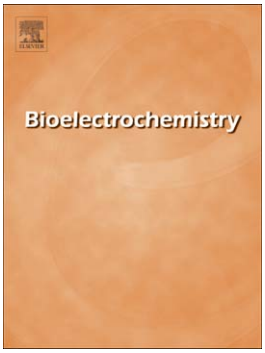

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Impedimetric DNA-biosensor for the study of dopamine induces DNA damage and investigation of inhibitory and repair effects of some antioxidants

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Abstract

A simple and inexpensive methodology was used to develop a new method in order to inspect the DNA damage due to dopamine and some ionic metals. In addition, the inhibitory and repair effects of some antioxidant such as glutathione and ascorbic acid were studied and compared with each other using electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV). In this work a pencil graphite electrode (PGE) was modified with multiwall carbon nanotubes (MWCNTs) and chitosan (CHIT), then it was decorated with a ds-DNA (ds-DNA/CHIT–MWCNTs/PGE). Due to interaction of ds-DNA and the damaging agents (dopamine + metallic ions), electrochemical and spectroscopy properties of ds-DNA at the surface of the modified electrode was changed, and these changes are followed with EIS and DPV methods. Our studied showed that dopamine, Cu(II) and Fe(III) alone could not destroy DNA, but dopamine + Cu(II) and dopamine + Fe(III) can damage DNA. In addition, the ability of dopamine-Cu(II) was greater than dopamine-Fe(III). Moreover, some antioxidant such as glutathione and ascorbic acid can overcome and/or minimize the influence of these damaging interactions.

Keywords: DNA damage; DNA-biosensor; Antioxidants; Electrochemical methods; Dopamine.

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1. Introduction

In recent years scientists have been focused on oxidative damage. In oxidative damage some tissues such as proteins, lipids and DNA are changed and destroyed [1]. These changes lead to aging brain and outbreak of some dangerous and incurable diseases such as cancer [2, 3], diabetes [4], parkinson [5], Down syndrome [6] and many other diseases. However, among the changes resulting from the oxidation of tissues, oxidization of DNA has greater concern because the DNA damage may cause serious disturbances of the cell life, including mutations or malignant transformations. Some compounds and metals ions such as chromium, lead, mercury, cobalt and copper ions have the ability to oxidize tissues. Oxidation of tissues (oxidation stress) leads to produce reactive oxygen species and reactive nitrogen species in vivo and vitro systems in experimental and clinical medicine [7-10].

Reactive oxygen species (ROS) can be beneficial for body and sometimes they are very dangerous [11]. One of the important roles of ROS is in cellular responses to anoxia, for example in protection against infectious agents and in the function of a number of cellular signaling systems. In addition, ROS at low concentration can cause a mutagenic response. High concentration of ROS can damage the structure of cell (including proteins, lipids and DNA) [12]. One particular source of ROS generation has attracted special attention: the path-way, which includes the reduction of metal ions by organic compounds. Immune systems of cells have diverse actions to counteract and diminish the destructive effect of ROS. Antioxidant with low molecular weight (such as ascorbic acid, vitamin E, and glutathione) and antioxidant enzymes (such as thioredoxins, superoxide dismutase (SOD), catalase, and glutathione peroxidase) play this role [13, 14]. According to what was mentioned and the importance of DNA damage study; it is necessary to find a simple and efficient method to evaluate the damaging agents and identification of the types of damage.

Endogenous catecholamines have high DNA damaging potential in the presence of essential trace metal ions such as Cu(II) and Fe(III). As the distribution of catecholamines and metals in cells is strictly regulated, their interactions should be controlled to avoid serious DNA damage under normal conditions. Catecholamines are derived from tyrosine hydroxylation and other structural modifications, and they are functionalized as neurotransmitters such as dopamine, adrenaline and nor-adrenaline [15,16]. Dopamine (3,4-dihydroxyphenethylamine) is a neuroendocrine transmitter in the catecholamine and phenethylamine families that plays important role in the brain and body of human [17, 18]. According to report of Spina and co-workers, production of ROS during the oxidation of dopamine leads to death of dopaminergic neurons in Parkinson disease [19]. Moreover, observation of Yoshie and Ohshima showed that the generated ROS from dopamine in the presence of some compounds can cause ds-DNA breakage [20]. Therefore, dopamine has been considered as contributing factor that generates ROS due to its unstable catechol moiety. Therefore, it is necessary to find a simple and efficient way to inspect the genotoxicity of dopamine and investigation of its mechanism.

To the best of our knowledge, there is not any report about the fabrication of electrochemical biosensors to detect dopamine induced DNA damage. Therefore, designing suitable electrode materials for fabricating electrochemical biosensors in detecting dopamine-induced DNA damage and offer an in vitro model to simulate the pathway of DNA damage and protection in living process is highly necessary.

In this study, a DNA-based biosensor was constructed using layer-by-layer technique. First, chitosan (CHIT) was used as dispersant of multiwall carbon nanotubes (MWCNTs). Then, CHIT–MWCNTs were immobilized on the surface of electrochemically pretreated pencil graphite electrode (PGE), to increase the electron transfer characteristics of the electrode surface. Finally, the ds-DNA polyanions were immobilized at the surface of CHIT–

MWCNTs/PGE. The DNA–modified PGE was used as an electrochemical sensor to evaluate the genotoxicity of dopamine plus Cu(II) and Fe(III). Oxidative DNA damage due to dopamine in the presence of the metal ions was investigated with electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV). In addition, the role of glutathione (GSH) and ascorbic acid, as antioxidants in DNA damage prevention, were assessed.

2. Material and methods

2.1. Reagents

All solutions were prepared using reagent grade chemicals. GSH, ascorbic acid (AA), $CuCl_2$ and $Fe_2(SO_4)_3$ were purchased from Merck (Darmstadt, Germany). Reagent grade Tris–HCl, CH_3COOH, CH_3COONa, EDTA, NaCl, $K_3Fe(CN)_6$, $K_4Fe(CN)_6$, dopamine and NaOH were purchased from Aldrich Chemicals (Milwaukee, USA). Doubly distilled water was used throughout the experiments.

MWCNTs (Fluka) with diameter ranging between 70 and 110 nm were employed in this study.

Chitosan (CHIT) was obtained from Acros Chemical. CHIT solution (0.10%) was prepared by dissolving 0.005 g CHIT in 5.0 mL CH_3COOH (2.0 mol L^{-1}) by the aid of sonication for 30 min. The CHIT solution was stored in refrigeration at 4 $^{\circ}$C when not in use.

Tris–HCl buffer (pH 7.0) contained 10.0 mmol L^{-1} Tris–HCl, 10.0 mmol L^{-1} NaCl and 1.0 mmol L^{-1} EDTA was prepared. Acetate buffer solution (pH 4.8) contained 50.0 mmol L^{-1} acetic acid, 50.0 mmol L^{-1} sodium acetate and 2.0 mmol L^{-1} NaCl was used as a buffer medium.

