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Determination of atropine sulfate using a novel sensitive DNA–biosensor based on its interaction on a modified pencil graphite electrode

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Abstract:

A novel, selective, rapid and simple electrochemical method is developed for the determination of atropine sulfate. UV–Vis and differential pulse voltammetry are used to study the interaction of atropine sulfate with salmon sperm ds-DNA on the surface of salmon sperm ds-DNA modified-pencil graphite electrode (PGE). For this purpose, a pencil graphite electrode (PGE) modified with multiwall carbon nanotubes (MWCNTs), titanium dioxide nano-particles (TiO_2NPs), and poly-dialyldimethylammonium chloride (PDDA) decorated with ds-DNA is tested for the determination of atropine sulfate. The electrochemical oxidation peak current of adenine and guanine bonded on the surface of ds-DNA/PDDA–TiO_2NPs–MWCNTs/PGE is used to obtain the analytical signal. Decreases in the intensities of guanine and adenine oxidation signals after their interaction with atropine sulfate are used as indicator signals for the sensitive determination of atropine sulfate. Using ds-DNA/PDDA–TiO_2NPs–MWCNTs/PGE and based on the guanine signal, linear calibration curves were obtained in the range of 0.6 to 30.0 μmol L^{-1} and 30.0 to 600.0 μmol L^{-1} atropine sulfate with low detection limits of 30.0 nmol L^{-1}. The biosensor shows a good selectivity for the determination of atropine sulfate. Finally, the

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applicability of the biosensor is evaluated by measuring atropine sulfate in real samples with good accuracy.

Keywords: DNA–biosensor; Atropine detection; Modified pencil graphite electrode; Voltammetry.

1. Introduction

Drug analysis is an important branch of analytical chemistry for its role in drug quality control and wide impact on public health. Developing sensitive, simple, rapid, and reliable methods for the determination of active ingredients is, therefore, valuable and necessary.

Atropine ($C_{17}H_{23}NO_3$), the tropane alkaloid is widely found in solanaceae plants, such as Flos daturae, Atropa, and Hyoscyamus [1] and it is generally used as antispasmodic, anticholinergic, and antidote effects [2]. The drug has found wide clinical applications because of its strong parasympatolytic and anticholinergic actions [3]. Atropine, the racemic mixture of (S)-hyoscyamine and (R)-hyoscyamine, acts pharmacologically via blocking acetylcholine receptors of the muscarine subtype, which causes not only peripheral symptoms such as tachycardia, dilated pupils, and decreased gastrointestinal motility, but also affects the central nervous system and causes agitation, disorientation, and hallucination [4]. The most common atropine compound used in medicine is atropine sulfate ($(C_{17}H_{23}NO_3)_2{\cdot}H_2SO_4{\cdot}H_2O$) or sulfate monohydrate. Atropine sulfate, an alkaloid extracted from belladonna herb, is a classic cholinergic drug that is widely used in all kinds of viscera colic and administered as the medicine given before systemic anesthesia, as an anti-arrhythmic medicine [5,6], or for organophosphate poisoning treatment [7]. An inadequately low dosage fails to yield perfect remedial effects, while blindfold over-commitment may cause atropism [8]. Many cases of atropism have been reported [9, 10]

with an occurrence rate of up to 40–60% and a mortality rate of around 18.3% [11]. Since most alkaloids have special and distinct physiological properties in addition to toxicity, the determination of atropine sulfate is of great importance not only in clinical applications but also for pharmaceutical analysis.

A variety of methods are employed for the determination of atropine such as spectrophotometry [11], ion-selective electrode [12] and liquid chromatography [13]. However, spectrophotometry suffers from low sensitivity while liquid chromatography, although more sensitive, requires expensive apparatus, long and time-consuming procedures and different chemicals. In ion-selective electrode, the response is affected by electrical properties of the film and electric double-layer capacitance while it also suffers from low sensitivity. Electroanalytical techniques, however, have been shown to be excellent for the determination of pharmaceutical compounds in different matrices. The advances made in the experimental electrochemical techniques used in the field of drug analysis owe much to their simplicity, low cost, and relatively short analysis time compared to other techniques [14, 15]. As yet, there is only one electrochemical method reported in the literature for the determination of atropine, which used multiwall carbon nanotube electrode with a linear calibration curve of 0.014 to 0.094 μmol L^{-1} [16]. However, the method does not enjoy adequate selectivity for the determination of atropine in real samples.

Over the last two decades, intense research has been devoted to DNA biosensors as useful tools for monitoring hybridization and DNA–molecule interactions [17–20]. The transduction of these events into measurable information has a great importance for pharmacological, medical and environmental analyses [19,21,22]. Electrochemical DNA–biosensors have attracted attention among the DNA biosensors because they allow for rapid and inexpensive detection of ingredients [23–27]. While different types of electrode

materials such as carbon paste [28], glassy carbon [29], carbon fiber [30], and graphite [23–27] have been used in their construction, pencil graphite electrode (PGE) was selected in this study for its cost effectiveness, commercial availability and disposability.

A key issue with electrochemical DNA–based biosensors is achieving enhanced DNA immobilization on the substrates. DNA immobilization on the surface plays an important role in the performance of DNA biosensors as the amount of immobilized DNA probe will directly influence the sensor's accuracy, sensitivity, selectivity, and effective life [31]. In this study, a mixture of nanomaterials (MWCNTs and titanium dioxide nanoparticles (TiO_2NPs)) and positively charged polyelectrolytes (poly dialyldimethylammonium chloride, PDDA) were immobilized on the surface of a pencil graphite electrode (PGE) to improve the immobilization of ds-DNA on the surface.

A PGE modified with MWCNTs, TiO_2NPs and PDDA decorated with a ds-DNA was fabricated (ds-DNA/PDDA–TiO_2NPs–MWCNTs/PGE) and the oxidation signals of adenine and guanine were obtained using differential pulse voltammetry (DPV). The decreases in the current intensities of guanine and adenine oxidation signals after interaction with atropine sulfate were used as indicators for the sensitive determination of atropine sulfate. The presence of both MWCNTs and TiO_2NPs was found to dramatically improve the immobilization rates by covalent and electrostatic immobilization. The new biosensor exhibited a better sensitivity for trace analysis than those reported previously. The new method is inexpensive, highly selective, rapid, and sensitive method for the direct determination of atropine sulfate in real samples.

2. Experimental

2.1. Chemicals

All solutions were prepared using reagent grade chemicals and doubly distilled water were used through.

Atropine sulfate was purchased from Aldrich Chemicals. Double stranded salmon sperm DNA (ds-DNA, catalog No. D8899) was purchased from Sigma-Aldrich (St. Louis, USA). Reagent grade Tris–HCl, CH_3COOH, CH_3COONa, H_3PO_4, EDTA, NaCl and NaOH were purchased from Aldrich Chemicals (Milwaukee, USA). PDDA (low molecular weight) was purchased from Sigma-Aldrich Chemicals (Milwaukee, USA).

Aqueous solution of PDDA was prepared with 0.5 mol L^{-1} NaCl. Multiwall carbon nanotubes (MWCNTs, diameter of 70–100 nm and length of 5–9 μm) was purchased from Fluka. Titanium dioxide nano particles (TiO_2NPs) powder (30 nm) was purchased from Fluka. The suspensions were prepared in PDDA solution after sonication for 3 h to obtain a homogeneous suspension.

