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Research Article

ELECTROCHEMICAL DETERMINATION OF THE INTERACTION BETWEEN ANTICANCER DRUG CAPECITABINE AND DNA BY CARBON PASTE ELECTRODE

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ABSTRACT

In this study, the interaction of an anticancer drug, Capecitabine with DNA was investigated by electrochemical methods using carbon paste electrode (CPE). The interaction of Capecitabine with single-stranded Calf thymus DNA (ss DNA) and double-stranded Calf thymus DNA (ds DNA) at electrode surface, or in solution phase was investigated by monitoring the changes at the oxidation signal of guanin base of DNA measured by differential pulse voltammetry (DPV) technique. Impedimetric measurements were performed by electrochemical impedance spectroscopy technique that also confirmed that ds DNA was immobilized onto the electrode surface. The detection limit (DL) in the case of interaction of Capecitabine with dsDNA and ssDNA interaction was calculated and found to be 17.35µg/mL and 17.12µg/mL, respectively.

Keywords: *DNA Biosensors, Capecitabine, CPE*

INTRODUCTION

Biosensors are obtained by incorporating biological substances such as enzymes, cells, tissues, antibodies, nucleic acids into the structure of an electrochemical sensor (Özsöz., et al 2002; Wang, 2002). DNA biosensors are biosensors in which DNA is used as the biological material that recognizes it by interacting with the substance to be analyzed (Wang, 1997; Erdem, 2007) . DNA biosensors are used to elucidate the effects of certain DNA - targeted drugs or substances on DNA and to determine the interaction mechanisms of these substances (Wang, 1997; Nawaz, et al., 2006). Drug interaction with nucleic acids is one of the most important factors in the design of drugs and the development of related processes (Erdem, 2002). Many different techniques are used to examine drug - DNA interaction, which have several advantages and disadvantages. Therefore, the demand for new techniques for drug design and process development based on drug-DNA interaction is increasing (Özsöz, et al., 2002; Nawaz, et al., 2006).

Electrochemical DNA biosensors consist of a nucleic acid recognition layer immobilized to the electrochemical transducer (Rauf, et al., 2005; Erdem and Özsöz, 2001). The nucleic acid recognition layer detects changes in DNA structure or the specific sequence of DNA during the interaction of the binding molecules with DNA. DNA - drug interaction mechanism can be explained using the difference between pre - and post - interaction measured signals (Erdem and Özsöz., 2001). This interaction is also used to determine the quantity of the drugs analyzed or to design new drugs (Rauf, et al., 2005). During the design of many newly synthesized substances, drugs and especially DNA - targeted carcinogenic drugs, rapid and effective clarification of the interaction of these substances with DNA will enable these studies to progress more rapidly for their purposes. DNA - drug interactions can be detected successfully using DNA biosensors. This detection can be achieved by the guanine/adenine signal, which is the electroactive bases of the DNA, or by the electrochemical signal of the drug to be analyzed. DNA - drug interactions can be interpreted according to the changes in these signals (Wang, et al., 1998). Biosensors containing the nucleic acid (DNA) recognition surface are used for purposes such as elucidating or quantifying the interaction mechanism of the substance to be analyzed (carcinogenic substances, drugs, etc.) that interact with this surface, or monitoring hybridization events in certain regions of the base sequence in DNA (Wang, et al., 1998). Interaction of DNA with certain drug molecules (especially interaction with drug molecules with anticancer properties) and determination of this interaction with new methods developed is highly important for new product designs. The rapid detection of by - products that may occur in DNA after the interaction of a chemical substance or metabolite with DNA is very important for cancer research (Mikkelsen, 1996). There are many publications in the literature on DNA – drug interaction. In one of them, in the study of anticancer herbal medicine emodin with differential pulse voltammetry technique and using alternating voltammetry technique (L. Wang et al., 2006). found that emodine intercalated into DNA double helix structure and interacted with DNA. In another study; according to the study of (H. Nawaz et al, 2006) with ciprofloxacin having antibacterial effect of quinoline derivative, it showed that ciprofloxacin binds to DNA electrostatically and by intercalation (Niu, et al., 2008).

Many antitumor drugs show their effect by binding to DNA (Wang, et al., 2006, Richardson and Springfield., 1981). This information provides an opportunity to examine whether many compounds have potential to be used as anticancer drugs. In similar studies based on drug - DNA interaction, electrochemical studies (Marin, et al., 1998) have shown that drugs that interact with DNA cause a decrease or increase in the electrochemical response (Wang, et al., 1996; Yan, et al., 2001). Changes in the signals of electroactive bases in DNA or changes in the electrochemical signal of the substance to be analyzed provide reliable interactions between the analyte and DNA. In this study, the interaction and electrochemical behavior of

the anticancer drug, capecitabine, with single - use CPE modified or unmodified by DNA were investigated. DNA – drug interactions can be classified in two ways as interaction in solution phase and interaction on the electrode surface (İnteraction on the DNA modified electrode surface, interaction on the surface of the drug modified electrode). In this study, the interactions in solution phase and DNA modified electrode surface will be examined. The amount of capecitabine will be calculated based on reductions in the signal of the electroactive guanine base of DNA. In addition, when the capecitabine concentration is changed, parameters such as the response of the guanine signal, duration of interactions, and reproducibility will be examined. Although there have been many studies on anticancer drugs in the literature, no studies have examined the capecitabine - DNA interaction with CPE. In this respect, our study will eliminate an important deficiency in the literature (Palecek, 1996).

MATERIALS and METHODS

Devices Used

Scales (Precisa XB 220A), Sound vibrating cleaner (Bandelin Sonorex), pH - meter (WTW series), Magnetic stirrer (AGE velp), Vortex (Velp scientifics), Potentiostat (AUTOLAB 302, GPRES 4.9 software, Eco Chemie), Ag/AgCl reference electrode, Platinum wire (used as auxiliary electrode).

Chemicals Used

Capecitabine (CPT) (Sigma), Acetic acid (99-100%) (Sigma-Aldrich), Hydrochloric acid (37%) (Sigma-Aldrich), Sodium Hydroxide (Sigma), Tris (hydroxymethyl) aminomethane hydrochloride (Sigma), Sodium chloride (Sigma), EDTA disodium salt (Sigma), *Calf thymus* ds-DNA (Sigma), *Calf thymus* ss-DNA (Sigma), 18 mega-ohm ultra-pure water was used in all studies. Experimental studies were performed at room temperature (25.0 ± 0.5 ° C).

Preparation of the solutions used

Preparation of buffer solutions:

Preparation of 0.05 M phosphate buffer solution (pH 7.4; PBS): The 0.05 M phosphate buffer solution used during the measurements contained 1.36g (0.01 mol) KH_2PO_4, 6.96 g (0.04 mol) KH_2PO_4 and 1.168 g NaCl (0.02 mol) per liter.