Salmon sperm ds-DNA was purchased from Sigma (catalog No. D1626). The molecular weight and sequence is not determined by Sigma. The G-C% content of the DNA is reported

as 41.2%. A salmon sperm ds-DNA stock solution (1000 mg L^{-1}) was prepared in Tris-buffer (pH 7.0) and kept in frozen form. More dilute solutions were prepared with the acetate buffer (pH 4.8).

2.2. Apparatus

Electrochemical measurements were performed using an Autolab PGSTAT 12, potentiostat/galvanostat connected to a three-electrode cell (Metrohm, Model 663 VA stand) with a GPES 4.9 software package (Eco Chemie, The Netherlands). The raw data was treated using Savitzky and Golay filter (level 2) of the GPES software, followed by the GPES software moving average baseline correction with a "peak width" of 0.01. Differential pulse voltammetry with a potential amplitude of 50 mV, a modulation time of 0.05 s and a step potential of 8 mV was used throughout the experiments. The reference electrode was Ag/AgCl (3 mol L^{-1} KCl) and the counter electrode was a platinum wire. All reported potentials were recorded *vs.* Ag/AgCl (3 mol L^{-1} KCl) reference electrode.

Electrochemical impedance spectroscopy was running with at a polarization potential of 0.10 V vs. Ag/AgCl (3 mol L^{-1} KCl) in the frequency range of 0.005 to 10^5 Hz. A standard one-compartment three–electrode cell of 10 mL capacity was used in all experiments. The renewable PGE, described in the study of Ozsoz and co-workers [21] was used in all experiments. The pencil graphite leads were used as received. A Noki pencil was used as a holder for Pentel graphite leads. The pencil graphite lead was a HB type spare lead (60×0.7 mm; Pentel; Japan). Electrical contact with the lead was obtained by soldering a metallic wire to the metallic part of the holder. The pencil was held vertically with 12 mm of the lead being extruded outside (9 mm of which was immersed in the solution). All electroanalytical measurements were performed at room temperature.

A Metrohm pH meter (Model 827) with a glass electrode (Corning) was used to adjust the solutions pH.

2.3. Functionalization and purification of MWCNTs

A mass of 1.2 g of MWCNTs was stirred in 10 mL of a 3 mol L^{-1} nitric acid solution for 20 h. The solid product was collected on a filter paper and washed several times with pure water until the filtrate solution was neutral (pH 7). The obtained functionalized MWCNTs were then dried in an oven at 80° C for 24 h. Nitric acid usually causes significant destruction in carbon nanotubes and introduces –COOH groups at the ends or at the sidewall defects of the nanotubes structure [22]. The presence of oxygen-containing groups facilitates the exfoliation of MWCNTs bundles [23]. This, in turn, affects the processing of MWCNTs and increases the possibility of further modification/functionalization depending on application [24].

2.4. Preparation of the DNA–based biosensor

The surface of PGE was pretreated by applying +1.40 V for 300 s in quiescent solution of 0.5 mol L^{-1} acetate buffer containing 20 mmol L^{-1} NaCl (pH 4.8). Then, 2.0 mg MWCNTs was dispersed into 1.0 mL of 0.10% CHIT solution. The mixture was sonicated for 3 h to obtain a homogeneous black suspension, which was sonicated for 15 min immediately before preparing the CHIT–MWCNTs films. PGEs were dipped into this composite for 90 s. Then, the electrode was washed with deionized water and dried. To prepare the DNA–modified electrode, the modified PGE was immersed into 1.0 mg mL^{-1} DNA solution (in 0.5 mol L^{-1} acetate buffer solution and 20 mmol L^{-1} NaCl, pH 4.8) under stirring (200 rpm) for 30 min at room temperature (25 ºC). After this time, the electrode was washed with the acetate buffer

(0.5 mol L^{-1} at pH 4.8) for 5 s to remove any unbounded ds-DNA on the electrode surface. This modified PGE was designated as ds-DNA/CHIT–MWCNTs/PGE.

2.5. Electrochemical impedance spectroscopy

EIS measurements were carried out in the presence of 3.0 mmol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ as a redox probe in 0.1 mol L^{-1} KCl at a polarization potential of 0.15 V in the frequency range of 0.005 to 10^5 Hz and at amplitude of 10 mV.

2.6. Procedure

Investigations of DNA damage induced by dopamine in the presence of the metal ions and some antioxidants were carried out using EIS and DPV methods. To detect the DNA damage induce by dopamine in the presence of some metallic ions such as copper(II) ions, ESI method was used. The constructed ds-DNA/CHIT–MWCNTs/PGE was immersed into Tris-buffer solution (pH 7.0) containing damaging agents (the metallic ions plus dopamine with certain concentration). To follow the DNA damage, impedance measurements were carried out for ds-DNA/CHIT–MWCNTs/PGE before and after immersing the electrode in the damaging solution. In the final stage of our study, the effect of GSH and ascorbic acid (as antioxidant) on the DNA damage were investigated simultaneously and separately. In simultaneous method, different concentrations of antioxidants were added to the damaging solution and the impedance spectra were recorded. In separate solution study, the ds-DNA/CHIT–MWCNTs/PGE, after immersion in the damaging agents, was transferred to the antioxidant solution and the impedance spectra of ds-DNA/CHIT–MWCNTs/PGE were recorded. Then, it compared with the impedance spectra of ds-DNA/CHIT–MWCNTs/PGE before immersion in the antioxidant solution.

In order to detect the DNA damage by DPV method, DPV signals of ds-DNA/Chit–MWCNTs/PGE before and after immersion in the damaging solutions (dopamine plus metallic ions) were recorded. Moreover, the effect of GSH and ascorbic acid were investigated simultaneously and separately. It should be noted that all experiments were repeated 4 times (n=4) and the average values were reported.

2.7. HPLC analysis of 8-oxo guanine (8-oxo G)

Several solutions containing ds-DNA (0.5 mg) + dopamine (30.0 mmol L^{-1}), ds-DNA (0.5 mg) + dopamine (30.0 mmol L^{-1}) + Cu(II) (30.0 mmol L^{-1}) and ds-DNA (0.5 mg) + dopamine (30.0 mmol L^{-1}) + Fe(III) (30.0 mmol L^{-1}) were prepared in the Tris buffer solution (pH 7.0). The components of each solutions were mixed at room temperature in interval time of 1 – 10 min. Then, the samples were centrifuged at 3000 rpm for 5 min. The supernatants were discarded, then 0.5 mL of 60% (v:v) formic acid was added to each of the solutions. The samples were hydrolyzed for 45 min at 150 °C. After the hydrolysis, the formic acid was removed by freeze-drying. The residues were solubilized in distilled water and then were used to HPLC analysis for 8-oxo G, using the method of Kaur and Halliwell (1996) with a minor modification [25]. The HPLC analysis was carried out on a Nucleocil 3 µm C_{18} column (30 cm×4.6 mm) with an eluent of 20 mmol L^{-1} sodium citrate, 17 mmol L^{-1} acetate buffer (pH 3.0) and methanol (9:1) at a flow rate of 1.0 mL min^{-1}. 8-oxo G was detected by electrochemical detector at a potential of 0.85 V.

