Cumhuriyet Science Journal

e-ISSN: 2587-246X
ISSN: 2587-2680

Cumhuriyet Sci. J., 42(4) (2021) 814-821
http://dx.doi.org/10.17776/csj.953419

Electrochemical investigation of DNA and Capecitabine interaction using glassy carbon electrode (GCE)

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Abstract

In recent years, studies with biosensors have increased in order to better understand the mechanisms of anticancer drug action. Thus, studies to examine interactions with DNA using biosensors have gained momentum. In our study, it was investigated that Capecitabine (CPT), an anticancer drug, and glassy carbon electrode (GCE) interaction by using electrochemical methods. The interaction of CPT with calf thymus DNAs (dsDNA, ssDNA) immobilized on the electrode surface was analyzed by exploiting changes in the oxidation signals of the guanine base. The immobilization of DNA on the electrode surface has been optimized. Optimal DNA concentration and optimal interaction times were found. Electrochemical impedance spectroscopy technique was used for impedimetric measurements.
The results obtained confirmed that the ct-DNAs were immobilized on the electrode surface. Detection limit (DL) was found as 17.54 µg / mL for interaction capecitabine with ds-DNA and was found 17.12 µg / mL for interaction capecitabine with ss-DNA.

Article info

History:
Received: 17.06.2021
Accepted: 03.10.2021

Keywords:
DNA biosensors, Capecitabine (CPT), Glassy carbon paste electrode (GCE), Differential pulse voltammetry (DPV), Electrochemical impedance spectroscopy (EIS).

1. Introduction

Structures obtained by adding biological substances to an electrochemical sensor device are called biosensors. [1-3]. Sensors using DNA as recognition surface are called DNA biosensors [3-5]. These biological structures are used to determine the interaction mechanisms of some drugs or substances that target DNA. It also helps to illuminate the effects of these drugs on DNA [3-7].

Analyzing of DNA - drug interaction using these new methods is important in terms of enabling new drug designs [7]. The rapid and reliable determination of the interactions of drug molecules has anticancer properties with DNA is of great importance for drug development studies [8]. Antitumor drugs that act by binding to DNA help to examine whether many different compounds have anticancer drug properties [9]. Many different techniques have been developed to examine the interaction of compounds with nucleic acids. This technique may allow some parts of the drug molecule to bind to DNA through hydrogen bonds and van der Waals interaction or other interactions. Drug molecules must be functional in order to interact with target DNA. The biological functions of the drug can be explained according to the functional groups it contains. It will be possible to devise a new class of compounds with the necessary structures to increase or change the activity of a drugs. [10-13].

Today, the determination of drug-DNA interaction can be successfully performed using electrochemical DNA biosensors. Studies examining the effect of drugs on DNA have analyzed drug-dependent changes in the measured signals. [3, 12]. This change in signal may be due to the DNA base, or it may be due to changes in the drug signal. This result indicates a reliable interaction between analyte and DNA [3, 5,13].

In our research, the interaction of the capecitabine with GCEs with or without DNA modification were investigated. Capecitabine binding capacity determined by reduction in guanine base signal [3,13-15]. Subsequently, by changing the capecitabine concentration, variables such as guanine signal response, interaction times and reproducibility were

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examined [16]. There is no study in the literature investigating the effect of capecitabine on DNA using GCE. It is believed that our study will complete the deficid in the literature.

2. Materials and Methods

2.1 Devices

All measurements were performed using the electrochemical workstation SP-150 from Bio132 Logic Science Instruments (France), connected to the EC-Lab software V.11.25. Conventional three-electrode system was used. Glassy carbon electrode (GCE) was used as indicator electrode; Ag/AgCl was used as reference electrode and platinum wire was used as counter electrode. Balance (Precisa XB 220A), Sound vibration cleaner (Bandelin Sonorex), pH-meter (WTW InolabpH 720), Magnetic stirrer (AGE velp), Vortex (Velp scientifics), Potentiostat μ-AUTOLAB type III (GPES ve FRA Modülleri – EcoChemie, Hollanda).

