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A powerful DNA-based voltammetric biosensor modified with Au nanoparticles, for the determination of Temodal; an electrochemical and docking investigation

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Abstract

Temodal is an anticancer drug with many side effects such as loss of appetite and fatigue. Given that the determination of Temodal in patients treated with is a necessity, this research was focused on developing a DNA-based sensor for the determination of Temodal. For this purpose, the pencil graphite electrode was modified with Au nanoparticle and ds-DNA (ds-DNA/Au-NPs/PGE). The resulting electrode (ds-DNA/Au-NPs/PGE) was found to produce a linear response to concentration Temodal in the range of 5.0 nM - 45.0 μ M with a detection limit of 1.0 nM. The response was attributed to the intercalation of Temodal with guanine species of the DNA species. The presence of Au nanoparticle at the surface of the modified PGE increases the active surface area to over 5.1-times and hence helps improve the detection limit of the voltammetric analysis. The factors affecting the performance of the biosensor (e.g. DNA concentration, ds-DNA/Temodal interaction time, and temperature) were optimized. A docking investigation was further used to evaluate the intercalation between guanine base with Temodal and the minor groove interaction of guanine bases of the ds-DNA with Temodal. The theoretical investigation confirmed that Temodal is stabilized by the intercalating site of DNA through π - π interactions and two hydrogen bonds with nucleotides and the binding energy was determined to be -5.75 Kcal/mol.

Keywords: Temodal, ds-DNA, intercalation, Au nanoparticle, docking investigation, Pencil graphite electrode

1. Introduction

Temozolomide, commonly known under the brand name Temodal, is a beneficial chemotherapy drug used in the treatment of brain cancer. The drug Temodal acts through stopping the synthesis of DNA, and hence preventing the growth, of cancer cells [1]. The use of Temodal as chemotherapy drug can lead to various side effects as hair thinning, loss of appetite, fatigue, itchy rashes and stopping of menstrual cycles. Temodal has showed higher chemotherapeutic activity after in vitro hydrolysis to 5-(3-methyltriazene-1-yl) imidazole-4-carboxamide (MTIC) in physiologic pH [2]. The selective trace analysis of chemotherapy drugs, especially Temodal, could create the possibility to better control of the treatment process [3]. Considering these, analytical sensors can be evaluate as an auxiliary device for such analysis in chemotherapy patients.

Rather recently, electrochemical biosensors have been introduced as fast, sensitive, powerful, selective and portable analytical tools for the determination of proteins, drugs, amino acids and other biological compounds in blood, tablets, and urine samples [4-12]. The modification of electrochemical biosensors with nanomaterials has been also found to improve the ability of these devices in the trace analysis of compounds in biological samples [13-18]. Accordingly, DNA-based biosensors have attracted more attention due their good time stability and ease of preparation [19-21]. As an example, the application of pencil graphite electrodes modified with ds-DNA, MWCNTs/COOH and polypyrrole as biosensors for the determination of 6-Mercaptopurine anticancer drug has been introduced by our research group [4]. Li et al.,

reported a DNA biosensor for the determination of mitoxantrone through electrochemical and spectroscopic methods [22].

On the other hand, docking studies constitute a powerful theoretical tool for studying the interactions of biological compounds such as DNA [23]. Coupling of docking and electrochemical investigations can serve as very useful complementary tools for a better understanding of the phenomena occurring in these measurement processes [23].

In this study, and in line with our previous works on the fabrication of DNA-based biosensors [4, 19, 23-30], our team tried to fabricate a new electrochemical biosensor based on the interactions of DNA guanine of calf thymus and Temodal as an analytical factor. The fabricated sensor was modified with Au-NPs and was used for nano-molar determination of Temodal. The intercalations between the guanine base of ds-DNA and Temodal were further evaluated by docking. The resulting electrode (ds-DNA/Au-NPs/PGE) showed good performance in the analysis of Temodal in some real samples including pharmaceutical capsules, urine and blood serum specimens.

2. Experimental

2.1. Materials and instruments

Ds-DNA from calf thymus, gold(III) chloride trihydrate, Temodal, acetic acid, Tris-HCl and potassium hexacyanoferrate(III) were purchased for Sigma-Aldrich. A 0.001 M stock solution of Temodal was prepared by dissolving of 0.005 g of Temodal in 25 mL ethanol/hot water (40 °C) solution.