3. Results and discussion

3.1. Electrochemical properties and surface characterizations

In order to study the surface morphology of thin films on a nano-scale, SEM is considered an excellent tool. The surface topographies of the stepwise fabrication processes of the DNA–

biosensor were studied using SEM (Fig. 1). Fig. 1A displays the SEM image of the unmodified-PGE. The graphite layers can be seen well in this figure. Fig. 1B shows that MWCNTs were well dispersed in the CHIT solution. When the ds-DNA was immobilized on the surface of CHIT–MWCNTs, the surface morphology was changed (Fig. 1C). The diameter of the nanotubes was slightly increased and the clear MWCNTs images appear darker. Coated and uncoated surfaces of the PGE and the interface between the PGE and the solution can be clearly seen in Fig. 1C. **"Here Fig. 1"**

Previous studies have revealed that the assembly of nucleic acids on supports can be followed by electrochemical impedance spectroscopy [26-28]. Fig. 2 shows a Nyquist plot of EIS for PGE (Fig. 2, a), PGE modified with CHIT–MWCNTs nanocomposite (Fig. 2, b) and ds-DNA/CHIT–MWCNTs/PGE (Fig. 2, c) in 3.0 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ (1:1) containing 0.10 mol L^{-1} KCl. It was found that the charge transfer resistance (R_{ct}) of the PGE drastically decreased in the presence of CHIT–MWCNTs nanocomposite. The nanocomposite of MWCNTs and CHIT promoted the electron exchange between $[Fe(CN)_6]^{3-/4-}$ and the electrode. As shown in Fig. 2, the R_{ct} increases after immobilization of negatively charged ds-DNA on the electrode surface. This is due to the fact that the negatively charged interface electrostatically repels the negatively charged redox probe $[Fe(CN)_6]^{3-/4-}$, and inhibits the interfacial charge-transfer. The optimum experimental conditions for the preparation of ds-DNA/CHIT-MWCNTs/PGE are described in Sec. 1 of the supplementary material.

"Here Fig. 2"

3.2. Investigation of ds-DNA damage due to dopamine in the presence of the metallic ions

The proposed method for assessing the effect of dopamine in the presence of the metallic ions was divided into four parts, which is given below:

I) Immobilization of the ds-DNA on the surface of CHIT–MWCNTs/PGE under the optimum conditions.

II) Demolition of the ds-DNA with placing of ds-DNA/Chit–MWCNs/PGE in dopamine solution plus Cu(II) and Fe(III).

III) Investigation of the behavior of some antioxidants such as GSH and ascorbic acid in the presence of damaging agents (dopamine plus Cu(II)) simultaneously. In this part of our investigation, the antioxidants were added to the damaging agents. Then, the ds-DNA/CHIT–MWCNTs/PGE was placed into the solution containing damaging agents and the antioxidant.

IV) Finally, study the effect of GSH and ascorbic acid on the DNA repairing, after ds-DNA damage. In this part, the ds-DNA/CHIT–MWCNTs/PGE was incubated in the damaging agents and then it was transferred into the antioxidant solutions for a certain time.

3.3. Initial study of the interaction between the ds-DNA and dopamine in the presence of metallic ions

The effect of dopamine plus Cu(II) and/or Fe(III), as a damaging agent, on the ds-DNA was studied with EIS and DPV methods. For this purpose firstly, the ds-DNA/CHIT–MWCNTs/PGE was transferred into 10.0 mmol L^{-1} Tris-buffer solution (pH 7.0) as a blank solution for a given time and the EIS and DPV response of ds-DNA/CHIT–MWCNTs/PGE was recorded. In second stage of our investigation, the ds-DNA/CHIT-MWCNTs/PGE was transferred into 10.0 mmol L^{-1} Tris-buffer solution (pH 7.0) containing a certain amount of dopamine plus Cu(II) and/or Fe(III) for a given time under string (150 rmp). Then, the ds-DNA/CHIT–MWCNTs/PGE was washed with the Tris-buffer and the EIS and DPV responses of the electrode were recorded. The difference in the numerical values of the electron transfer resistance (R_{ct}) in the EIS method before and after immersion of ds-DNA/CHIT–MWCNTs/PGE is chosen as the criterion of the DNA damage. In addition, the difference between the oxidation peaks current of adenine and guanine in DPV method is proportional to the amount of DNA damage. Moreover, due to damaging of the DNA

(oxidation of DNA), a new potential peak can be seen in a potential range of 0.45 – 0.55, that is related to 8-oxo guanine (8-oxo G; as a biomarker of DNA oxidation).

3.4. Investigation of DNA damage using EIS technique

In order to inspect the DNA damage due to dopamine plus Cu(II) and/or Fe(III), six separate solutions (10 mL) including the blank solution and/or Cu(II), Fe(III), dopamine, and/or a mixture of them (Sec. 2, supplementary material) were prepared. Then, ds-DNA/CHIT–MWCNTs/PGE was used to study the damage. The results (Fig. 3A) showed that after immersion of ds-DNA/CHIT–MWCNTs/PGE in dopamine, Cu(II) and Fe(III) solutions separately, their Rct were the same as for Rct of the blank solution (Tris-buffer solution, pH 7.0). In addition, the R_{ct} decreased after immersion of ds-DNA/CHIT–MWCNTs/PGE in solutions containing dopamine plus Cu(II) and/or dopamine plus Fe(III). These results prove that Cu(II) or Fe(II) and/or dopamine, each one separately, unable to damage the DNA, whereas dopamine + Cu(II) or dopamine + Fe(III) have abilities to destroy the DNA. The interaction of ds-DNA and dopamine + Cu(II) or dopamine + Fe(III) tends to decrease the amount of negative charges of the ds-DNA. Decreasing the effective negative charge of ds-DNA causes the facility of redox reactions of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$, as a redox probe, and decreasing the R_{ct}. Comparison between R_{ct} related to dopamine plus Fe(III) or Cu(II) showed that the R_{ct} of ds-DNA/CHIT− MWCNTs/PGE in dopamine + Cu(II) solution *vs.* dopamine + Fe(III) was much less. This proves that strength of Cu(II) for destruction of DNA is much greater than Fe(III) in the presence of dopamine.