Preparation of 0.50 M acetate buffer solution (pH 4.81; ABS): The 0.5 M acetate buffer solution contains about 29 mL of concentrated acetic acid per L and 1.168 g NaCl. The pH of the buffer solution was adjusted to 4.81 with 0.1 M NaOH solution.

Preparation of 0.01 M Tris-HCl, 1 mM EDTA buffer solution (pH 8.0;Tris-EDTA): The 0.01 M Tris-HCl, 1 mM EDTA buffer solution used contains 1.576 g Tris HCl and 0.372 g EDTA per liter. The pH of the solution was adjusted to 8.0 by adding 0.1 N NaOH and/or 0.1 N HCl measured with the pH meter.

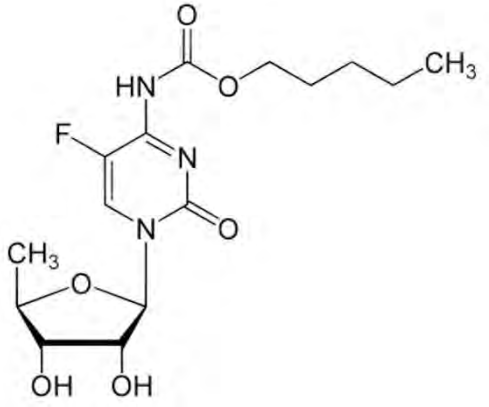
Preparation of the Capecitabine solution: The stock solutions of the purchased 100% pure Capecitabine with ABS buffer were prepared and stored at 4˚C by placing them in eppendorf tubes in volumes of 50µL for later use.

Preparation of DNA solutions: DNA from *Calf thymus* gland (= *Calf thymus* DNA); double-stranded DNA (ds DNA) stock solutions; 1000µg/mL was prepared with TE solution (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and stored below zero [21]. The Ct - ds DNA dilute solution was prepared with 0.5M Acetate buffer (pH 4.8). To minimize the exposure of the solution to light, it was stored in the refrigerator at -20˚C in a non-light box. The single stranded DNA (ss DNA) solution was also prepared as described above for ct - ds DNA and was stored

in the refrigerator at -20°C. The single-stranded DNA (ss DNA) solution was also prepared as described above for ct - ds DNA and stored in the refrigerator at -20 °C.

General information about Capecitabine (CPT)

Figure 1: Structural formula of Capecitabine ($C_{15}H_{22}FN_3O_6$).



Pharmacological Properties: Capecitabine is a cancer (chemotherapeutic) drug. It prevents the prolifereation of cancer cells. Slows down their development. And slows down their spread in the body. Capecitabine is a non-cytotoxic fluoropyrimidine carbamate that acts as an orally administered prodrug of the cytotoxic 5-fluorouracil (5-FU). Capecitabine is activated through several enzymatic steps. In the last step, thymidine phosphorylase (ThyPase), an enzyme involved in its conversion to 5-FU, is found in tumor tissues; this enzyme is also found in normal tissues but is usually at lower levels. In human cancer xenograft models, capecitabine showed a synergistic effect in combination with docetaxel; this effect may be due to the increase in thymidine phosphorylase functions by docetaxel. There is evidence that the metabolism of 5- FU in the anabolic pathway blocks the methylation reaction of deoxyuridylic acid to thymidilic acid, thereby inhibiting deoxyribonucleic acid (DNA) synthesis. 5-FU formation also results in inhibition of RNA and protein synthesis. Since DNA and RNA are essential for cell division and growth, the effect of 5-FU may be to produce a thymidine insufficiency that causes cell unstable growth and death. The effects of blocking DNA and RNA synthesis are evident on cells that proliferate and metabolize 5-FU faster. Studies have supported the use of Xeloda as a first-line treatment for metastatic colorectal cancer. Data from a multicentered, randomized, controlled phase III trial support the use of Xeloda in combination with docetaxel after the failure of anthracycline-containing cytotoxic chemotherapy to treat patients with locally advanced or metastatic breast cancer. In addition, data from multicentered two-phase II studies support the use of Xeloda monotherapy for the treatment of patients after failure of the taxanes and anthracycline-containing chemotherapy regimen, or for whom further anthracycline treatment is not indicated (Karadeniz, et al., 2007).

Method Used

For the steps regarding the activation of the electrodes used and attachment of ct - ds DNA and ct - ss DNA to the electrode surface, interaction of DNA material with the material examined on the electrode surface, the path reported in the current literature (Erdem, et al., 1999; Kuralay, et al., 2009; Wang, et al., 2001) was followed. The electrode was renewed each time and repeated 5 times in succession and current values were measured.

Preparation of electrodes used

In this study, using the differential pulse voltammetry technique, μ-AUTOLAB III (Eco Chemie, Netherlands) was used as the potentiostat device and GPES 4,9 was used as the software program. As the triple electrode system, carbon paste electrode was used as working electrode, Ag/AgCl was used as reference electrode and platinum wire was used as counter electrode. Each electrode was connected to the system by metal connections and this triple electrode was adjusted to a volume of 6 mL of the measuring solution into which the system was immersed.

Carbon Paste Electrode (CPE) Preparation: The working electrode is made of 3 mm diameter glass tube and contains carbon paste inside, and electrical conductivity is provided by copper wire. The carbon paste was prepared by homogeneous mixing of graphite powder and mineral oil at a ratio of 70: 30. After the electrode (CPE) was prepared, the electrode surface was turned into a homogeneous surface with parchment paper (Wang, 1997; Erdem and Özsöz, 2001; Wang, et al., 1996).

Electrochemical Impedance Spectroscopy (EIS) Measurements: After the DNA (ct - ds DNA and ct – ss DNA) immobilization optimization and DNA - CPT interaction optimization for the working electrode, necessary solutions were prepared and measurements were taken for EIS experiments. Solutions were prepared containing 164.5mg $K_3Fe(CN)_6$ (molecular mass: 329.243 g) and 208.13 mg $K_4Fe(CN)_6.3H_2O$ (molecular mass: 422,38 g) redox probes in which measurement would be made with different pH values. To the prepared redox solution probe [Fe $(CN)_6^{3-/4-}$1.49 g of KCl was added (for 200 mL) to keep the ionic strength constant. The redox pair probe was adjusted in this way in all experiments. In electrochemical impedance spectroscopy experiments, Ag/AgCl was used as reference electrode and Pt wire as auxiliary electrode. Using the Frequency Analyzer (FRA) software, the impedance of the solutions was obtained by electrochemical impedance spectroscopy technique. The impedance measurements were fixed at a wave height of 10 mV in the prepared redox solution so that the system properties were kept in equilibrium. In order to obtain Nyquist curves, the applied frequency was adjusted between 0.1 Hz and 100 kHz. As with all previous studies, the surface of the electrodes was refreshed each time, repeated 5 times in succession, and the surface resistance of the electrode was measured.

