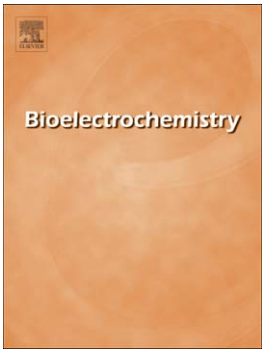


Electrochemical investigation of interaction between topotecan and DNA at disposable graphite electrodes

Gulsah Congur, Arzum Erdem, Fehmi Mese

PII: S1567-5394(14)00189-3
DOI: doi: 10.1016/j.bioelechem.2014.11.003
Reference: BIOJEC 6804

To appear in: *Bioelectrochemistry*

Received date: 13 April 2014
Revised date: 9 November 2014
Accepted date: 16 November 2014

Please cite this article as: Gulsah Congur, Arzum Erdem, Fehmi Mese, Electrochemical investigation of interaction between topotecan and DNA at disposable graphite electrodes, *Bioelectrochemistry* (2014), doi: 10.1016/j.bioelechem.2014.11.003

ELECTROCHEMICAL INVESTIGATION OF INTERACTION BETWEEN TOPOTECAN AND DNA AT DISPOSABLE GRAPHITE ELECTRODES

Gulsah Congur, Arzum Erdem* and Fehmi Mese

Ege University, Faculty of Pharmacy, Analytical Chemistry Department,
35100 Bornova, Izmir, Turkey

***Corresponding author (A. Erdem):** Tel.: +90 232 311 5131; fax: +90 232 388 5258.
E-mail address: arzum.erdem@ege.edu.tr and arzume@hotmail.com

Abstract

Topotecan (TPT) is a semisynthetic, water soluble analog of the plant alkaloid camptothecin which has been widely used for the treatment of ovarian and cervical cancers. To obtain better understanding how it can affect DNA structure, electrochemical biosensor platforms for investigation of TPT-double stranded DNA (dsDNA) interaction were developed for the first time in this study. The electrochemical detection of TPT, and TPT-dsDNA interaction were investigated at the surface of pencil graphite electrodes (PGEs) and single-walled carbon nanotubes (SWCNTs) modified PGEs by using differential pulse voltammetry (DPV). The changes at the oxidation signals of TPT and guanine were evaluated before/after each modification/immobilization step. An enhanced sensor response was obtained by using SWCNT-PGEs compared to unmodified PGEs with resulting limits of detection (LODs) for TPT as 0.51 µg/mL, 0.45 µg/mL, 0.37 µg/mL (130 pmol, 117 pmol, 96.5 pmol in 110 µL sample, respectively) by using electrochemically pretreated PGE, unmodified PGE and SWCNT modified PGE. In addition, electrochemical impedance spectroscopy (EIS) measurements were performed for the purpose of modification of PGEs by using SWCNTs and the interaction process at the surface of SWCNT-PGEs by evaluating the changes at the charge transfer resistance (R_{ct}).

Keywords: Topotecan, Drug-DNA interactions, Pencil graphite electrode, Single walled carbon nanotubes, Differential pulse voltammetry, Electrochemical impedance spectroscopy.

1. Introduction

Nucleic acids recognition technologies combined with electrochemical transducers have been started to be applied in diagnostic areas after the discovery of electroactivity of nucleic acids by Palecek [1]. To understand interaction between DNA binding (bio)molecules (i.e., anticancer drugs) and DNA has a vital importance and there are some progress on design DNA biosensors developed for DNA interactions [2-9]. There are several interactions mechanisms but binding of small molecules to DNA primarily occurs in three modes: *(i)* electrostatic interactions of molecule with the negative-charged sugar-phosphate backbone DNA, *(ii)* binding with two grooves of double helix structure of DNA, and *(iii)* intercalation between the stacked base pairs of DNA [10].

Topotecan ((*S*)-10-[(dimethylamino)methyl]-4-ethyl-4,9-dihydroxy-1*H*-pyrano[3',4',:6,7]indolinizo[1,2,*b*] quinolone-3,14(4*H*,12*H*)-dione monohydrochloride, TPT) is a semisynthetic, water soluble analog of the plant alkaloid camptothecin. There have wide range applications for the treatment of ovarian and cervical cancers. It inhibits specifically the intranuclear enzyme topoisomerase I [11-13], which is resulting in enzyme linked DNA cleavage and single strand breaks [13]. TPT has the planar 5-membered polycyclic ring system and mimics a DNA base pair in the DNA duplex [11]. Thus, TPT intercalates at the site of DNA cleavage, forming base-stacking interactions with both the -1 (upstream) and +1 (downstream) base pairs [11,12]. There have been some reports about determination of TPT which were mostly focused of detection by using as high-performance liquid chromatography (HPLC) [14,15]. Craig et al. [14] studied about the stability and compatibility of topotecan hydrochloride and they prepared the common infusion solutions and containers before analyzing the samples by using HPLC. It was reported that topotecan hydrochloride was stable for up to 24 h at room temperature and for up to 7 days at 5°C in the prepared containers [14]. In another study performed by using reversed-phase HPLC method to determinate the total TPT in both human whole blood and unwashed erythrocytes [15], 80% of topotecan was extracted out of whole blood and consequenlty, a low limit of quantitation in whole blood was found as 0.20 ng/mL. Susloparova and coworkers [16] were investigated the adhesion status of cancer cells at a single cell level by using impedance spectroscopy. For this purpose, topotecan hydrochloride was used and analyzed the effect of this drug onto cancer cells on based open-gate field-effect transistor (FET) devices. It was reported that the whole cell FET chips could be utilized for testing of specifity of novel anti-cancer drugs in cancer studies [16].

Developing novel nanomaterials-based biosensing strategies is the most popular area recently to create more sensitive and selective devices and also miniaturize them to the hand-held devices. For this purpose, carbon nanotubes (CNTs) have been widely used after they had been discovered in 1991 by Iijima [17,18]. They have excellent physical properties such as unique size, shape, electronic and thermal properties which depend on nanotube types; single walled (SWCNTs) or multiple walled (MWCNTs). There have been many reports in the literature about sensing for different molecules by using carbon nanotubes modified biosensors [4,5,19-26]. For example, Manoso et al [27] developed a quartz crystal microbalance (QCM) biosensor modified with multi-walled carbon nanotubes (MWCNTs) for monitoring of volatile organic compounds (VOCs), aldehydes and amines with high sensitivity and the low limit of detection (LOD). In another study, poly(anilineboronic acid)/single walled-carbon nanotube (SWCNT) composite modified gold substrates were prepared for detection of dopamine by Ali and coworkers [28]. The determination of dopamine was performed by using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques. After optimization of experimental conditions, the selectivity of this biosensor was tested in the presence of ascorbic acid by using a nafion film. For electrochemical monitoring of drug-DNA interaction, Erdem et al. [4] performed the

SWCNTs modification onto the surfaces of disposable pencil graphite electrodes (PGEs) and they applied these biosensors for monitoring of interaction of an anticancer drug daunorubicin with double stranded DNA. It was presented that SWCNT provided a higher surface area and enhanced guanine oxidation signal was measured by using differential pulse voltammetry (DPV). Similarly, SWCNT-PGEs were tested to monitor impedimetrically the interaction between cis diamminedichloroplatinum(II) and oxaliplatin with calf thymus double-stranded DNA (ctdsDNA) by Yapasan et al. [5].

The aim of this present study is to investigate firstly the detection of TPT, and the interaction between TPT and double stranded DNA (dsDNA) by using electrochemical techniques in combination with disposable electrodes. The oxidation signals of TPT and guanine were measured in the same voltammetric scale by using PGEs and SWCNTs modified PGEs were used. An enhanced sensor response was obtained by using SWCNT-PGE in comparison to the one obtained by unmodified PGE. Electrochemical impedance spectroscopy (EIS) was also used for the purpose of characterization of the stepwise modification/immobilization process at the surface of PGEs.

2. Experimental

2.1. Apparatus

All experimental measurements were carried by using AUTOLAB – PGSTAT 302 electrochemical analysis system supplied with a FRA 2.0 module for impedance measurements, and GPES 4.9006 software package (Eco Chemie, The Netherlands). For electrochemical measurements, the DPV and EIS were used. The raw datas of voltammetric measurements were also treated using the Savitzky and Golay filter (level 2) of the GPES software, followed by the moving average baseline correction with a "peak width" of 0.03.
The three electrode system was consisted of the PGE, an Ag/AgCl/3 M KCl reference electrode and a platinum wire as the auxiliary electrode. The EIS measurements were performed in the Faraday cage (Eco Chemie, The Netherlands).