According to the results, our studies were focused on the DNA damage due to dopamine plus Cu(II). In the presence of O_2, dopamine can redox cycle producing quinone metabolites, which can bind directly to DNA and forming covalent DNA adducts via semi-quinone intermediates while simultaneously reducing the metal ions. Superoxide is then generated by

the reduction of molecular oxygen followed by the re-generation of the metal to its oxidized state. Superoxide can also react with Cu(I) to produce H_2O_2, which then can complex with either Cu(I) or Cu(II) bound to DNA to form DNA–Cu(I)OOH. Following the formation of the DNA–Cu(I)OOH, a variety of oxidative DNA lesions including the unidentified oxidation DNA products. Singlet oxygen may also be produced from this complex yielding 8-oxo G [29].

In order to compare the results of the DNA based sensor with a standard method, the produced 8-oxo G of these samples were determined by a HPLC assay (Fig. 3B). After interacting $HO^{\bullet}$ with the DNA, guanine yields an oxidation product of 8-oxo G. The most common procedure employed to assay oxidative DNA damage is the measurement of 8-oxo G by HPLC method with electrochemical detection (HPLC-ECD), after hydrolysis of DNA [30, 31]. This procedure has the advantage that 8-oxo G is a major product of DNA damage, generated by attack of several reactive oxygen species. As shown in Fig. 3B, the HPLC results are in good agreement with the impedance spectra obtained under similar conditions.

"Here Fig. 3"

3.5. Optimization of the affecting parameters

In order to obtain the highest sensitivity, some variables such as dopamine concentration, mole ratio of dopamine and Cu(II) and incubation time of ds-DNA/CHIT–MWCNTs/PGE in the damaging solution were studied and optimized.

To find the best dopamine concentration for investigation of the DNA damage, a series of ds-DNA/CHIT–MWCNT/PGEs were prepared according to the optimum conditions in section 3.2 and transferred to the Tris-buffer solution (pH 7.0) containing 80.0 mmol L^{-1} Cu(II) and 1.0 to 150.0 mmol L^{-1} dopamine under string (150 rmp). Then, the ds-DNA/CHIT–MWCNTs/PGEs were washed with the Tris-buffer solution (pH 7.0) and transferred into the electrochemical cell and their impedimetric responses (R_{ct}) were

recorded. The R_{ct} of ds-DNA/CHIT–MWCNTs/PGEs in the blank solution (Tris-buffer solution (pH 7.0) was named as R_1 and the R_{ct} of ds-DNA/CHIT–MWCNTs/PGE after incubation in dopamine + Cu(II) solution was named R_2. The difference between R_1 and R_2 ($\Delta R = R_2 - R_1$) was used as a final impedimetric signal to evaluate the best dopamine concentration. The obtained results (Fig. 4A) showed that increasing the dopamine concentration tends to increase the DNA damage and consequently increased of ΔR.

"Here Fig. 4"

In order to find the best mole ratio of dopamine/Cu(II), different solutions of dopamine (with constant concentration of 100.0 mmol L^{-1}) and variable concentrations of Cu(II) were prepared in the Tris-buffer solution (pH 7.0), so that the mole ratio of dopamine/Cu(II) be in the range of 0.5 to 3.0. The ds-DNA/CHIT–MWCNTs/PGEs were inserted in these solutions for 10 min, under stirring (150 rmp). Then, the ds-DNA/CHIT–MWCNT/PGEs were washed with the Tris-buffer solution (pH 7.0) and transferred to the electrochemical cell to record the impedimetric responses (R_{ct}). The R_{ct} of ds-DNA/CHIT–MWCNTs/PGEs in the blank solution (Tris-buffer solution, pH 7.0) was recorded and named R_1. The R_{ct} of ds-DNA/CHIT–MWCNTs/PGE after incubation in dopamine + Cu(II) solution was named R_2. Then, ($\Delta R = R_2 - R_1$) was plotted *vs.* the mole ratio of dopamine/Cu(II), to evaluate the best mole ratio of the damaging agent. The results showed that in dopamine/Cu(II) mole ratio of 1.0, the signal (ΔR) was maximum (Fig. 4B). Therefore, this mole ratio was used in all subsequent steps of our investigation.

In order to achieve the best incubation time of ds-DNA/CHIT–MWCNTs/PGEs in dopamine + Cu(II) solution, the ds-DNA/CHIT–MWCNT/PGEs were incubated in solutions containing the buffer solution (pH 7.0) and 100.0 mmol dopamine + 100.0 mmol Cu(II) for interval time of 0.0 to 60 min, under stirring (150 rmp) conditions. Then, the ds-DNA/CHIT–MWCNTs/PGEs were washed with the Tris-buffer (pH 7.0) and transferred to the

electrochemical cell to record the impedimetric responses (R_2). In addition, for each time, the blank signal (in the Tris-buffer solution, pH 7.0) was recorded for interval time of 0.0 to 60 min, under stirring (150 rmp) conditions. Finally, the impedimetric responses of the ds-DNA/CHIT–MWCNTs/PGEs in the blank solution were recorded and named as R_1. For each time, the (ΔR) was calculated and plotted *vs.* time. The results of our studied (Fig 4C) conformed that in 5 min, as incubation time, the (ΔR) reaches to maximum value. So, this time was selected to investigate the DNA damage (Fig. 4C).

3.6. Influence of GSH as an antioxidant on the DNA damage

Investigation of GSH effect was divided into two sections as below:

I) The inhibitory effect of GSH (simultaneous investigation).

II) The repair effect of GSH (separate solution investigation).

3.6.1. Investigation of inhibitory effect of GSH

In order to inspect the inhibitory effect of GSH on DNA damage, EIS method was used. The ds-DNA/CHIT–MWCNTs/PGE was incubated in the Tris-buffer solution (pH 7.0) and/or in dopamine + Cu(II) (100.0 mmol L^{-1} dopamine and Cu(II)) and/or GSH, as described in Sec. 3 of the supplementary material. The results of our studied showed that with increasing GSH concentration till 1.0 mmol L^{-1}, the R_{ct} did not changed and it was equal to the R_{ct} of ds-DNA/CHIT–MWCNTs/PGE in the blank solution (Tris-buffer solution (pH 7.0)) and the two graphs have been superposed (Fig. 5A). This is due to the fact that GSH keeps dopamine in the reduced state, thereby shutting down the initial step of the dopamine-mediated Fenton cascade [32]. For GSH concentration more than 1.0 mmol L^{-1}, the R_{ct} was greater than R_{ct} for the ds-DNA/CHIT–MWCNTs/PGE in the blank solution, which may be due to the intercalation of GSH in the ds-DNA and thereby decreasing its conductivity.