2.2 Chemicals

Acetic acid (CH_3COOH, 98%), Hydrochloric acid (HCl, 37%), Sodium chloride (NaCl), Capecitabine (CPT), Sodium Hydroxide (NaOH), Calf thymus ds DNA, Calf thymus ss DNA were obtained from Sigma-Aldrich (Missouri, USA). Potassium ferrocyanide ($K_4[Fe(CN)_6]$), Potassium ferricyanide ($K_3[Fe(CN)_6]$), Sodium phosphate dibasic (Na_2HPO_4), Potassium hydrogen phosphate (KH_2PO_4), Di potassium monophosphate (K_2HPO_4), Ethanol (C_2H_6O, 98%), Tris(hydroxymethyl)aminomethane hydrochloride ($C_4H_{11}NO_3$), Trisma hydrochloric acid (Trisma-HCl), EDTA ($C_{10}H_{16}N_2O_8$) disodium salt were purchased from Merck-sigma Aldrich (Saint-Quentin-Fallavier, France). All solutions in the study were prepared in deionized Milli- Q water (Millipore, Bedford, MA, USA) (18 Mega-ohms). Experimental studies were carried out at room temperature (25.0 ± 0.5) °C.

2.2.1. Preparation of the solutions

18 ohm ultrapure water was used in the preparation of all buffer solutions. After the buffer solutions were prepared, they were stored in plastic bottles in the refrigerator.

Preparation of 0.05 M phosphate buffer solution (pH 7.4; PBS):

The 0.05 M phosphate buffer solution used during the measurements contained 1.36 g (0.01 mol) KH_2PO_4, 6.96 g (0.04 mol) K_2HPO_4 and 1.168 g NaCl (0.02 mol) per liter. The pH value of the prepared buffer solution is about 7.4. If necessary, the pH of the solution was adjusted to 7.4 with a pH meter by the addition of 0.1 M NaOH and/or 0.1 M HCl. Then, 5 mM phosphate buffer solution was prepared by diluting the 0.05 M phosphate buffer solution prepared as a stock [3].

Preparation of 0.50 M acetate buffer solution (pH 4.8; ABS, from liquid):

Some ultrapure water was added to the 500mL flask. 28.9 mL of concentrated acetic acid solution was taken. The balloon was added onto the water in the flask. It was made up to 500 mL with ultrapure water and poured into a 1 liter beaker with a stirrer. The mixer was started and the pH meter was started. 1 M NaOH solution was added until the pH was 4.81. Then 1.168 g of NaCl was weighed and added. When the pH was 4.8, the solution was poured into a 1L flask and made up to 1L with ultrapure water [3].

Preparation of 0.02 M Tris HCl buffer solution (pH 7.0; TBS):

The 0.02 M Tris HCl buffer solution used contains 3.152 g Trisma HCl and 1.168 g NaCl (0.02 mol) per liter. Adjustment of the pH of the solution to 7.0 was accomplished by adding 0.1 M NaOH and/or 0.1 M HCl, by measuring with a pH meter [3].

Preparation of 0.01 M Tris-HCl, 1 mM EDTA buffer solution (pH 8.0; Tris-EDTA):

The 0.01 M Tris-HCl used contains 1.576 g Trisma HCl and 0.372 g EDTA per liter of 1 mM EDTA buffer solution. Adjustment of the pH of the solution to 8.0 was accomplished by adding 0.1 M NaOH and/or 0.1 M HCl, by measuring with a pH meter [3].

Preparation Capecitabine solution:

Until the purchased Capecitabine was completely dissolved, the previously prepared buffer solution ABS was added to the bottle containing the drug, when the dissolution was complete, the stock concentration of the drug was calculated and put into eppendorf tubes in 50 μL volumes for later use and stored at 4°C. In order to minimize the exposure of the drug to light, it was worked as quickly as possible during preparation and the prepared drug solution was kept in a refrigerator in a box that will not be exposed to light [3].

Preparation DNA solutions:

DNA from calf thymus gland (= Calf Thymus DNA); double-stranded DNA (dsDNA) stock solutions; 1000 μg/mL was prepared with TE solution (10 mM Tris-HCl, 1mM EDTA, pH 8.0) and stored below zero. Ct dsDNA dilute solution was prepared with 0.5 M Acetate buffer (pH 4.8). In order to minimize the exposure of the solution to light, it was stored in a refrigerator at -20 °C in an opaque box [3, 5].

Single-stranded DNA (= ssDNA) solution was also prepared as described above for ct dsDNA and stored in the refrigerator at -20 ˚C.

2.3. Method

Preparation of electrodes and immobilization of DNA on electrodes were performed as reported in the current literature [3]. In addition, the interaction of DNA with the drug was also made as reported in the current literature [3, 4]. Each study was repeated at least 5 times.

Glassy carbon electrode was polished with pure water include small alumina powders until a mirror-like bright image was formed. Afterwards, carbon dust and polishing dust were removed from the surface by ultrasonication. Surface activation of the glassy carbon electrode was carried out in ABS (acetate buffer solution) by applying +0.50 V for 60 seconds. The buffer solution was changed by aborting the system for the last 5 seconds and the measurement was continued in PBS (phosphate buffer solution) [1,2,11]. These prepared electrodes are suitable for one use only. For this reason, the electrodes are prepared and activated fresh each time before use.