Experiments were done utilizing potentiostat/galvanostat (Autolab). The EIS spectroscopy was done utilizing potentiostat/galvanostat (PGSTAT 302N) and NOVA software. The

Ag/AgCl/KCl_{sat} was used as reference electrode in all of the voltammetric investigations. The canonical DNA (PDB code: 1BNA) with program AutoDock 4.2 (ADT) [31] was selected for docking procedure and more detail for theoretical investigation described in supplementary information data section S1.

2.2. Fabrication of ds-DNA/Au-NPs/PGE

The ds-DNA/Au-NPs/PGE was fabricated in two steps. In the first step, a PGE (HB; d=0.5 mm and L=30 mm; cleaned in solution ethanol/water 1:1 for 2 min) was immersed into a 6.0 mM gold (III) chloride trihydrate solution in 0.1 M KNO₃. The Au nanoparticles were synthesized through an electrodeposition approach at -0.4 V for 7.0 min.

In the second step, the Au-NPs/PGE assembly was immersed into in 10 mL of a solution containing; 0.5 M acetate buffer (pH = 4.8) + 0.02 M KNO₃ + 30.0 mg/mL ds-DNA, where ds-DNA was electrodeposited on its surface of electrode by applying a potential of +0.50 V for 8.0 min. The ds-DNA/Au-NPs/PGE was then removed from the preparation solution, and washed with the distilled water for 30s to remove of unbounded ds-DNA.

2.3. Real sample analysis

Five capsules Temodal capsules (SP Europe, rue de Stalle 73, B-1180 Bruxelles, Belgium) were opened and their contents were mixed, before being dissolved in a hot ethanol/water solution and used as a real sample.

The urine samples were collected from a healthy man (the corresponding author) and was centrifuged for 5 min at 5000 rpm. After filtration with whatman membrane filters (0.2 µm), the sample was diluted 5 folds using a Tris-HCl buffer (pH=7.4) and then subjected to the analyses.

3. Results and discussion

3.1. *ds-DNA/Au-NPs/PGE surface characterization*

The modification process of the PGE was evaluated by atomic force microscopy (AFM) and electrochemical impedance spectroscopy (EIS). The PGE proved to have a flat surface with a low active surface area (figure 1A (a)). After the modification of the PGE with Au nanoparticle, however, much raggedness was created at the PGE surface which can naturally increase the surface area of electrode (figure 1A (b)). **“Here Figure 1”**

The electrical conductivity of PGE, before and after modification with Au nanoparticle, was characterized by EIS (DC=0.35 V and frequency range of 10 kHz to 1 Hz) and the results are presented in Figure 1 B. Moving from PGE (curve a) to Au-NPs/PGE (curve b), R_{ct} values were reduced confirming the good electrical conductivity of the Au nanoparticle as the modifier. After the deposition of ds-DNA (curve c), R_{ct} increased from 55.3 K Ω to 338.1 K Ω , confirming that the non-conductive ds-DNA was deposited on the surface Au-NPs/PGE.

The active surface area of Au-NPs/PGE and PGE were obtained to be 0.018 cm² and 0.092 cm² from the Randles-Sevcik equation using the data from a 1.0 mM [Fe(CN)₆]^{3-,4-} solution in 0.1 M KCl as the electrolyte. The results showed that the presence of Au-NPs increases the active surface area PGE 5.1-times.

3.2. *Interaction investigation*

The differential pulse voltammetric signal (at the respective pulse height and width values of 50 mV and 5 ms) using the ds-DNA/Au-NPs/PGE was recorded in the absence (figure 2 curve a) and presence of 12.0 μ M Temodal (figure 2 curve b). As can be seen in the curve a, ds-DNA/Au-

NPs/PGE produced an oxidation current signal of 7.69 μA at 781 mV corresponding to the oxidation of guanine in ds-DNA. In the presence of 12.0 μM Temodal, the oxidation current was reduced to 4.93 μA and the oxidation potential of the peak increased to 800 mV, which confirms the intercalations between Temodal and guanine base in the ds-DNA structure.

“Here Figure 2”

3.3. Optimization of sensor

The optimization of sensor response is an important part in fabrication of new analytical sensors. The oxidation current of guanine as functions of the ds-DNA concentration in sensor fabrication process is presented in Figure S1 (supplementary information data). As can be observed, the guanine signal of ds-DNA/Au-NPs/PGE was increased with increasing of ds-DNA concentration in sensor fabrication step from 10.0 to 30.0 mg/mL and then stable after 30.0 mg/mL. This results confirm that 30.0 mg/mL can be saturated the electrode surface of Au-NPs/PGE.

