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Research Article

ELECTROCHEMICAL BIOSENSOR FOR BRCA1 GENE AND TAMOXIFEN INTERACTION

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ABSTRACT

The electrochemical nanobiosensor was designed for the determination of specific DNA sequences related to breast cancer 1 (BRCA1) gene and interaction between Anticancer Drug Tamoxifen (TAM) and related DNA sequences by using pencil graphite electrode (PGE), bare and multi-walled carbon nanotube (MWCNT) contained screen printed carbon electrodes (SPEs) for the first time. Here, biomolecular interaction between TAM and DNA was investigated differential pulse voltammetry (DPV) based on not only guanine signal but also TAM oxidation response. It was obtained that the guanine signal at about +1.00V obtained from probe DNA or hybrid DNA shows a remerkable increase after the interaction with TAM. Additionally, it was found that TAM interact with guanine bases and TAM signal which is near the guanine oxidation area also increase after the interaction with DNA. Consequently, the prepared biosensor offer suitable platform for the analysis of DNA hybridization and TAM-DNA interaction sensitively.

Keywords: *Tamoxifen, BRCA1, Electrochemical DNA nanobiosensor, carbon nanotube, Differential Pulse Voltammetry, Drug-DNA interaction, Pencil Graphite Electrode, Screen Printed Electrode.*

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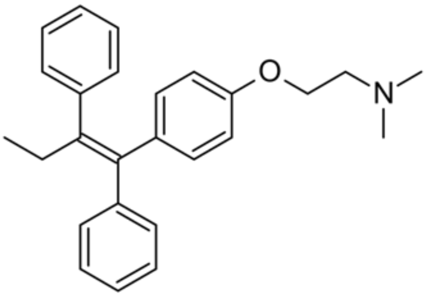
1. INTRODUCTION

Tamoxifen (TAM, Fig. 1), [Z-1-[4-(2-dimethylamino)ethoxy] phenyl-1,2-diphenylbut-1-ene], the class representative of triphenylethylenes, is a Selective Estrogen Receptor Modulator (SERM), one of a group of drugs that block the effect of estrogen on breast tissue (Kelsey and Bernstein, 1996). Tamoxifen is approved for use in both pre- and post-menopausal women. When taken for five years, it reduces breast cancer risk by up to 40%; this protective effect continues beyond the five-year treatment period. Tamoxifen offers additional benefits beyond treatment. In studies of BRCA1 mutation carriers who were diagnosed with cancer in one breast and took tamoxifen, the risk for breast cancer in their healthy breast was reduced by up to 50% (Hurtado-Monroy et al., 2007)

Figure-1. The chemical formula of Tamoxifen



It is well known that several drugs (especially anticancer drugs) and chemicals have a damaging effect on DNA. These molecules are generally interacting with DNA non-covalent or covalent ways (Bagni and Mascini, 2010). Applications of electrochemical techniques used to study interactions between DNA and drugs or small ligand molecules that are potentially of pharmaceutical interest and has an important place during the treatment (Ozkan et al., 2004).

In recent years, various biosensor development techniqueshas been applied alongwith the nanotechnology. The new generation of nanomaterials has provided significant benefits in qualitative and quantitative analysis. Biosensors based on nanomaterials exhibit advantages such as high surface-to-volume ratio, thus reducing the detection limit (Aydinlik et al., 2011), (Ktaz et al., 2004), (Patolsky and Lieber, 2004), (Pumera et al., 2007), (Wang, 2005).

The detection, identification and quantification of Tamoxifen has limited investigation such as; amperometric biosensor enzyme-based electrode was developed and studied for the first time (Keisham et al., 2012), electrochemical MIP sensorby using GCE (Yarman and Scheller, 2014) and determination of tamoxifen in urine and plasma and formulation by fast Fourier transform square wave voltammetry (SWV) by using gold microelectrode (Daneshgaret al., 2009).

There have not yet been any reports related to the voltammetric analysis of the interaction between DNA and TAM by using PGE, SPE and carbon nanotube-contained SPE based on the changes of guanine and TAM signal which is very close to area of guanine oxidation signal. These nanobiosensor systems providean information about DNA-TAM interaction and their mechanisms. The features of the detection procedure are discussed in the following parts.

2. MATERIAL AND METHOD

2.1 Apparatus

AUTOLAB 12 potatiostat/galvanostat device (Eco Chemie, Netherlands) were used for all electrochemical measurements. GPES4.9 software program and raw voltammograms were smoothed peak width of 0.01 V and raw voltammograms were treated with a Savicky and Golay algorithm using GPES 4.9 software program by moving average method (peak width 0.01 V). The analysis electrochemical cell was consisted of three electrode system in which are pencil graphite electrode (PGE), screen printed electrode (SPE) and multi walled modified screen printed electrode (MWCNT) were used as the working electrode, a reference electrode (Ag/AgCl) and a platinum wire as the auxiliary electrode. Disposable SPEs were purchased from DropSens (Spain). Two types of commercially available Carbon and MWCNT modified SPEs were used during the study. These electrodes incorporate a conventional three-electrode configuration, printed on ceramic substrates (L33× W10×H0.5 mm). Bare SPE-Working electrode is made of Carbon (4 mm diameter) and MWCNT SPE-Working electrode is composed of MWCNT/Carbon. Ultrapure water (18Ω) was used for the preparation of all DNA stock solutions and buffer solutions. Ultra-pure water (UPW) obtained from Sartorius Arium model Ultra-Pure Water Systems was used to prepare the supporting electrolyte solutions. AR grade chemicals were used in all the experiments and all tests were performed at room temperature (22.0-25.0˚C).