Glassy carbon electrode was prepared for the experiment with the differential pulse voltammetry technique using a potentiostat device, as stated in the current

2.4. Electrochemical impedance spectroscopy (EIS) measurements

Optimization steps for DNA immobilization on the GCE surface and optimization steps for the interaction of DNA immobilized GCEs with CPT were performed as stated in the current literature [3]. In addition, the preparation of the solutions in the EIS experiments and the measurement technique were also performed as reported in the existing literature [3].

3. Results and Discussion

3.1. DNA immobilization to the active GCE surface

Passive adsorption for GCE was chosen as the immobilization technique. The ct ds-DNA and ct ss-DNA were interacted with the activated GCE. The concentrations of the immobilized ct ds-DNA were kept constant and the most appropriate time for immobilization was optimized (Figure 1). The optimum amount of ct ds-DNA for immobilization keeping the optimal interaction time of the immobilized DNA constant was optimized (Figure 2). As stated in the literature measurements were made over guanine signals [3,4].

The immobilization parameters (time and concentration) on the GCE surface for ct ds DNA and ct ssDNA were separately optimized.

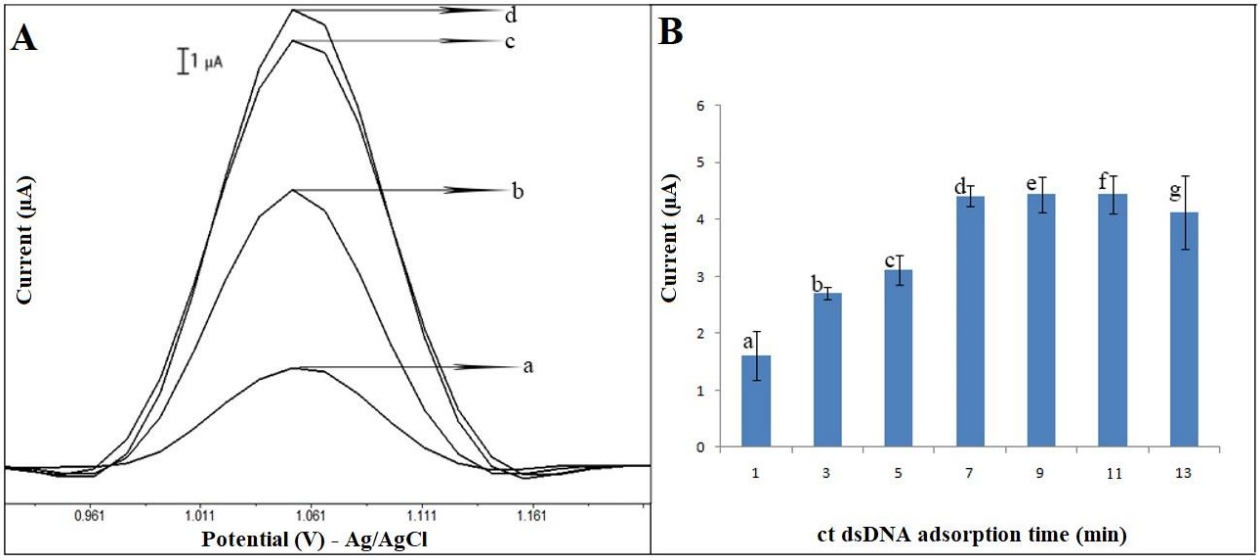

Figure 1. (A) Voltammogram and (B) Histogram for ct ds-DNA immobilized on the GCE surface at different time

When the voltammogram and histogram in Figure 1 is examined, it is seen that the interaction times for immobilization of ct ds-DNA on the GCE surface are compared. When the voltammogram of different interaction times such as a)1, b)3, c)5, d)7, e)9, f)11, g)13 min was examined, it was found that the most appropriate interaction time in terms of reproducibiliy to be 9 min.

