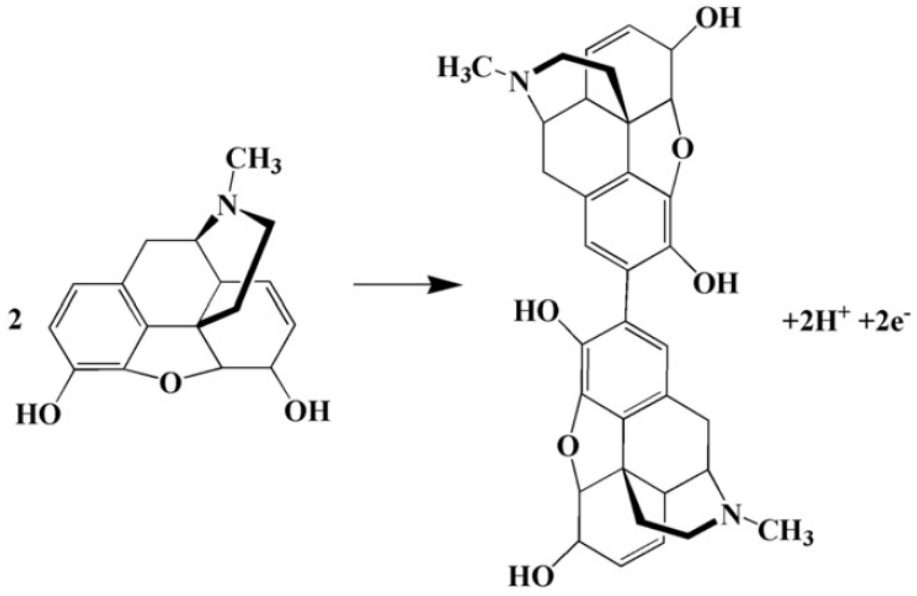


Figure 6.



Scheme 1. The mechanism for electrooxidation of morphine at a surface of modified electrode.

Table 1 Comparison of the analytical parameters with some previously reported morphine sensors.

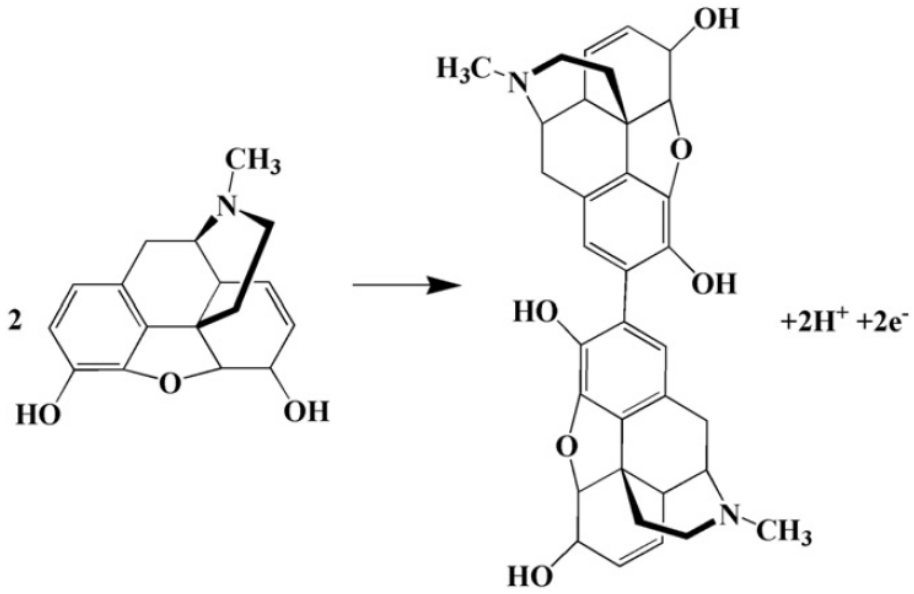
Ref no	Modification method	Detection method	Linear range ($\mu mol\ L^{-1}$)	Detection limit ($\mu mol\ L^{-1}$)
47	Prussian blue film modified-palladized aluminum electrode	hydrodynamic amperometry	2-50	0.8
44	ZnO/CNT nanocomposite room temperature ionic liquid modified carbon paste electrode	square wave voltammetry	0.1-700	0.06
45	NiO/CNTs ionic liquid carbon paste electrode	square wave voltammetry	0.05–520	0.01
48	Gold Nanoparticles–Ferrocene Modified Carbon Paste Electrode	differential pulse voltammetry	1-1800	0.003
49	Multiwall carbon nanotubes paste electrode	square wave voltammetry	0.2-250	0.09
50	N-hexyl-3-methylimidazolium hexafluoro phosphate/multiwall carbon nanotubes paste electrode	differential pulse voltammetry	0.6–600	0.02
This work	Mercaptobenzaldehyde and ds-DNA modified Au electrode	differential pulse voltammetry	0.05-500	0.01

Table 2 Analytical results of spiked morphine to urine and blood plasma samples[a].

Sample	Added (μmol L^{-1})	Found (μmol L^{-1})	Recovery (%)
Urine	-	-	-
	50.0	48.5 (±0.1)	97.0
	100.0	104.9 (±0.2)	104.9
Blood plasma	-	-	-
	50.0	51.6 (±0.2)	103.2
	100.0	104.8 (±0.2)	105.8

[a]Values in parentheses are RSDs based on three replicates.

Graphical abstract



The mechanism for electrooxidation of Morphine at a surface of ds-DNA modified electrode.

Highlights

- A novel electrochemical biosensor for determination of Morphine was reported.
- Different interaction of Morphine with double-stranded DNA was engaged for designing a sensitive biosensor.
- A linear dependence of the Morphine oxidation signals was observed in the range of 0.05-500 μmol L^{-1}.