2.2 Reagents and materials

In this study, NaOH (Riedel-de Haen), NaCl (Sigma) were used for the buffer solution preparation. 0.2 M of pH=4.80 Acetate Buffer Solution (ABS) was used for pretreatment of the electrodes. 0.2 M, pH=7.40, Phosphate Buffer Solution (PBS) was prepared by using K_2HPO_4 (Merck) and KH_2PO_4 (Merck). The drug active substance tamoxifen as Tamoxifen citrate salt obtained from the Sigma-Aldrich. The stock solution of TAM was prepared in CH_3OH (Merck, 99.5 %) and stored at -20 ◦C. The working solutions for the voltammetric investigations were prepared by dilution of the stock solution. All solutions were prepared using ultrapure water (18ohm) and protected from light. Britton–Robinson buffer solutions (B–R) were prepared by mixing the mixed acid composed of H3PO4 (Sigma), CH_3COOH (Riedel-de Haen, 99 %) and H_3BO_3 (Sigma) with NaOH (Sigma) in proportion. 0.04 M Britton-Robinson (B-R) buffer (pH=4.50) were used as the support electrolyte solutions. All of the oligo stock solutions were prepared with ultrapure water and stored at −20◦C. and buffer solutions were stored at +4˚C until use. All chemicals provided were supplied in as an analytical reagent grade.

2.3 DNA materials

The synthetic sequences used in the study and the nucleotide sequence of these sequences are given in Table 1. The probe and target DNA was designed based on E908X WT breast cancer 1. Other sequences were used as non complementary DNA.

Table-1. Synthetic oligonucleotides and their base sequences.

Synthetic Oligonucleotides	Number of Bases	Name
5'- GATTTT CTTCCT TTTGTT C-3'	19	BRCA-1 PROBE
5'- GAACAA AAG GAA GAA AAT C -3'	19	BRCA-1 TARGET
5’-TTG TCCTTCATG CCA GCG AA-3’	20	PROBE-1
5’-TTCGCTGGCATGAAGGACAA-3’	20	TARGET-1
5' –ACC TTC GGC AAA AGC TTC AAT ACT CCA–3'	27	PROBE-2
5'–TGG AGT ATT GAA GCT TTT GCC GAA GGT–3'	27	TARGET-2

2.4 TAM stock solution preperation

The drug active substance of tamoxifen obtained from the Sigma-Aldrich Company. The stock solution of 5mM TAM was prepared by dissolving 46 mg TAM in 5 mL of methanol and stored at -20°C. The working solutions for the voltammetric investigations were prepared by dilution of the stock solution. The stock solution was taken in appropriate amounts and the selected support electrolytes (pH=4.50 ABS for interaction on the surface and in solution medium) were completed in the required volumes in the eppendorf tube. The voltamograms of the prepared solutions were taken in B-R buffer.

2.5 Analytical procedure

2.5.1 Electrochemical oxidation of tamoxifen

PGEs was activated with an electrochemical cell which contains 4 mL of an acetate buffer solution (ABS; pH=4.80). Their 1 cm surface has applied the buffer solution for a potential of +1.40 V for 30 seconds. The activated PGE was modified with DNA and Drug by passive adsorbtion technique. After activation, 5µM TAM was immobilized to the electrodes and mesasured respectively to investigate the interaction mechanism of the drug with DNA.

2.5.1 The preparation of sensorsurface

PGEs was activated in an electrochemical cell which contains 4 mL of an acetate buffer solution (ABS; pH=4.80). The 1 cm surfaces of the electrodes were placed in ABS under the application of +1.40 V potential for 30 seconds.

SPEs were also electrochemically pretreated by applying positive potential to the electrodes. For pretreatment, 70 ml ABS was dropped on the sensor surface containing the triple electrode system. Subsequently, a potential of +1.8 V was applied to the working electrode surface for 1 minute to form a layer of carboxyl groups on the carbon electrode surface and to remove any contaminants that may be caused by the manufacturing process (Topkaya and Ozkan-Ariksoysal, 2016).

2.5.2 Surface phase procedure

For surface phase interaction, 50 µl of probe solution (for probe-1: 10 µg/ml, for probe-2: 7 µg /ml, for BRCA-1 probe: 4 µg/ml) was immobilized for 30 min. After the probe immobilization, the electrodes were rinsed with ABS. The hybridization was carried out by passive adsorbtion. 50 µl of target solution (for target-1: 15 µg/ml, for target-2: 12 µg/ml, for BRCA-1 target: 6 µg/ml) onto the probe coated working electrode surface. Hybridization process was occured between the probe and the target sequence during 45 minutes. Following the hybridization, the electrodes were rinsed with PBS buffer to prevent unspecific adsorption to the sensor surface. DNA modified PGEs and SPEs were interacted with drug (TAM) for an hour by wet-adsortion technique. The electrode surface was then rinsed with B-R buffer for 5 seconds in order to eliminate nonspecific binding of TAM. Finally, the oxidation signals of TAM and guanine were then recorded.

2.5.3 Solution phase procedure

For the solution phase interaction; TAM, probe and target DNA sequences interacted for an hour. Than 1 hour interacted TAM/probe/target (TAM/probe-1/target-1, TAM/probe-2/target-2, TAM/BRCA-1 probe/BRCA-1 target) immobilised to the electrode surface by adsorption technique for 20 minutes. The bare PGEs were immersed into the vials containing 50 µL of mixture of required amount of TAM/probe/target solution in B-R buffer (pH=4.50). Each of the electrodes then rinsed with B-R buffer (pH=4.50) to prevent unspecific adsorption of DNA sequences or TAM on PGEs. On the other hand, 70µl of the mixture of

TAM/probe/target was also immobilised to the screen printed electrode surface by adsorption technique for 20 minutes. SPEs were then washed with 80µl of B-R buffer (pH=4.50) solution to prevent unspecific adsorption.