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 the 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. The reference electrode was Ag/AgCl (3 mol L^{-1} KCl) and the counter electrode was a platinum wire. A standard one-compartment three-electrode cell of 20 mL capacity was used in all experiments. PGE and/or modified–PGE was the working electrode. A Noki pencil was used as a holder for Pentel graphite leads. Electrical contact with the lead was

obtained by soldering a metallic wire to the metallic part. The pencil was hold vertically with 12 mm of the lead extruded outside (9 mm was immersed in the solution). The pencil leads were used as received. All the electroanalytical measurements were performed at room temperature.

Electrochemical Impedance spectroscopy was carried out in the presence of 5.0 mmol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ as a redox probe in 0.1 mol L^{-1} KCl (0.1 mol L^{-1} PBS pH 4.0) 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.

The differential pulse voltammetry was performed by employing a positive-going differential pulse potential scan (from 0.40 to 1.40), using a pulse amplitude of 50 mV, a pulse with of 50.0 ms and a scan rate of 10 mV s^{-1}. The oxidation signals of guanine and adenine were recorded. The raw data were treated using the Savitzky and Golay filter (level 2) of the GPES software, followed by the GPES software moving average baseline correction, using a "peak width" of 0.01.

UV-Vis spectra were recorded with a double beam spectrophotometer, Jasco Model V–750, using 1.0 cm quartz cells.

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

2.3. Functionalization and purification of MWCNTs

MWCNTs were purified and functionalized as described elsewhere [32]. A mass of 120 mg 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 distilled water until the filtrate solution became neutral (pH 7). The functionalized MWCNTs thus obtained were then dried in an oven at 80 °C for 24 h. Nitric acid usually causes a

significant destruction in carbon nanotubes and introduces –COOH groups at the ends or at the sidewall defects of the nanotube structure.

2.4. Preparation of free DNA and DNA modified–PGE

Initially, the surface of PGE was pretreated by applying +1.40 V for 300 s in a quiescent solution of 0.5 mol L^{-1} acetate buffer containing 0.02 mol L^{-1} NaCl (pH 4.8). Then, 5.0 mg of MWCNTs and 5.0 mg of TiO_2NPs were dispersed into 5.0 mL of 1.0 mg mL^{-1} PDDA solution. The mixture was sonicated for 3 h to obtain a homogeneous suspension. PGE was dipped into this composite for 2 h. To prepare the DNA–modified electrode, the modified PGE (PDDA–TiO_2NPs–MWCNTs/PGE) was immersed into the ds-DNA solution (1.0 mg mL^{-1} buffered with Tris–HCl, pH 7.0) for approximately 30 min and allowed to dry for 1 h at room temperature. This modified PGE was designated as ds-DNA/PDDA–TiO_2NPs–MWCNTs/PGE.

2.5. Interaction between ds-DNA and atropine sulfate at the ds-DNA modified electrode

In order to investigate the interaction between atropine sulfate and ds-DNA, the PDDA–TiO_2NPs–MWCNTs/PGE and/or ds-DNA/PDDA–TiO_2NPs–MWCNTs/PGE was initially placed in a quiescent solution (20 mL) of 0.5 mol L^{-1} acetate buffer containing 0.02 mol L^{-1} NaCl (pH 4.8). The DP voltammograms were recorded to get the blank signals of the electrodes. Then, the PDDA–TiO_2NPs–MWCNTs/PGE and/or ds-DNA/PDDA–TiO_2NPs–MWCNTs/PGE was immersed into the Tris-buffer solution (pH 7.0) containing different concentrations of atropine sulfate while the solution was stirred at 200 rpm for 25 min in an open circuit system. After accumulation of atropine sulfate, the ds-DNA/PDDA–TiO_2NPs–MWCNTs/PGE was rinsed and placed in the acetate buffer solution (pH 4.8) containing 0.02 mol L^{-1} NaCl. Finally, differential pulse voltammograms were recorded to get the sample signals.

2.6. Preparation of real samples

Written informed consent was obtained from all participants prior to the collection of the blood and urine samples.

Blood serum and urine samples were obtained from hospitalized patients, stored at −20 °C pending analysis, and allowed to thaw at ambient temperature prior to use. Different amounts of atropine sulfate were spiked into the samples before the treatment. Acetonitrile was added to the samples, to separate any present proteins, before they were centrifuged and filtered. An amount of 2.0 mL of the filtered solution was mixed thoroughly with 8.0 mL of the Tris-buffer solution. The atropine sulfate content was measured according to the recommended procedure.

3. Results and discussion

3.1. SEM characterization

The surface topographies of the stepwise DNA biosensor fabrication processes were investigated using SEM (Fig. 1). The surface roughness of the unmodified PGE can be clearly seen in Fig. 1A, in which the graphite layers are also seen. The electrode coverage morphology also reveals that the effective surface area of the modified working electrodes is significantly increased as a result of using highly conductive MWCNTs and TiO_2NPs, which were well dispersed in the PDDA solution (Fig. 1B). The surface morphology, however, changed when ds-DNA was immobilized on the surface of MWCNTs–TiO_2NPs–PDDA/PGE (Fig. 1C). **"Here Fig. 1"**

3.2. Electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) is a valuable technique to investigate the interfacial characterization of an electrode surface, such as charge transfer resistance (R_{ct}) during the oxidation process. The value of the electron transfer resistance (semicircle diameter) depends on the dielectric and insulating features at the electrode–electrolyte interface. An increase in R_{ct} value indicates a resistance or hindrance of electron flow due to the addition of a substance on the surface of the electrode, which leads to an increase in R_{ct} value. A solution containing 5.0 mmol L^{-1} $Fe(CN)_6^{3-/4-}$ in 0.1 mol L^{-1} KNO_3 (pH 4.0, phosphate buffer) was used as a probe. Fig. 2 displays Nyquist plot of the imaginary impedance (Z_{im}) *vs.* the real impedance (Z_{re}) of the EIS obtained from an unmodified–PGE (curve a), PDDA–MWCNTs–TiO_2NPs/PGE (curve b) and dsDNA/PDDA–MWCNTs–TiO_2NPs/PGE (curve c). As shown in Fig. 2 (curve b), the R_{ct} (the diameter of the semicircle) decreases after modification of PGE by PDDA–MWCNTs–TiO_2NPs, which is due to the acceleration of electron transfer. It was found that the impedance of the electrode drastically decreased in the presence of PDDA–MWCNTs–TiO_2NPs. It may be due to promotion of electron transfer rate between $[Fe(CN)_6]^{3-/4-}$ and the electrode surface. Furthermore, as shown in Fig. 2 (curve c) by addition of ds-DNA at the surface of PDDA–MWCNTs–TiO_2NPs, the amount of R_{ct} was increased, because the DNA reduces conductivity of PDDA–MWCNTs–TiO_2NPs and therefore the R_{ct} increases. **"Here Fig. 2"**