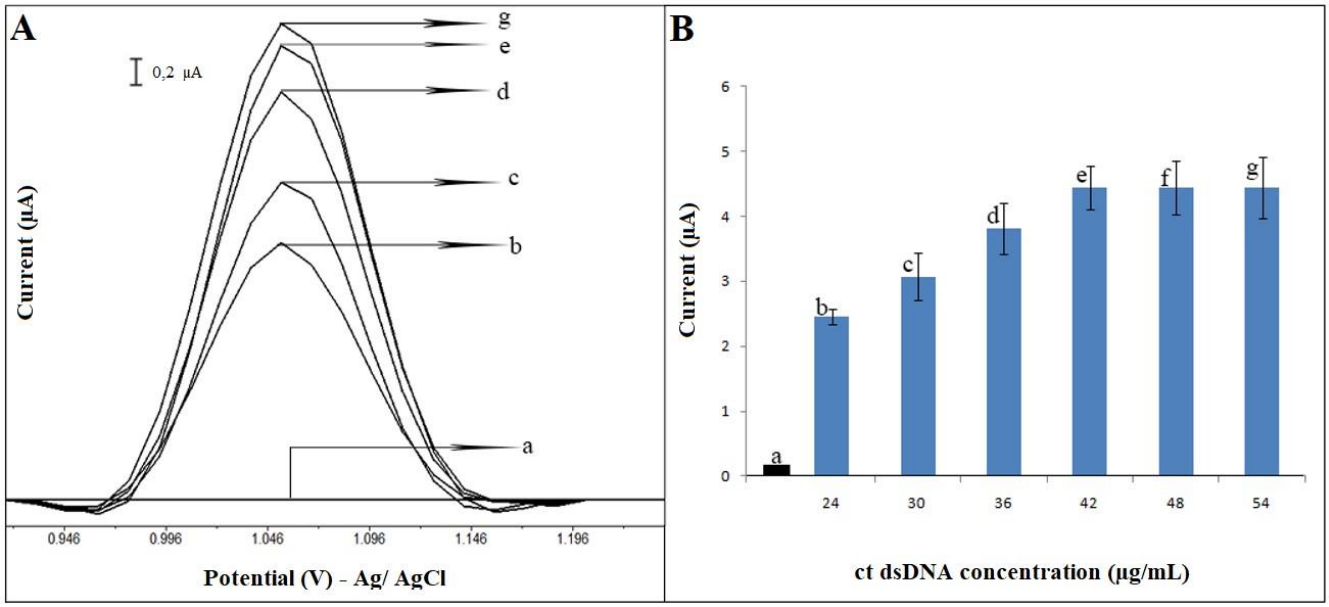

Figure 2. (A) Voltammogram and (B) Histogram for ct ds-DNA immobilized on the GCE surface at different concentration.

When the voltammogram and histogram in Figure 2 is examined, it is seen that the interaction concentrations for the immobilization of ct ds-DNA on the GCE surface are compared. When the voltammogram of ct ds-DNA prepared at different concentrations such as a) without DNA, b)24, c)30, d)36, e)42, f)48, g)54 µg /mL is examined, it was concluded that the most appropriate interaction concentration in terms of reproducibility to be 42 µg /mL. The signal marked with (a) in the histogram is the received signal (without DNA) for GCE with no DNA immobilized.

When the optimization procedures were repeated for ct ss-DNA immobilization on the GCE surface, it was determined that the ct ss-DNA was immobilized on the GCE surface in an optimal 9 min. And the concentration of ct ss-DNA was determined to be 36 µg/mL (also not shown in the figure).

3.2. Interaction between immobilized DNA and CPT

DNA (ct ds-DNA and ct ss-DNA) immobilized GCE was kept at different times in CPT solutions at constant volume and different concentrations. Optimum interaction time (Figure 3) and optimum interaction amount (Figure 4) for ct ds-DNA –CPT interaction were found as indicated in the current literatüre [3].