The intercalation time between of ds-DNA at surface ds-DNA/Au-NPs/PGE and 12.0 μM Temodal was optimized (figure S2). The figure S2 showed the oxidation signal of ds-DNA at surface of ds-DNA/Au-NPs/PGE in the presence of 12.0 μM Temodal vs. different incubation time. With moving of 1.0 min to 8.0 min in incubation time process, the oxidation signal of guanine reduced and then stable after 8.0 min. This point showed that at 8.0 min of incubation time, the intercalation of Temodal and guanine base at surface of ds-DNA/Au-NPs/PGE completed and this time can be select as optimum condition for the next experiments (Figure S2).

The interaction temperature between Temodal and ds-DNA was optimized in the temperature ranges between 15 $^{\circ}\text{C}$ -35 $^{\circ}\text{C}$. The intercalation between anticancer drug and ds-DNA increased with moving 15 $^{\circ}\text{C}$ towards 25 $^{\circ}\text{C}$ and then reduced (figure S3). The reducing in

intercalation process can be relative to the cleavage of ds-DNA at surface of ds-DNA/Au-NPs/PGE.

The reduction in guanine signal of ds-DNA at a surface of ds-DNA/Au-NPs/PGE in the presence of Temodal was choice as analytical factor for determination of anticancer drug (figure 6 insert). The different between oxidation signal of guanine at a surface of ds-DNA/Au-NPs/PGE in the absence and the presence of Temodal showed a linear relation with drug concentrations in the range of 5.0 nM - 45.0 μ M with detection limit 1.0 nM (figure 3). The ds-DNA/Au-NPs/PGE showed better dynamic range or limit of detection for determination of Temodal compared to another electrochemical sensors and biosensors (table 1) [32-35] **“Here Table 1& Figure 3”**

3.4. Reproducibility and interference tests

The reproducibility of ds-DNA/Au-NPs/PGE was investigated in the presence of 12.0 μ M Temodal. The results showed a R.S.D~3.9% in oxidation current of guanine in the presence of 12.0 μ M Temodal using ten different ds-DNA/Au-NPs/PGE that are an acceptable value for the new analytical sensor.

The selectivity of the ds-DNA/Au-NPs/PGE for determination of Temodal was investigated by recording of DPVs of ds-DNA/Au-NPs/PGE in the solution containing 12.0 μ M Temodal in absence and presence of different potential interferences. The results confirm that 1000-fold Mg^{2+} , Li^+ , Br^- , glucose, and alanine and 400-fold of uric acid have not noticeable interference in determination of Temodal at surface of ds-DNA/Au-NPs/PGE.

3.5. ds-DNA/Au-NPs/PGE ability for real sample analysis

The ability of ds-DNA/Au-NPs/PGE as a sensitive analytical biosensor was checked for determination of Temodal in capsule and urine samples. The recovery data for determination of Temodal are between 98.45-105.2 % that confirms high performance ability of ds-DNA/Au-NPs/PGE for determination of Temodal in real samples (table 2). **“Here table 2”**

3.6. Docking investigation

Experimental docking observations for Temodal (figure 4 A) were followed up with docking studies in two modes; groove interaction (figure 4 B) and intercalation (figure 8 C) studies.

“Here Figure 4”

3.6.1. Minor groove docking study

In order to investigate groove interaction a canonical DNA (PDB code: 1BNA) was selected for positioning docking in the minor groove (figure 5). Blind docking was performed on the DNA duplex. **“Here Figure 5”**

Results obtained shown that hydrogen bond has a main role for interaction of this molecule with DNA and Temodal is accommodated in minor groove through seven hydrogen bonds interaction with nucleotides and the binding energy was -5.48 kcal/mol.

The hydrogen bonds are as followed: Hydrogen (H) 60 of cytosine 9(DAC9) interacts with nitrogen (N) 10 from Temodal (bond length: O-H...N 2.72 Å, bond angle: 72.3 °), H 85 and H 47 of guanine 10(DAG10) interacts with N 14 and oxygen (O) 4 of Temodal (bond length: N-H...N and O-H...H 2.89 Å and 2.85 Å, bond angle: N-H...N and O-H...H 137.6 ° and 148.5 ° respectively). H 39 and H41 of Temodal interacts with O 4' from cytosine 11 (DAC11 bond length: N-H...O 2.63 Å and 2.19 Å; bond angle: N-H...O 69.8°, 43.8° respectively). H 83 from guanine 16 interacts with O 13 of Temodal (DBG16, bond length: N-H...O 2.17 Å, bond angle:

152.6°) and H 81 from guanine 16 interacts with N 10 of Temodal. The docking results revealed foremost interaction between Temodal and DNA when fitted into the minor groove of DNA.