3.3. Interaction between ds-DNA and atropine sulfate at ds-DNA/PDDA–TiO_2NPs–MWCNTs/PGE

DPV oxidation signals of guanine and adenine at the ds-DNA/PDDA–TiO_2NPs–MWCNTs/PGE before and after interaction with atropine sulfate are shown in Fig. 3. Different concentrations of atropine sulfate (0 to 600 μmol L^{-1}) were used to interact with

guanine and adenine at the surface of ds-DNA/PDDA–TiO_2NPs–MWCNTs/PGE. It was found that the oxidation peaks current decreased with increasing atropine sulfate concentration. To investigate the electroactivity of atropine sulfate, the DNA free modified–PGE (PDDA–TiO_2NPs–MWCNTs/PGE) and/or unmodified–PGE were immersed into a 100.0 μmol L^{-1} of atropine sulfate solution while the solution was being stirred at 200 rpm for 25 min in an open circuit. No remarkable oxidation signal of atropine sulfate was obtained at the PDDA–TiO_2NPs–MWCNTs/PGE and/or unmodified–PGE in the potential range of +0.40 and +1.40 V (dash lines, Fig. 3). Thus, it was concluded that atropine sulfate was not able to interact with the electrodes surface to can produce an oxidation signal. The decreases in the oxidation signals of guanine and adenine bases were attributed to the binding of atropine sulfate to these electroactive DNA bases. This could be explained as a possible damage to, or shielding of, the oxidizable groups of guanine and adenine bases while atropine sulfate interacted with the ds-DNA at the PGE surface. The results obtained showed that ds-DNA modified PGE might be used for the detection of atropine sulfate interaction with the ds-DNA. **"Here Fig. 3"**

3.4. UV–Vis spectrophotometric study for the interaction of atropine sulfate and ds-DNA

UV–vis absorption spectrophotometry is a technique generally employed to investigate drug–DNA interactions. The UV–vis absorption peak positions of atropine sulfate are around at 275 and 390 nm (Fig. 4b), and that of ds-DNA is at 285 nm (Fig. 4a). As is evident from Fig. 4c, the absorbance of atropine sulfate decreases at 390 nm and vanishes at 275 nm as a result of adding ds-DNA. These results reveal that there is a strong interaction between atropine sulfate and the ds-DNA. The intercalative binding of molecules to the ds-DNA helix has been characterized by an appreciable shift in wavelength (red shift ≥15 nm) due to the interaction of a ds-DNA π stack with the ligand,

while the outside binders (groove binders) display a smaller red shift (red shift ≤ 8 nm) [33]. The red shift for absorption peaks at 390 nm is about 40 nm. UV–vis spectrophotometry combined with DPV is used to propose the most plausible mechanism for the interaction between atropine sulfate and ds-DNA. The decrease in the UV–vis absorption spectrum of atropine sulfate after interaction with ds-DNA, and the decrease in the DPV oxidation signal intensity of ds-DNA at PGE after interaction with atropine sulfate provide potent evidence for possible intercalation of atropine sulfate.**"Here Fig. 4"**

3.5. Effect of ds-DNA concentration on immobilization at PGE surface

The DNA biosensor was prepared by immobilizing salmon sperm ds-DNA on the PGE surface (Fig. 5). A linear relationship was observed between ds-DNA concentration and DVP signals of guanine and adenine bases in the concentration range of 0.05 to 1.0 mg mL^{-1}, beyond which it leveled off. Therefore, 1.0 mg mL^{-1} of ds-DNA was selected and used in all the further experiments.

"Here Fig. 5"

3.6. Effect of accumulation time on immobilization of ds-DNA at the PGE surface

One important parameter for the immobilization of ds-DNA at an electrode surface is the accumulation time. It can be seen in Fig. 6 that the oxidation peaks current of guanine and adenine bases (of ds-DNA) on the ds-DNA/PDDA–TiO_2NPs–MWCNTs/PGE surface increased up to 30 min before they leveled off over longer times. At this accumulation time, the sites on the film surface were filled with the ds-DNA such that no more ds-DNA diffused onto the film surface. Therefore, 30 min was considered as an optimum accumulation time of ds-DNA for preparing the ds-DNA/PDDA–TiO_2NPs–MWCNTs/PGE. **"Here Fig. 6"**

3.7. Effect of incubation time of atropine sulfate on guanine and adenine signals

Binding of atropine sulfate to ds-DNA depends on their incubation time. Therefore, the incubation time for the interaction of atropine sulfate with the ds-DNA/PDDA–TiO_2NPs–MWCNTs/PGE surface was optimized. For this purpose, electrochemical detection was obtained and assessed for an incubation time of atropine sulfate in the range of 5–40 min. The results (Fig. 7) showed dramatic decreases in the oxidation signals of guanine and adenine with increasing interaction time up to 25 min beyond, which the signals almost leveled off for longer incubation times. Accordingly, 25 min was selected as an optimum time for the interaction of atropine sulfate with the ds-DNA/PDDA–TiO_2NPs–MWCNTs/PGE. **"Here Fig. 7"**

4. Figures of merit

DPV was used at a pulse amplitude of 50 mV, a pulse width of 50.0 ms, and a scan rate of 10 mV s^{-1}. A linear dependency was observed between the analytical signals (considered as the decrease in guanine and adenine signals before and after interaction with atropine sulfate) and atropine sulfate concentrations. Using DPV and following the changes in the oxidation signal intensity of guanine after interaction with atropine sulfate concentration, the linear dependency was detected in the two regions in the ranges of 0.6 – 30.0 and 30.0 – 600.0 μmol L^{-1}. The regression equations were $I(\mu A) = 0.2054(\pm 0.0028)C_{Atropine} + 0.0594(\pm 0.0364)$ with $R^2 = 0.9982$ and $I(\mu A) = 0.0043(\pm 0.0004)C_{Atropine} + 6.1364(\pm 0.1251)$ with $R^2 = 0.9952$, respectively, where C is concentration of atropine sulfate in μmol L^{-1}.

The detection limit ($3S_b/m$, three times of the standard deviation of the blank divided by the slope of the calibration curve) and the limit of quantitation ($10S_b/m$) of the proposed method were found to be 0.03 and 0.10 μmol L^{-1} of atropine sulfate. Fig. 8 shows the

calibration curve obtained by plotting the changes in the oxidation signal of guanine after interaction of atropine with the sulfate of ds-DNA/PDDA–TiO_2NPs–MWCNTs/PGE with respect to atropine sulfate concentration. **"Here Fig. 8"**

To check the reproducibility of the biosensor, changes in the oxidation peak current of guanine after interaction of ds-DNA/PDDA–TiO_2NPs–MWCNTs/PGE with atropine sulfate (at concentrations of 1.0 and 100.0 μmol L^{-1}) were measured by repeating the tests within several days using the same biosensor. Between the measurements, the sensor was stored in dry conditions in an open air at room temperature. The biosensor's stability was tested during a period of 20 days by measuring guanine signals every 4-day and characterized by relative standard deviation (RSD%) values of the guanine peak currents. The RSD values for guanine signals after interaction with 1.0 μmol L^{-1} atropine sulfate on interval times of 4-day were recorded as 3.9%, 3.1%, 3.6%, 4.1%, and 3.5% (n=5) and with an atropine sulfate concentration of 100.0 μmol L^{-1}, they were 4.2%, 3.4%, 3.8%, 3.3% and 3.7% (n=5).