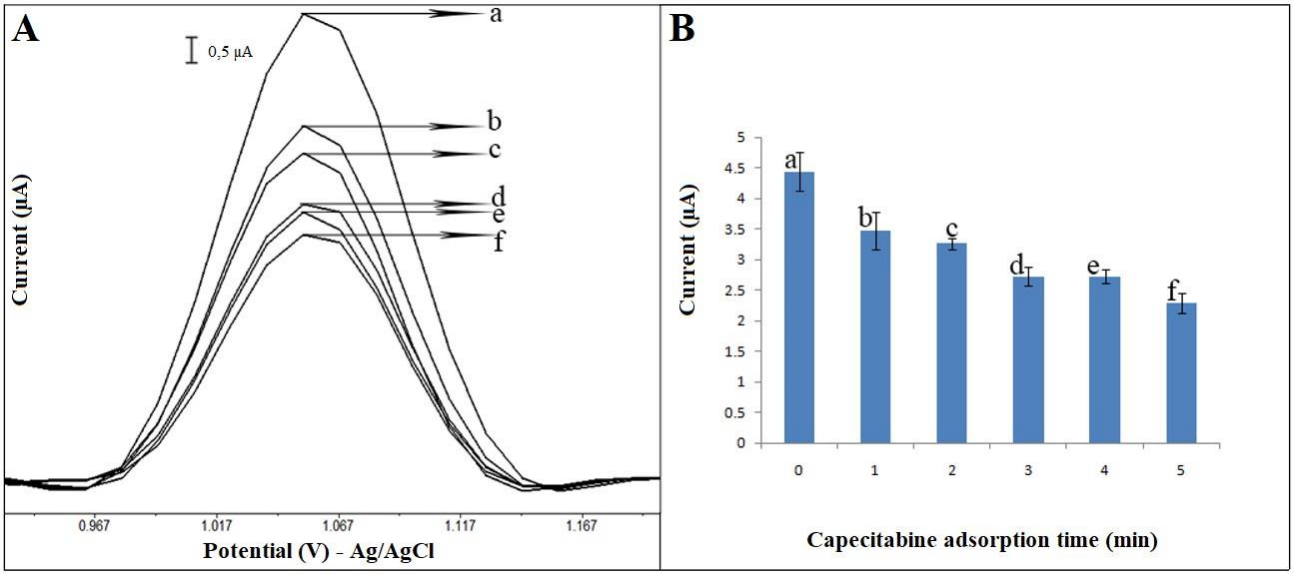

Figure 3. (A) Voltammogram and (B) Histogram of the effect of capecitabine immobilization time on response.

When the voltammogram and histogram in Figure 3 are examined, it is seen the interaction of ct ds DNA immobilized GCEs in CPT solution at different times such as a)0, b)1, c)2, c)3, d)4, e)5min. The signal marked with (a) in the histogram is only that of ct ds-DNA immobilized GCE. In other words, it is the measurement signal taken without interacting with the electrode CPT (without CPT, with ct- dsDNA). When the measured guanine signals were compared, the optimum interaction time was found to be 4 min.

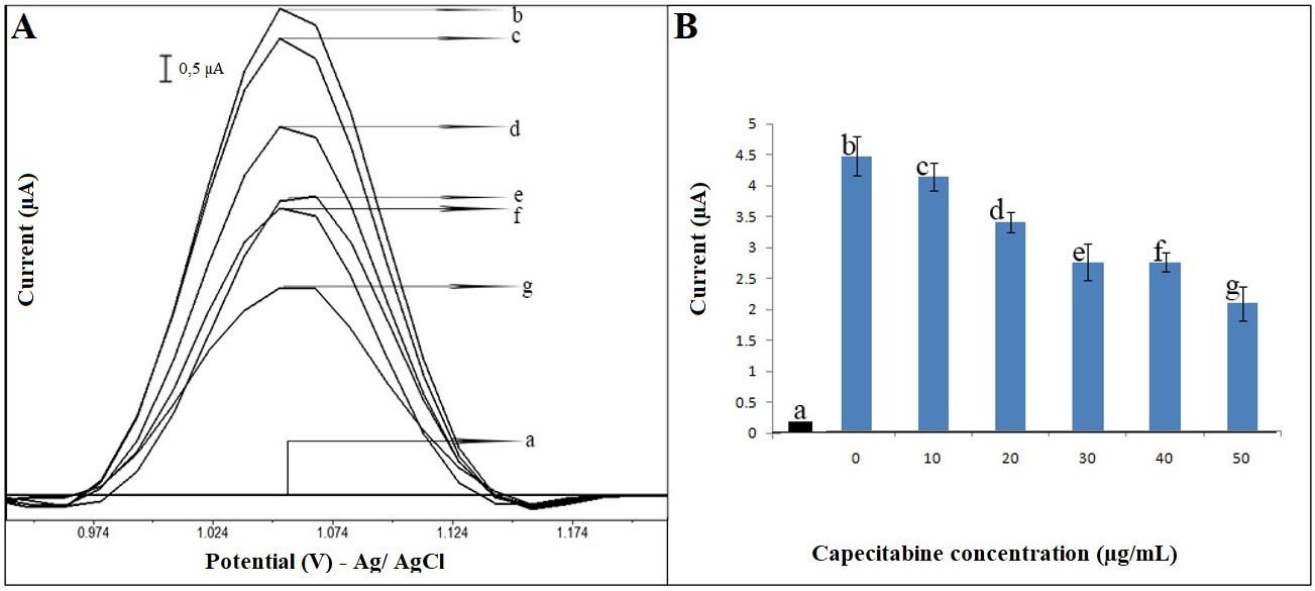

Figure 4. (A) Voltammogram and (B) Histogram of the effect of capecitabine concentration on response.