RESULTS AND DISCUSSION

DNA Immobilization on Activated CPE elektrod

Passive adsorption was selected for CPE as the immobilization technique. Separate experiments were performed for ct - ds DNA and ct - ss DNA with activated carbon paste electrodes. Firstly, the optimal time of immobilization (**Figure 2**) and then the optimal DNA concentration for that time were optimized (**Figure 3**) by keeping the concentrations of DNA to be immobilized constant. Guanine peak current values obtained from DPV measurements were used to optimize both time and DNA concentration. To do this, the triple electrode system was immersed into the electrochemical cell and then voltammetric measurement was performed. The oxidation signals of guanine were measured in ABS (pH 4.8) by DPV (Differential Pulse Voltammetry) technique with a scanning speed of 50 mV/s and pulse amplitude of 50 mV between 0.2 V and 1.4 V.

Figure 2: Voltamograms (A) and histograms (B) showing guanine signals measured by DPV technique after interaction of ct - ds DNAs immobilized on CPE surface at different times: (a) 1 (b) 3 (c) 5 (d) 7 (e) 9 (f) 11 min

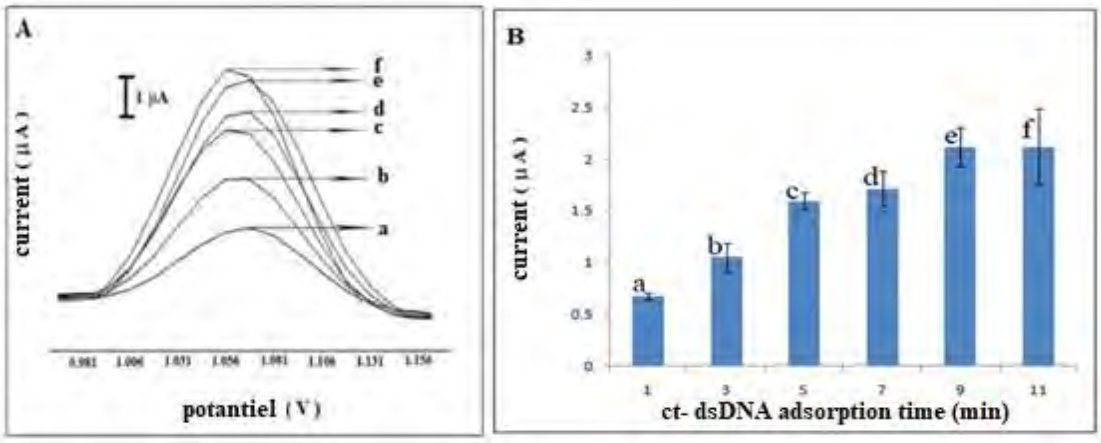
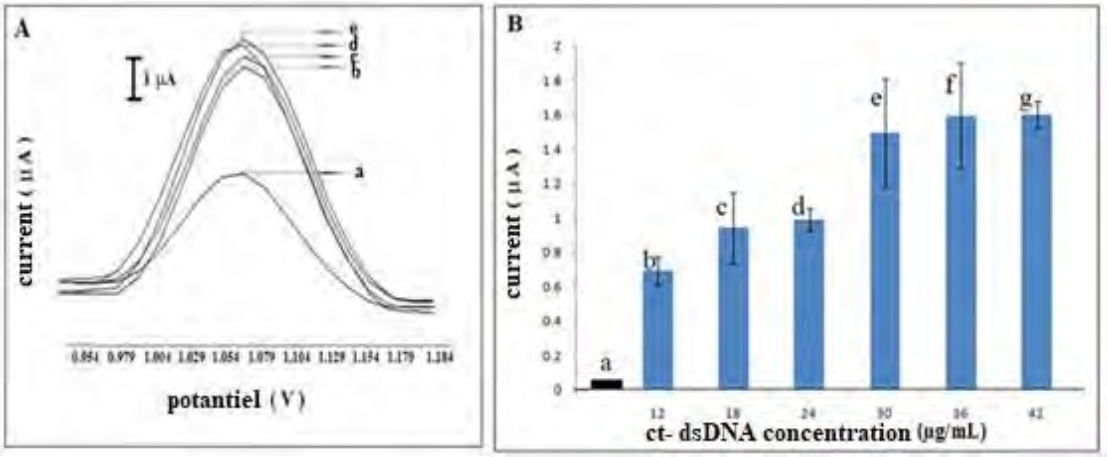


Figure 3: In the absence of (a) ct - ds DNA immobilized on the CPE surface, voltamograms (A) and histograms (B) showing guanine signals measured by DPV technique of ct - ds DNA at different concentrations: (b) 12, (c) 18, (d) 24, (e) 30, (f) 36, (g) 42 µg/mL



Time and concentration optimization in DNA immobilization was performed separately for both ct - ds DNA and ct - ss DNA. As shown in **Figure 2** and **Figure 3**, while the optimum interaction time for ct - ds DNA was found to be 9 minutes most favorable for reproducibility and 42µg/mL for ct - ds DNA concentration, this time was determined as 9 minutes for ct - ss DNA and 36 µg/mL for ct ss-DNA (not shown).

Optimization studies of CPT (Capecitabine) interaction with immobilized DNA (ct-dsDNA and ct- ssDNA) on CPE surface

Ct - ds DNA and ct - ss DNA immobilized CPEs were incubated at 110 ’µL of solutions containing Capecitabine at various concentrations for various periods of time, and the optimal time for DNA-drug interaction (**Figure 4**) and then the optimal drug concentration for this time (Figure 5) was determined. Reductions in guanine peak currents after DNA - drug interaction were utilized in the optimization processes. The oxidation signals of guanine were measured in ABS (pH 4.8) by DPV technique with a scanning speed of 50 mV/s and pulse amplitude of 50 mV between 0.2 V and 1.4 V.

Figure 4: Response effect of CPT immobilization time: Voltamogram (A) and histogram (B) showing guanine signals measured by DPV technique by immersing at different times: (a) without CPT , with ct - ds DNA, (b) 1, (c) 2, (d) 3, (e) 4 ,(f) 5 min interacting whit ct - ds DNA immobilized CPEs in CPT solution

