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Electrochemical biosensor for simultaneously detection of Tamoxifen

Hasret Subak

Yuzuncu Yil University, Department of Analytical Chemistry, Faculty of Pharmacy, Van, Türkiye,
hasretsubak@yyu.edu.tr, ORCID: 0000-0003-0100-2529

ABSTRACT

Cancer is described as the uncontrollably multiplying abnormal proliferation of cells. Cancer can affect everyone, and risk of which rises with age, lifestyle, and environmental toxins. Tamoxifen (TAM) which is a selective estrogen receptor modulator, has estrogenic or antiestrogenic effects on the breast tissue by binding to the estrogen receptors. The current study presents a voltammetric biosensor to identify the effect of Tamoxifen on DNA structure. In this study, the effect of TAM on the double-stranded DNA (dsDNA) was investigated electrochemically in both the presence and absence of antioxidants. For this purpose, TAM-dsDNA-antioxidant interaction investigated by using the pencil graphite electrode (PGE). The DNA modified sensor was created simply by wet-adsorbtion method. The prepared biosensor was examined electrochemically by square wave voltammety (SWV) method, and its lowest concentration and optimum pH range were determined. The effect of TAM on dsDNA was investigated simultaneously for the first time in the literature.

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1. Introduction

The anti-estrogen tamoxifen was found by Beatson as an antineoplastic agent in the late nineteenth century. The anti-estrogen tamoxifen (TAM) found by Beatson is used in the treatment of breast cancer in pre-and post-menopausal women [1]. TAM which is a Selective Estrogen Receptor Modulator (SERM) has been used safely for more than 50 years as a single drug in the treatment of hormone-dependent breast cancer [2,3]. The chemical structure presented in fig 1 TAM has a benzothiophene core which is familiar with estrogen structure. Hence TAM effects the estrogen receptor like an agonist molecule [1,4]. There are two important pathways for using TAM in treatment which are blocking estrogen effect on cancer tissue and antiosteoporosis effect cause by increased bone mineral density. In light of TAM's effect mechanism, provide an agonistic effect as well as an antagonist effect by binding to estrogen receptors and causing estrogen-like effects [4,5]. In addition to these, TAM, known as a hormonal drug, is used in some hormone-sensitive cancers (pancreatic carcinoma, and in the treatment of benign breast disease) and in the treatment of male/female infertility due to this effect [2,6]. The certaindverse efeffectsf TAM are hot flushes, nausea and/or vomiting, vaginal bleeding or discharge, and menstrual disturbances which are based on antiestrogenic activity of TAM. Besides these, according to cancer drugs and their side effects, TAM is a very well tolerated drug. In addition, due to the side effects of tamoxifen, discontinuation of the drug was not common [2,6,7]. The cumulative use of TAM, which has been used by millions of women in cancer treatment and postmenopausal period due to its bone protection, makes it attractive to investigate. TAM's blockage by binding to the receptors and its agonist/antagonist effect allows it to be investigated with biological sensors. Chromatographic methods for TAM molecule determination [8,10], and electrochemical [4,11,12] determination methods were used. A reported study was used horseradish peroxidase immobilized on modified platinum electrode to define TAM by using CV and the EIS [11]. The antioxidants recommended together with chemotherapy in cancer treatment on tamoxifen, a chemotherapeutic, was investigated for the first time in our study. It is known that antioxidants reduce oxidative stress-induced carcinogenesis with their radical scavenging effect. For this reason, there are always questions about whether it is a correct practice for patients undergoing chemotherapy to take antioxidant-based food supplements during the treatment [13,15]. This is due to the fact that it is not known how the food supplements with high antioxidant content will affect the treatment protocol. If there is a patient in your neighborhood who is being treated for cancer or any diseases, you may have come across a patient's relative who says that you should eat these. The intense consumption of plants in the treatment of many diseases by the local people both in the world and in Turkey, and the centuries-old experience of folk medicine can guide various scientific studies. For this reason, the effect of the interaction of endemic antioxidant species that can be used as potential supplements by people who are treated for breast

cancer was investigated. In this study, the full electrochemical methods used in cancer treatment were investigated. In addition, it was desired to investigate how the anticancer effect changes or affects in an environment with antioxidants. For this, the effect on DNA and the change of its electrochemical behavior were investigated by using antioxidants.