When the voltammogram and histogram in Figure 4 are examined, it is seen that the interaction signals of ct ds-DNA immobilized GCEs with different concentrations of CPT solutions such as (a) without both ct dsDNA and CPT (b)0 (c) 10 (d) 20 (e) 30 (f) 40 µg/mL are compared. The signal marked by (a) in the histogram is the signal of GCE that has not been immobilized ct ds-DNA and that has not interacted with the CPT. Likewise, the signal marked (b) in the histogram is the signal before to interaction with CPT of DNA immobilized GCEs (without CPT, with ct-ds DNA). When the measured guanine signals were compared, the optimum CPT concentration was found to be 40 µg/mL.

When optimization procedures were repeated for CPT interaction with ct ss-DNA immobilized GCEs, it was determined that the optimum CPT concentration was 40µg/mL and the optimum CPT interaction time was 3 min (also not shown in the figure).

Standard graphs of the interaction of ct ds-DNA and ct ss - DNA with capecitabine are given in Figure 5 and Figre 6.

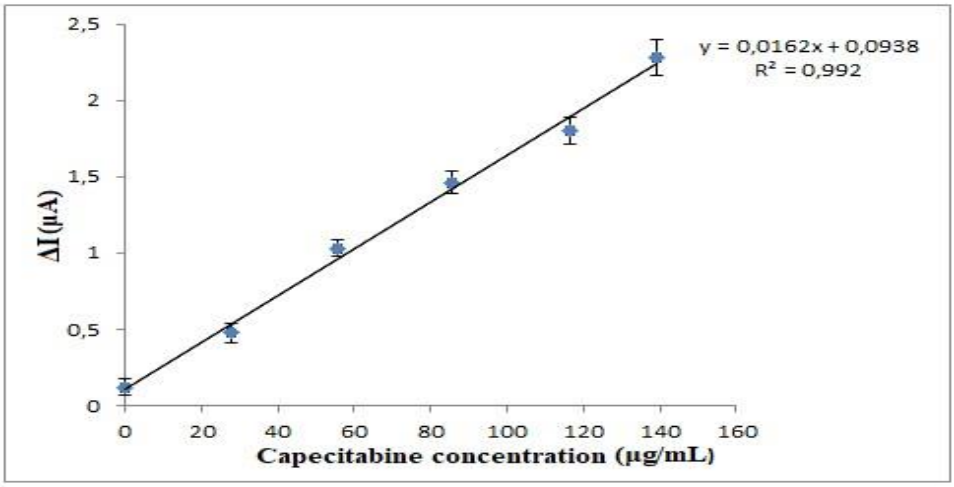

Figure 5. Calibration curve of CPT concentration change upon interaction of ct - ds DNA immobilized GCE and CPT

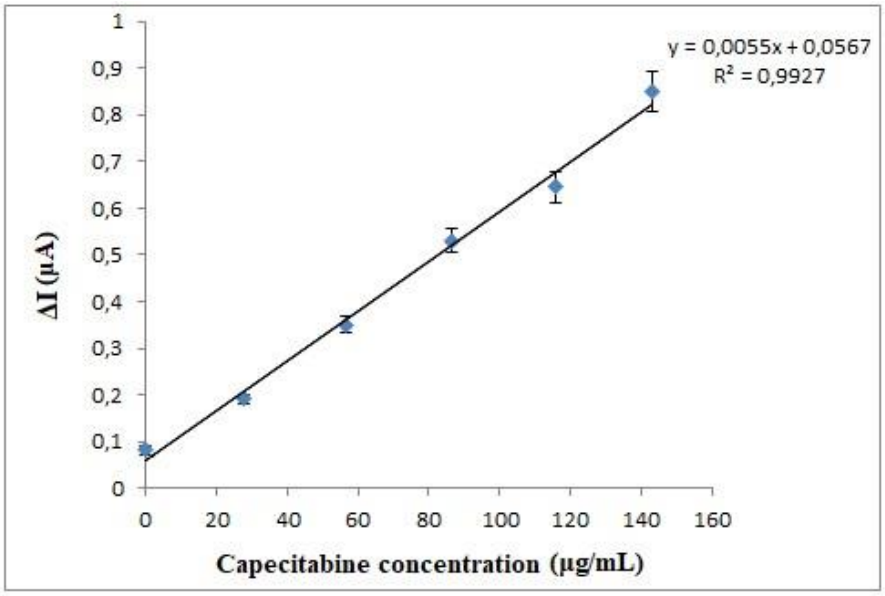

Figure 6. Calibration curve of CPT concentration change upon interaction of ct - ss DNA immobilized GCE and CPT

Detection limits were calculated as reported in the available literature [3].The lowest detection limit for ct ds-DNA-CPT interaction was 17.54 µg/mL, and the lowest detection limit for ct ss-DNA-CPT interaction was found as 17.12 µg/mL [3].