2.2. Chemicals

The stock solution of double stranded fish sperm DNA (dsDNA; 2000 µg/mL) were prepared with Tris-EDTA buffer (TE buffer; 10 mM Tris-HCl, 1 mM EDTA, pH=8.0) and kept frozen. The double stranded fish sperm DNA (dsDNA) is obtained from herring sperm (Sigma-Aldrich, catalogue no: D3159). It consists of less than 50 base pairs and defined as crude oligonucleotides. More dilute solutions of dsDNA were prepared with 0.50 M acetate buffer containing 20 mM NaCl (pH=4.8, ABS). Stock solution of TPT (100 µg/mL, Sigma) was prepared in 50 mM phosphate buffer solution containing 20 mM NaCl (pH=7.4, PBS). More diluted solutions of TPT were prepared with PBS (pH=7.4). Single-walled carboxylic acid (80–90%) functionalized carbon nanotubes (SWCNTs, diameter 4 – 5 nm; length 500 – 1500 nm bundles) were purchased from Sigma Aldrich. Other chemicals were in analytical reagent grade and they were supplied from Sigma Aldrich and Merck. All solutions were prepared by using ultrapure water.

2.3. TPT detection and investigation of TPT-dsDNA interaction at the surface of electrochemically pretreated PGEs

The detection of TPT and the interaction between TPT and dsDNA by using electrochemically pretreated PGEs, unmodified PGEs and SWCNT modified PGEs was performed (represented in Scheme 1).

Here Scheme 1

2.3.1. Electrode preparation

A Tombow pencil was used as a holder for the graphite lead. Electrical contact with the lead was obtained by soldering a metallic wire to the metallic part. The pencil was hold vertically with 14 mm of the lead extruded outside (10 mm of which was immersed in the solution). PGEs were pretreated by applying +1.40 V for 30 s in ABS (pH=4.8).

2.3.2. Investigation of the electrochemical behavior of TPT-immobilized electrochemically pretreated PGEs

The PGEs were immersed into the vials containing 110 µL of various concentrations of TPT between 0.05 to 10 µg/mL. The electrodes were then kept in these vials during 30 min followed by passive adsorption procedure [29,30]. The electrodes were washed with PBS (pH=7.4) during 5 s to eliminate unspecific binding.

2.3.3. Detection of TPT-dsDNA interaction by using electrochemically pretreated PGEs

The PGEs were immersed into the vials containing 110 µL of 16 µg/mL dsDNA solution. The electrodes were kept in these vials during 7.5 min followed by passive adsorption procedure

[4,5] and washed into ABS (pH=4.8) for 5 s to inhibit unspecific binding. Then, dsDNA immobilized PGEs were immersed into the vials containing 110 µL of 0.5 µg/mL (equals to 1187 nM) TPT solution by using different interaction times and washed into PBS (pH=7.4) for 5 s.

2.4. TPT detection and investigation of TPT-dsDNA interaction at the surface of SWCNT-PGEs

Preparation of SWCNT solution, PGE modification by using SWCNT and dsDNA immobilization onto SWCNT-PGE surface were performed as previously reported studies [4,5].

2.4.1. Preparation of SWCNT solution

The samples of SWCNT were suspended in organic solvent N,N-dimethylformamide (DMF) and sonicated during an hour in the room temperature.

2.4.2. Preparation of SWCNT modified PGEs

Each unpreated pencil lead was immersed into eppendorf tubes containing 110 µL of 3000 µg/mL SWCNT solution during an hour in order to form a thin SWCNT layer on the surface of electrodes [4,5]. Then, CNT modified PGEs were allowed to dry for 15 min. at upside down position.

2.4.3. Investigation of the electrochemical behavior of TPT-immobilized unmodified (bare) PGEs and TPT immobilized SWCNT-PGEs

The unmodified/SWCNTs modified PGEs were immersed into the vials containing 110 µL of various concentrations of TPT between 0.05 to 10 µg/mL. The electrodes were then kept in these vials during 30 min. followed by passive adsorption procedure [4,5]. The electrodes were washed with PBS (pH=7.4) during 5 s. to eliminate unspesific binding.

2.4.4. Detection of TPT-dsDNA interaction by using SWCNT-PGEs

2.4.4.1. Surface confined interaction of TPT and dsDNA at SWNCT-PGEs

A higher DNA concentration was chosen for immobilization of DNA onto the surface of SWCNT-PGE due to the higher surface area of SWCNT modified PGE [4,5]. The unmodified/SWCNTs modified PGEs were immersed into the vials containing 110 µL of 50 µg/mL dsDNA solution. The electrodes were kept in these vials during 1 h followed by passive adsorption procedure [4,5] and washed into ABS (pH=4.8) for 5 s. Then, dsDNA immobilized PGEs were immersed into the vials containing 110 µL of 0.5 µg/mL TPT solution at required times and washed into PBS (pH=7.4) for 5 s.

2.4.4.2. *Interaction of TPT and dsDNA in solution phase and detection by using SWCNT-PGEs*

50 µg/mL dsDNA solution was immobilized onto the surface of SWCNT-PGE as above. 1 mL 0.5 µg/mL TPT (1187 nM) was prepared in PBS (pH=7.4) and transferred into an electrochemical cell. Then, dsDNA immobilized SWCNT-PGE was transferred into stirring electrochemical cell containing TPT solution and the interaction of TPT with dsDNA was performed during 5 min. The electrodes were then washed with PBS (pH=7.4) and the DPV measurements were done in ABS (pH=4.8).

2.5. Differential pulse voltammetry transduction

The oxidation signals of TPT and guanine were measured by using DPV in ABS (pH=4.8) by scanning from +0.20 to +1.40 V at the pulse amplitude; 50 mV and the scan rate; 50 mV/s.

The raw datas were also treated using the Savitzky and Golay filter (level 2) of the GPES software, followed by the moving average baseline correction with a peak width of 0.03.

2.6. Impedimetric measurements

The EIS measurements were performed in the presence of 2.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture as a redox probe prepared in 0.1 M KCl. The impedance was measured in the frequency range from 100 mHz to 100 kHz in a potential of + 0.23 V versus Ag/AgCl (sat. KCl), with a sinusoidal signal of 10 mV. The frequency interval divided into 98 logarithmically equidistant measure points. The respective semicircle diameter corresponds to the charge-transfer resistance, R_{ct}, the values of which are calculated using the fitting program AUTOLAB 302-FRA, version 4.9006 (Eco Chemie, The Netherlands).

3. Results and discussion

The concentration dependent behaviour of TPT was firstly investigated at the surface of pretreated PGEs by using DPV (Fig. 1). The concentration of TPT was changed between 0.05 and 10 µg/mL (118.7-23740 nM) due to IC_{50} value of TPT was reported as 1-800 nM [31]. The oxidation signal of TPT observed at +0.75 V gradually increased till 2 µg/mL then it levelled off (Fig. 1A and B-f). After three repetitive measurements, the TPT signal at the concentration level of 2 µg/mL was measured as 12.1 ± 1.6 µA (relative standart deviation (RSD) %= 13.1 %). The linear graph based on the TPT signal was shown in Fig. 1B, inset. The limit of detection (LOD) was calculated as 0.51 µg/mL (130 pmol in 110 µL sample) with coefficient of determination (R^2)=0.9947 according to the method describing by Miller and Miller [32]. Although the highest TPT signal was obtained at the concentration level of 2 µg/mL, the effect of TPT towards to the structure of dsDNA was evaluated in the presence of 0.5 µg/mL (1187 nM) TPT due to this concentration was closer to concentration range of IC_{50} value and repeatable TPT signal was obtained at this concentration level (5.9 ±0.9 µA, RSD %= 14.6 %, *n*=3).

Here Fig. 1

Next, the voltammetric detection of interaction of 0.5 µg/mL TPT and 16 µg/mL dsDNA was performed by using electrochemically pretreated PGEs in different interaction times ranging from 5 to 30 min (Fig. 2). The decrease % of TPT and guanine signals were given in Table S1. It was shown that most decrase at guanine signals was obtained by using 5 min as 38.4 % (Fig. 2A-a to B-a). Moreover, TPT had the same behaviour by means of decreasing and the most decrease % was observed by using 5 min interaction time as 80 % (Fig.2A-b to B-b). These results may be attributed that TPT could intercalate the structure of double-stranded DNA, even if interaction was performed during 5 min due to its planar aromatic groups (shown in Scheme S1) [11,12].