5. Interference study

Interference studies were carried out with several species prior to the application of the proposed method for the assay of atropine sulfate in real samples. Potential interfering substances were chosen from the group of substances commonly found with atropine sulfate in pharmaceuticals and biological fluids. Tolerance limit was defined as the maximum concentration of the potential interfering substance causing an error less than 5% in the determination of 20.0 μmol L^{-1} atropine sulfate. The atropine sulfate was measured in the absence and presence of the potential interfering compounds, based on the recommended procedure. A substance was considered not to interfere if the variation in the peak current of atropine sulfate was less than 5%. The results are shown in Fig. 9 and are

also presented in Table 1, indicating the high selectivity of the proposed method for atropine sulfate determination. **"Here Table 1"**

6. Analytical performance

In order to evaluate the performance of the proposed method for real sample analysis, blood serum and urine samples were used for atropine sulfate analysis after preparation as described above. Five ds-DNA/PDDA–TiO_2NPs–MWCNTs/PGEs were also prepared to determine the recovery of atropine sulfate in blood serum and urine samples. The oxidation signals of guanine and adenine were obtained using DPV at the optimized conditions. A new ds-DNA/PDDA–TiO_2NPs–MWCNTs/PGE was prepared, and immersed in the real sample solutions containing atropine sulfate and stirred at 200 rpm for 25 min in an open circuit. The differential pulse voltammogram was then recorded. The peaks current of guanine and adenine were compared before and after interaction with the samples containing atropine sulfate to obtain the analytical signal. The results are given in Table 2. In addition, the accuracy of the biosensor was checked using a standard HPLC method. Student t-test and F-test were used to evaluate the accuracy and precision of the proposed method. As can be seen in Table 2, the biosensor exhibited a good accuracy and precision for the determination of atropine sulfate.

"Here Table 2"

7. Conclusion

In the present work, a sensitive DNA–biosensor was developed for the determination of atropine sulfate. The interaction of atropine sulfate with ds-DNA was initially investigated using electrochemical and UV–vis spectroscopy. The decreases observed in the intensities of the oxidation signals of guanine and adenine before and after interaction with atropine sulfate were used for the determination of atropine sulfate. PDDA as a

polycation and MWCNTs plus TiO_2NPs of small sizes provide a surface with positive charges and a high surface area for the immobilization of ds-DNA as a polyanion. Using the ds-DNA/PDDA–TiO_2NPs–MWCNTs/PG electrode, we were able to detect the interaction of atropine sulfate with ds-DNA, which allowed us to apply the DNA–modified electrode for the determination of atropine sulfate. The advantages of the proposed method are low limit of detection, wide linear dynamic range, ease of application, sensitivity and its selectivity.

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Table 1. Interference study for determination of 20.0 µmol L^{-1} atropine sulfate.

Species	Tolerance limit (w/w)
Lactose, Fructose, Glucose, Sucrose, Methanol, Ethanol, Ca^{2+}, Mg^{2+}, NH_4^+, Ascorbic acid	500*
Citric acid	250
Urea, Uric acid	100

*maximum ratio checked.

Table 2. Recovery of atropine sulfate in blood serum and urine samples (n = 5).

Sample	Sample No.	Atropine added (μmol L^{-1})	Found by biosensor* (μmol L^{-1})	Recovery (%)	Found by HPLC* (μmol L^{-1})	$t_{calculated}$**	$F_{calculated}$**
Blood serum	1	---	<Detection limit	---	---	---	---
	2	3.00	2.90±0.15	96.7	2.95±0.19	1.02	2.98
	3	8.00	7.82±0.40	97.7	7.89±0.48	1.33	3.44
Urine	1	---	<Detection limit	---	---	---	---
	2	6.00	5.88±0.22	98.0	5.82±0.31	1.24	3.20
	3	10.00	9.60±0.69	96.0	9.67±0.63	1.49	3.65

*average of five replicate measurements.

**theoretical value of t-student test = 2.78 (for n = 5, p = 0.05) and theoretical value for F-test = 6.39 (for n = 5, p = 0.05).

Figures caption:

Figure 1. SEM images of (A) unmodified-PGE, (B) PDDA-MWCNTs-TiO_2NPs/PGE and (C) ds-DNA/PDDA-MWCNTs-TiO_2NPs/PGE.

Figure 2. Impedance spectra of a) bare PGE; b) PDDA-MWCNTs-TiO_2NPs/PGE; and c) dsDNA/PDDA-MWCNTs-TiO_2NPs/PGE in 5.0 mmol L^{-1} $Fe(CN)_6^{3-/4-}$ containing 0.10 mol L^{-1} KCl.

Figure 3. Differential pulse voltammograms for the interaction of atropine sulfate with ds-ds-DNA/PDDA-MWCNTs-TiO_2NPs/PGE; Oxidation signals of guanine and adenine after interaction with atropine sulfate at the surface of ds-DNA/PDDA-MWCNTs-TiO_2NPs/PGE (from up to down) 0.0, 0.6, 0.8, 1.0, 3.0, 6.0, 8.0, 10.0, 20.0, 30.0, 60.0, 80.0, 100.0, 300.0, 500.0 and 600.0 μmol L^{-1} atropine sulfate. Dash lines show the unmodified-PGE and PDDA-MWCNTs-TiO_2NPs/PGE signal in 100.0 μmol L^{-1} atropine sulfate (pH 4.8). Conditions: scanning potential between +0.40 and +1.40 V in acetate buffer (pH 4.8).

Figure 4. UV–vis spectrogram of 100.0 μmol L^{-1} atropine sulfate before (a) and after (c) the interaction with 1.0 mg mL^{-1} ds-DNA; and (b) ds-DNA (1.0 mg mL^{-1}).

Figure 5. Effect of the ds-DNA concentration on the guanine and adenine oxidation signals at the surface of ds-DNA/PDDA-MWCNTs-TiO_2NPs/PGE. Conditions are as in Fig. 3.

Figure 6. Effect of accumulation time for immobilization of ds-DNA at PDDA-MWCNTs-TiO_2NPs/PGE on the guanine and adenine oxidation signals. Conditions are as in Fig. 3.

Figure 7. Effect of incubation time of atropine sulfate at ds-DNA/PDDA-MWCNTs-TiO_2NPs/PGE surface on the guanine and adenine oxidation signals. Conditions are as in Fig. 3.

Figure 8. Calibration curve for determination of atropine sulfate based on the changes of the oxidation signal of guanine after interaction with atropine sulfate. Conditions are as in Fig. 3.

Figure 9. A): Differential pulse voltammograms of 20.0 μmol L^{-1} atropine sulfate (alone) and the atropine sulfate (APS) containing (from up to down) 10.0 mmol L^{-1} Mg^{2+}, 10.0 mmol L^{-1} Ca^{2+}, 10.0 mmol L^{-1} ethanol, 10.0 mmol L^{-1} methanol, 10.0 mmol L^{-1} ascorbic acid, 5.0 mmol L^{-1} citric acid, 2.0 mmol L^{-1} uric acid, 10.0 mmol L^{-1} NH_4^+ and 2.0 mmol L^{-1} urea. B): Schematic diagram of the selectivity study. Conditions are as in Fig. 3 in acetate buffer (pH 4.8).

Highlights

→ A biosensor was developed based on ds-DNA-modified pencil graphite electrode.

→ The new biosensor was used for the determination of atropine using voltammetry.

→ MWCNTs and titanium dioxide nano particles were used to modified the graphite electrode.

→ Decreasing in the intensities of the guanine and adenine oxidation signals were used as probes.

→ Atropine could be detected as low as 30.0 nM based on guanine oxidation signal.