1. Materials and methods

2.1 Chemicals

Tamoxifen citrate salt was obtained from the Sigma-Aldrich. were obtained from Sigma-Aldrich. Ultra-pure water was used for all buffer, stock and support buffer solutions preparations. In this work; NaOH (Riedel-de Haen), NaCl (Sigma), K_2HPO_4 (Merck), KH_2PO_4 (Merck), H_3PO_4 (Sigma), CH_3COOH (Riedel-de Haen, 99 %), tablet forms of CF (Sigma-Aldrich), H_3BO_3 (Sigma), fish sperm (dsDNA) (Sigma-Aldrich), single stranded from calf thymus (ssDNA) (Sigma) were used for analysing. The fish sperm dsDNA stock solutions were prepared as high concentration (such as 1000 µg/mL) and stored at -20°C. The antioxidant substance used in our study was obtained from Hypericum species found around the province of Van, which has a rich ethnomedical diversity. This species is still used by the local people for various medicinal purposes today.

2.2. Aparatus

All of the electrochemical measurements were performed with using an AUTOLAB 12 potentiostat/galvanostat analyzer (Eco Chemie, Netherlands). The voltammogram which obtained from the raw differential pulse (DP) was smoothed by Savitzky and Golay filter (level 2) of the General-Purpose Electrochemical Software (GPES) of Eco Chemie with moving average baseline correction, using a "peak width" of 0.01 V. PGE was used as a "working electrode" that con- tains 3 cm of graphite rod (0.5 mm diameter, Tombo, Japan) and a rotring model pencil was used as a holder. The 3-electrode system that includes of a pencil graphite electrode (GE, as a working electrode), an Ag/AgCl (KCl, 3M), (as a reference electrode) and a platinum wire auxiliary electrode obtained from Basi (USA).

2.3 Methods

In the study, all electrochemical analyzes of tamoxifen and antioxidant were carried out using the square wave stripping [16] voltammetry method. First, pencil graphite electrodes were activated in ABS buffer under 1.4 V for 30 seconds to form surface-active –COOH functional groups. All measurements were made with activated electrodes. In the method, electrochemical studies of tamoxifen and antioxidant were firstly performed. In this direction, pH scanning, frequency scanning, amplitude and scan rate measurements were made. In the experiment performed for stripping voltammetry , after the activated electrodes were immersed in the cell containing 10 ml of support solution , the appropriate concentration of tamoxifen was transferred to the cell . After adding tamoxifen, it was mixed for a certain time (30 sec). At the end of the period, measurements were made with square wave voltammetry. The general outline of the experiment is thus provided. Concentration study of tamoxifen Concentration study was carried out by measuring with 0.625/ 1.25/ 2.5/ 5/ 7.5/ 10/ 20 µg/mL tamoxifen. All electrochemical optimization studies TAM were performed using PGE and square wave voltammetry. pH scanning, frequency, amplitut, scan rate scans were performed as optimization parameters. After the most suitable conditions were provided, calibration study was carried out. In the experiments, the change of the electrochemical signal depending on antioxidants was also examined.

2.4 Measurement conditions

The electrochemcal measurement was performed by using SWV with 0 to +1.4 mV potential range; 5 mV step potential; 70 mV modulation amplitude; 0.05 s modulation time; 0.15 s interval time; 4 mV/s scan rate. The method indirectly detects the effect of the drug on the DNA structure.

2. Results and discussions

3.1 pH Scanning

Scanning was performed between pH 2/8 with BR buffer using PGE. The most suitable pH was determined as 5 in the obtained data. The voltammogram shows pH between 2 to 8.

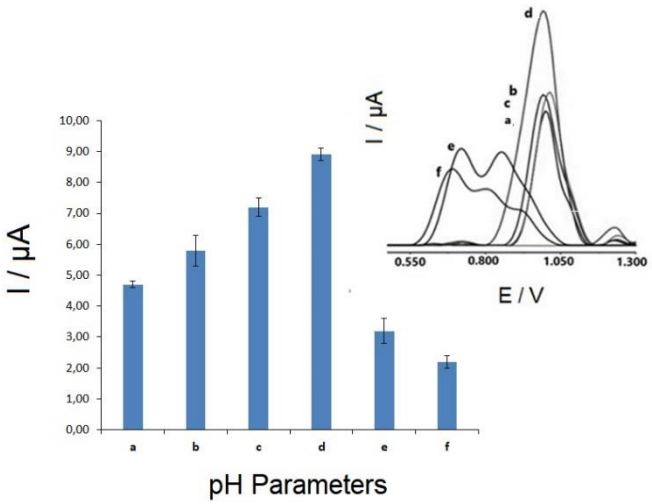

Figure 1. *The oxidation peak current values obtained from DPV voltammograms of 100µM TAM in the range changed from pH(a-d): 2.00 to 8.00 in 0.04 M B-R buffers at PGE*