Here Fig. 2

In the second part of this study, the interaction between TPT and dsDNA was investigated by using SWCNT-PGEs. For this purpose, TPT at different concentrations ranging from 0.05 to 2 µg/mL was firstly immobilized onto the surfaces of unmodified PGEs and SWCNT modified PGEs, and the voltammetric measurements were then performed (Fig. 3). The highest TPT signals were obtained by using SWCNT-PGEs (Fig. 3B) and the most repeatable TPT signals were obtained by using SWCNT-PGEs compared to ones obtained by using pretreated PGEs, or unmodified PGEs (shown in Table S2). The average TPT signal as 13.5 ± 0.2 µA (RSD %= 1.8 %, *n*=3) was obtained in the presence of 0.5 µg/mL TPT by using SWCNT-PGEs, that was 2-folds higher than the one obtained by unmodified PGEs (i.e., 4.5 ± 0.9 µA, RSD%=20.6 %, *n*=3).The LOD was calculated in linear concentration range varying from 0.05 to 0.2 µg/mL (Fig. S2) and found to be 0.37 µg/mL (96.5 pmol in 110 µL sample) with R^2= 0.9639 according to the method described by Miller and Miller [32]. This LOD value was 0.2-folds lower than the one obtained by unmodified PGEs (shown in Fig. S1; i.e, found to be 0.45 µg/mL and equals to 117 pmol in 110 µL sample).

Here Fig. 3

The surface confined interaction of TPT and dsDNA was then investigated by using unmodified and SWCNT modified PGEs during 5 min (Fig. 4 and 5). TPT and guanine signals were measured by using DPV before/after interaction of 0.5 µg/mL TPT and 50 µg/mL dsDNA was performed. The voltammetric detection of the interaction process was succesfully achieved by using SWCNT-PGEs based on the signal enhancement observed at the signals of TPT and guanine. The average signals of TPT and guanine were respectively found to be 3.3± 0.9 µA (Fig 5B-a) and 4.9 ± 0.9 µA (*n*=3) (Fig. 5B-b) measured at +0.79 V

(Fig. 4B-a) and +1.0 V (Fig. 4B-b) by using SWCNT-PGEs, that were 0.3 and 0.8 folds higher than the ones obtained by unmodified PGEs (Fig. 4,5A-a and b, respectively). These results were consistent with the reports presenting the evaluation of the biomolecular recognitions at the surface of CNT, or graphene oxide modified sensor platforms [4, 5, 33, 34].

Here Fig. 4

Here Fig. 5

Next, the interaction between 0.5 µg/mL TPT and 50 µg/mL dsDNA was performed at the surface of SWCNT-PGEs in different interaction times (Fig. 6). The TPT and guanine signals were evaluated by means of decrease %. The highest decrease % were obtained for TPT (Fig.6A-b to C-b') and guanine signals (Fig.6A-d to C-d') (77.5 % and 26 %, respectively) after 15 min interaction time (Table S3). After three repetitive measurements, the average signals of TPT and guanine were respectively measured as 2165 ± 106.1 nA (RSD %= 4.9 %) and 3675 ±247.5 (RSD %= 6.7 %) after interaction of 0.5 µg/mL TPT and 50 µg/mL dsDNA during 15 min at the surface of SWCNT-PGEs. This result may be attributed that SWCNTs provided a larger surface area, which caused by the requirement of longer duration for adsorption of DNA and drug molecules [4,5].

The interaction of 0.5 µg/mL TPT with 50 µg/mL dsDNA was also investigated in solution phase using SWCNT-PGEs (Fig. S3). The average signals of TPT and guanine were found to be 10.2 ± 2.8 µA (RSD%=27.5%, *n*=3) and 5.6 ±1.5 µA (RSD%=26.7%, *n*=3) respectively before interaction process (Fig. S3-A). There were 42 % and 88 % decreases at the TPT and guanine signals after interaction in the solution phase. Concerning the decrease % at the TPT signal, 5 min interaction time had a similiar behaviour by means of the interaction performed both at the electrode surface and in the solution phase. However, the surface-confined interaction was required a less sample solution (i.e, 110 µL sample) comparison to the interaction performed in solution phase (i.e., required 1 mL sample).

Here Fig. 6

The surface confined interaction between TPT and dsDNA at the surface of SWCNT-PGEs was then investigated by using electrochemical impedance spectroscopy (EIS), which was a useful technique especially for the surface characterization. The complex impedance is presented as the sum of the real and imaginary components (*Z*' and *Z*'') . The value of the charge transfer resistance "R_{ct}" depends on the dielectric and insulating features at the electrode/electrolyte interface. A simple equivalent circuit model (inset in Fig. 7A) also used to fit the impedance data. In the Nyquist plot of impedance spectra, the semicircle section at higher frequencies characterize the electron transfer limited process and the linear section seen at lower frequencies may be attributed to the diffusion [35]. The average R_{ct} value was found to be 36.5 ± 21.8 Ohm (*n*=3) using unmodified PGEs (Fig. 7A and B-a). After CNT modification, there was 2-folds increase obtained at R_{ct} due to the repulsive interaction between carboxylated CNT modified electrode surface and negatively charged ferricyanide ions (Fig. 7A,B-b) [4, 5, 36]. After immobilization of dsDNA, a 6-folds increase was observed at the average R_{ct} (i.e, 837.7 ± 42.3 Ohm (*n*=3) (Fig.7A,B-c)). This result may be due to the fact that the negatively charged phosphate backbone of double stranded DNA prevented redox couple, $[Fe(CN)_6]^{3-/4-}$ from reaching the electrode surface. The R_{ct} value 0.34 times decreased after interaction process (Fig 7.A,B-d). The intercalation of TPT into dsDNA

resulted by the damage of double helix form of DNA, and consequently the decrease was recorded at R_{ct}. Accordingly, these impedimetric results were consistent with voltammetric results and parallel with the other studies performed by using CNT modified electrodes [4,5].

Here Fig. 7

4. Conclusion

The interaction of an anticancer drug, TPT and dsDNA was investigated at the surface of pretreated PGEs, unmodified PGEs and SWCNT modified PGEs by using electrochemical techniques for the first time in the literature. The oxidation signals of TPT and guanine were measured before/after each modification/immobilization/interaction step and the changes at the signals of TPT and guanine were evaluated by means of interaction process. An enhanced sensor response was obtained after SWCNT modification of disposable PGEs due to SWCNTs provided a larger surface area for immobilization of DNA and drug molecules [4,5] comparison to unmodified ones. The lower limit of detection (LOD) (0.37 µg/mL, 96.5 pmol in 110 µL sample) was obtained by using SWCNT-PGEs for detection of TPT in comparison to the ones obtained by pretreated and unmodified PGEs (0.51 µg/mL and 0.45 µg/mL, equal to 130 pmol and 117 pmol in 110 µL sample, respectively). After TPT detection at various concentrations, the interaction of TPT and dsDNA was performed in various interaction times. Impedimetric measurements were also performed under the optimum experimental conditions and the changes at R_{ct} value were evaluated by means of SWCNT modification, immobilization and drug-DNA interaction process.

Our electrochemical DNA biosensor has presented some advantages, such as easy-to-use, cheaper, sensitive and less time-consuming in comparison to the other types of biosensor platforms developed for analyzing of the interactions of some other anticancer drugs with nucleic acids [2,7,8,30,37]. These advanced biosensor platforms successfully detected TPT in our study compared to the one developed in more labor intensive and time-consuming study [38]. Moreover, they could be utilized for understanding and evaluating the mechanism of TPT even if cancer cells and detect TPT sensitively in a shorter time compared to the conventional techniques.

As a conclusion, our disposable DNA biosensor platforms will be able to be implemented for development of chip technologies, which will be developed for recognition of biomolecules as drugs and nucleic acids and drug-DNA interactions in the future.

Acknowledgements

A.E acknowledges the financial support from Turkish Scientific and Technological Research Council (TUBITAK; Project No.111T073), and she also would like to express her gratitude to the Turkish Academy of Sciences (TUBA) as an Associate member for its partial support. G.C. and F.M. acknowledge a master project scholarship through by TUBITAK project (Project No.111T073).

References

[1] E. Palecek, Oscillographic polarography of highly polymerized deoxyribonucleic acid, Nature 188 (1960) 656-657.

[2] D. Marin, P. Perez, C. Teijeiro, E. Palecek, Interactions of surface-confined DNA with acid-activated mitomycin C, Biophys. Chem. 75 (1998) 87-95.