"Here Fig. 5"

The inhibitory effect of GSH on DNA damage due to dopamine + Cu(II) was investigated by DPV. In DPV investigation, three solutions were prepared with and without dopamine, Cu(II), or mixture of them, as described in Sec. 3 of the supplementary material. The results (Fig. 5B) showed that after interaction of the ds-DNA with dopamine and Cu(II), the adenine and guanine signals diminished and a new peak at potential around 0.50 V emerged related to the oxidation of guanine (called 8-oxo guanine, 8-oxo G). It should be noted that after adding GSH into dopamine + Cu(II) solution, the adenine and guanine signals increased and the peak corresponds to 8-oxo G was decreased. Comparing the results of our studies conducted by the two methods (EIS and DPV), they were totally agreed with each other and the inhibitory effect of GSH on the degradation of ds-DNA was proved.

3.6.2. The repair effect of GSH

Before investigate the repair effect of GSH, first, the incubation time of ds-DNA/CHIT–MWCNTs/PGE in GSH after damage was optimized as described in Sec. 4 of the supplementary material. The obtained results showed that the damage percentage decreased till 10 min and then leveled off. So this time was selected as an optimum time for the incubation of the ds-DNA/CHIT–MWCNTs/PGEs in GSH solution (figure not shown).

In order to study the repair effect of GSH by EIS method, the ds-DNA/CHIT–MWCNT/PGEs were incubated in the Tris-buffer and/or solution (pH 7.0) free of dopamine + Cu(II) for 15 min under the stirring (150 rmp) conditions. Then, they were transferred into the electrochemical cell for recording the impedimetric responses (R_{ct}). In second stage, the ds-DNA/CHIT–MWCNT/PGEs were incubated for 5 min in the Tris-buffer solution (pH 7.0) containing certain amounts of dopamine + Cu(II) (100.0 mmol L^{-1} dopamine and Cu(II)) under stirring (150 rmp) conditions. Then, the ds-DNA/CHIT–MWCNTs/PGEs were

incubated in 100.0 mmol L^{-1} GSH solution. Finally, the ds-DNA/CHIT–MWCNTs/PGEs were washed with the Tris-buffer solution (pH 7.0) and they were transferred into the electrochemical cell for recording the impedimetric responses (R_{ct}). Fig. 5C shows the impedance spectra of the damaged ds-DNA/CHIT–MWCNTs/PGEs before and after immersion in the GSH solution. As shown in this figure, the R_{ct} decreases after immersion of ds-DNA/CHIT–MWCNTs/PGEs in GSH solution, proves that the repair effect of GSH.

In order to study the repair effect of GSH using DPV technique, three types of signals were recorded as described in Sec. 5 of the supplementary material. The results (Fig. 5D) showed that after immersion of ds-DNA/CHIT–MWCNTs/PGE in GSH solution, the peaks current of adenine and guanine increased and the peak current of 8-oxo G decreased, which confirms that GSH has ability to repair and deterrence of the DNA damage.

3.7. *Effect of ascorbic acid as an antioxidant*

In this section the inhibitory and repair effect of ascorbic acid, as an antioxidant, were studied similar to section 3.7. The results are illustrated in the supplementary material. The impedance spectra and DPV signals of ds-DNA/CHIT–MWCNTs/PGE after immersion in the Tris-buffer solution (pH 7.0), 100.0 mmol L^{-1} dopamine + Cu(II) and 1.0 mmol L^{-1} ascorbic acid at pH 7.0 (for simultaneously investigation) are shown in Figs. S2-A and S2-C, respectively. Moreover, the impedance spectra and DPV signals of ds-DNA/CHIT–MWCNTs/PGE in the Tris-buffer solution (pH 7.0) and in 100.0 mmol L^{-1} of dopamine + Cu(II) and in 100.0 mmol L^{-1} dopamine and Cu(II) (at pH 7.0), after addition of 100.0 mmol L^{-1} ascorbic acid, are shown in Figs. S2-B and S2-D, respectively (separate solution investigation).

3.8. Reproducibility and repeatability of the ds-DNA/CHIT–MWCNTs/PG electrode

In order to test the reproducibility in the fabrication of ds-DNA/CHIT–MWCNTs/PGE, twenty four ds-DNA/CHIT–MWCNTs/PGEs were prepared on different days and transferred into the electrochemical cell to record their impedimetric responses (R_{ct}). ANOVA test was used to evaluate the reproducibility of the fabricated ds-DNA/CHIT–MWCNTs/PGEs. The calculated F_{cal} between days was 3.186, whereas F_{crit} was equal to 4.011. In addition, F_{cal} for within days was 3.013, whereas F_{crit} was found to be 3.514. The F-test showed that there were not any significant differences between the fabricated ds-DNA/CHIT–MWCNTs/PGEs on a day and different days. In addition, to evaluate the repeatability of the measured resistance (R_{ct}), using ds-DNA/CHIT–MWCNTs/PGEs, three solutions were prepared as follow: I) 100.0 mmol L^{-1} dopamine; II) 100.0 mmol L^{-1} dopamine + Cu(II); III) 100.0 mmol L^{-1} dopamine + Fe(III). Then, four EIS measurements (n=4) were performed for each of the solutions. The RSD% values for the above solutions (from I to III) were obtained as 5.6%, 6.2% and 5.8%, respectively.

3.9. General comments

According to research of Schweigert and coworkers [33,34], it can be concluded that when catecholamine is oxidized enzymatically or in the presence of oxygen and heavy metals ions, one electron is transferred to the molecular oxygen, and consequently a superoxide ($O_2^{\bullet}$) is formed. In the presence of heavy metals ions (e.g., copper, iron ions), the superoxide is further reduced to hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$) [33,34]. DNA strand breaks are caused by the redox reaction of Cu(II) and catecholamine to yield Cu(I) and semiquinone radical, and the subsequent copper catalyzed the reduction of molecular oxygen to form superoxide and hydrogen peroxide. A DNA–copper–ox complex [DNA–Cu(I)–OOH] finally causes the DNA strand breaks by splitting hydroxyl radicals in the vicinity of the DNA [35]. The possible mechanism of 8-oxo G generation is shown in Scheme 1.

GSH, as an antioxidant, diminishes the DNA damage induced by dopamine + Cu(II)). Degradation of ds-DNA due to dopamine + Cu(II) is a type of oxidative damage and in this case, $OH^{\bullet}$ radical attacks guanine to form 8-oxo G, so GSH acts in order to reduce the amount of $OH^{\bullet}$. In the presence of cellular reductants such as GSH, depending on the amount of the reducer and the ratio of the reactants, dopamine can reduced. So the Fenton reaction and production of $OH^{\bullet}$ radical diminishes. Moreover, the $OH^{\bullet}$ radical can be snatched by reduced GSH. On the other hand, according to our results in separate solution investigation, GSH can diminish the oxidation current of 8-oxo G, due to the reduction of 8-oxo G. In the final stage of our investigation, the role of ascorbic acid to prevent DNA damage was evaluated. Ascorbic acid is oxidized with loss of one electron to form a radical cation and then with loss of a second electron to form of dehydroascorbic acid. It typically reacts with the oxidants of the reactive oxygen species, such as hydroxyl radical. In this case, ascorbic acid, as an antioxidant, can reduce Cu(II) to Cu(I) and cause to generate high amount of free radical and consequently high amount of DNA damage [36,37].