2.6 Determination of the appropriate hybridization conditions of the olignucleotide solutions of the BRCA-1 gene region

The hybridization concentrations of synthetic sequences 1 and 2 were previously optimized in our previous works reported in the literature (Topkaya and Ozkan-Ariksoysal, 2016) and (Subak and Ozkan-Ariksoysal, 2018). According to these literatures, the guanine-containing probe coated electrode gave the highest signal, while after hybridization, the peak from the hybrid showed a decrease. The binding of the guanine bases to complementary cytosine bases in the hybrid structure is also proved that observed guanine response was not caused by non-specific binding of target sequence to the electrode.

Synthetic BRCA-1 oligonucleotides which is the breast cancer disease gene region and other synthetic sequences were evaluated and investigated under the same experimental conditions.

2.6.1 Salt Concentration

In order to find the optimal salt concentration for hybridization, a salt ratio study was carried out in the hybridization buffer. Hybridization of target and probe sequences with different ionic strength and base conformation with optimal salt concentration in the buffer is required. In low ionic strength solutions, less probe adsorption observe because of the larger electrostatic repulsion between probe strands. In high ionic strength solutions, the electrostatic repulsions between probe and target molecules are effectively can be reached higher probe coverage. These results are consistent with observations made by Subak and Ozkan-Ariksoysal, 2018. For this purpose various salt concentrations prepeared with hybridization buffer and optimum salt concentration selected according to maximum change of guanine oxidation signal. Hybridization was performed in PBS with 20mM, 50mM, 130mM, 500mM, 1000mM NaCl containing target sequence and probe sequence of BRCA-1 oligonucleotide for 45 min. DNA modified electrodes were then rinsed 5 sec. with the B-R buffer.

2.6.2 Probe/Target Concentrations

Synthetic DNA probe BRCA-1 was hybridized with the synthetic target DNA to determine the optimal probe concentration for hybridization. For this purpose, the probe is immobilized to the electrode surface at various concentrations and oxidized by DPV technique. The maximum signal of minimum probe concentration was selected. Increasing concentrations of BRCA-1 target solution began to interact with the selected prob modified PGE. Guanine oxidation signals in B-R pH=4.50 buffer were evaluated and the difference in the highest guanine oxidation signal was targeted for the experiment.

3. RESULTS AND DISCUSSION

The electrochemical method involves not only monitoring the oxidation signal of guanine, but also monitoring the TAM oxidation signal, both of which increase in these experiments after the interaction between DNA and TAM.

3.1 Electrochemical behaviour of tamoxifen

The electrochemical behavior of TAM on the PGE was investigated in several buffers. As the highest TAM signals were recorded at an acidic pH value (Fig. 2), the BR buffer with pH=4.50 was chosen for further studies as the supporting electrolyt.

Figure-2. The CV voltammograms of TAM

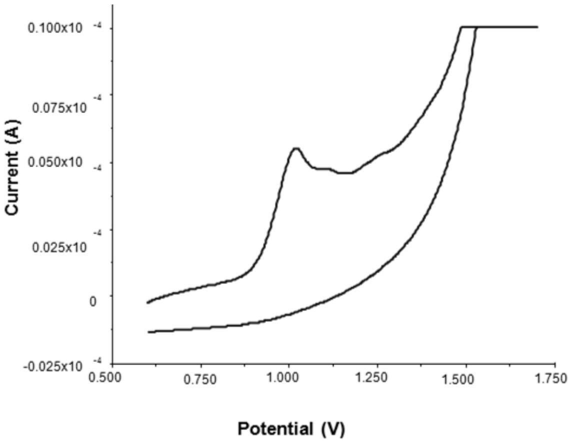
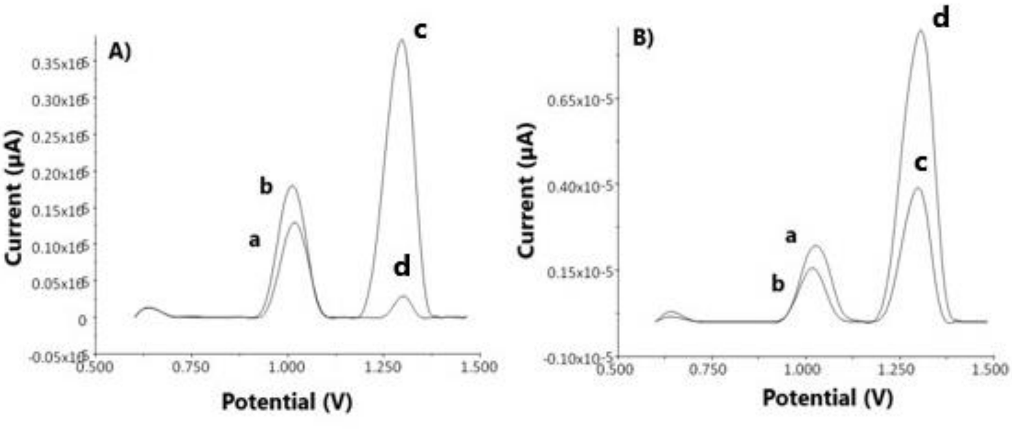


Figure-2 represented the oxidation peak current values obtained from CV voltammograms of 100µM TAM at pH=4.50 in 0.04 M B-R buffers at PGE (In experimental conditions: Potential range between +0.50 V and +1.6 V, pulse amplitude 50 mV and scan rate 16 mV/s) and shows that 100µM TAM gives a single anodic oxidation peak. Obtained voltammogram showed that the TAM peak was affected by anodic peak. According to the CV voltammogram, there is no reduction peak, in the reverse scan showing the oxidation process.