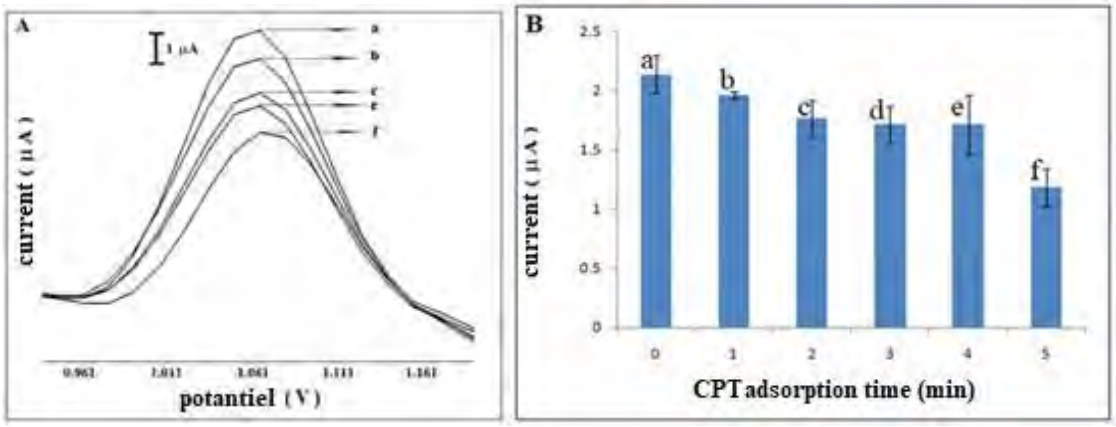
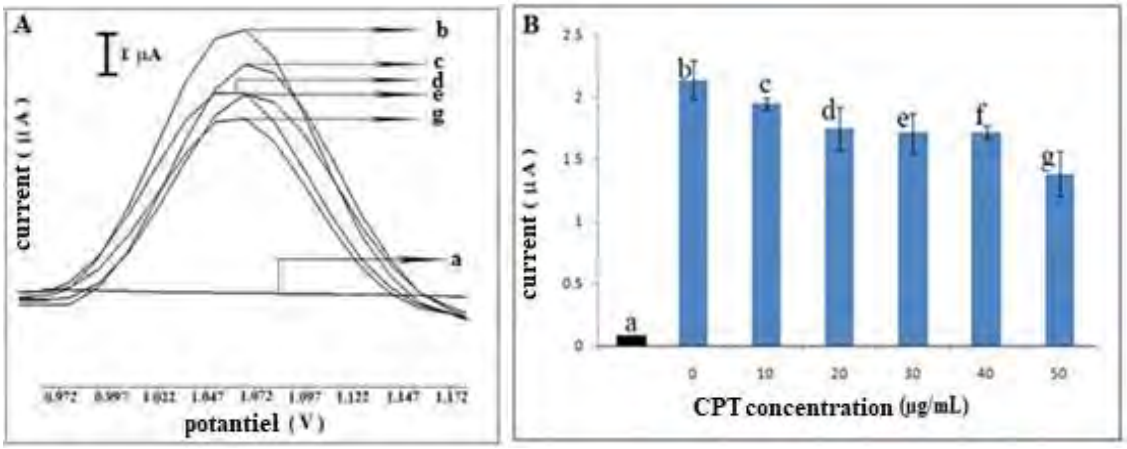


Figure 5: Response effect of CPT concentration: Voltamogram (A) and histogram (B) showing guanine signals measured by DPV technique by immersing in CPT solutions of different concentrations such as ct - ds DNA immobilized CPE's (a) without ct - ds DNA (b) without CPT, with ct - ds DNA (c) 10 (d) 20 (e) 30 (f) 40 (g) 50 mg/ml.



Time and concentration optimization in DNA - CPT interaction was performed separately for both ct - ds DNA and ct - ss DNA. As shown in **Figure 4** and **Figure 5**, the optimal interaction time for ct - ds DNA with CPT was found to be optimal 3 minutes for reproducibility and the CPT concentration was 40 µg/mL, while this time was 3 minutes and the CPT concentration was 30 µg/mL for for ct - ss DNA-CPT interaction (not shown). Calibration curves for both ct - ds DNA - CPT and ct - ss DNA - CPT interactions using the data obtained are shown in **Figure 6** and **Figure 7**.

Figure 6: Calibration curve of CPT concentration change in CPT interaction with ct - ds DNA immobilized on CPE surface.

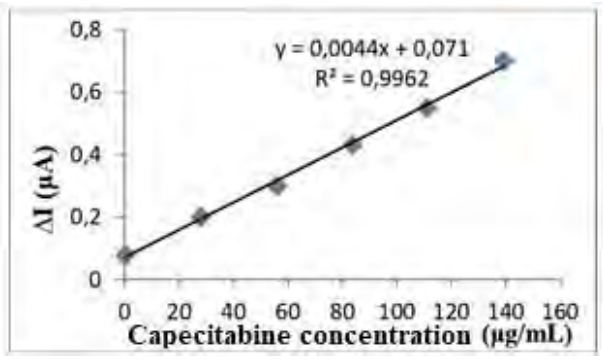
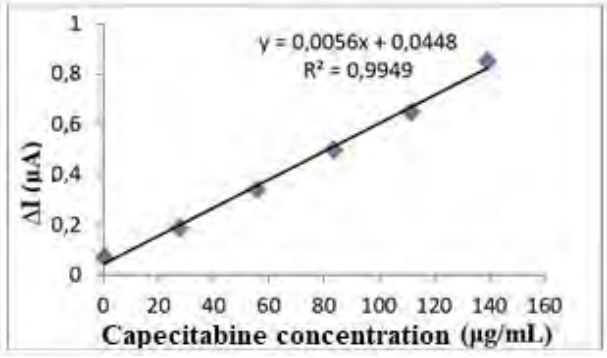


Figure 7: Calibration curve of CPT concentration change in CPT interaction with ct - ss DNA immobilized on CPE surface.

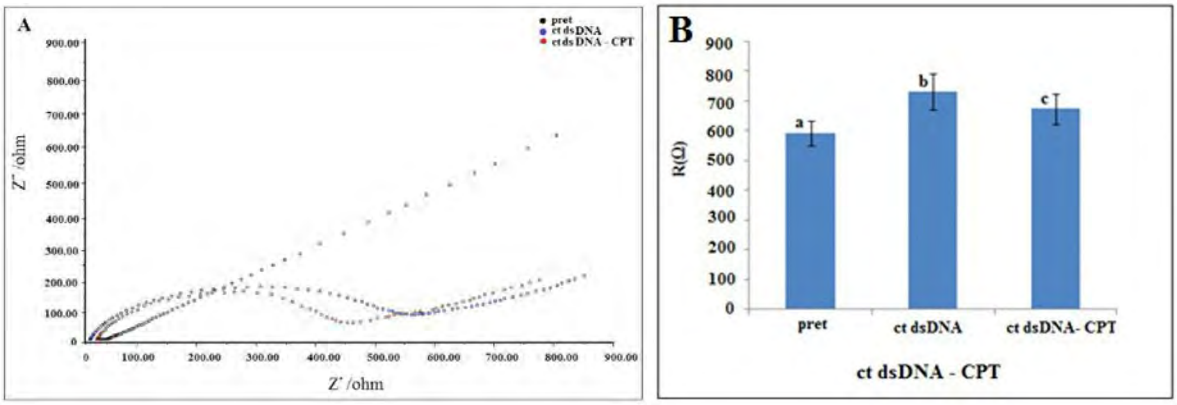


Determination limits were calculated according to the regression equation (y=0,044x + 0.071) of the calibration plot plotted for ct - ds DNA - CPT interaction with DL = yB + 3 sB correlation (yB: blind signal; sB: blind standard deviation; n = 4) and the regression equation (y=0.0056x + 0.0448) of the calibration plot plotted for ct - ss DNA - CPT interaction. The minimum detection limit for ct - ds DNA - CPT interaction was 17.35 μg/mL and the lowest detection limit for ct – ss DNA - CPT interaction was 17.12 mg/mL.