"Here Scheme 1"

4. Conclusion

In this study we constructed a DNA-based biosensor for monitoring the DNA damage due to dopamine in the presence of some metallic ions such as Cu(II) and Fe(III). In addition, we investigated the effect of some antioxidant such as GSH and ascorbic acid, using EIS and DPV methods. The results of our studied showed that dopamine, Cu(II) and Fe(III) alone could not destroy DNA, but dopamine + Cu(II) and dopamine + Fe(III) can damage DNA. Our observations proved that the ability of Cu(II) was greater than Fe(III) in the presence of dopamine and therefore, the investigation was concentrated on damage due to Cu(II) + dopamine. In addition, GSH and ascorbic acid have the ability to repair and preclude the DNA damage, but the ability of GSH is more than ascorbic acid (twice) to decrease the

damaging rate. This simple method could help us to distinguish which substances can damage DNA or which of them can repair and/or have inhibitory effect on DNA damage. Moreover, by this method the antioxidants ability and power of them, such as flavonoid compounds, can be measure.

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Figures caption

Fig. 1. SEM images of A): bare pretreated PGE; B): CHIT–MWCNTs modified–PGE; and C): ds-DNA/CHIT–MWCNTs modified PGE.

Fig. 2. Impedance spectra of a): unmodified PGE; b): CHIT–MWCNTs/PGE; and c): ds-DNA/CHIT–MWCNTs/PGE.

Fig. 3. A) Impedance spectra of ds-DNA/CHIT–MWCNTs/PGE after immersion in a): 10.0 mmol L^{-1} Tris-buffer solution (pH 7.0); b): Tris-buffer solution (pH 7.0) containing 30.0 mmol L^{-1} Cu(II) and/or 30.0 mmol L^{-1} Fe(III); c): Tris-buffer solution containing 30.0 mmol L^{-1} dopamine; d): Tris-buffer solution (pH 7.0) containing 30.0 mmol L^{-1} Fe(III) and dopamine; and e): Tris-buffer solution (pH7.0) containing 30.0 mmol L^{-1} Cu(II) and dopamine. Impedance conditions: 3.0 mmol L^{-1} $Fe(CN)_6^{3-/4-}$ containing 0.1 mol L^{-1} KCl at a polarization potential of 0.10 V in the frequency range of 0.005 to 10^5 Hz and at amplitude of 10 mV. B) Formation of 8-oxo G induced by Tris buffer solution (pH 7.0) containing: a) 0.5 mg ds-DNA + 30.0 mmol L^{-1} dopamine, b) 0.5 mg ds-DNA + 30.0 mmol L^{-1} dopamine and Fe(III), and c) 0.5 mg ds-DNA + 30.0 mmol L^{-1} dopamine and Cu(II) in time interval between 1-10 min.

Fig. 4. A) Influence of the dopamine concentration on the impedimetric response of ds-DNA/CHIT-MWCNTs/PGE, B) Influence of the mol ratio of dopamine/Cu(II) on the impedimetric response of ds-DNA/CHIT-MWCNTs/PGE; C) Influence of the incubation time of ds-DNA/CHIT-MWCNTs/PGE in damage solution on the impedimetric response.

Fig. 5. A): Impedance spectra and B): DPV signals of ds-DNA/CHIT–MWCNTs/PGE in a): Tris-buffer solution, b): Tris-buffer solution (pH 7.0) containing 100.0 mmol L^{-1} of dopamine + Cu(II); c): Tris-buffer solution (pH 7.0) containing 100.0 mmol L^{-1} of dopamine + Cu(II)

and 1.0 mmol L^{-1} GSH (simultaneously investigation). C): Impedance spectra and D): DPV signals of ds-DNA/CHIT– MWCNTs/PGE in a): Tris-buffer solution (pH 7.0); b): Tris-buffer solution (pH 7.0) containing 100.0 mmol L^{-1} of dopamine + Cu(II); and c): Tris-buffer solution (pH 7.0) containing 100.0 mmol L^{-1} of dopamine + Cu(II) and then 100.0 mmol L^{-1} GSH (separate solution investigation).

Scheme 1. Possible mechanism for 8-oxo G generation during the DNA damaging.