3.2 Electrochemical optimisation of BRCA-1 oligonucleotides

To assess the TAM-DNA interaction better, an electrochemical biosensor was developed. First of all, to investigate of the DNA signal with the proposed method, the voltammetric oxidation of TAM signal (Figure-2), appropriate BRCA-1 probe/BRCA-1 target concentration for hybridization and optium salt concentration of the hybridization buffer were selected. Briefly, electrodes were first activated and after that probe sequences were immobilized onto the electrodes by dipping electrodes into the probe solution. Probe coated electrodes were later interacted with its target sequence to create hybrid form on the surface of electrodes. The same procedure was also repeated to control hybridization, ie, by sending a probe onto the target DNA modified PGE. The changes in the oxidation signal of the guanine bases of DNA were measured with DPV. Figure-3. shows the hybridization efficiency of BRCA-1 synthetic oligonucleotides.

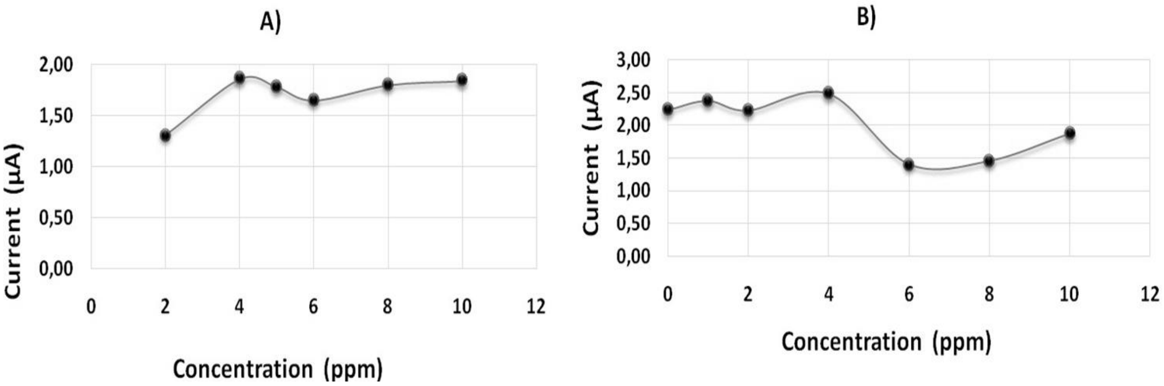
Figure-3. Voltamograms that show the hybridization result with BRCA-1 A) probe sequence to target hybridization; (a) only the probe signal of guanine, (b) hybridization signal of guanine (c) hybridization signal of adenine, (d) only probe signal of adenine; B) target sequence to probe hybridization; (a) only the target signal, (b) hybridization signal, (c) hybridization signal of adenine, (d) only the probe signal of adenine.



(In experimental conditions: Potential range was between +0.50 V and +1.6 V, pulse amplitude of 50 mV and scan rate of 16 mV/s). When hybridization was performed on the probe, an increase in the current values of the guanine oxidation signals was expected at the end of the hybridization (2 guanine base in the target sequence, 1 guanine base in the probe sequence). The only guanine-containing probe coated electrode gave lower signal (Figure3A-a) than that obtained hybrid modified one, because after the hybridization, a high signal is obtained because of the high number of guanine originating from the target (Figure 3A-b). On the contrary the target coated electrode signal (Figure 3B-a) showed a decrease (Figure2B-b) after hybridization. The increased signal (Figure 3A) is indicative for hybridization, while decreased signal (Figure 3B) is also evidence for hybridization. Also the verification of the hybridization was controlled using the adenine oxidation signals because probe sequence has 1 adenine base and target sequence has 12 adenine bases. The signal obtained from 1 adenine base contained probe showed an increase after the hybridization of 12 adenine base contained target as expected. For example, according to the Figure 3A, when adenine response increases, hybridization has occurred on the surface of the electrode. Thus, differences between both guanine oxidation of probe and hybrid signals or adenine oxidation of probe and hybrid signals prove the hybridization of BRCA-1 oligonucleotides.

Figure-4. represents the synthetic oligonucleotide BRCA-1 probe hybridized with the synthetic target oligonucleotide to determine the optimal probe concentration for the hybridization. The electrochemical response of the prepared working electrodes was evaluated using a solution phase hybridization protocol. For this purpose, various probe concentrations of the previously prepared BRCA-1 oligonucleotide solution and the target BRCA-1 oligonucleotide solution at different concentrations were evaluated to find the optimal hybridization conditions.

Figure-4. Line graph showing A) probe concentration B) target concentration of BRCA-1 oligonucleotide based on guanine oxidation signal



The line graph of the guanine oxidation signals measured at +1.00 V using PGE electrodes at various BRCA-1 probe concentrations (2-4-5-6-8-10 µg/ml) and BRCA-1 target concentrations (1-2-4-6-8-10 µg/ml) are represented in Figure-4. (In experimental conditions: Potential range between +0.50 V and +1.6 V, pulse amplitude 50 mV and scan rate 16 mV/s).

Accordingly, DPV method; in guanine signals obtained by screening between +0.5/+1.60 V potential. Since PGE reaches surface saturation after a gradual increase, there is no significant difference observed in the signal as expected. Therefore, the synthetic probe DNA concentration value was determined as 4µg/ml (Figure 4-A) and the optimal target concentration was found as 6 µg/ml (Figure 4-B) which is the minimum value of the guanine signal obtained from the electrode surface.

The electrostatic repulsion caused by the phosphate backbone in the probe and target DNA molecules is reduced under high ionic strength conditions, resulting in hybridization of these two molecules. For this purpose, the salt concentration of the hybridization buffer was examined and obtained results were shown in Figure-5.