[3] J.Wang, M. Ozsoz, X. Cai, G. Rivas, H. Shiraishi, D.H. Grant, M. Chicharro, J. Fernandes, E. Palecek, Interactions of antitumor drug daunomycin with DNA in solution and at the surface, Bioelectrochem. Bioenerg. 45 (1998) 33–40.

[4] A. Erdem, H. Karadeniz, A. Caliskan, Single walled carbon nanotubes modified graphite electrodes for electrochemical monitoring of nucleic acids and biomolecular interactions, Electroanalysis 21 (2009) 464-471.

[5] E. Yapasan, A. Caliskan, H. Karadeniz, A. Erdem, Electrochemical investigation of biomolecular interactions between platinum derivatives and DNA by carbon nanotubes modified sensors, Mater. Sci. Eng. B 169 (2010) 169-173.

[6] A. Erdem, Nanomaterial based electrochemical DNA sensing strategies, Talanta 74 (2007) 318-325.

[7] I. C. Gherghi, S. T. Girousi, A. N. Voulgaropoulos, R. Tzimou-Tsitouridou, Study of interactions between actinomycin D and DNA on carbon paste electrode (CPE) and on the hanging mercury drop (HMDE) surface, J. Pharm. Biomed. Anal. 31 (2003) 1065-1078.

[8] X. Tian, Y. Song, H. Dong, B. Ye, Interaction of anticancer herbal drug berberine with DNA immobilized on the glassy carbon electrode, Bioelectrochem. 73 (2008) 18-22.

[9] B.C. Yin, D. Wu, B.C. Ye, Sensitive DNA-Based Electrochemical Strategy for Trace Bleomycin Detection, Anal. Chem. 82 (2010) 8272–8277.

[10] Q. Feng, N.Q. Li, Y.Y. Jiang, Enhanced sensitivity for deoxyribonucleic acid electrochemical impedance sensor: Gold nanoparticle/polyaniline nanotube membranes, Anal. Chim. Acta 344 (1997) 97-104.

[11] B. L. Staker, K. Hjerrild, M. D. Feese, C. A. Behnke, A. B. Burgin Jr., L. Stewart, The mechanism of topoisomerase I poisoning by a camptothecin analog, Proc. Nat. Acad. Sci. U.S.A 99 (2002) 15387–15392.

[12] B. L. Staker, M. D. Feese, M. Cushman, Y. Pommier, D. Zembower, L. Stewart, A.B. Burgin, Structures of three classes of anticancer agents bound to the human topoisomerase I-DNA covalent complex, J. Med. Chem. 48 (2005) 2336–2345.

[13] Y.H. Hsiang, R. Hertzberg, S. Hecht, L.F. Liu, Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I, J. Biol. Chem. 260 (1985) 14873–14 878.

[14] S.B. Craig, U.H. Bhatt, K. Patel, Stability and compatibility of topotecan hydrochloride for injection with common infusion solutions and containers, J. Pharm. Biomed. Anal. 16 (1997) 199–205.

[15] W.J. Loos, D.M. Zomeren, H. Gelderblom, J. Verweij, K. Nooter, G. Stoter, A. Sparreboom, Determination of topotecan in human whole blood and unwashed erythrocytes by high-performance liquid chromatography, J. Chromatog. B 766 (2001) 99–105.

[16] A. Susloparova, D. Koppenhofer, X.T. Vu, M. Weil, S. Ingebrandt, Impedance spectroscopy with field-effect transistor arrays for the analysis of anti-cancer drug action on individual cells, Biosens. Bioelectron 40 (2013) 50–56.

[17] S. Iijima, Nature Helical microtubules of graphitic carbon, 354 (1991) 56-58.

[18] S. Iijima, T. Ichihashi, Single-shell carbon nanotubes of 1-nm diameter, Nature 363 (1993) 603-605.

[19] E. Palecek, M. Bartosik, Electrochemistry of nucleic acids, Chem.Rev. 112 (2012) 3427–3481.
[20] J. Wang, Carbon-Nanotube Based Electrochemical Biosensors, Electroanalysis 17 (2005) 7–14.
[21] L. Agui, P. Yanez-Sedeno, J. M. Pingarron, Role of carbon nanotubes in electroanalytical chemistry, Anal. Chim. Acta 622 (2008) 11-47.
[22] S. Kruss, A.J. Hilmer, J. Zhang, N.F. Reuel, B. Mu, M.S. Strano, Carbon nanotubes as optical biomedical sensors, Adv. Drug Delivery Rev. 65 (2013) 1933-1950.
[23] A. Erdem, P. Papakonstantinou, H. Murphy, Direct DNA Hybridization at Disposable Graphite Electrodes Modified with Carbon Nanotubes, Anal. Chem. 78 (2006) 6656-6659.
[24] C.B. Jacobs, M.J. Peairs, B.J. Venton, Carbon nanotube based electrochemical sensors for biomolecules Anal. Chim. Acta 62 (2010) 105-127.
[25] G.A. Rivas, M.D. Rubianes, M.C. Rodriguez, N.E. Ferreyra, G.L. Luque, M.L. Pedano, S.A. Miscoria, C. Parrado, Carbon nanotubes for electrochemical biosensing, Talanta 74 (2007) 291-307.
[26] G. Sanchez-Pomales, L. Santiago-Rodriguez, C.R Cabrera, DNA-Functionalized Carbon Nanotubes for Biosensing Applications, J. Nanosci. Nanotechnol. 9 (2009) 2175-2188.
[27] E.S. Manoso, R. Herrera-Basurto, B.M. Simonet, M. Valcárcel, A quartz crystal microbalance modified with carbon nanotubes as a sensor for volatile organic compounds, Sens. Act. B 186 (2013) 811-816.
[28] S.R. Ali, Y. Ma, R.R. Parajuli, Y. Balogun, W.Y.C. Lai, H. He, A Nonoxidative Sensor Based on a Self-Doped Polyaniline/Carbon Nanotube Composite for Sensitive and Selective Detection of the Neurotransmitter Dopamine, Anal. Chem. 79 (2007) 2583-2587.
[29] J. Wang, A.N. Kawde, Pencil-based renewable biosensor for label-free electrochemical detection of DNA hybridization, Anal. Chim. Acta 431 (2001) 219–224.
[30] A. Erdem, M. Ozsoz, Interaction of anticancer drug, Epirubicin with DNA, Anal. Chim. Acta 437 (2001) 107-114.
[31] European Medicine Agency, Topotecan Eagle, 2011. http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002261/smops/Positive/human_smop_000302.jsp&mid=WC0b01ac058001d127&murl=menus/medicines/medicines.jsp (access June 13, 2013).
[32] J.N. Miller, J.C. Miller, Statistics and Chemometrics for Analytical Chemistry, 5th edPearson Education, Essex, UK, 2005.
[33] A. Erdem, M. Muti, P. Papakonstantinou, E. Canavar, H. Karadeniz, G. Congur, S. Sharma, Graphene oxide integrated sensor for electrochemical monitoring of mitomycin C–DNA interaction, Analyst 137 (2012) 2129-2135.
[34] E. Canavar, F. Kuralay, A. Erdem, Interaction of Mitomycin C with DNA Immobilized onto Single-walled Carbon Nanotube/Polymer Modified Pencil Graphite Electrode, Electroanalysis 23 (2011) 2343-2349.
[35] E. Barsoukov, J.R. MacDonald, Impedance Spectroscopy Theory, Experiment, and Applications, Wiley-Interscience, USA, 2005.
[36] A. Caliskan, A. Erdem, H. Karadeniz, Direct DNA Hybridization on the Single-Walled Carbon Nanotubes Modified Sensors Detected by Voltammetry and Electrochemical Impedance Spectroscopy, Electroanalysis 21 (2009) 2116-2124.

[37] M. S. Ibrahim, Voltammetric studies of the interaction of nogalamycin antitumor drug with DNA, Anal. Chim. Acta 443 (2001) 63-72.

[38] Q. Cheng, Y. Duc, B. Wu, J. Chen, Y. Zhou, Electrochemical detection of anticancer drug topotecan using nano-acetylene black film, Colloids Surf. B. 84 (2011) 135–139.

The Captions of Figures:

Figure 1. DPV voltammograms **(I)**, histograms **(II)** representing the oxidation signals (*I*/µA) of 0.05 **(a)**, 0.1 **(b)**, 0.2 **(c)**, 0.5 **(d)**, 1 **(e)**, 2 **(f)**, 5 **(g)** and 10 µg/mL **(h)** topotecan (TPT) by using pretreated pencil graphite electrodes (PGEs) (*n*=3). Control experiment done in 0.50 M acetate buffer containing 20 mM NaCl (pH=4.8, ABS) **(i)**. **Inset** was the linear graph obtained after immobilization of TPT at different concentrations varying from 0.1 to 1 µg/mL.