Results of Electrochemical Impedance (EIS) Tests

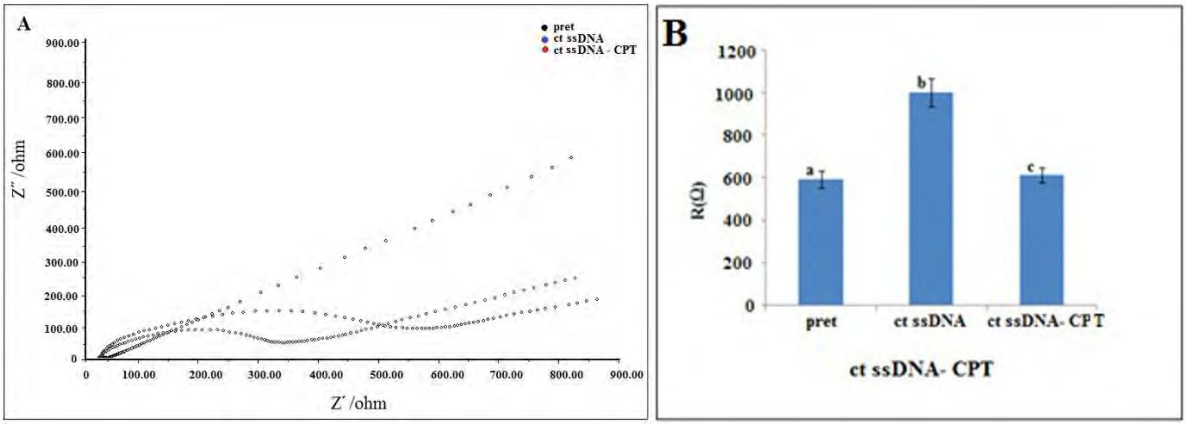
In this method, CPEs were activated by 1.4 V 60 seconds activation process applied in other studies. The only different step is the step where measurements are taken for EIS experiments using Frequency Analyzer (FRA) software instead of differential pulse voltammetry. After ct - ds DNA immobilization to the CPE surface at optimum conditions (42 μg / mL, 9 minutes), EIS experiments were performed in redox solution. Ct - ds DNA - CPE interaction on the surface of the CPE after immersion of ct - ds DNA immobilized on the surface of the CPE was again measured for its resistance to the transferred current in the redox solution and shown in **Figure 8** with the Nyquist curve generated from values close to the mean values.

Figure 8: (A) Niquist curve (B) Resistance of ct - ds DNA - CPT interaction to the transferred current load on the surface of the CPE; (a) resistance of activated CPEs, (b) resistance of ct - ds DNA immobilized CPEs, (c) resistance of ct - ds DNA - CPT interaction immobilized on CPE surface



After ct – ss DNA was immobilized to the CPE surface at optimum conditions (36 µg/mL, 9 minutes), all experiments on ct – ds DNA were repeated for ct – ss DNA. The resistance of the Ct - ss DNA - CPT interaction to the transferred current was measured and is shown in **Figure 9** with the Nyquist curve generated from values close to the mean values.

Figure 9: (A) Niquist curve **(B)** The resistance of the ct - ss DNA - CPT interaction to the transferred current load on the surface of the CPE; (a) resistance of activated CPEs, (b) resistance of ct - ss DNA immobilized CPEs, (c) Ct - ss DNA - CPT interaction immobilized on CPE surface



According to electrochemical impedance measurements in DNA immobilization or after DNA - CPT interactions, electrode surface differentiated and load transfer resistances increased.

CONCLUSION

In our study for sensor-based DNA analysis, activation of carbon paste electrodes (CPE), optimization for double stranded DNA (ct – ds DNA) and single stranded DNA (ct – ss DNA) analysis and its interaction with capecitabine (CPT), an anticancer drug, were investigated with electrochemical methods. In this study, where carbon paste electrodes were used as working electrodes, electrochemical behavior was investigated by differential pulse voltammetry (DPV) technique. Electrochemical determination of DNA immobilized on the

CPE surface was performed by measuring the oxidation signal of guanine, the electroactive base of DNA by DPV technique. Moreover, the effect of CPT concentration, change in DNA concentration and surface activation on the response was investigated and highly good results were obtained in terms of reproducibility and sensitivity. Before examining the interaction of anti-cancer drug CPT with DNA, electrochemical behavior of the drug in the studied potential range was examined and no electroactive species was observed in this range. After the interaction of CPT with DNA, it was observed that the oxidation signal of the guanine base, an electroactive group of DNA, decreased due to increased drug concentration in DNA - drug interaction. This reduction in guanine signals is a result of the interaction of CPT with double - stranded DNA, in alignment with the results of similar studies in the literature (Nawaz, et al., 2006), and may be explained by the fact that groups suitable for oxidation in the structure of this molecule are partly present for the redox reaction after interaction.

For immobilization of ct – ds DNA and ct – ss DNA on CPT surface, the optimized interaction time was 9 minutes for ct – ds DNA and 9 minutes for ct – ss DNA, the optimized amount of interaction was found to be 42 µg/mL for ct – ds DNA and 36 µg/mL for ct – ss DNA. In the examination of CPT - DNA interaction with DNA immobilized CPTs, the optimized interaction time was 3 minutes for ct – ds DNA and ct – ss DNA, the optimized amount of interaction was found to be 40 µg/mL for ct – ds DNA and 30 µg/mL for ct – ss DNA. In the examination of pH effect on CPE surface interactions, the pH of ct – ds DNA and ct – ss DNA was 4.8 and the guanine signal of ABS buffer was the highest.

For immobilization of ct – ds DNA to the activated CPE surface, after the optimum concentration of ct – ds DNA was determined as 42 µg/mL and the optimum interaction time was determined as 9 minutes and ct – ds DNA - CPT interaction at the activated CPE surface was determined to have an optimal CPT concentration of 40 µg/mL and an optimum interaction time of 3 minutes, EIS measurements were taken for activated CPE, ct ds-DNA immobilized CPE and CPE after ct ds-DNA - CPE interaction. After ct – ds DNA immobilization, an increase in the resistance of the CPE surface compared to the previous state was observed and a decrease in the resistance after ct–ds DNA - CPT interaction was observed. As is known, the resistance and conductivity are inversely proportional, that is to say, the higher the resistance, the lower the conductivity. When the histogram is examined with this in mind, we can say that the activated CPE conductivity is higher than the CPE after ct – dsDNA immobilized CPE and ct – dsDNA - CPT interaction. In the immobilization of ct–ssDNA to the activated CPE surface, the optimum concentration of ct – ss DNA was determined as 36 µg/mL and the optimum interaction time was determined as 9 minutes. And after ct – ss DNA - CPT interaction at the activated CPE surface was determined as 30 µg/mL of optimum CPT concentration and 3 minutes of optimum interaction time, EIS measurements were taken for activated CPE, ct – ss DNA immobilized CPE and CPE after ct – ssDNA - CPT interaction. After ct – ssDNA immobilization, it was observed that there was an increase in the resistance of the CPE surface compared to the previous state and a decrease in the resistance after ct – ss DNA - CPT interaction. Activated CPE conductivity was found to be higher than the CPE after ct – ss DNA immobilized CPE and ct – ss DNA - CPT interaction.