Figure-5. The effect of the salt concentration on the guanine signal and the hybridization that occurred between the probe and the target BRCA1 sequences

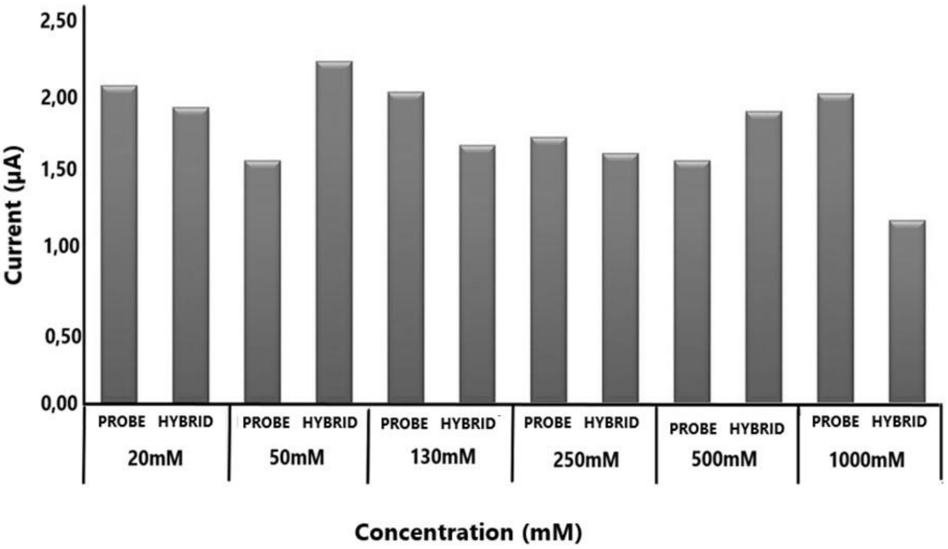


Figure-5. shows the effect of salt concentration on hybridization formed between BRCA-1 probe and target sequences (In experimental conditions: potential range between +0.50 V and +1.6 V, pulse amplitude 50 mV and scan rate 16 mV/s). The highest difference in the guanine signal between the probe and the target DNA was achieved when the hybridization buffer contained 1000 mM salt.

3.3 Solution/Surface interaction of TAM with DNA at PGE

After selecting the appropriate probe/target concentration for hybridization and optimizing the salt concentration of the hybridization buffer, the biosensor system was investigatedfor three different syntetic oligonucleotides as can be seen in Figure-6. For this purpose, the target sequence on the probe sequence and the probe sequence on the target sequence were hybridized. Change in current values of guanine oxidation were monitored after the electrode surface interaction and solution phase interaction methods.

Figure-6. Histograms showing comparison of 5µM TAM and guanine oxidation signals of oligonucleotides interacted in solution phase and surface phase; A) Probe-1/Target-1 syntetic oligonucleotid probe/target concentration 10/15 µg/mL (a:drug, b:probe, c:probe interacted with TAM, d:hybrid, e:hybrid interacted with TAM; B) Probe-2/Target-2 syntetic oligonucleotid probe/target concentration 7/12 µg/mL (a:drug, b:probe, c:probe interacted with TAM, d:hybrid, e:hybrid interacted with TAM); C) BRCA-1 syntetic oligonucleotid probe/target concentration 4/6 µg/mL (a:drug, b:probe, c:probe interacted with TAM, d: hybrid, e: hybrid interacted with TAM)

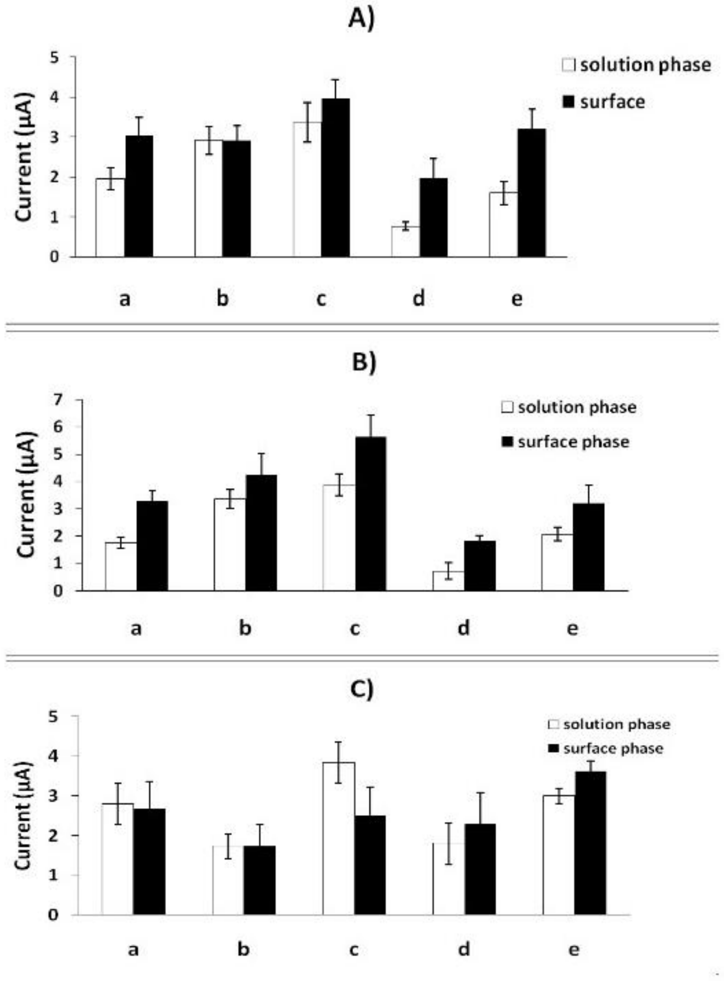


Figure-6 shows the comparison of TAM/DNA interaction in solution phase and surface phase with three different oligonucleotides by using PGE as sensor surfaces (In experimental conditions: potential range between +0.50 V and +1.6 V, pulse amplitude 50 mV and scan rate 16 mV/s). Figure-5A shows the guanine and TAM oxidation peak current after Probe-1/Target-1 hybridization. The anodic peak current of guanine oxidation signal increases about %13 (n=5) for the solution phase in presence of probe-1 (6A-c) and hybrid-1 increases about (6A-e) %52 (n=5) after the interaction with TAM. For the surface phase interaction signal increases about (n=5) %26 in presence of probe-1 (6A-c) and in presence of hybrid-1 (6A-e) (n=5) %38 after the interaction with TAM. Comparison of the after interaction of probe-1 signal and hybrid-1 signal at pH=4.50 indicated that the increase in peak current of guanine oxidation signal more sharply with the addition of target-1. In the presence of only probe-1 the interaction signal was displayed relatively lower signal increase as shown in Fig. 5A.