3.6.2. Intercalation docking study

As Temodal known as an intercalator, a preformed intercalation DNA complex (PDB code: 1Z3F), was used for investigating the Temodal-DNA interaction (figure 6). The selection was according to the literature [36]. Docking results revealed that Temodal is stabilized in intercalating site of DNA through one π - π interactions and two hydrogen bonds with nucleotides and the binding energy was -5.75 Kcal/mol. The interactions are as followed: O2P and O5*of guanine 2(DAG2) formed hydrogen bond with H 39 and H 41 Temodal (DAG2, bond length: N-H...O and N-H...O 2.89 Å and 2.09 Å; bond angle: 82.5° and 37.9° respectively). There is a π - π interaction between ring B of Temodal and (DAG2). **“Here Figure 6”**

According to the literature Temodal interact with DNA through intercalation stabilization at DNA intercalation also the intercalation ΔG is less than the minor groove interaction ΔG , therefore intercalation docking studies is more reliable and rational.

4. Conclusion

In the presence research, a pencil graphite electrode amplified with Au nanoparticle was modified with ds-DNA was suggested a new biosensor for determination of Temodal. The intercalation process between guanine and Temodal was selected as analytical factor for determination of anticancer drug in concentration range of 5.0 nM-45.0 μ M with detection limit 1.0 nM. The docking investigation confirm the intercalation process between guanine base at surface of ds-DNA/Au-NPs/PGE and Temodal.

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Figure Captions

Figure 1. A) AFM image of (a) bare PGE and (b) Au-NPs/PGE. B) EIS responses of (a) bare PGE, (b) Au-NPs/PGE, and (c) ds-DNA/Au-NPs/PGE in the presence of 1.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl.

Figure 2. DPVs of ds-DNA/Au-NPs/PGE a) in the absence and b) in the presence of 12.0 μM Temodal.

Figure 3. Relation between net oxidation current of guanine (difference between guanine current in the absence and presence of Temodal) vs. concentration of Temodal. Inset: DPVs of ds-DNA/Au-NPs/PGE in the absence (a) and in the presence of (b) 0.005; (c) 1.0; (d) 6.0; (e) 12.0; (f) 20.0; (g) 25.0; (h) 32.0; (i) 37.0, (j) 40.0 and k) 45.0 μM Temodal.

Figure 4. A) Atomic numbering scheme of Temodal. B) Temodal DNA minor groove interaction and C) Temodal DNA intercalation.

Figure 5. Geometrical disposition of Temodal in DNA minor groove distances in $\text{\AA}$.

Figure 6. Geometrical disposition of Temodal in DNA intercalation distances in $\text{\AA}$.