Figure 2. Histograms representing the topotecan (TPT) and guanine signals (*I*/µA) before **(A)** /after interaction during 5 min **(B)**, 15 min **(C)** and 30 min **(D)** by using DPV. TPT signals obtained before/after interaction between 0.5 µg/mL TPT and 16 µg/mL double stranded DNA (dsDNA) during 5 min **(a)**, 15 min **(b)** and 30 min **(c)** and guanine signal **(d)** at surface of pretreated pencil graphite electrodes (PGEs) (*n*=3). **Inset:** Differential pulse voltammograms representing the TPT and guanine signals obtained before/after interaction between 0.5 µg/mL TPT and 16 µg/mL dsDNA during 5 min. TPT **(a)** and guanine **(b)** signal obtained before interaction, TPT **(c)** and guanine **(d)** signal after interaction. Control experiment done in 0.50 M acetate buffer containing 20 mM NaCl (pH=4.8, ABS) **(e)**.

Figure 3. Histograms representing the topotecan (TPT) signals (*I*/µA) obtained after immobilization of TPT at different concentrations varying from 0.05 to 1 µg/mL at the surface of unmodified **(A)**, single walled carbon nanotube (SWCNT) modified PGEs **(B)** (*n*=3). Measurements were performed by using DPV.

Figure 4. DPV voltammograms representing the topotecan (TPT) and guanine signals (*I*/µA) observed before/ after interaction of 0.5 µg/mL TPT and 50 µg/mL dsDNA during 5 min by using unmodified **(I)** and single walled carbon nanotube (SWCNT) modified PGEs **(II)**. The oxidation signals of TPT obtained before **(a)** and after **(a')** interaction. The oxidation signals of guanine obtained before **(b)** and after **(b')** interaction. Control experiments done in 0.50 M acetate buffer containing 20 mM NaCl (pH=4.8, ABS) by using unmodified **(A-c)** and SWCNT modified **(B-c)** PGEs.

Figure 5. Histograms representing the topotecan (TPT) and guanine signals (*I*/µA) measured before/ after interaction of 0.5 µg/mL TPT and 50 µg/mL double stranded DNA (dsDNA) during 5 min by using unmodified **(A)** and single walled carbon nanotube (SWCNT) modified PGEs **(B)** (*n*=3). The oxidation signals of TPT obtained before **(a)** and after **(a')** interaction by using DPV. The oxidation signals of guanine obtained before **(b)** and after **(b')** interaction by using DPV.

Figure 6. Histograms representing the topotecan (TPT) and guanine signals (*I*/µA) measured before **(A)**/ after interaction of 0.5 µg/mL TPT and 50 µg/mL double stranded DNA (dsDNA) during 5 **(B)**, 15 **(C)** and 30 min **(D)** by using single walled carbon nanotube (SWCNT) modified PGEs (*n*=3). The TPT signals obtained after immobilization during 5 **(a)**, 15 **(b)**, 30 **(c)** and guanine signal **(d)** by using DPV**.** The TPT signals obtained after interaction during 5 **(a')**, 15 **(b')**, 30 **(c')** and guanine signal **(d')** by using DPV**.**

Figure 7. Nyquist diagrams **(I),** histograms **(II)** representing the charge transfer resistance (R_{ct}/Ω) values obtained after interaction of 0.5 µg/mL TPT and 50 µg/mL double stranded DNA (dsDNA) at the surface of single walled carbon nanotube (SWCNT)-PGEs by using electrochemical impedance spectroscopy (EIS). Unmodified PGE **(a)**, SWCNT modified PGE **(b)**, dsDNA immobilized SWCNT-PGE **(c)**, interaction between TPT and dsDNA at the

surface of SWCNT-PGE **(d)**. **Inset:** The equivalent circuit model used to fit the impedance data, the parameters of which are listed in the text; R_S is the solution resistance. The constant phase element Q is then related to the space charge capacitance at the DNA–electrolyte interface. R_{ct} is related to the charge transfer resistance at the DNA– electrolyte interface. The constant phase element W is the Warburg impedance due to mass transfer to the electrode surface.

Scheme Caption:

Scheme 1: Detection of topotecan (TPT) **(a)** and the interaction between TPT and double stranded DNA (dsDNA) **(b)** at the surface of electrochemically pretreated pencil graphite electrodes (PGEs), unmodified PGEs and single walled carbon nanotube (SWCNT) modified PGEs by using DPV technique.

Gulsah Congur received a B. Sc. degree in Bioengineering from Faculty of Engineering, Ege University and a M.Sc. in biotechnology from Natural and Applied Sciences, Ege University, Izmir, Turkey. Currently, she has been studying Ph.D. in Biotechnology from Natural and Applied Sciences, Ege University. Her current research interests include development of electrochemical biosensors for monitoring of DNA interactions and nucleic acid hybridization related to genetic diseases.

Arzum Erdem is a Professor at the Analytical Chemistry Department in the Faculty of Pharmacy of Ege University in Turkey. She received her PhD in Analytical Chemistry from Ege University in 2000. Dr. Erdem was awarded by the Turkish Academy of Sciences (TUBA) as the one of highly skilled young scientists selected in 2001, and she also received Juniour Science Award 2006 given by The Scientific and Technological Research Council of Turkey (TUBITAK). She has initiated many international collaborative research on development and applications of electrochemical (bio)sensors based on drug, enzyme and nucleic acids. Her recent research is centred on the development of novel transducers and chemical and biological recognition systems by using different nanomaterials (e.g, magnetic nanoparticles, carbon nanotubes, graphene, dendrimers, gold and silver nanoparticles, nanowires, nanorods etc.) designed for electrochemical sensing of nucleic acid (DNA, RNA) hybridization, and also the specific interactions between drug and DNA, or protein and DNA, aptamer-protein and also the development of integrated analytical systems for environmental, industry and biomedical monitoring.

Fehmi Mese has a B.Sc. degree in Chemistry in the Faculty of Science from Adnan Menderes University, Aydın, Turkey, and a M.Sc degree in Analytical Chemistry from the Institute of Health Sciences at Ege University, Izmir, Turkey. His field of interest is the development of electrochemical DNA biosensors for monitoring of nucleic acid hybridization related to genetic diseases.