Similarly, comparison of TAM/DNA interaction at solution phase and surface phase by using the Probe-2/Target-2 sequences gave parallel results. Guanine oxidation signal increases about (n=5) %14 for the solution phase in presence of probe-2 (6B-c) and in presence of hybrid-2 (6B-e) (n=5) %65 after the interaction with TAM. For the surface phase interaction signal increases about (n=5) %25 in presence of probe-2 (6B-c) and in presence of hybrid-2 (6B-e) (n=5) %43 after the interaction with TAM.

The gene region BRCA-1 oligonucleotides gave relatively similar results with the other synthetic oligonucleotides (probe1/target-1, probe-2/target-2), although there was no big difference between the number of guanine bases (the probe has 2 Guanine and the target has 4 Guanine). Guanine oxidation signal increases about (n=5) %65 for the solution phase of BRCA-1 probe (6C-c) and increases about (6C-e) (n=5) %40 for the solution phase of BRCA-1 hybrid after the interaction with TAM. For the surface phase interaction guanine oxidation peak current increases about (n=5) %30 in BRCA-1 probe (6A-c) and in BRCA-1 hybrid (6C-e) (n=5) %37 after the interaction with TAM.

In this context the apparentin the voltamograms in Figure-6., the increase in the TAM interacted hybrid guanine oxidation signal is greater than the increase in the TAM interacted probe guanine oxidation signal as expected. The results obtained with the DNA sequences BRCA1 were found to be consistent with the results obtained with other DNA sequences. Significant increase in signal was observed with the interaction of hybrid DNA with TAM with all three sequences.

3.3 SPE /MWCNT SPE investigation of TAM

As can be seen the Figure-6., there is no meaningful difference between both solution phase and electrode surface phase interaction. It was also observed that there is no remarkable difference between the solution medium and electrode surface phase hybridization results. For this reason and also for shorten the analysis time, SPE investigations were performed in the solution phase. Figure-7. representing the guanine oxidation signal obtained from 5µM TAM and Probe-1/Target-1 (10/15 µg/mL) after 1 hour interaction at solution phase protocol.

Figure-7. Differential pulse voltammograms and histograms representing the guanine oxidation signal obtained 1 hour interaction in solution phase of 5µM TAM and probe-1/target-1 concentration 10/15 µg/mL (A) MWCNT-SPE voltammogram; a:drug, b:hybrid, c:probe interacted with TAM, d:probe, e:hybrid interacted with TAM and (B) Bare SPE/MWCNT-SPE histogram.

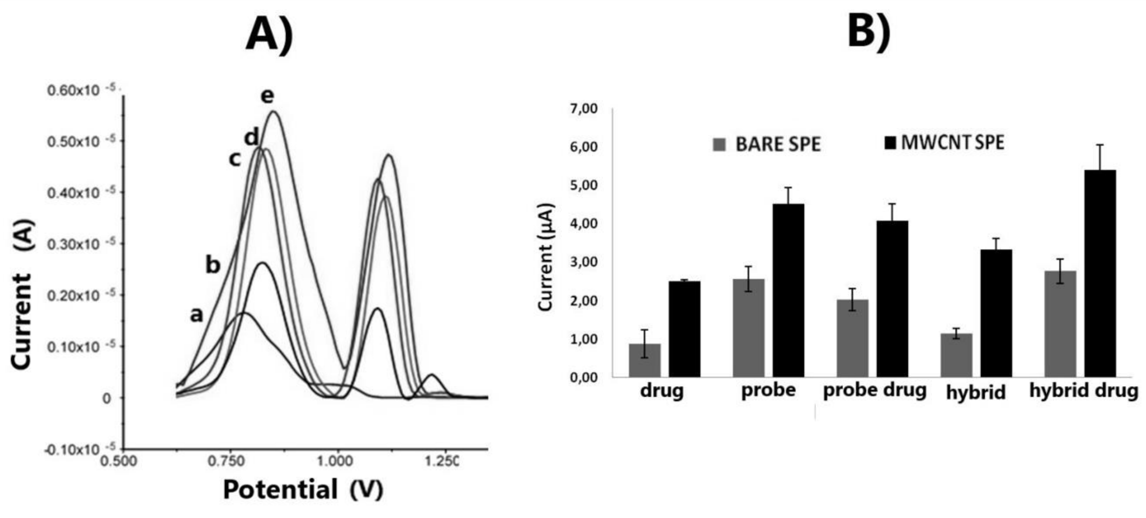


Figure-7 shows the interaction between DNA and TAM resulted in an increase of the guanine oxidation signal with SPE and MWCNT SPE (In experimental conditions: potential range between +0.50 V and +1.6 V, pulse amplitude 50 mV and scan rate 16 mV/s). MWCNT

SPEs gave greater guanine signal than normal SPE (nanomaterial-free electrode) as expected. In Figure 7A and B, DPV measurements of the guanine and TAM oxidation peak current after Probe-1/Target-1 hybridization were monitored. The anodic peak current of guanine oxidation signals obtained from MWCNT SPE as about 2.52µA for only TAM, 4.51µA for only probe, 4.08µA probe interacted TAM, 3.33µA for only hybrid and 5.39 µA for hybrid interacted TAM respectively. It can be clearly seen from the figure that guanine oxidation signal obtained from hybrid show are markable increase after the interaction with TAM. Also bare SPE results were showed in Fig.7-B that 0.88µA for only TAM, 2.57 µA for probe, 2.03 µA probe interacted TAM, 1.15 µA hybrid and 2.77 µA for hybrid interacted TAM respectively. Probe guanine oxidation signals were obtained approximately the same on both the SPE electrode and the MWCNT SPE electrode before and after interaction with TAM. However, when the obtained guanine oxidation signals in hybrid DNA modified SPE and MWCNT SPEs were examined, the guanine signal increased as a result of interaction with TAM with both electrodes. Here, the increase rate in MWSPE is calculated as (7B) (n=5) %38 and the increase rate in SPE is calculated as (7B) (n=5) %53 .