Figure 1

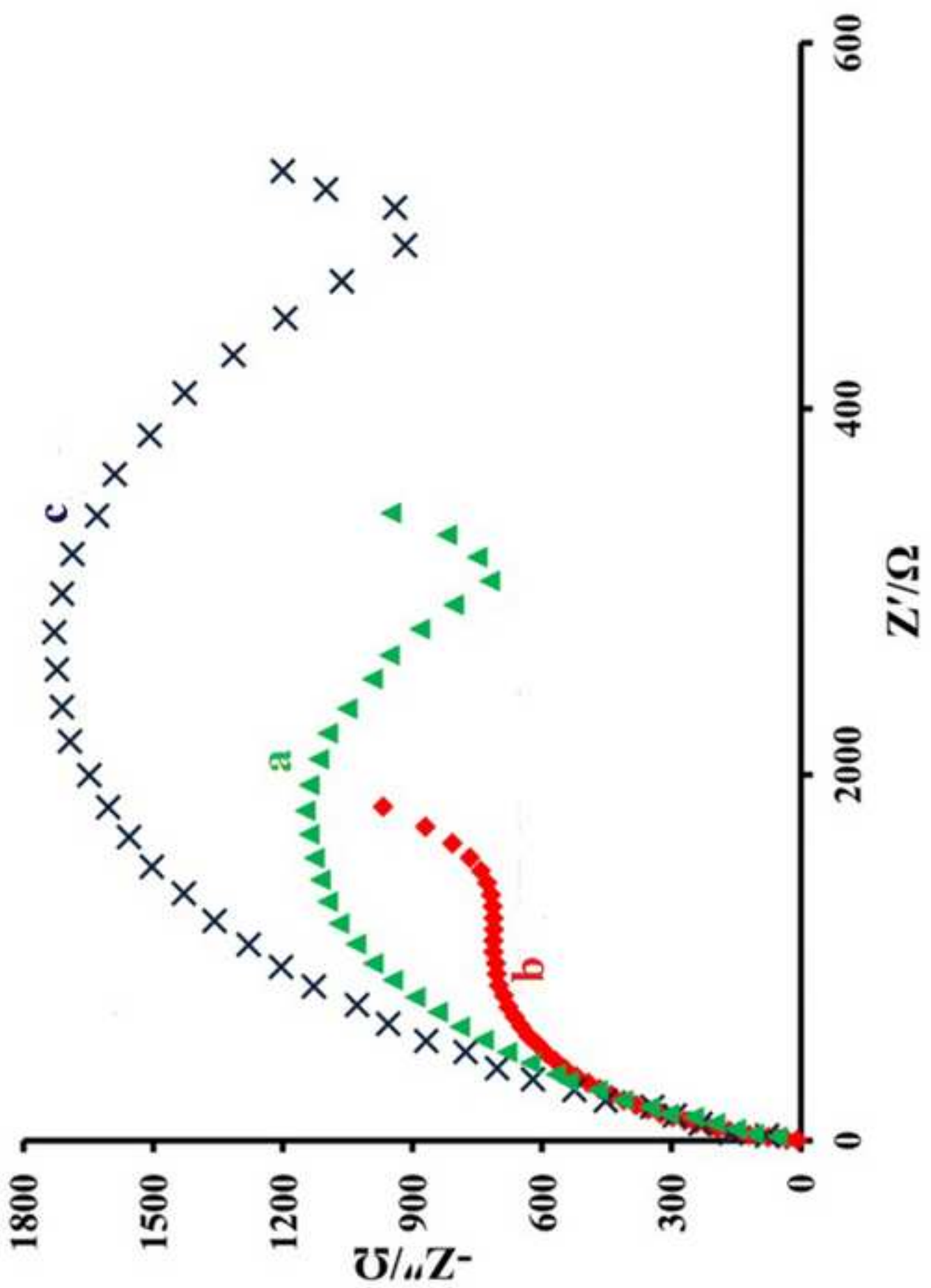


Figure 2

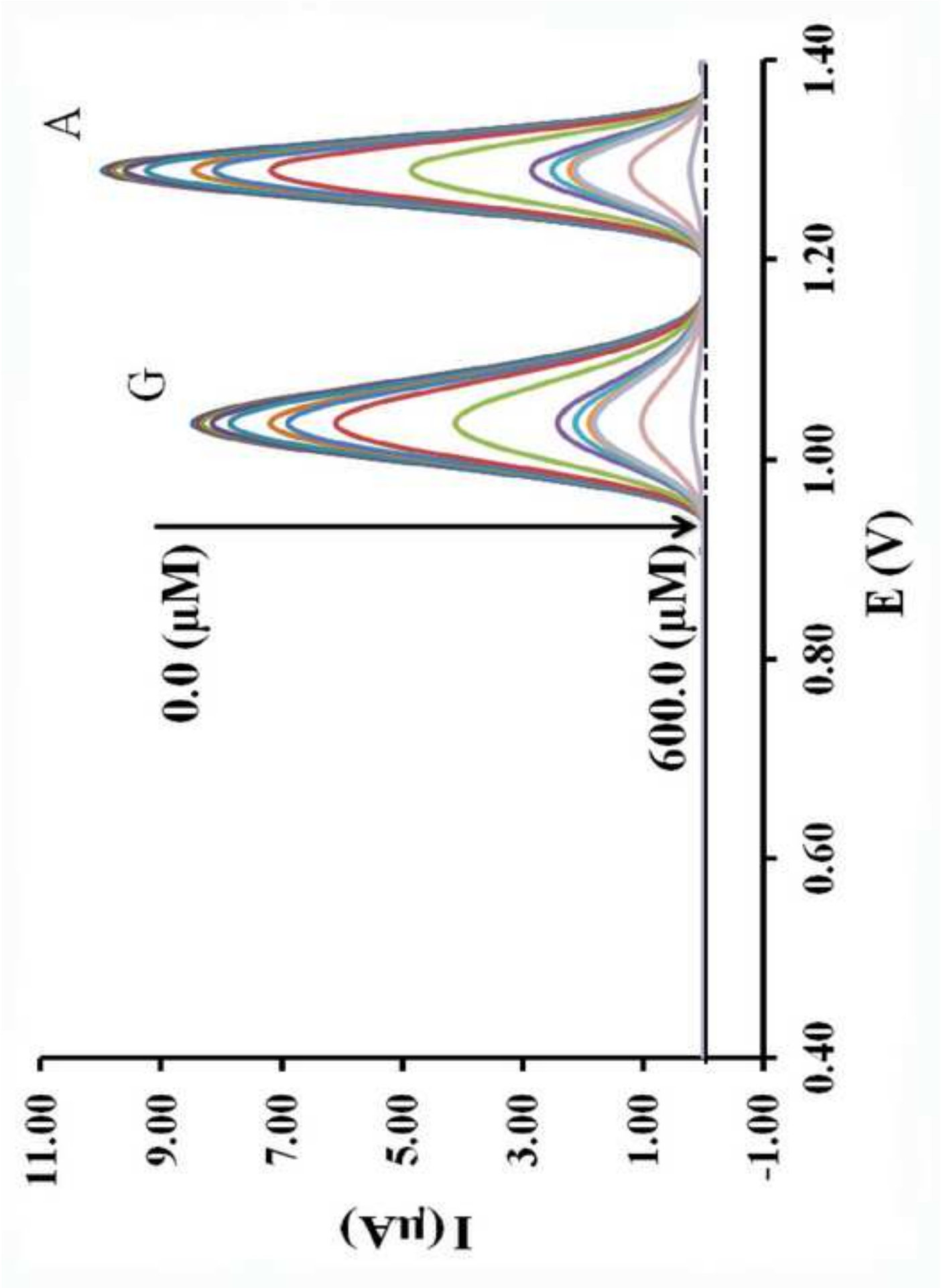

A
G
0.0 (μM)
600.0 (μM)
I (μA)
E (V)
11.00
9.00
7.00
5.00
3.00
1.00
-1.00
0.40
0.60
0.80
1.00
1.20
1.40