As a result, it is seen that electrochemical sensors developed using designed DNA biosensors can provide more sensitive, reliable and selective results in the determination of drug - DNA interactions. It is important to investigate whether Capecitabine, a commonly used anticancer drug, has an interaction with DNA and to develop an inexpensive and inexpensive electrochemical method for the determination of such drugs in biological samples.

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Research Article

VOLTAMMETRIC ANALYSIS OF TAMOXIFEN RECOVERY AND ITS INTERACTION WITH DNA

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ABSTRACT

The design of biosensors is one of the most important areas of analytical chemistry today, and interest in DNA-based bio (nano) sensors developed to examine applications related to compound-DNA interaction has been increasing in the last two decades. Changing in DNA structure, even for therapeutic purposes, can have serious effects on human health. The detection of any chemical substances in DNA sutructure is very important. In this study, an anticancer drug Tamoxifen (TAM) is used in the treatment of cancer since the early 1970s was identifying the possible DNA interaction during treatment by using differential pulse voltammetry (DPV) based on both TAM and guanine oxidation signals at the disposable pencil graphite electrode (PGE). The effect of TAM on single stranded (ss)-DNA and double stranded (ds)-DNA showed differences, depending on the double helix and single stranded structure. It was found that TAM interacting to ds-DNA more strongly than ss-DNA. Thus, Drug-DNA interaction analysis has been investigated for the first time under optimized conditions with the Tamoxifen which, gave an oxidation peak potential near the guanine oxidation area. These results presented that the developed DNA biosensor could be detected TAM-DNA interaction as a sensitive, rapid and cost effective way. Electrochemical detectionTamoxifen recovery from commercial tablets was also studied..

Keywords: *Electrochemical DNA Biosensor, Tamoxifen, Differential Pulse Voltammetry (DPV), Commercial Drug Tablet, Recovery Work.*

1. INTRODUCTION

Breast cancer is the most common cancer in women, accounting for 32% of all newly diagnosed cancers (Kelsey and Bernstein, 1996), because breast cancer is estrogen-dependent, reducing estrogen secretion by oophorectomy, hypophysectomy, or adrenalectomy can cause the cancer to regress. The need for these surgical procedures was reduced by the introduction of tamoxifen, which acts as an antiestrogen by inhibiting the binding of estrogen to estrogen receptors (Osborn, 1998).

Tamoxifen (TAM) is an oral antiestrogen, first used in metastatic breast cancer in the early 1970s. Large clinical trials were initiated in the late 1970s and early 1980s to test the drug's role as adjuvant therapy in early stage breast cancer (Hurtado et al., 2007). There are several analytical methods to determine the concentrations of TAM in biological fluids and pharmaceutical preparations. The developed methods for TAM analysis include capillary electrophoresis (Bagni et al., 2010) and chromatography (Yang et al., 1999). Electrochemical techniques, especially as a biosensor, have received significant attention in the analysis of pharmaceuticals, due to their low detection limits and rapid analysis time. It provides various benefits to the analyzer in the analysis of fast, simple and low-cost drugs (Ozkan et al., 2004).

It is well known that several drugs and chemicals have a damaging effect on DNA. These molecules are generally interacting with DNA non-covalent or covalent ways such as groove binding, electrostatic binding or intercalative mode (Gupta et al., 2011). Therefore, it is important to detect the interaction way of DNA with the drug. The most common approach is to use electrochemical DNA biosensors containing a nucleic acid element to be immobilized on an electrochemical transducer for recognition. This recognition features helps to selectively detect a specific DNA sequence or to monitor changes in DNA structure during interaction with the drug molecule. Studying and investigating the interactions of drugs and DNA as a biosensor has an important place during the treatment (Jahandari et al., 2019). So far there has been only one voltammetric biosensor based TAM-DNA interaction which carbon paste electrode modified with graphene reported for TAM determination (Moghaddam et al., 2017).

In this study, TAM -DNA interaction analysis was performed and at the same time with DNA under optimized conditions using the TAM that gave an oxidation peak close to an area of guanine oxidation signal at disposable pencil graphite electrode (PGEs) for the first time. The developed electrochemical method for the determination of TAM contains the DPV technique, Britton Robinson (B-R) Buffer (pH=4.50) media. Additionally, the amount of TAM in commercial tablets was also determined with the developed technique.

2. MATERIAL AND METHOD

2.1 Apparatus

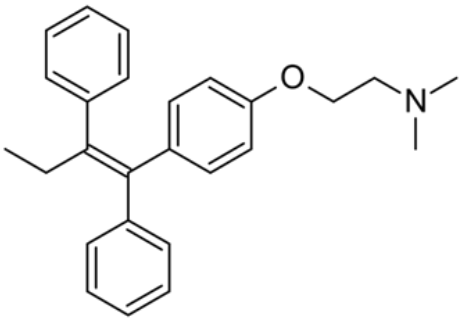
AUTOLAB 12 potentiostat/galvanostat device (Eco Chemie, Netherlands) was used for all electrochemical measurements and raw voltammograms were treated with a Savicky and Golay algorithm using GPES 4.9 software program by moving average method (peak width 0.01 V). The three electrode system was comprised of a pencil graphite electrode (PGE), Ag/AgCl/3M KCl reference electrode and a platinum wire as the auxiliary electrode. The Tombow 2B pencil lead of 0.5 mm diameter and length of 60 mm was used as PGE for the investigation. All experiments were performed at room temperature (22.0-25.0˚C).

2.2 Reagents and materials

In this study, CH_3COOH (Riedel-de Haen, 99 %), CH_3OH (Merck, 99.5 %), NaOH (Riedel-de Haen), NaCl (Sigma), K_2HPO_4 (Merck), KH_2PO_4 (Merck), H_3BO_3 (Sigma), H_3PO_4 (Sigma) were used. Double stranded calf thymus DNA and single stranded calf thymus DNA

were purchased from Sigma. The drug active ingredient tamoxifen which chemical formula stated in Figure-1 was obtained from Sigma. The stock solution of 5mM TAM was prepared by dissolving 46 mg TAM in 5 ml of methanol and stored at -20°C. The supporting electrolyte solution for the voltammetric investigations were prepared by dilution of the stock solution. Commercial form of TAM obtained from the AstraZeneca Company. All solutions were stored in the dark and were used within 24 hours to avoid decomposition. 0.067 M phosphate buffer (PBS, pH: 4.50-7.50), 0.2 M acetate buffer (ABS, pH: 3.50- 5.50) and 0.04 M B-R buffer (pH: 2.00-10.00) were selected as the support electrolyte solutions. The cyclic voltammetry (CV) and DPV voltammograms of TAM were recorded to determine the supporting electrolyte type and optimum conditions. Ultra-pure water (UPW) obtained from Sartorius Arium model Ultra-Pure Water Systems was used to prepare the supporting electrolyte solutions. All chemicals were of analytical reagent grade and were supplied from Sigma-Aldrich, Merck, and Riedel-de Haen.