Figure-8 shows the guanine oxidation signal obtained from 1 hour interaction in solution phase of 5µM TAM and Probe-2/Target-2 (concentration 7/12 µg/mL) as the same condition with Figure-7.

Figure-8. Differential pulse voltammograms and histograms representing the guanine oxidation signal obtained 1 hour interaction in solution phase of 5µM TAM and probe-2/target-2 concentration 7/12 µg/mL (A) MWCNT-SPE voltammogram; a:drug, b:hybrid, c:hybrid interacted with TAM, d:target interacted with TAM, e:probe and (B) Bare SPE/MWCNT-SPE histogram

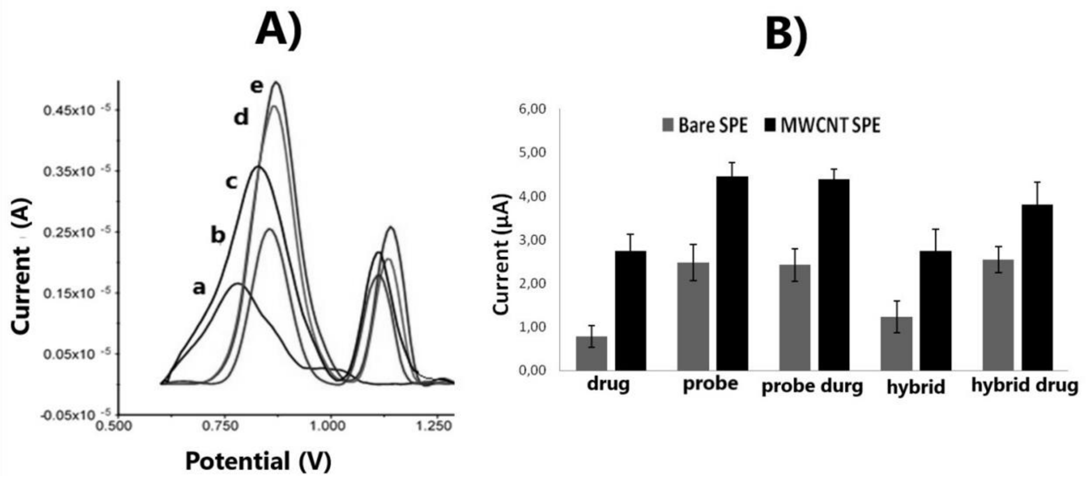


Figure-8 shows that interaction resulted in an increase of the guanine oxidation signal with SPE and MWCNT SPE (In experimental conditions: potential range between +0.50 V and +1.6 V, pulse amplitude 50 mV and scan rate 16 mV/s). Figure-8-B shows the interaction resulted in an increase of the guanine oxidation signal with SPE and MWCNT SPE. MWCNT SPEs gave greater guanine signal than normal SPE as expected. In Figure 8-A DPV measurements of the guanine and TAM oxidation peak current after Probe-2/Target-2 hybridization shown. The anodic peak current of guanine oxidation signals by MWCNT SPE 2.75µA for only TAM, 4.46µA for probe 4.38µA probe interacted TAM, 2.74µA hybrid 3.80µA for hybrid interacted TAM respectively. It can also be seen that the guanine oxidation signals in probe-2/target-2 is different between hybrid signal after the interaction of TAM. Also bare SPE results indicated in Fig.8-B 0.78µA for only TAM, 2.48 µA for probe 2.42 µA probe interacted TAM, 1.23 µA hybrid 2.55µA for hybrid interacted TAM respectively. Thus, there is a guanine oxidation signal increase of hybrid after the interaction with TAM. As a result in bare SPEs after interaction with TAM, the guanine oxidation signal increased about (n=5) %3 in precences probe-2 and (Fig.8-B) (n=5) 52% in the presence of hybrid-2. Following the guanine oxidation signals in MWCNT SPEs increased about %2 in precences of probe-2 and %28 precences of hybrid-2 after interaction with TAM. When MWCNT SPEs are used, guanine oxidation signals are increased by 57% for probe-2 and %34 for hybrid-2 compared to bare SPEs signal response after interaction.

Figure-9 shows the differential pulse voltammogram and histogram of guanine oxidation signals obtained after1 hour interaction between BRCA-1 sequences and TAM at solution phase protocol (BRCA-1 probe/target concentration 4/6 µg/mL).