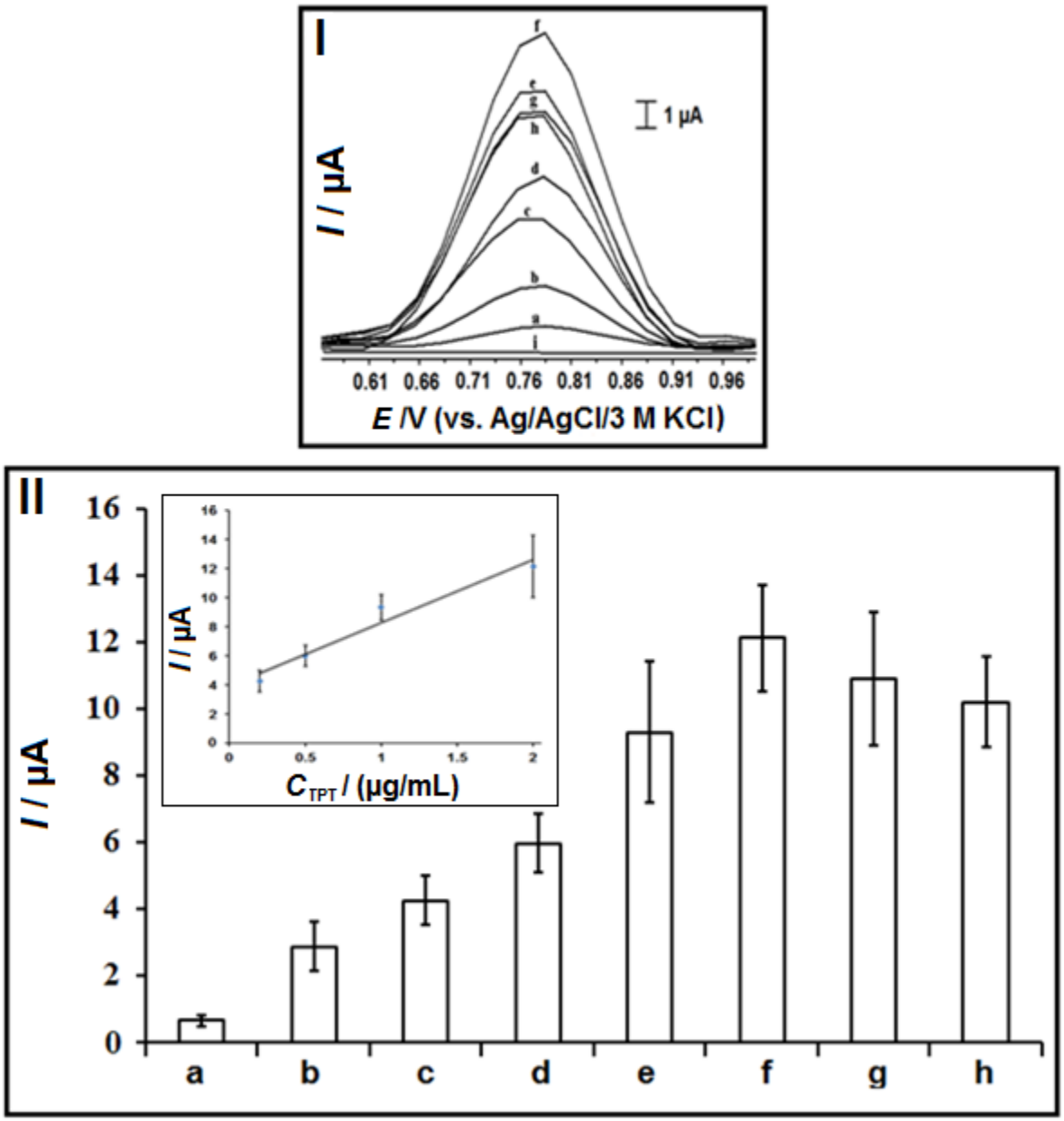


Figure 1

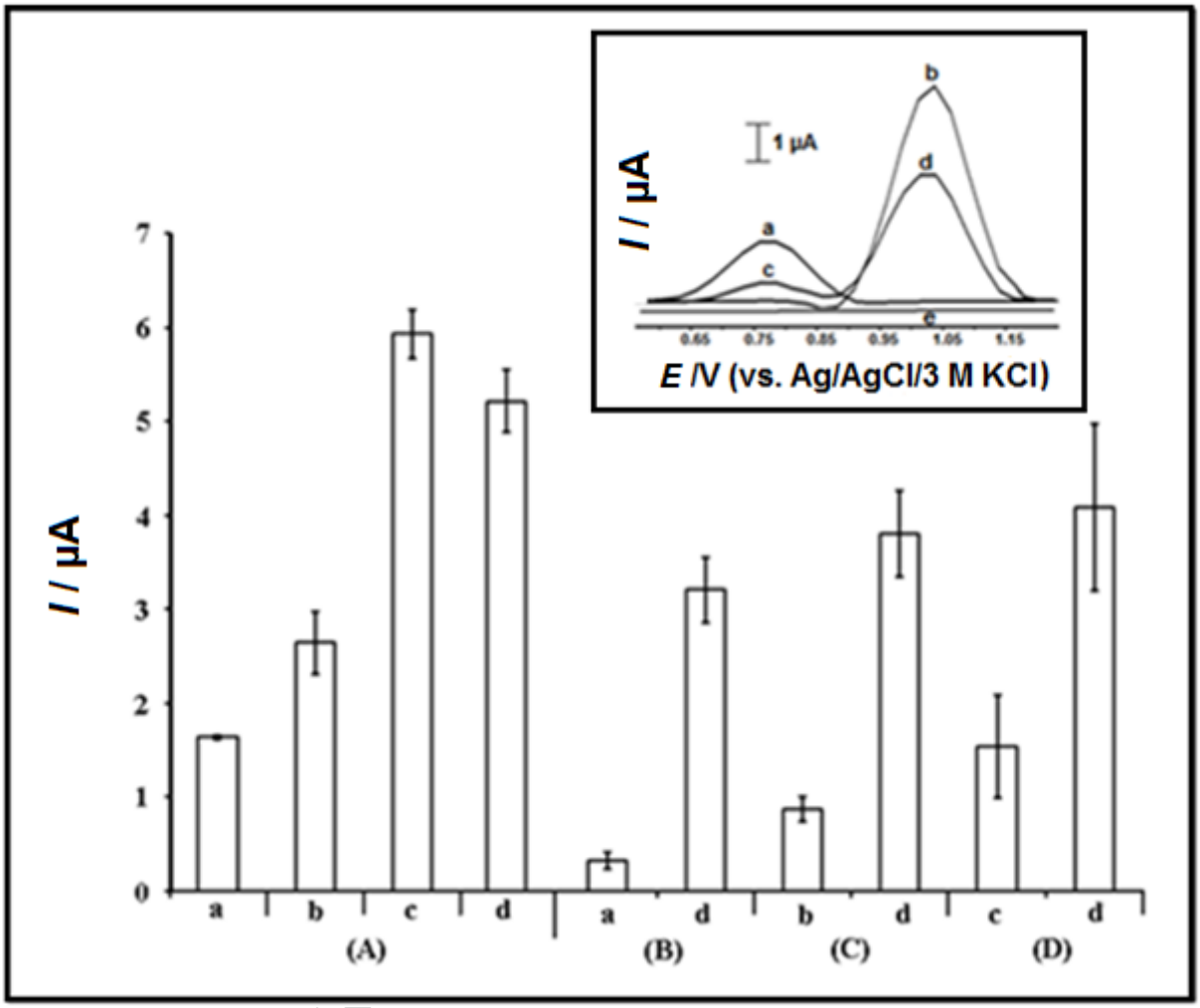


Figure 2

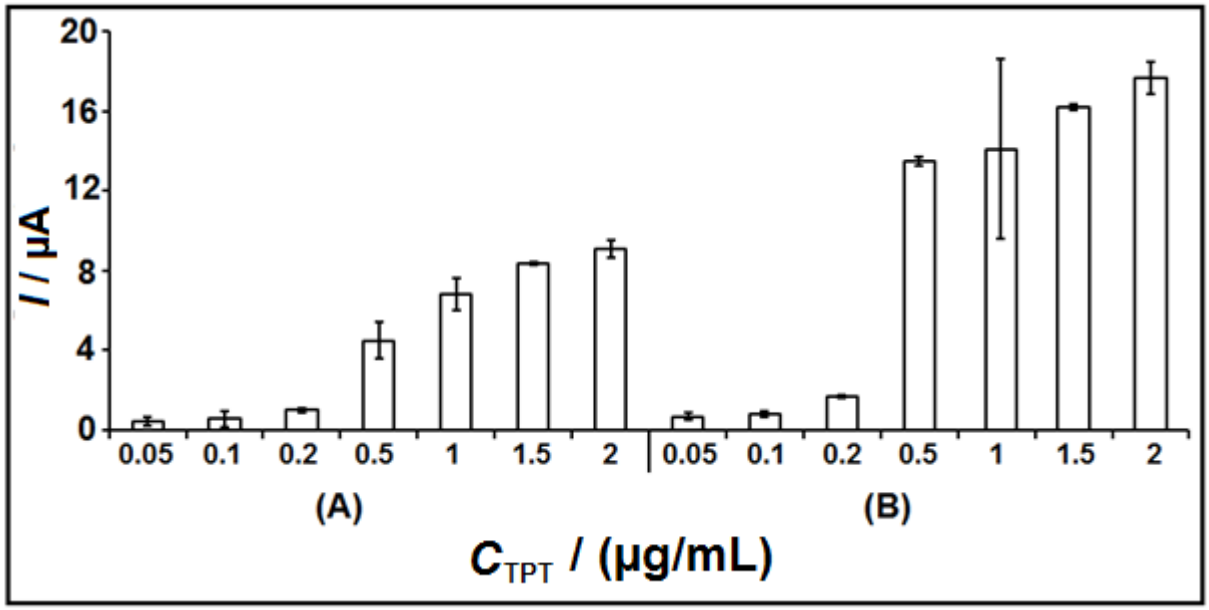


Figure 3