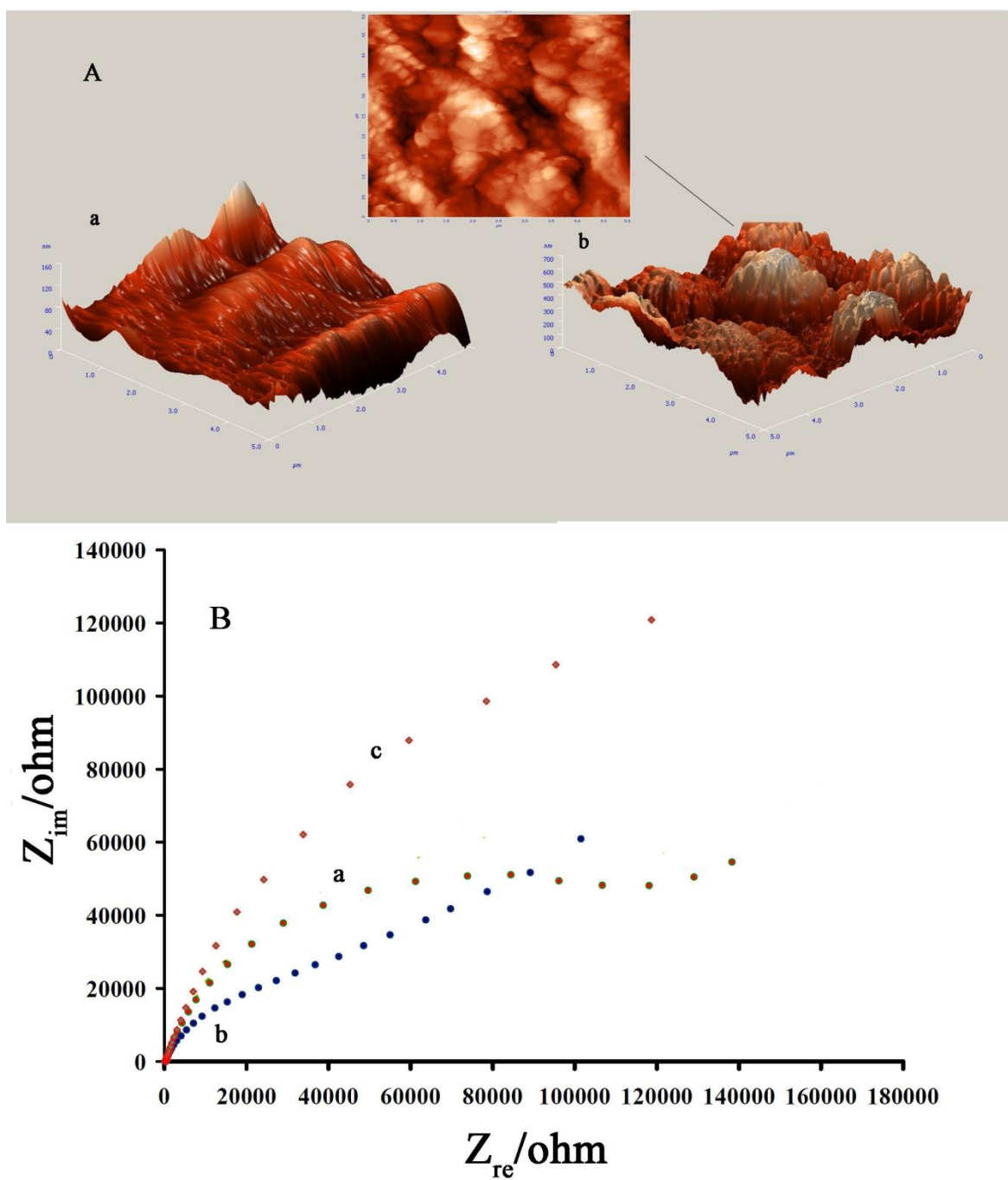
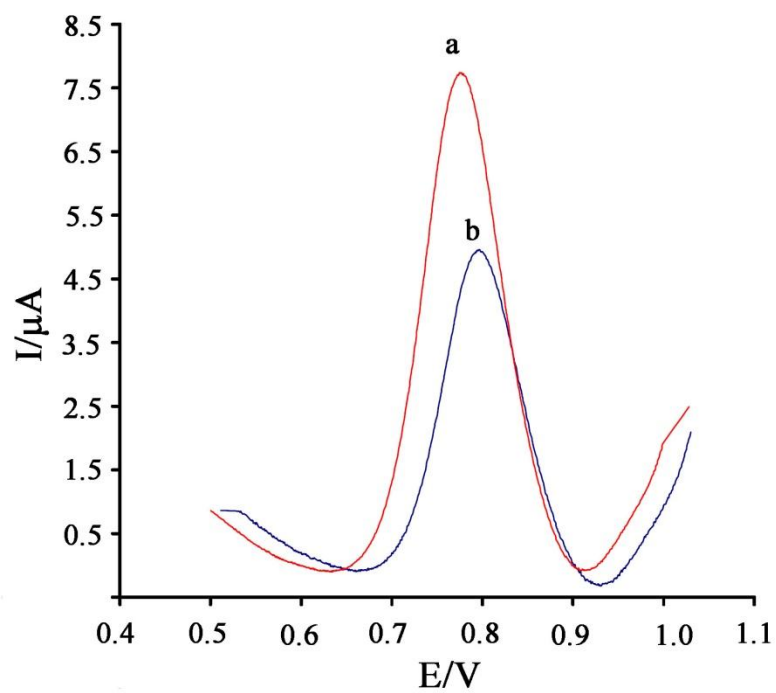