Figure-1. The chemical formula of Tamoxifen



2.3 Calibration graph for the quantitative determination of TAM

The diluted TAM solutions were prepared by using diluting with B-R buffer solution from the stock solution. In B-R buffer (pH=4.50) medium, a linear calibration curve was performed. in the concentration range of TAM from 2.5 μM to 70 μM with DPV method.

2.4 Recovery works

Five tablets were weighed and powdered in a mortar to determine the amount of tamoxifen from Nolvadex tablets. Each tablet contains 30.4 mg of tamoxifen citrate which is equivalent to 20 mg of tamoxifen (TAM). A suitable amount of each sample was solubilized and sonicated for an hour, in order to prepare equivalent molar stock solutions. The recovery works were constructed by using proper aliquots, respecting the linear response of calibration graphs.

2.5 Sensor 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 (Subak and Ozkan-Ariksoysal, 2018). The activated PGE was modified with DNA and TAM by wet-adsorption technique.

The experimental procedure for the surface and solution phase interaction process by using DPV. In the first step, the electrode surface was activated by using ABS (pH=4.80). Then for the solution phase interaction, the mixture of TAM and double stranded DNA (dsDNA) / TAM and single stranded DNA (ssDNA)/TAM was prepared at room temperature and waited for an hour. After this incubation step, electrode surfaces were covered with these solutions by adsorption technique for 20 minutes. dsDNA/ssDNA immobilized to the electrode surface by adsorption technique for the surface phase. The TAM interacted with DNA by adsorption

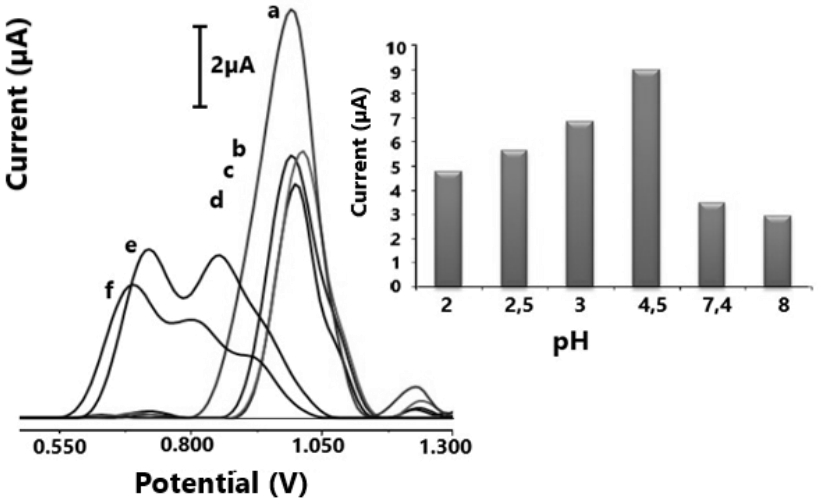
technique in different periods. The next step was to inhibition of nonspecific materials from the surface and then rinsed with ABS for 5 seconds to move unbound DNA from the electrode. The guanine oxidation signal and TAM signal were measured to determine the reaction process by DPV method after optimization of the immobilization time period of TAM/dsDNA/ssDNA for surface phase interaction.

3. RESULTS AND DISCUSSION

3.1 Electrochemical oxidation of tamoxifen

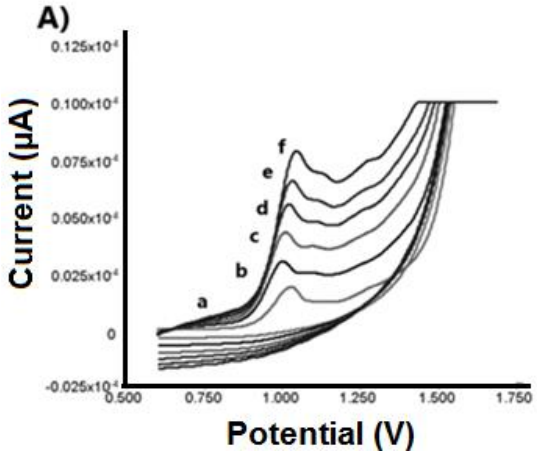
To determine optimal oxidation conditions of TAM, B-R buffers (pH: 2.00 to 8.00) were used as supporting electrolytes. DPV voltammograms of the 100µM TAM in B-R electrolytes at different pH were recorded. The highest current value related to the TAM oxidation was obtained in B-R buffer at pH=4.50 and 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.2.

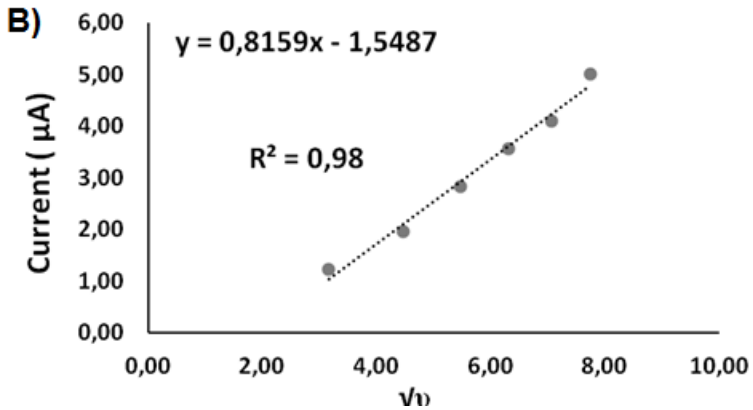
Figure-2. The oxidation peak current values obtained from DPV voltammograms of 100µM TAM in the range changed from pH: 2.00 to 8.00 in 0.04 M B-R buffers at PGE.

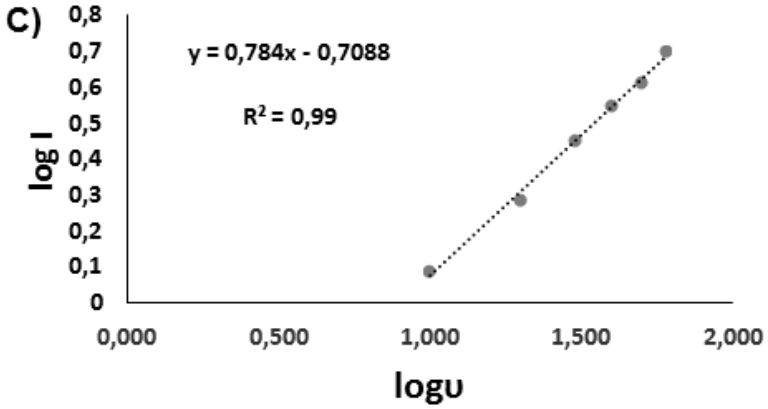


CV voltammograms of TAM in B-R buffer (pH=4.50) in scan rates between 10 to 60 mVs^{-1} at PGE were recorded in Figure-3 (Potential range between +0.40 V and +1.4 V, pulse amplitude 50 mV and scan rate 16 mV/s). According to the results, the anodic peak at the voltammogram increase with the rising of the scan rate value. There was no peak in the cathodic region. Additionally, the positive shift was observed in the anodic peak potential of TAM.