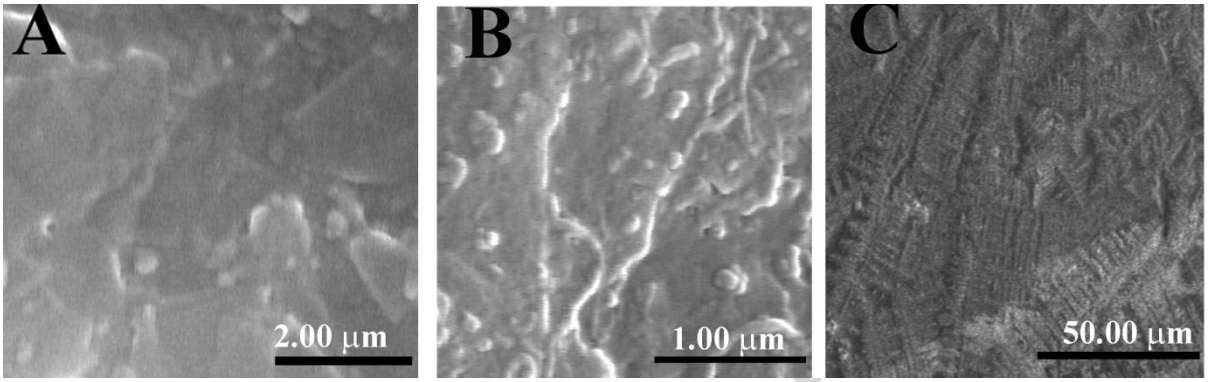


Figure 1

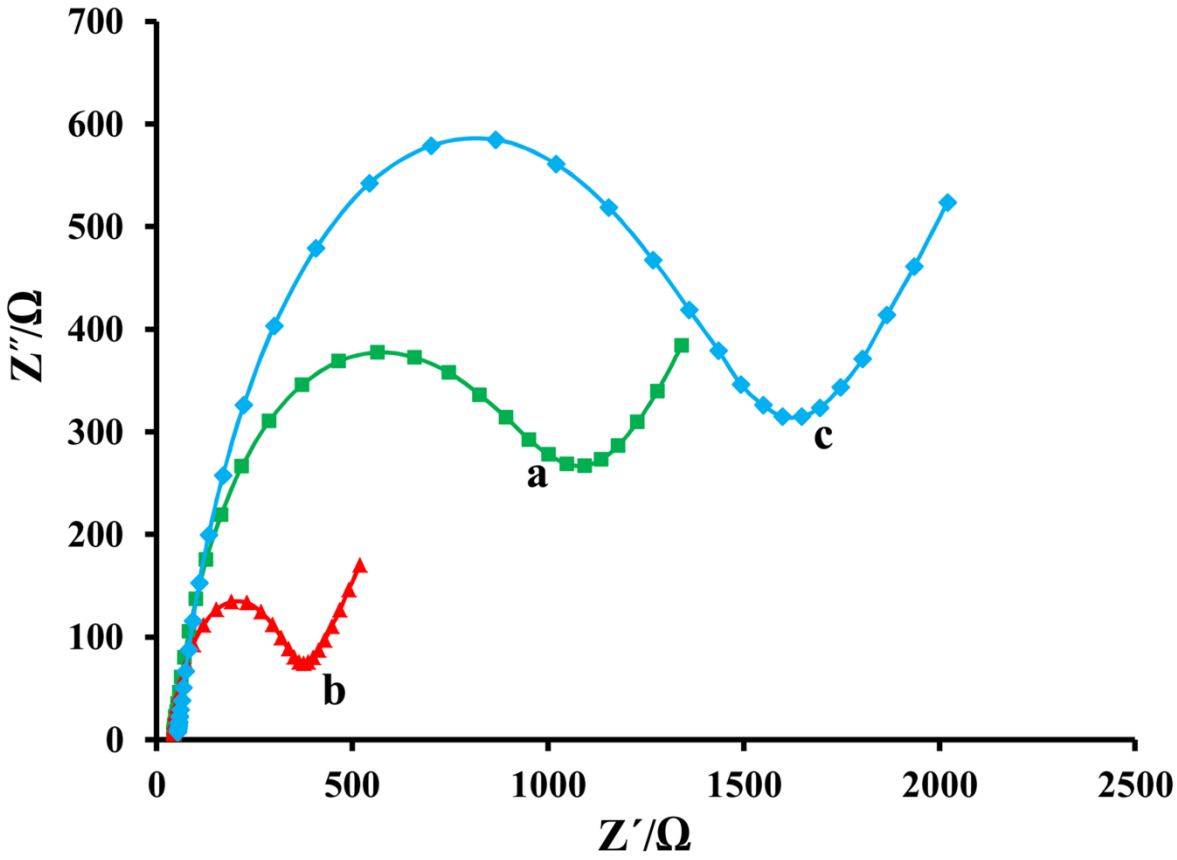


Figure 2

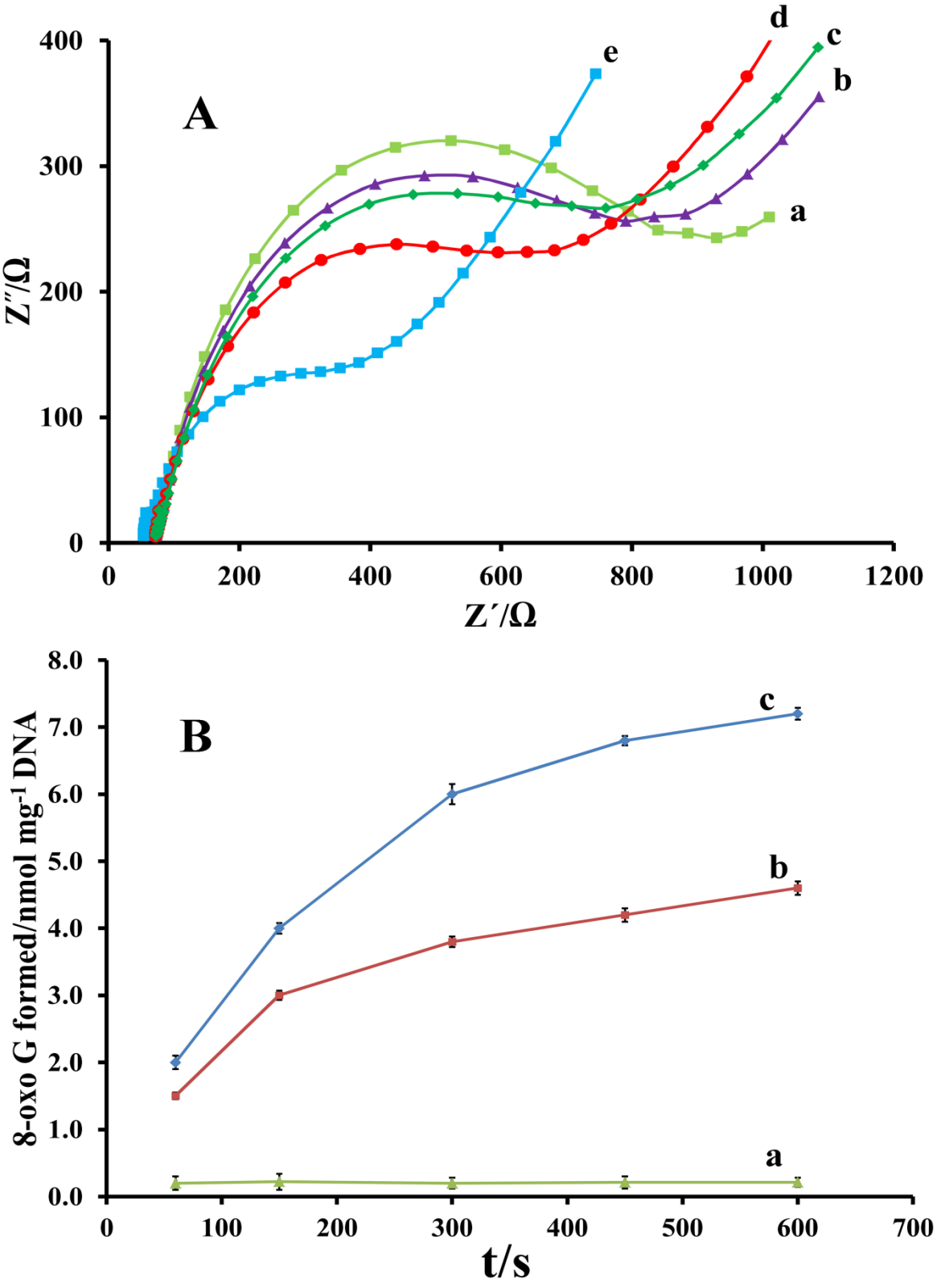


Figure 3

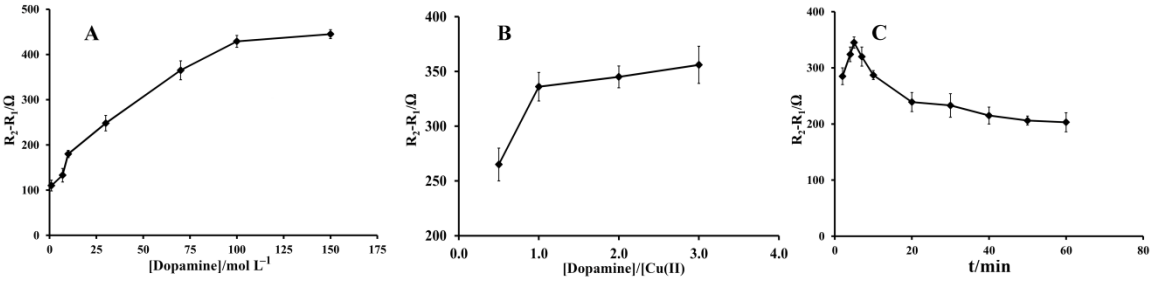


Figure 4

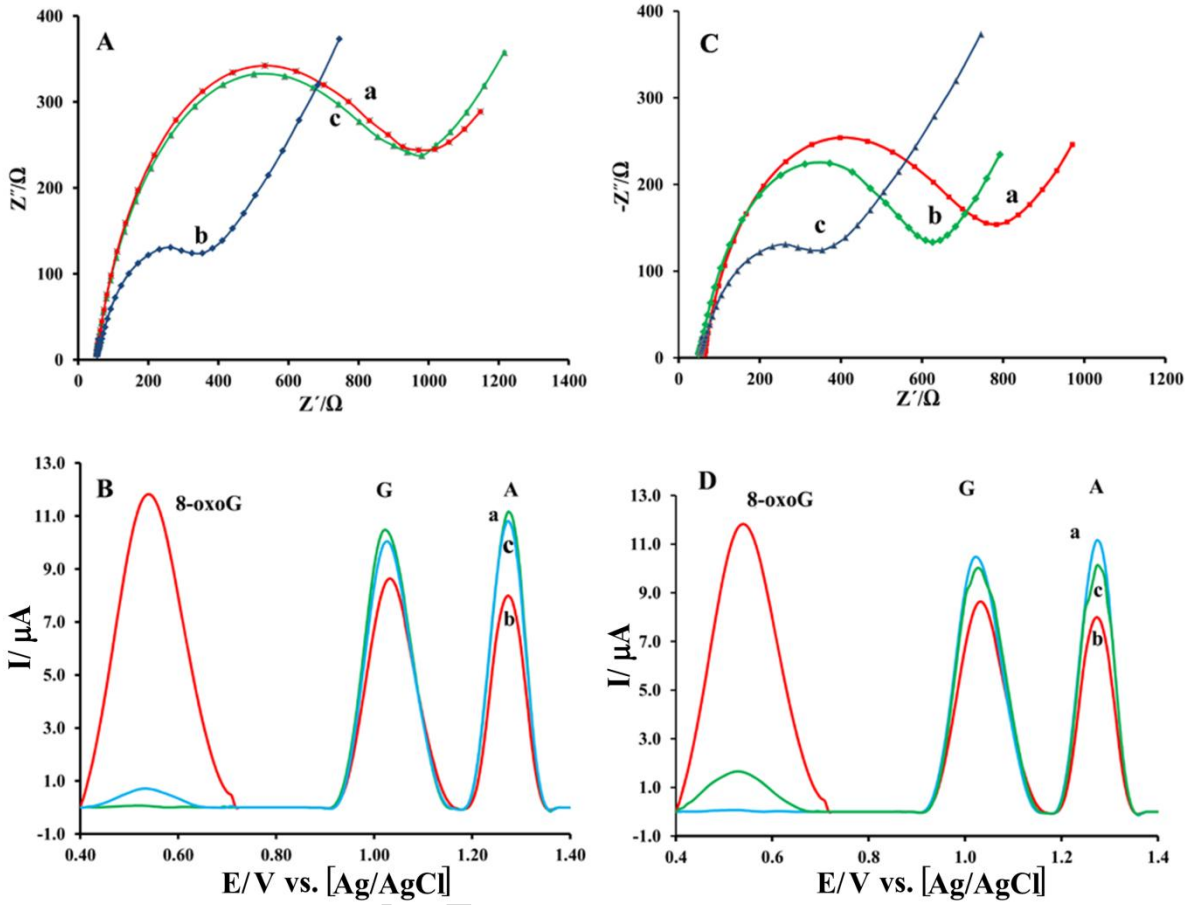
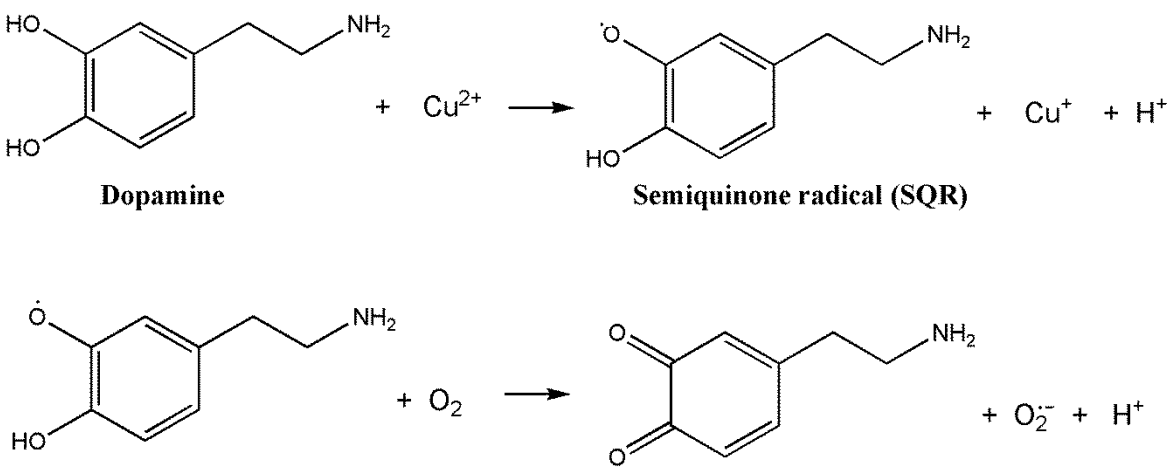


Figure 5



Scheme 1

Highlights

- A DNA-biosensor was introduced to study DNA damage using impedance spectroscopy.

- Dopamine, Cu(II) and/or Fe(III) alone could not destroy DNA.

- The charge transfer resistance of the electrode in the above separate solutions were > 1100 Ω.

- Dopamine plus Cu(II) and/or dopamine plus Fe(III) can damage DNA.

- Charge transfer resistance of the electrode in the mixture solution was < 1100 Ω.

- The damage ability of Cu(II)-dopamine was greater than Fe(III)-dopamine.

- The ability of glutathione is more than ascorbic acid (twice) to repair DNA damage.