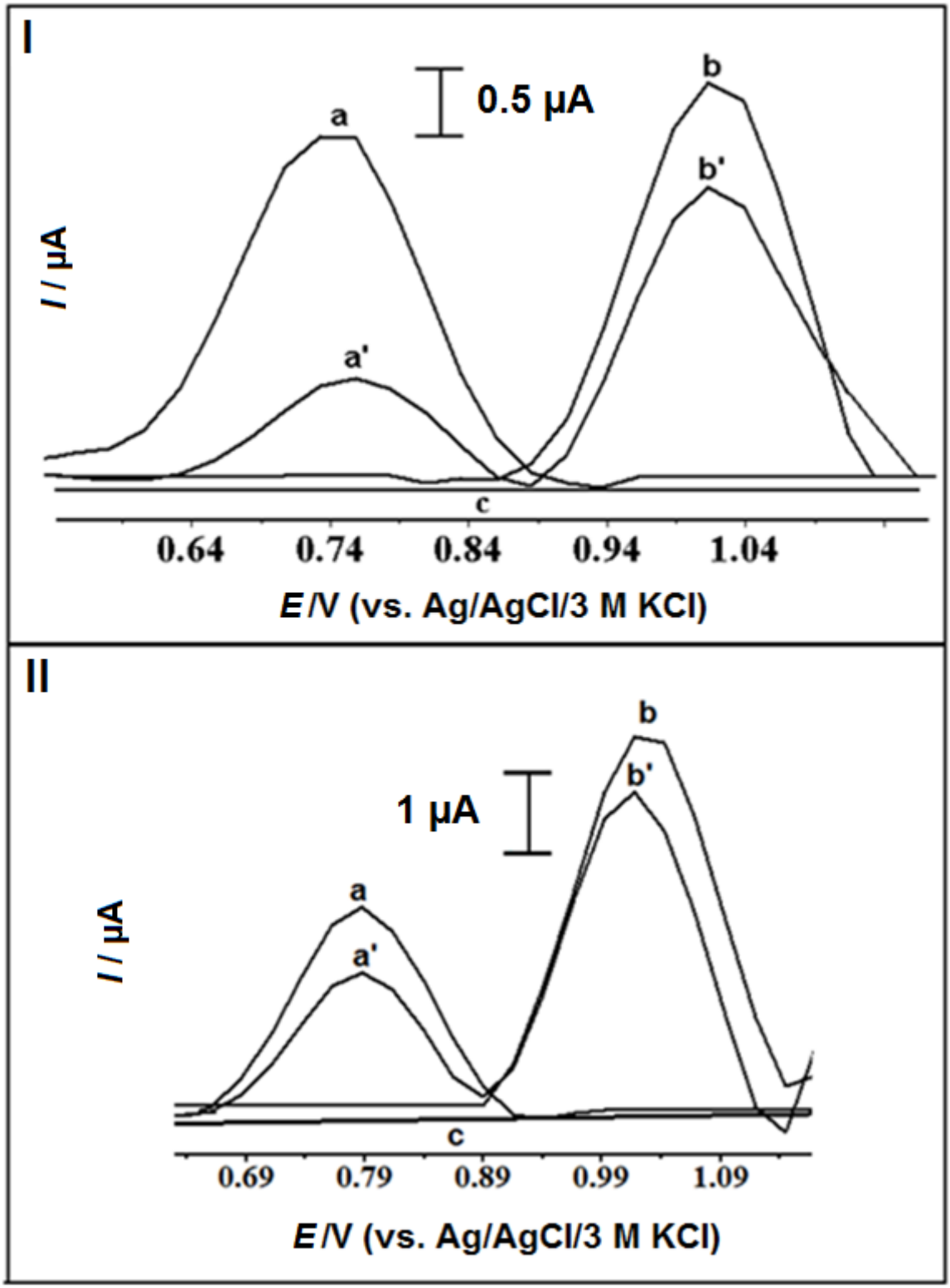

I
0.5 μA
a
b
b'
a'
c
I / μA
0.64
0.74
0.84
0.94
1.04
E/V (vs. Ag/AgCl/3 M KCl)
II
1 μA
b
b'
a
a'
c
I / μA
0.69
0.79
0.89
0.99
1.09
E/V (vs. Ag/AgCl/3 M KCl)