To determine optimal oxidation conditions of TAM, B-R buffers (pH: 2.00 to 8.00) were used as supporting electrolytes. DPV voltammograms of the 100µM TAM in B-R electrolytes at different pH were recorded. The highest

current value related to the TAM oxidation was obtained in B-R buffer at pH=4.50 and this medium was chosen for further experiments. The oxidation peak current value of the 100 µM TAM obtained with the buffer solutions at different pH in the range changed from pH: 2.00 to 8.00 as shown in Fig.1.

3.2 Optimization parameters of TAM

After TAM was added to the measuring cell, it was mixed for a certain time (30 sec). At the end of the period, measurements were made with square wave voltammetry. The general outline of the experiment is thus provided. Concentration study of tamoxifen Concentration study was carried out by measuring with 0.725/1.25/2.5/5/10/20/30/40 µg/mL tamoxifen amounts Fig.2.

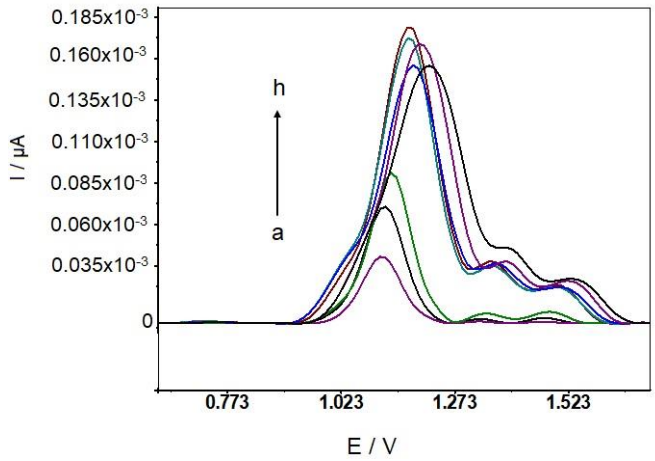

Figure 2. *Concentration study of tamoxifen which were presented a: 0.725/b:1.25/c:2.5/d:5/e:10/f:1 20/g:30/h:40 µg/mL*

Since the electrode surface reaches saturation after 5 µg/mL, the signal did not change or even decreased despite increasing concentration Fig 3.

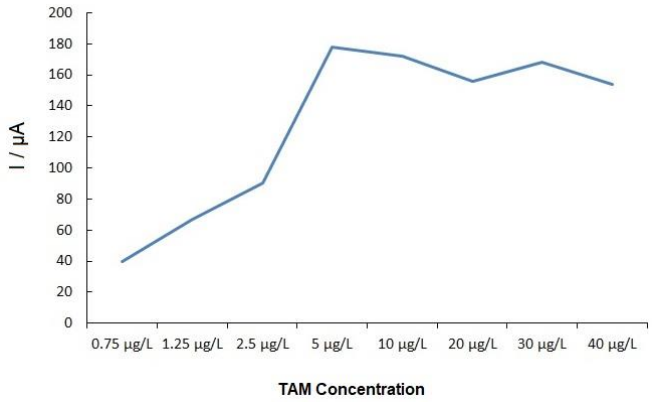

Figure 3. *In the given histogram, signal changes are seen due to increasing TAM concentrations. The drug solutions which increasing concentration prepared were immobilized on the electrode surface by stripping method.*

3.3 Biosensor preparation

PGEs were activated in an electrochemical cell which contains 4 mL of an acetate buffer solution (ABS; pH=4.80). Their 1 cm surface has applied to the buffer solution for 30 seconds at a potential of +1.40 V[17]. The activated PGE was modified with DNA by wet-adsorption technique. For the preparation of a biosensor under optimal conditions, time and concentration study was carried out with dsDNA. (Data not shown). The optimum condition for obtaining a DNA-modified biosensor was determined as a 20-mınute adsorptıon of 20µg/mL dsDNA-actıvated electrodes.