3.3. Result of electrochemical impedance (EIS) experiments

The electrode was activated according to the method described in 2.3 (electrode activation process). Differently, EIS measurements were made using FRA (Frequency Analyzer) software instead of DPV technique. Analyzes based on impedance values obtained by measuring current in the cell at constant potential and at different frequencies with the impedimetric technique were performed. Measures were taken by EIS for activated GCE, ct dsDNA immobilized GCE, and GCE after ct dsDNA – CPT interaction. Nyquist curves were drawn using values close to the mean value.

The procedures described above were repeated for ct ss-DNA. The results obtained are given in Figure 7 and Figure 8.

When the Histogram in Figure 7 is examined, it is seen that a) pret (activated) GCE resistance, b) ct dsDNA immobilized GCE resistance, and c) GCE resistance after interaction CPT with ct dsDNA immobilized to GCE surface are compared.

It was observed that there was an increase in the resistance of the GCE surface after ct dsDNA was immobilized compared to its previous state (only after surface activation).

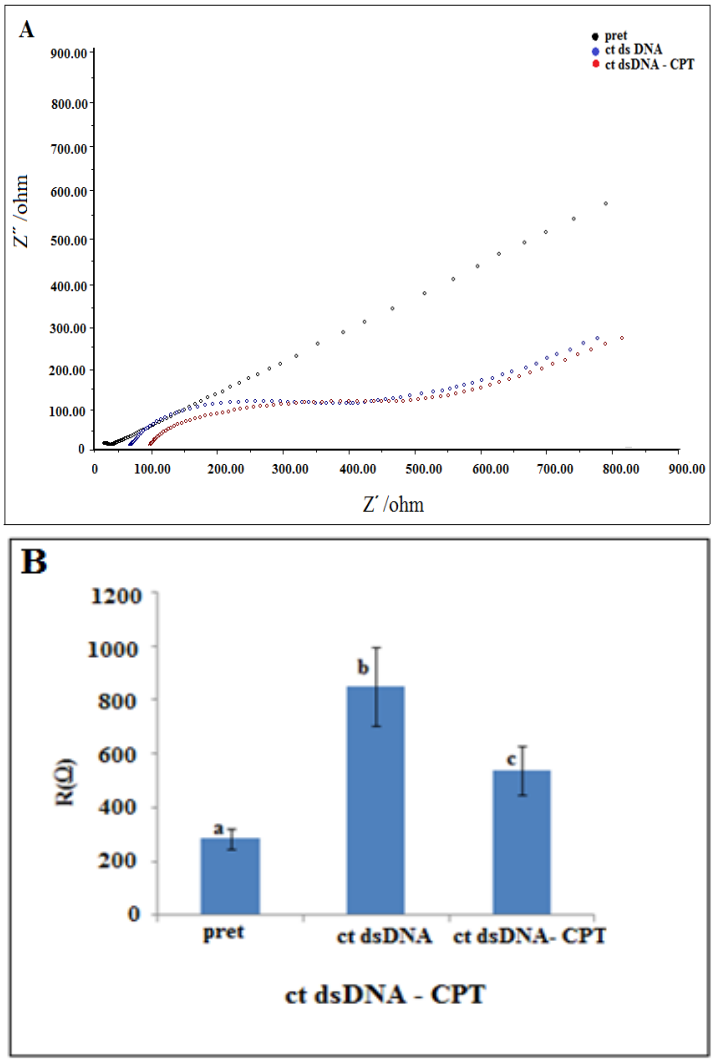

Figure 7. (A) Nyquist curve (B) Histogram of resistance of ct ds- DNA immobilized GCE and CPT interaction to transferred current load

Afterwards, a decrease in electrode resistance was observed after the interaction of CPT with ct dsDNA on the GCE surface. So, we can say that the conductivity of activated GCE is higher than that of ct dsDNA immobilized GCE and than that of GCE that happening interaction ct dsDNA with CPT on its surface.