Figure-9. Differential pulse voltammograms and histograms representing the guanine oxidation signal obtained 1 hour interaction in solution phase of 5µM TAM and BRCA-1 probe/ BRCA-1 target (concentration 4/6 µg/mL respectively) (A) MWCNT-SPE voltammogram; a:drug, b:probe, c:probe interacted with TAM, d:hybrid, e:hybrid interacted with TAM and (B) Bare SPE/MWCNT-SPE histogram

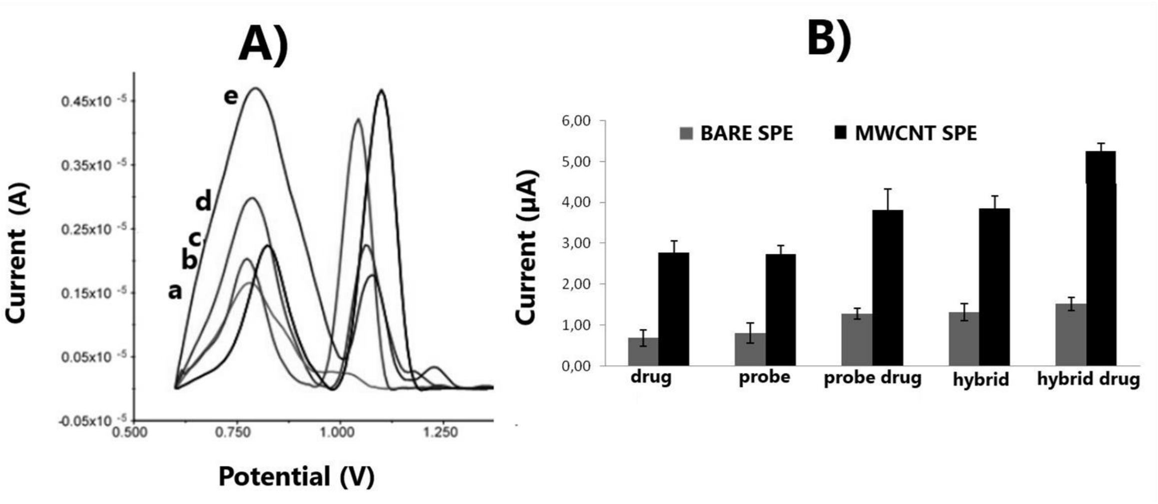


Figure-9 represents that interaction between BRCA1 seguences and TAM resulted in an increase of the guanine oxidation signal with SPE and MWCNT SPE (In experimental conditions: potential range between +0.50 V and +1.6 V, pulse amplitude 50 mV and scan rate 16 mV/s). MWCNT SPEs gave higher guanine signal than nanomaterial-free SPE as expected. Figure 9-A displays DPV measurements of guanine and TAM oxidation peak current after the hybridization of BRCA-1 probe with BRCA-1 target. The anodic peak current of guanine oxidation signals by MWCNT SPE 2.76µA for only TAM, 2.74µA for probe 3.82µA probe interacted TAM, 3.86µA hybrid 5.25µA for hybrid interacted TAM respectively. It can also be seen that the guanine oxidation signals changed after the interaction of TAM with BRCA-1 probe or BRCA-1 hybrid. Bare SPE results (Fig.9-B) indicated that 0.68µA of response obtained from only TAM modified electrode, 0.81µA for probe, 1.28µA probe interacted TAM, 1.31µA hybrid 1.52µA for hybrid interacted TAM respectively. The gene region BRCA-1 oligonucleotides guanin oxidation signals gave similar results with probe and hybrid as PGE. As can be seen in Fig.9-B the histogram in guanine oxidation signal increases about (n=5) %37 after the interaction with TAM in presence of BRCA-1 probe with BARE SPE. In MWCNT SPE the guanine oxidation signal increases about %28 in precence of BRCA-1 probe after the interaction with TAM. Also guanine oxidation signal of BRCA-1 hybrid increases (n=5) %14 for bare SPE and %27 for MWCNT SPE after the interaction with TAM.

When the signal response of BARE SPE and MWCNT SPE was compared, the interaction of BRCA-1 probe signal and BRCA-1 hybrid signal with TAM was determined to be 30% and 34%, respectively. As a result, TAM interaction with both the probe and hybrid sequence was achieved to a certain extent with the BRCA-1 sequences as in other sequences.

After the hybridization there is a signal increase observed on guanine oxidation peak current. To our knowledge, the increase in guanine signal may be observed as a result of opening or disruption of the G-C bonds at double helix DNA structure after interaction with TAM. It should be noted that TAM also interacts with guanine in probe DNA. In other words, when the results of all experiments are evaluated together, the interaction of TAM and guanine base is clearly observed. Therefore, G-C parts of the hybridization is likely to be hindered or impaired after the interaction between TAM and hybrid DNA. In addition, the interaction between TAM and double-stranded DNA can be evaluated as intercalation mode which is explained as the intercalation of TAM into G-C triple bonds.

On the other hand, both the adenine-thymine bond in the helix structure and the adenine signal in the probe do not differ before and after interaction with TAM. In summary, TAM does not interact with adenine bases in both probe and hybrid DNA.

When all the electrodes and different interaction conditions were evaluated, various oligonucleotides gave a similar interaction results with TAM. According to these results, when TAM interacted with double-stranded hybrid oligonucleotides, it gave a higher guanine signal than the signal obtained after interaction with single-strand probe.

4. CONCLUSIONS

In this study, the reproducible and sensitive electrochemical detection of biomolecular interaction between TAM and syntetic oligonucleotides by the advantages of nanomaterial (MWCNTs) modified SPE and surface/solution phase PGE resulting with an enhancement at the guanine signal. There was not any report in the literature for electrochemical investigation of interaction between TAM and oligonucletides using SPEs and PGEs. Also both methods (both surface and solution medium) interaction and hybridization resulted in an increase in the guanine oxidation signal. As a result of the literature and the researches carried out within the scope of the project, it was concluded that the increase in the guanine oxidation signal interacts with TAM both in electrochemical and intercalative directions. Also it has been concluded that the developed biosensor has the potential to be used in the analysis of other drug-DNA interactions. A disposable biosensor was developed with pencil graphite electrodes in the prepared DNA biosensor and new biosensor was used for each measurement, resulting in more reproducible results. This results could have important practical applications in the monitoring DNA modification or damage induced by anticancer drugs and thus have the potential for utilization in the development of new cancer therapies. We have also shown that a possible TAM intercalation into a DNA double helix can be assessed using label-less with electrochemical nanobiosensors.

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