3.4 TAM-DNA interaction

The selective analysis of cancer therapy drugs, especially TAM, could create the possibility to better control of the treatment process. Considering these, analytical sensors can be evaluated as an auxiliary device for such analysis in cancer patients. For this purpose, DNA modified electrodes were added at increased concentrations of TAM in the same solution medium and mixed for 30 seconds to interact. At the end of 20 minutes adsorption of dsDNA 2.5-5-10-10-10-10-10 µl 1000 mL is added to br buffer and mixed for 30 seconds, and then the measurement is taken in the same cell (4 mL B-R buffer medium). The interaction will also be evaluated in the dsDNA solution environment in the continuation of the study.

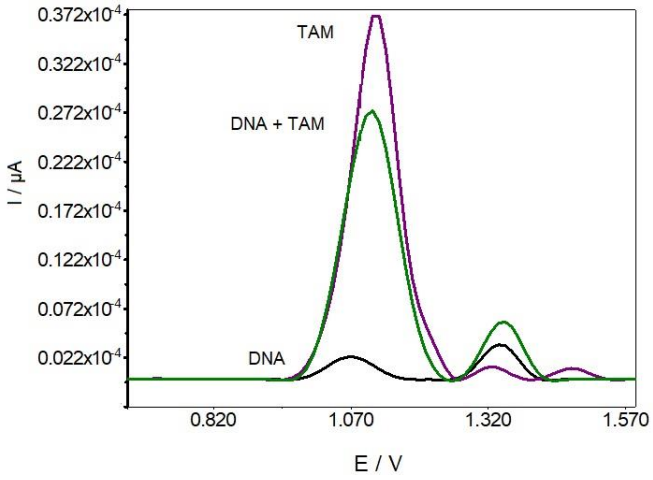

Figure 4. *TAM-DNA interaction. Before the interaction dsDNA solution was analysed alone. After that dsDNA was modified at the electrode surface and interact with TAM. The electrochemical investigation of dsDNA and the TAM was detected.*

Concentration study was carried out by square wave voltammetry method in B-R pH 5 buffer with PGE. Optimum concentration was find as 20µg/mL for DNA. % signal reduction was observed as increasing concentrations of TAM were added to the 20µg/mL DNA solution (Fig 4.).

3.5 Antioxidants – DNA interaction

The effect of antioxidant on tamoxifen and DNA interaction was also investigated. In this direction, the effect of antioxidant on DNA in the presence and absence of tamoxifen was investigated.

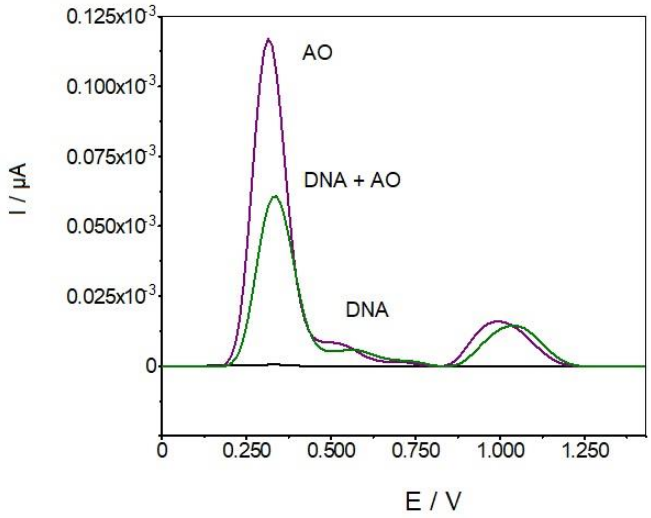

Figure 5. *Antioxidant-DNA interaction. Before and after interaction The change in the oxidation signal of guanine (+1.0 V) and the extract (0.35 V)were measured using SWV method.*

The experiment was carried out by applying the optimum conditions obtained with the PG electrode. AO gives signals at two potential area. Here is the effect with DNA at 1.0V which guanine oxidation potential area. There was a decrease in the signal around 0.30v and a shift towards the anodic region in the other signal. It is thought that there may be a reducing effect on the guanine oxidation signal by intercalation Fig 5.

3.6 Antioxidants – TAM interaction

In addition, the effect of antioxidant on the electrochemical behavior of tamoxifen in the absence of DNA was investigated in the study. In this direction, the signal change with increasing concentration of tamoxifen was investigated by measuring the AO and TAM oxidation potential points (Fig 6.).