Figure 3

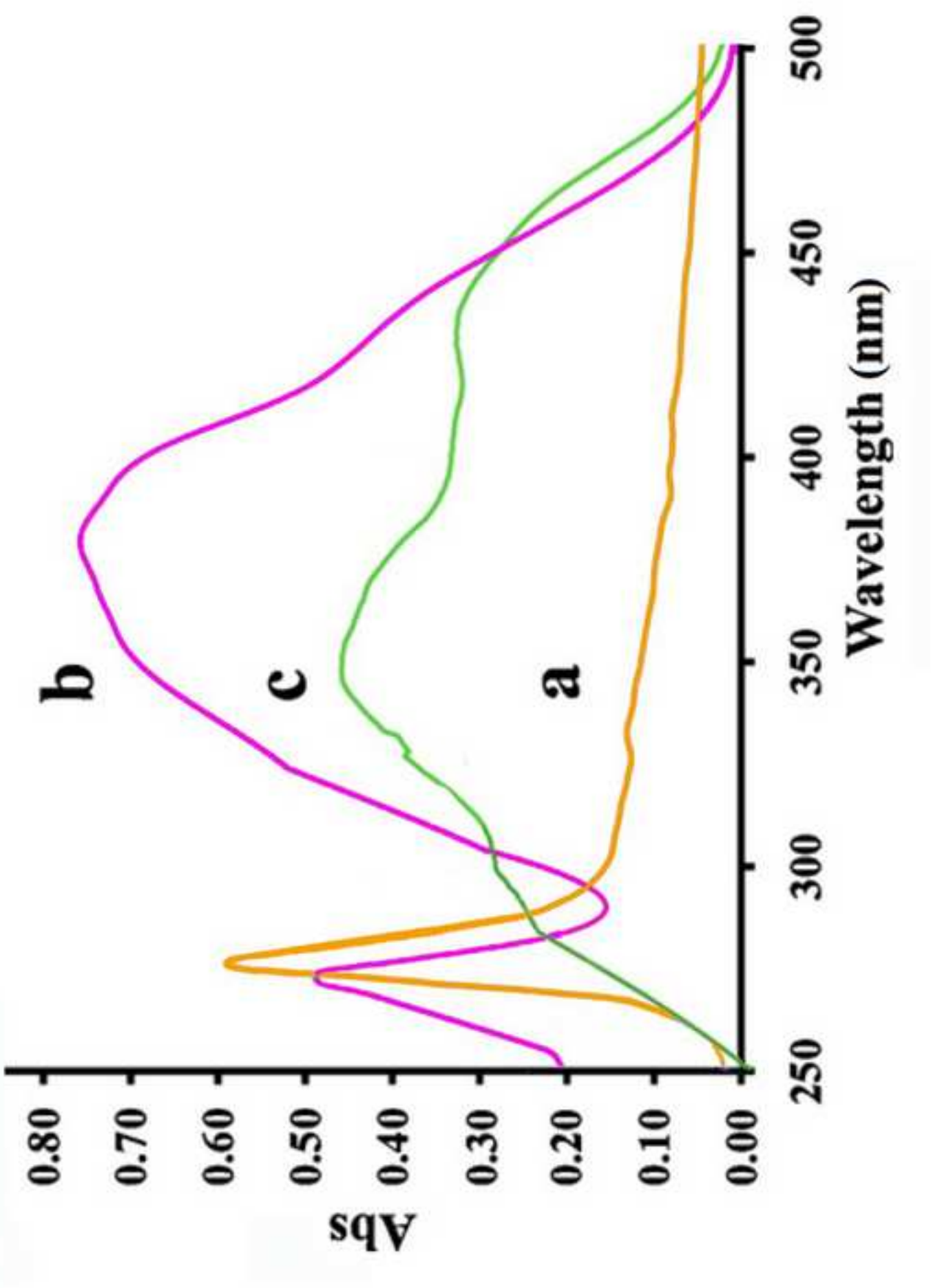


Figure 4

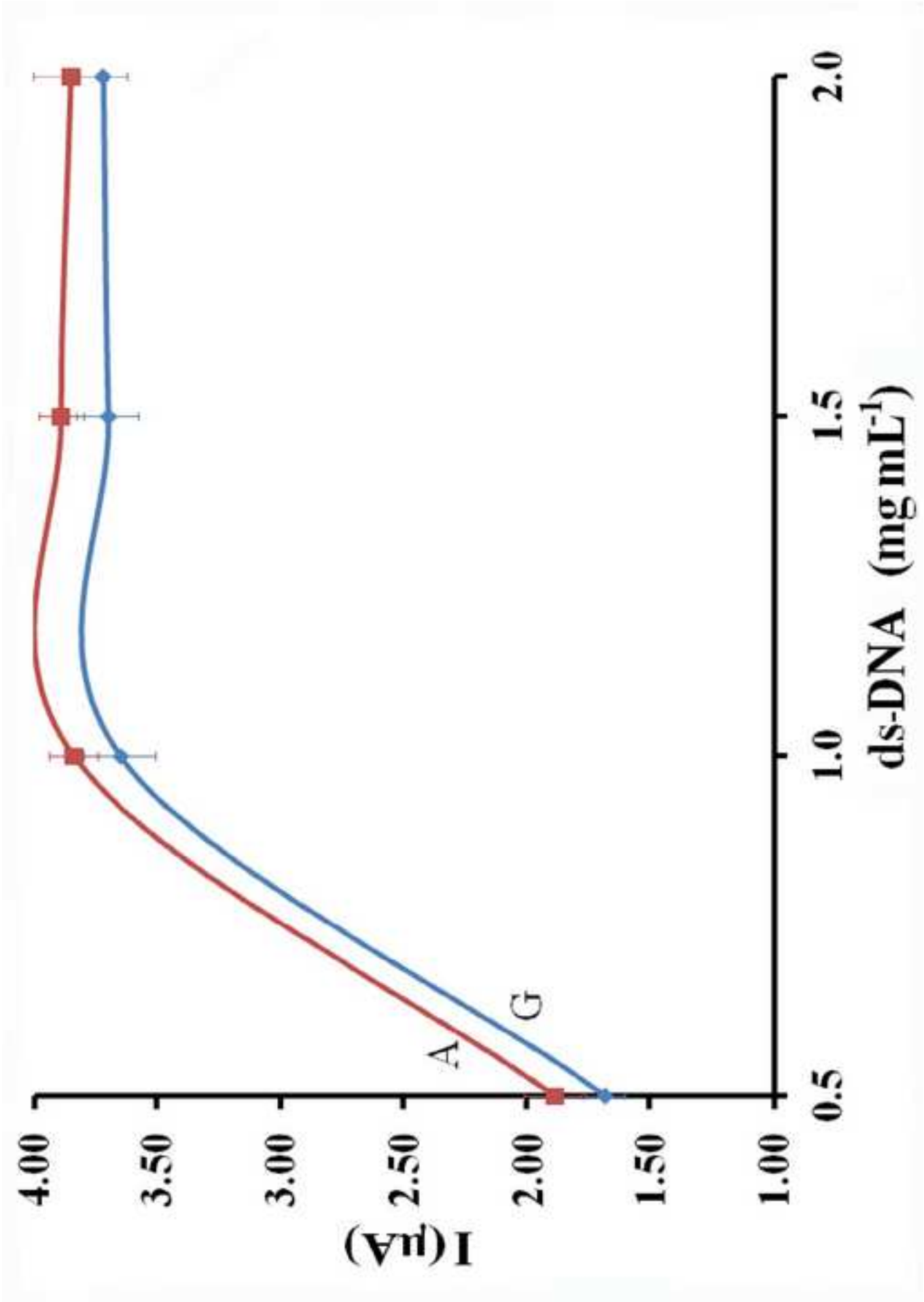


Figure 5