Figure 4

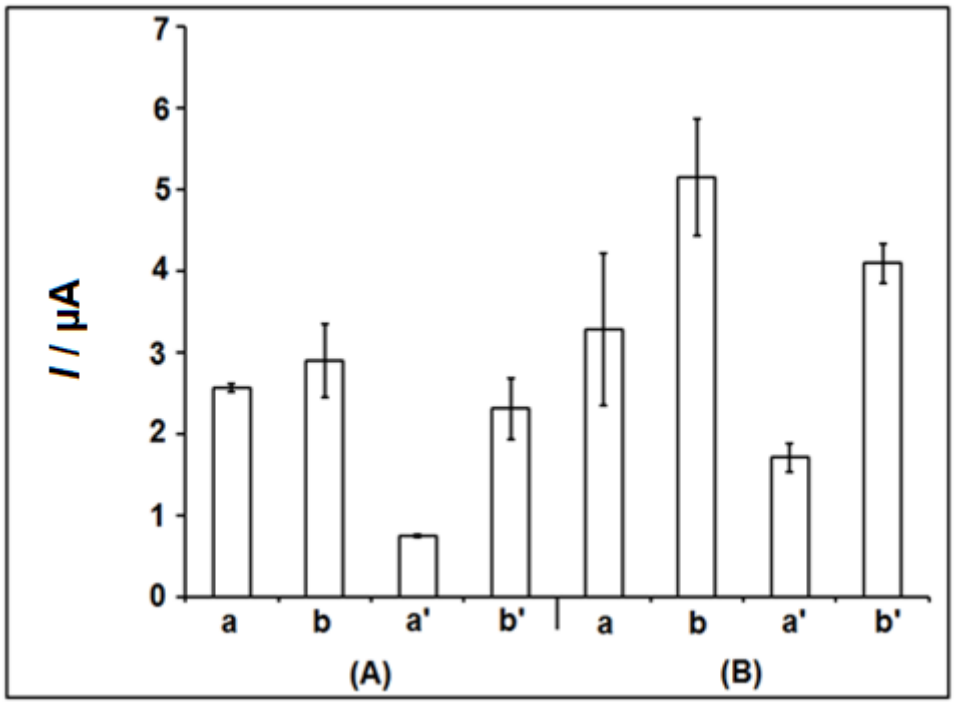


Figure 5

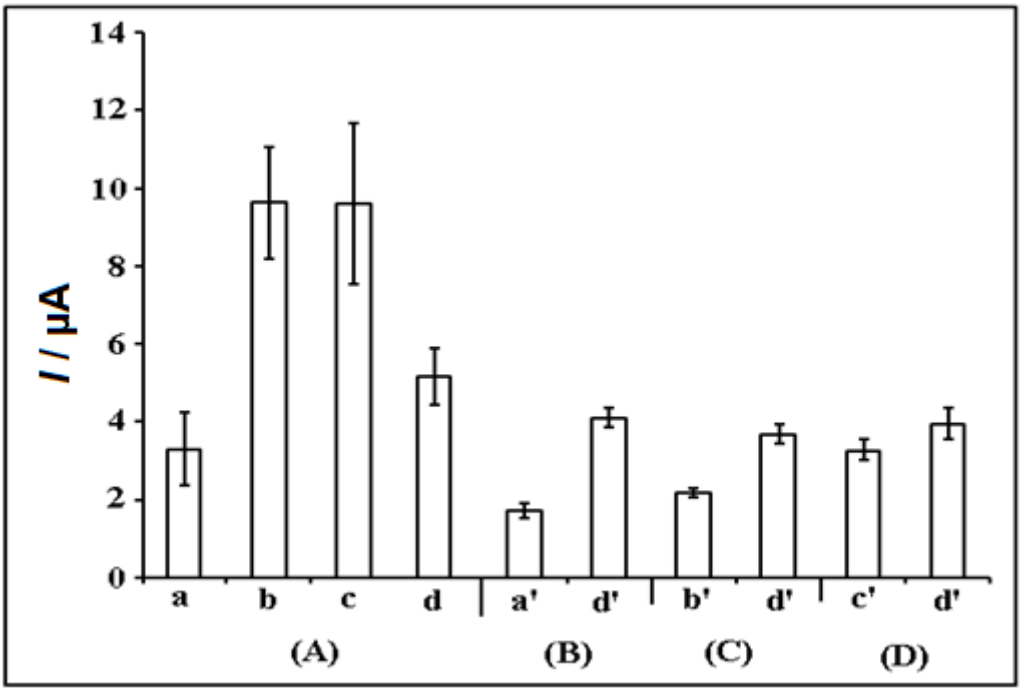


Figure 6

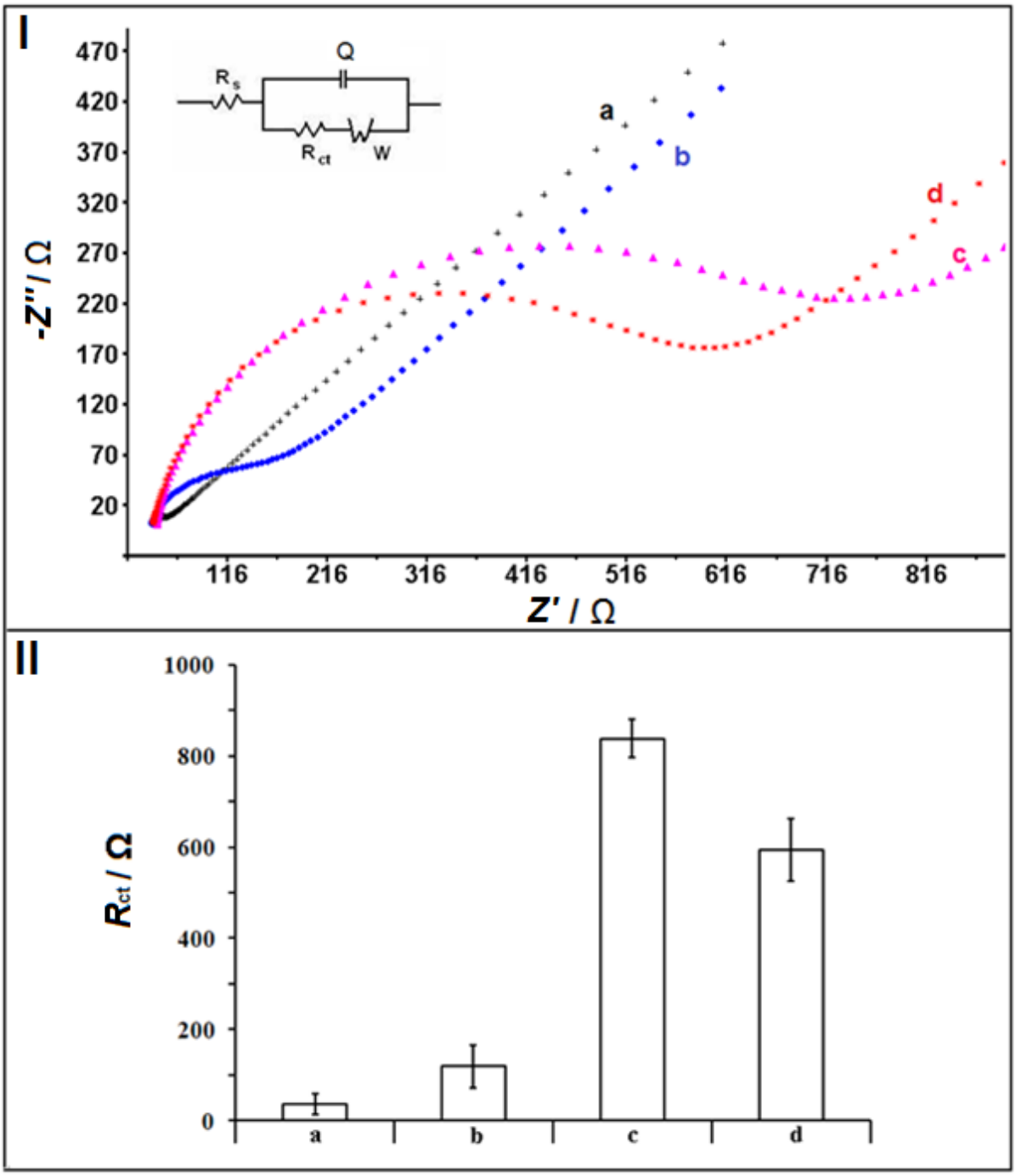
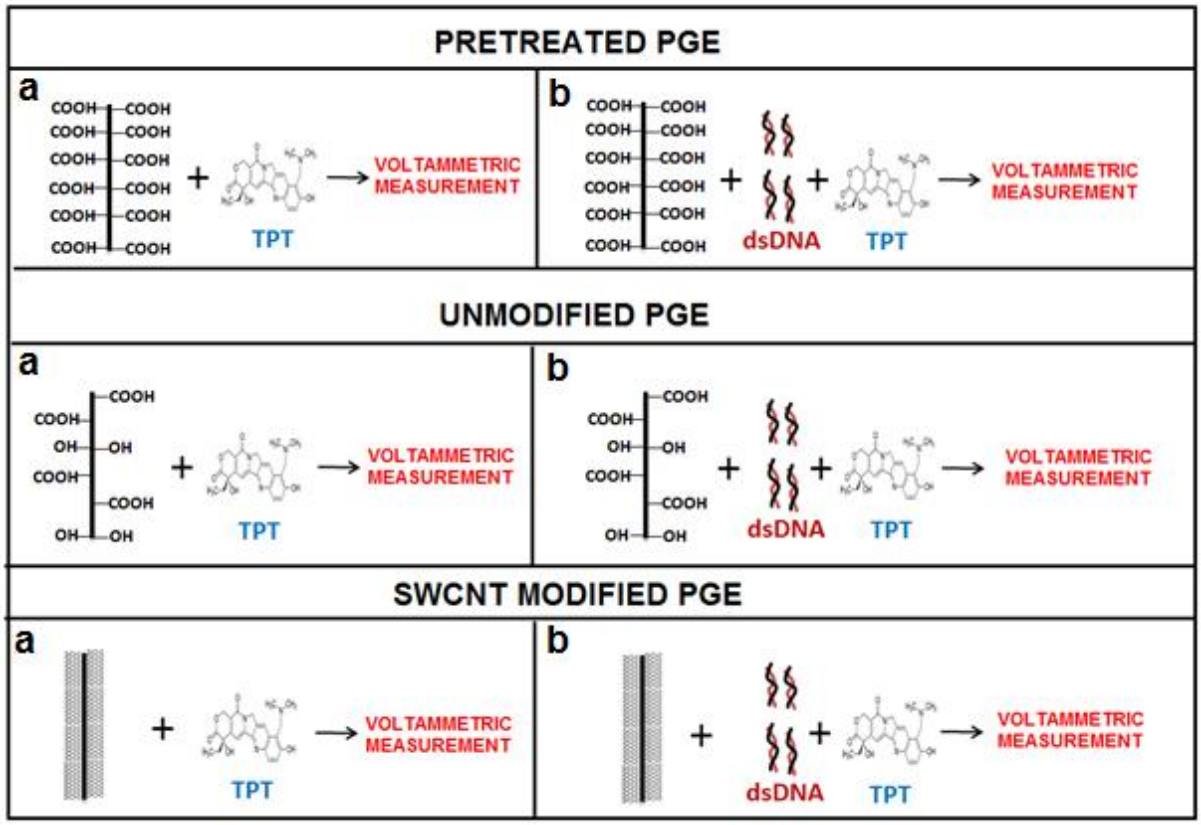
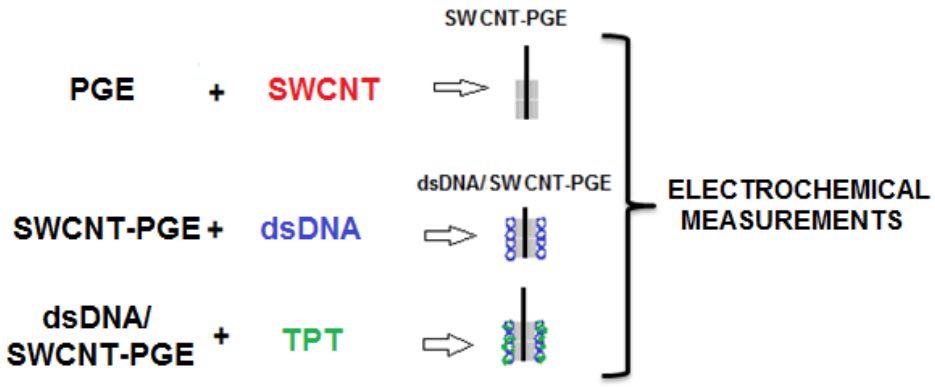


Figure 7



Scheme 1

GRAPHICAL ABSTRACT



HIGHLIGHTS

(i) TPT-DNA interaction was investigated based on the changes at TPT and guanine signals.

(ii) The LODs of TPT were obtained as 0.51 and 0.37 µg/mL by PGEs and SWCNT-PGEs.

(iii) After TPT-dsDNA interaction, there was a decrease at R_{ct} obtained by EIS technique.

(iv) TPT-DNA interaction was investigated for the first time herein by using DPV and EIS techniques.