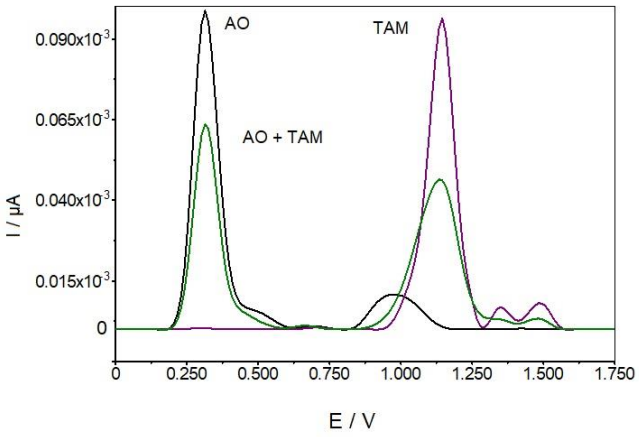

Figure 6. *Antioxidant-TAM interaction. The interaction AO with TAM was examined in the study. The evaluated data show that there are different changes in both signals.*

Despite the increasing concentration, the antioxidant signal reaches a maximum level of 120 µA and saturates on the electrode surface. When TAM is added to the environment, signal increase is observed. Concentration Studies of AO and TAM were also carried out separately. The concentration studies of TAM and AO were evaluated together with the concentration studies conducted separately. As a result of this study, a change was observed in the electrochemical behavior of AO in the presence of TAM. (Data not shown). Depending on the preliminary studies prepared for publication, the change in AO is indicated on the voltammogram (Fig 6.). Accordingly, AO and TAM were interacted electrochemically. The effects on the DNA concentration in the same medium were also be examined.

3.7 Antioxidants - TAM – DNA simultaneously interaction

Three electroactive materials, DNA, AO and TAM, were studied seperately. In this step, these three electroactive substances were examined in the same electrochemical cell. In the measurement, the effect of AO and TAM on DNA was examined. The results of this examination were compared with the data obtained from the effect of both TAM and AO on DNA seperately and together.

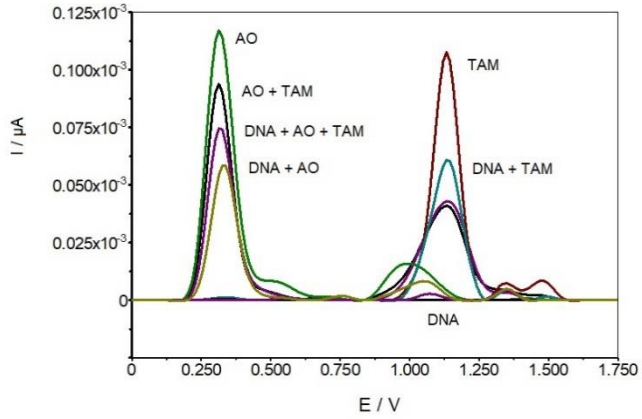

Figure 7. *Antioxidant-TAM-DNA interaction. In the given voltammogram showed the effect of TAM and AO on DNA in the same time and seperately.*

Evaluation was made at three different oxidation points. The effects of AO and TAM on in the DNA medium were examined on the electrode surface separately and together. As a result of this; after interaction with DNA, the TAM and AO signal decreased seperately. Here, the evaluation was made at two different points of the potential area. The decrease in the signal at 0.35 V is most distinct potential point which is belong AO interaction with DNA. The interaction of AO alone and wıth DNA has been compared. According to this, the change in the electrochemical signal of AO when it interacts with dsDNA alone is greater than the change obtained in the TAM environment. When the result is compared, it shows that AO interacts competitively with TAM in interaction with DNA. In the second signal, the overlap of TAM-AO and TAM-DNA-AO signals shows that the interaction with AO gives the same signal independent of DNA. The second electrochemical evaluation point, AO and TAM, was evaluated at the guanine oxidation area of DNA (1.0V). Accordingly, while TAM and AO do not change the guanine oxidation signal of DNA, it has been observed that AO alone changes the guanine oxidation signal of DNA. When TAM and AO were together, their signals at their oxidation sites did not change before and after the DNA interaction.

3. Conclusions

In this article, the simultaneously interaction of TAM with DNA and AO was investigated by electrochemical voltametric studies. The study of the interaction between the anticancer drug Tamoxifen and DNA is crucial to identify possible DNA damage during treatment. The research will also be valuable in the design of the molecule-specific electrochemical biosensor to be applied in diagnostic tests and the development of drugs for cancer treatment patients. A simple, fast and precise SWV method is recommended for the determination of Tamoxifen in pharmaceutical formulations. In conclusion, these studies in a new biosensor may play an important role in the development of unknown drug-DNA interaction mechanisms.

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