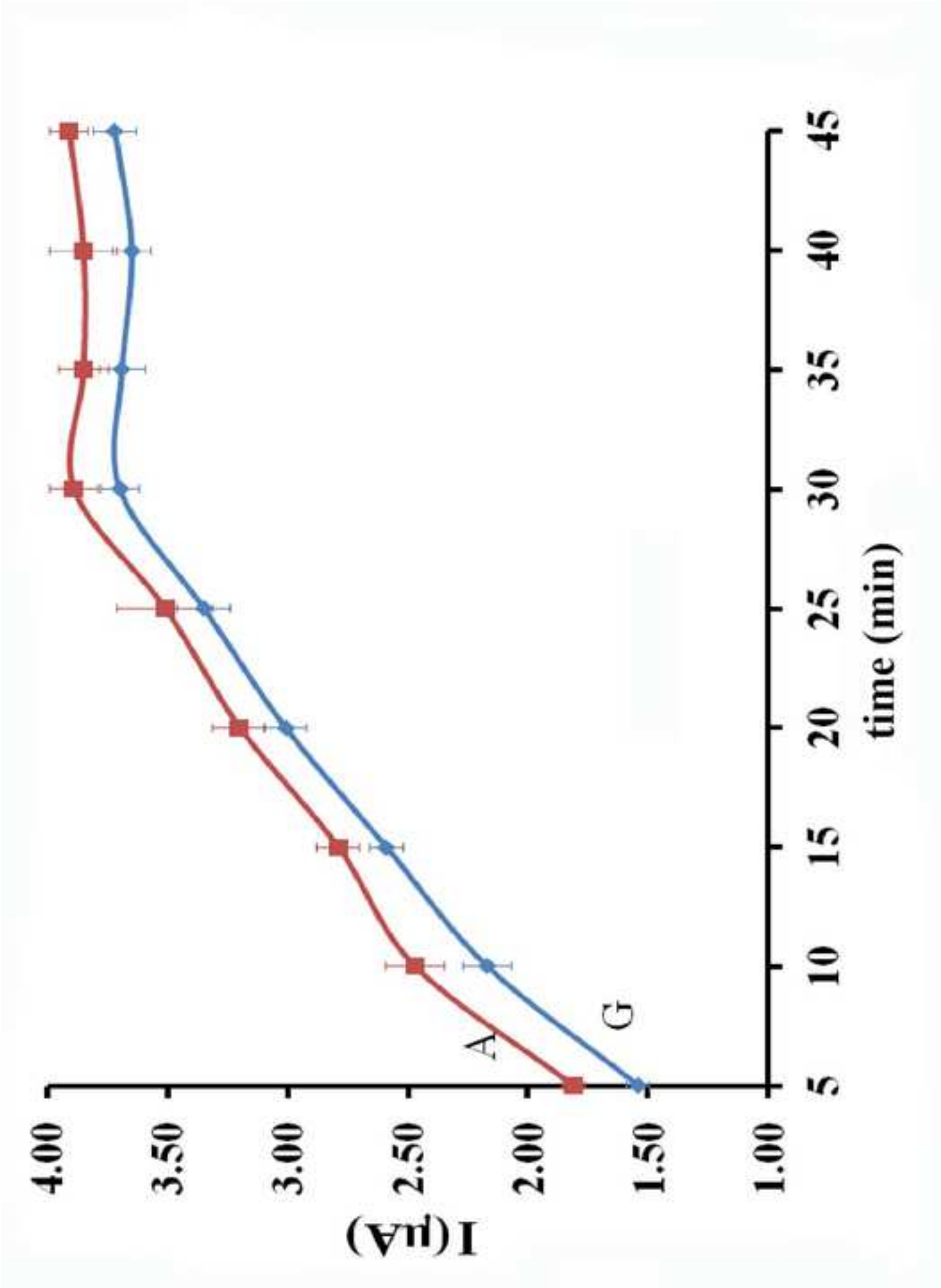

I (μA)
4.00
3.50
3.00
2.50
2.00
1.50
1.00
5
10
15
20
25
30
35
40
45
time (min)
A
G