Figure 1

**Figure 2**

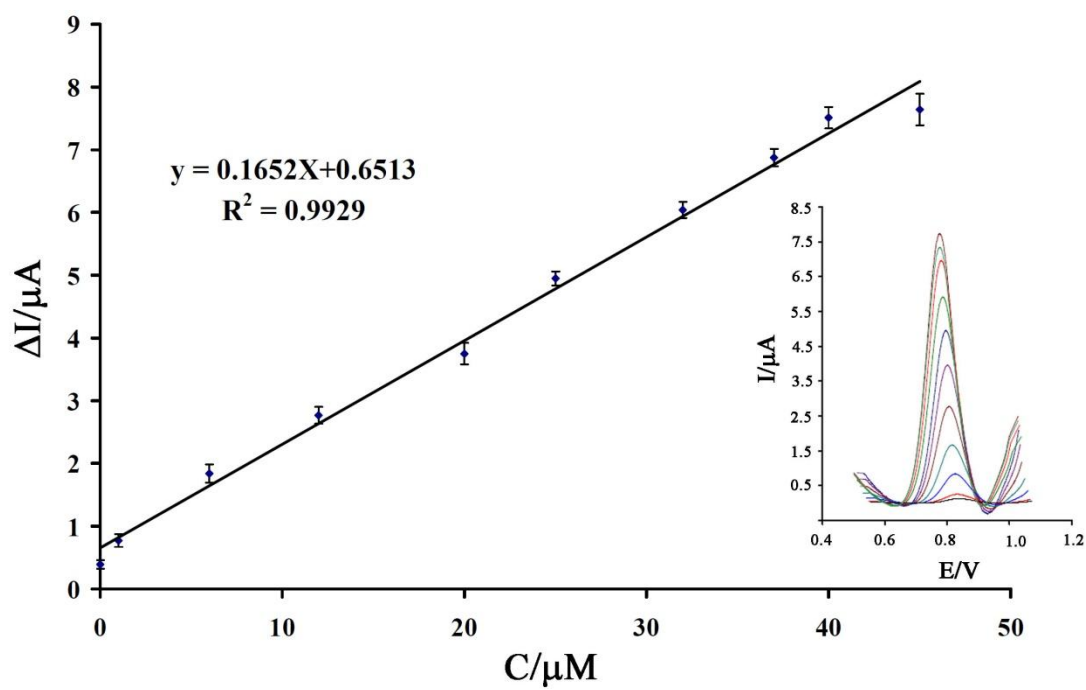
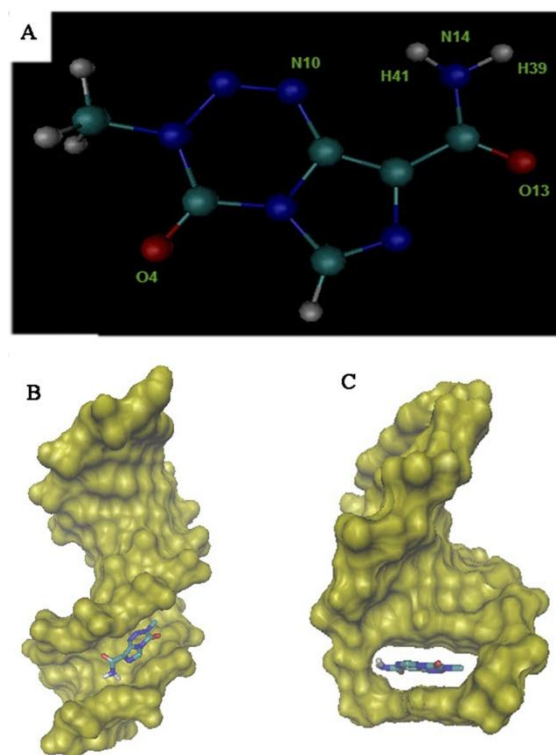