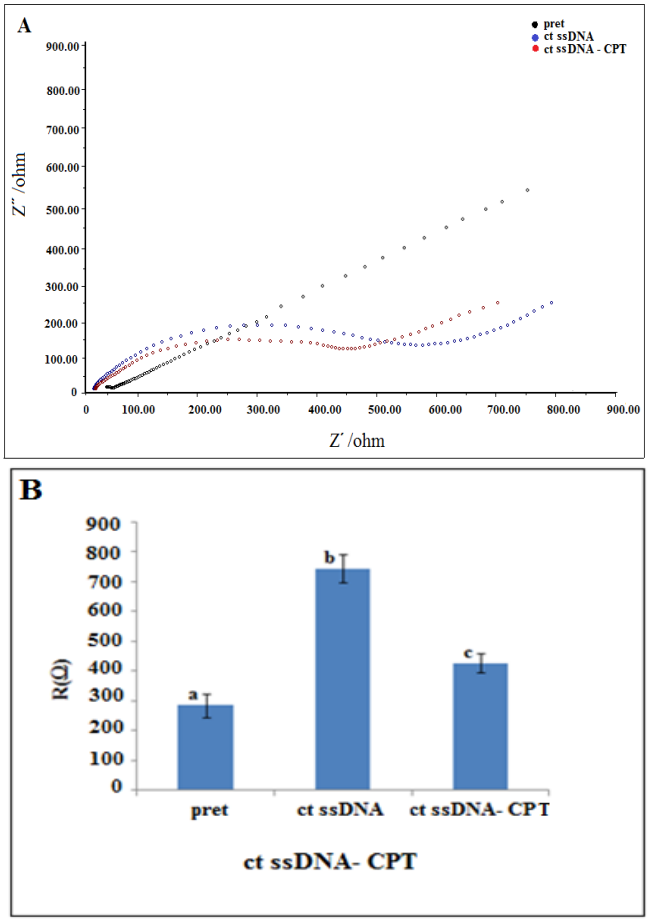

Figure 8. (A) Nyquist curve (B) Histogram of resistance of ct ss- DNA immobilized GCE and CPT interaction to transferred current load

When the Histogram in Figure 8 is examined, it is seen that a) pret (activated) GCE resistance, b) ct ssDNA immobilized GCE resistance, and c) GCE resistance after interaction CPT with ct ssDNA immobilized to GCE surface are compared. It was observed that there was an increase in the resistance of the GCE surface after ct ssDNA was immobilized compared to its previous state (only after surface activation). Afterwards, a decrease in electrode resistance was observed after the interaction of CPT with ct ssDNA on the GCE surface So, we can say that the conductivity of activated GCE is higher than that of ct ssDNA immobilized GCE and than that of GCE that happening interaction ct ssDNA with CPT on its surface.

When the measurements made with the impedimetric technique for both ct dsDNA and ct ssDNA are examined, it is seen that similar results are obtained. In addition, an increase in load transfer resistance was observed (Figure 7B and Figure 8B). We can say that in both types of DNA (ct dsDNA, ct ssDNA) DNA immobilization on the electrode surface and then interaction with the drug (CPT) cause differentiations on the electrode surface.

EIS experiments have shown that the interactions on the GCE face depend on two parameters (conductivity and resistance) that are inversely proportional to each other. As it is known, there is an inverse relationship between resistance and conductivity. Therefore, we can say that the conductivity decreases with increasing resistance.

If the histograms are interpreted in the light of this information; An increase in resistance was observed after DNAs (ct ds-DNA and ct ss-DNA) were immobilized on the GCE surface. And then, a decrease in resistance was observed with DNA-CPT interaction on the DNA-immobilized GCE surface (same results were found for both DNAs). Therefore, we can interpret that the conductivity of activated GCE is higher than that of DNA (ct ds-DNA and ct ss-DNA) immobilized GCE, and the conductivity of the DNA immobilized GCE surface decreases with CPT interaction.

In other words, by using these measurements, we can get information about whether the electrode surface is covered with DNA and whether the DNA-CPT interaction has taken place. Therefore, a decrease in the oxidation signal of guanine base was observed as the CPT concentration increased. This reduction indicates that CPT interacts with DNA, which is consistent with studies in the literature [11].

The optimal immobilization conditions of DNA to the GCE surface were determined. The optimal interaction time was found for 9 min (ct ds-DNA and ct ss-DNA), optimal DNA concentration 42 µg / mL (ct ds-DNA) and 36 µg / mL (ct ss-DNA).

Optimization experiments of DNA immobilized GCE and CPT interaction were performed. The optimized interaction time was 4 min and 3 min, respectively, and the optimized interaction amount was found to be 40 µg / mL for both DNAs.

As a result, we can say that capecitabine has an effect on DNA. We also believe that it will be an important electrochemical method for quantification of drugs such as capecitabine in biological materials.

Acknowledgment

This work is supported by the Scientific Research Project Fund of Sivas Cumhuriyet University (CÜBAP) under the project number F-387.

Conflicts of interest

The authors state that did not have conflict of interests.

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