Figure 6

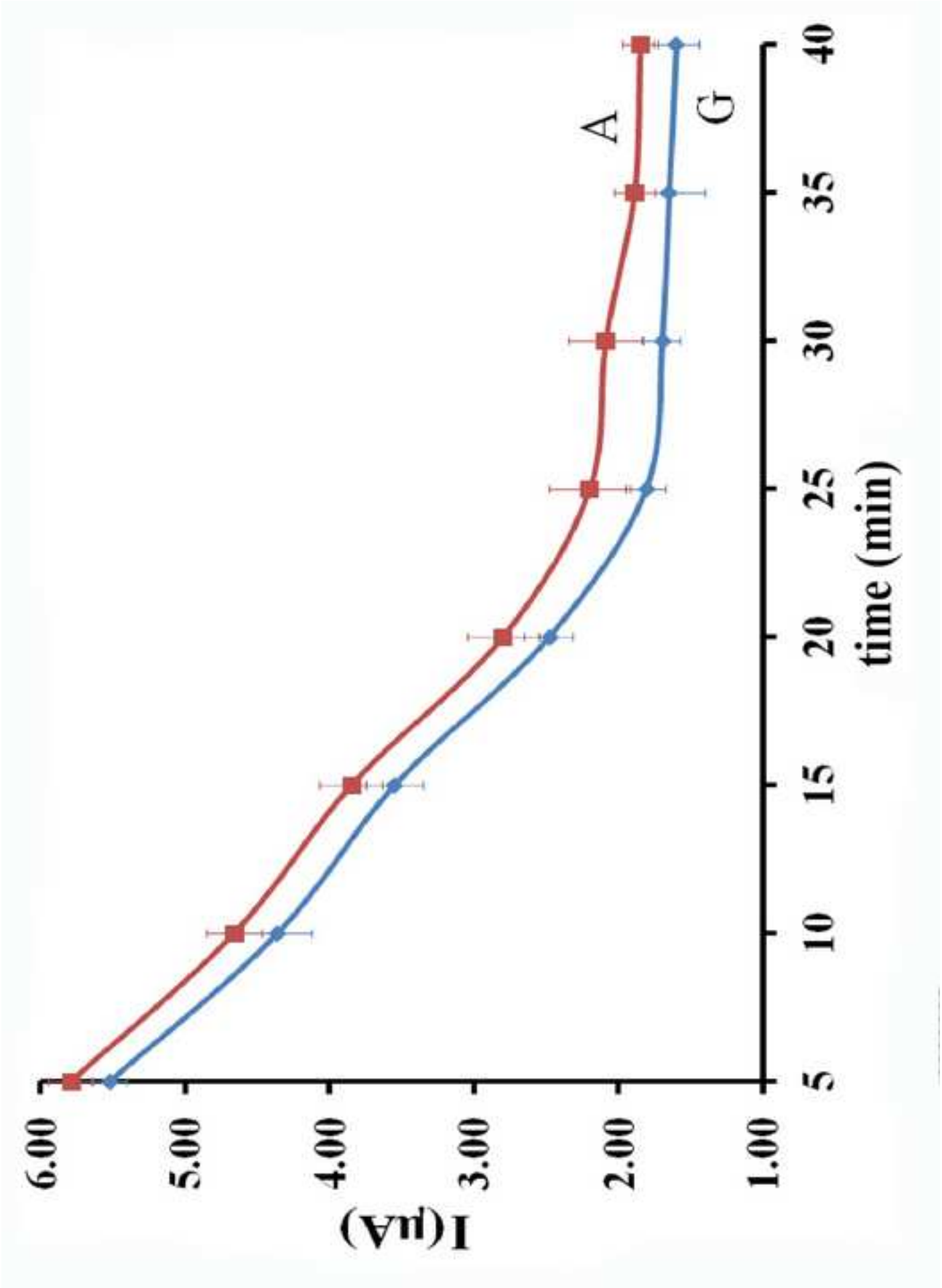


Figure 7

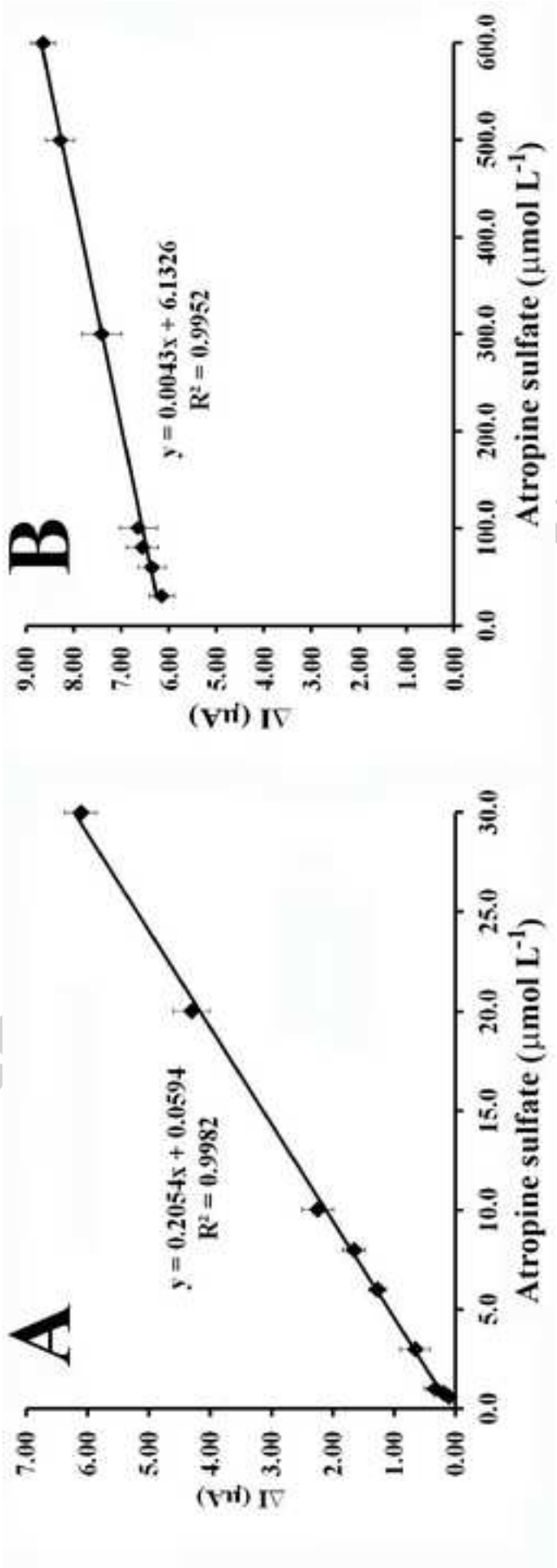
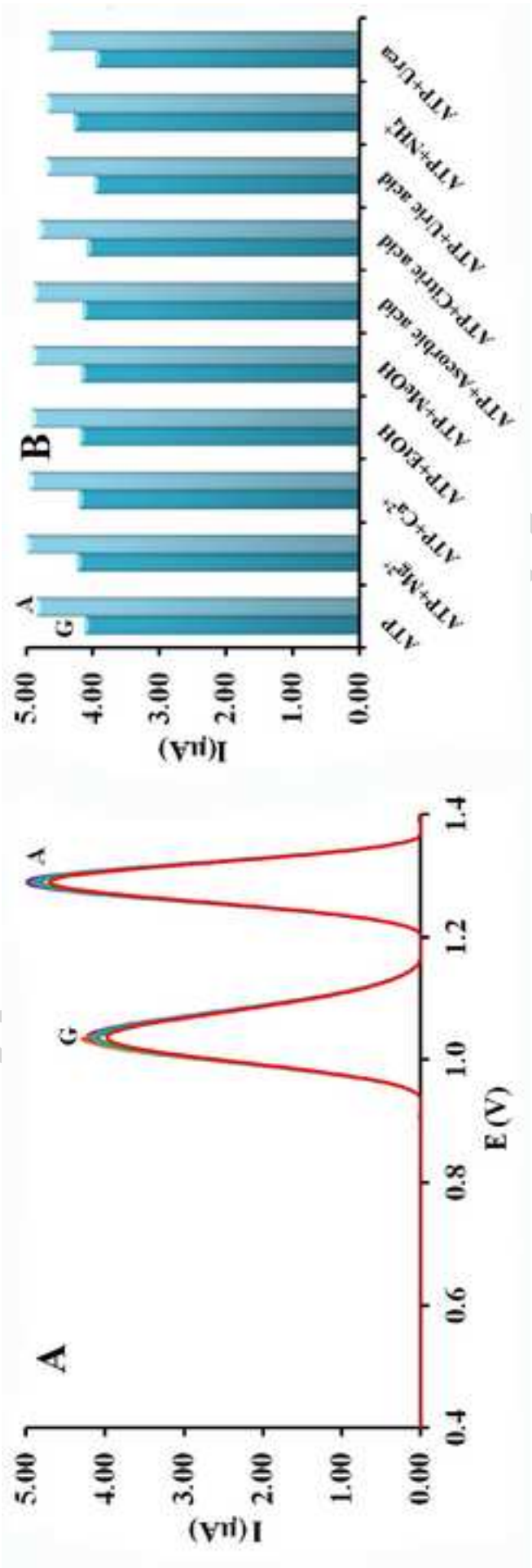


Figure 8


A
G
A
I (μA)
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4.00
3.00
2.00
1.00
0.00
0.4
0.6
0.8
1.0
1.2
1.4
E (V)
B
G
A
I(μA)
5.00
4.00
3.00
2.00
1.00
0.00
ATP
ATP+Mg2+
ATP+Ca2+
ATP+EtOH
ATP+MeOH
ATP+Ascorbic acid
ATP+Citric acid
ATP+Uric acid
ATP+NH4+
ATP+Urea

Figure 9

A pencil graphite electrode (PGE) modified with MWCNTs, titanium dioxide nano particles and poly dialyldimethylammonium chloride decorated with the ds-DNA were used in order to determine atropine sulfate. The electrochemical oxidation of atropine sulfate bonded on the modified biosensor was used to obtain the analytical signal.