Figure 3

**Figure 4**

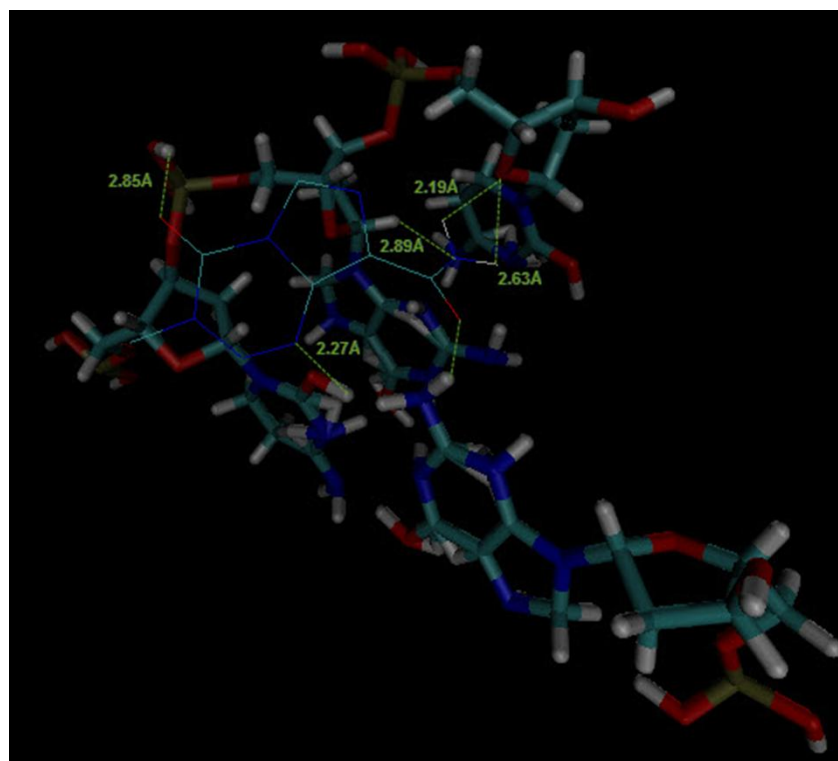


Figure 5

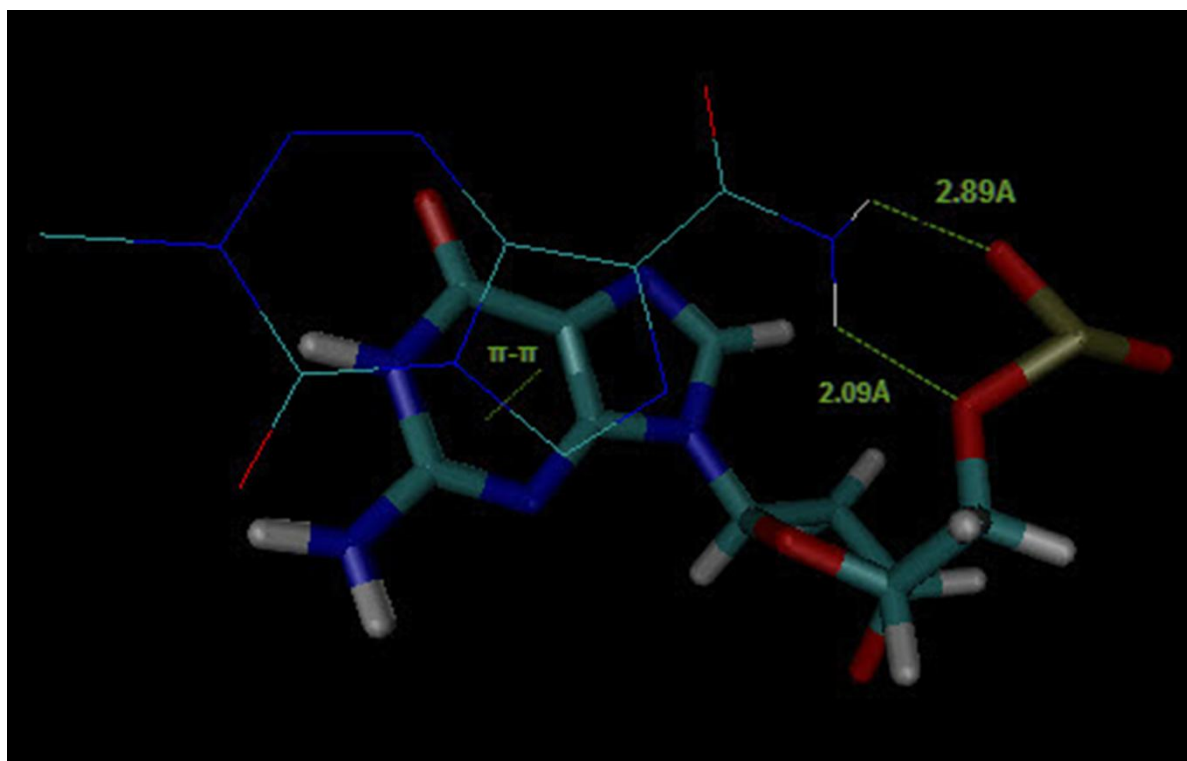


Figure 6

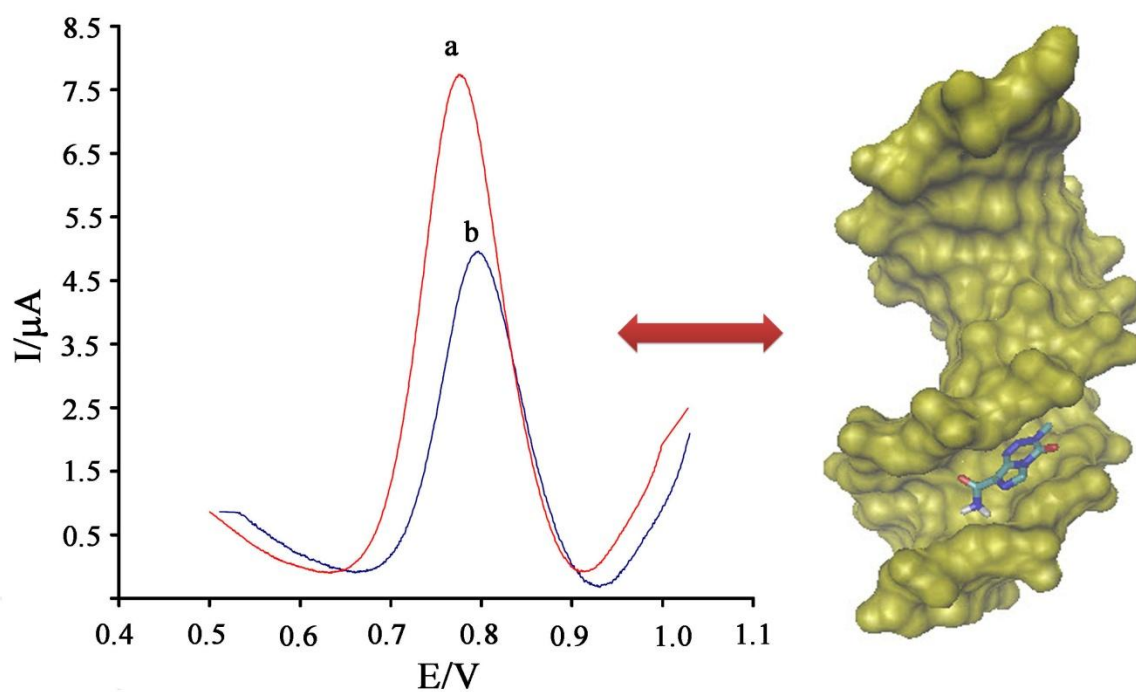
Table 1 Comparison of the efficiency of some electrochemical sensors in the determination of Temodal

Electrode	Modifier	Linear dynamic range (μM)	Limit of detection (μM)	Ref.
Glassy carbon	NiFe ₂ O ₄ /graphene nanoparticles	0.01-9.0	0.0036	[32]
Carbon paste	MIP/MWCNTs	0.01-20.0	0.004	[33]
Glassy carbon	Dowex50wx2 and gold nanoparticles	0.034-42.2	0.012	[34]
Glassy carbon	carbon nanoparticles	10-1000	1.0	[35]
PGE	Au nanoparticle	0.005-45.0	0.001	This work

Table 2 The investigation ability of ds-DNA/Au-NPs/PGE as biosensor for determination of Temodal in real samples

Samples	Added (μM)	Expected (μM)	Founded (μM)	Recovery%
Capsule	---	5.00	4.93 $\pm$ 0.12	98.6
	10.00	15.00	15.57 $\pm$ 0.68	103.8
Urine	---	---	<LOD	---
	15.00	15.00	15.78 $\pm$ 0.97	105.2
Blood Serum	---	---	<LOD	---
	20.00	20.00	19.69 $\pm$ 0.73	98.45

Graphical Abstract



In this study we suggested a highly sensitive DNA based biosensor amplified with Au nanoparticle for determination of temadol in pharmaceutical and biological samples

Research highlights

- ▶ Pencil graphite electrode modified with Au nanoparticle and ds-DNA as new biosensor
- ▶ The determination of temadol anticancer drug in nano-molar level using a new biosensor
- ▶ Confirming of fabricated biosensor ability for temadol analysis by docking investigation