Figure-3. A) CV voltammograms of TAM in B-R buffer (pH=4.50) at PGE (Scan rates; 10, b) 20, c) 30, d) 40, e) 50, f) 60m Vs^{-1}).
B) The peak current values plotted against $v^{1/2}$ obtained from the CV voltammograms of $5x10^{-5}$ M TAM in B-R buffer (pH=4.50) at PGE.
C) The logarithm of peak current (log *I*) against the logarithm of scan rate (log *v*) obtained from the CV voltammograms of $5x10^{-5}$ M TAM in B-R buffer (pH=4.50) at PGE.).



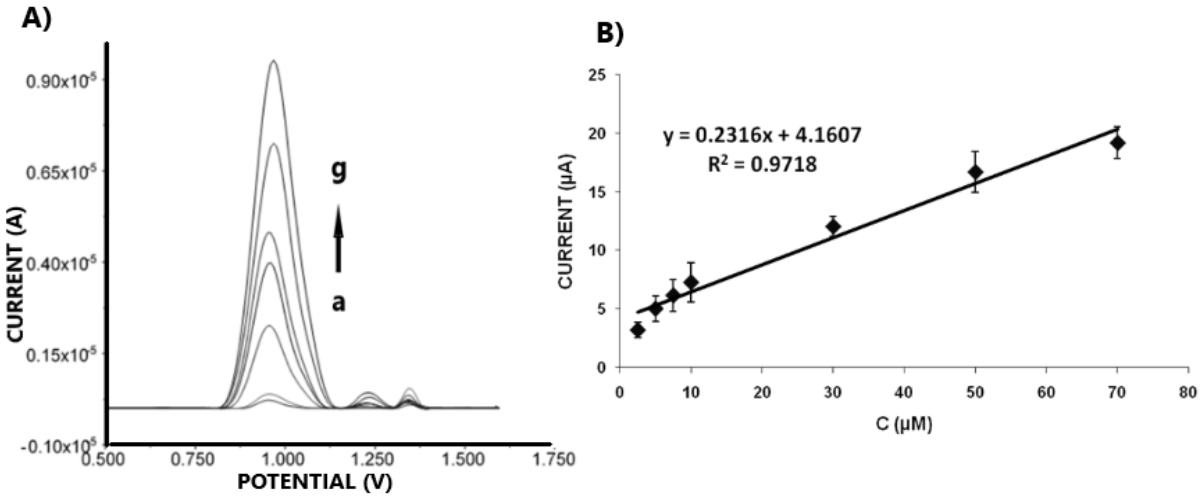




The slope is between 0.50-0.75 indicates that the current is diffusion controlled and that it is 0.75-1.00 indicates that the current is adsorption controlled (Sadikoglu et al., 2016), (Moghaddam et al., 2017). The logarithm of peak current (log I) against the logarithm of scan rate (log v) was shown in (Fig. 3B). According to the results, the slope was found as 0.784 which is indicated that the current type of the TAM was adsorption controlled.

The calibration graph of Tamoxifen active ingredient which was determined to have adsorption controlled current type were measured by DPV with the PGE electrode at +0.75 V for 60 sec. in the range of +0.6 / +1.5 V. Calibration graph of Tamoxifen active ingredient was indicated in the Figure-4.

Figure-4. The plot of concentration versus current obtained from DPV voltammograms of TAM in the concentration range from 2.5µM to 70µM in B-R buffer (pH=4.50) at PGE. ((a) 2.5µM, (b) 5µM, (c) 7.5µM, (d) 10µM, (e) 30µM, (f) 50µM, (g)70µM TAM)



As shown in Fig. 4, the plot was obtained linear in the concentration range of 2.5 to 70µM TAM (AdsDPV technique for 60 sec at +0.75 V Potential range between +0.60 V and +1.5 V, pulse amplitude 50 mV and scan rate 16 mV/s). For the regression plot of the peak current versus TAM concentration, the slope was 0.2316µA/M, the intercept was 4.1607µA and the correlation coefficient was R^2=0.9718. Limit of detection (LOD) and limit of quantification (LOQ) values was calculated using the following equations (Can et al., 2015), (Sadikoglu et al., 2016).

$$LOD = 3\ s/m,\ LOQ = 10\ s/m$$

Where is the standard deviation of the peak currents (n=5) and m is the slope of the calibration curve. Accordingly, the standard deviation of the current values was found as $4.46x10^{-3}$ by taking the voltammogram at 5µM, which is the concentration above the lowest concentration in the calibration graph. The achieved LOD and LOQ were recorded $8.66x10^{-8}$ M and 2.88 x 10^{-7}M at PGE, respectively.

To investigate the effect of the additives contained in the commercial tablet on the optimized biosensor system, tablets containing the active ingredient on the market have been provided. Then, 5 mM TAM solution with the adequate amount of this powder was prepared and the DPV voltammogram of the sample was recorded. The equation of the calibration curve obtained from the DPV voltammograms. Different concentrations of TAM were recorded for the equation y = 0.2316x + 4.1607. According to this equation, the amount of TAM in one tablet

was determined to be 20.55 mg. Table.1, the recovery results obtained from the market tablets are listed. As seen on Table.1, the amount of pure active substances from commercial tablets calculated and compared with the value indicated on the tablets.

Table.1 The assay of TAM in tablets by the DPV technique and recovery of TAM

Parameters	**Results**
TAM amount in commercial drug, mg	20.00
Amount found, mg	20.55
Relative Standard deviation(*RSD* / %)	3.99
Bias, %	1.04
Added TAM, mg	20.00
Found TAM, mg	21.15
Average recovery, %	105.74
The relative standard deviation of recovery (*RSD* / %),	2.17
Bias, %	0.90

3.2 Investigating of 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 (Teunissens et al., 2010).

The interaction time between DNA at solution phase interaction procedure was investigated at different times of periods such as 20min., 60min., 24 hrs. in Figure-5. To keep the analysis time short, it was decided to continue the experimental applications with 20 minutes interaction since there was no significant difference at the end of 60 minutes and 20 minutes interaction.

Figure-5. Voltamogram of the interaction time period TAM with dsDNA; (a) 10ppm dsDNA, (b) 5µM TAM (c) 20 min. interaction, (d) 60 min. interaction, (e) 24 hrs, voltamograms obtained after interaction in solution phase.

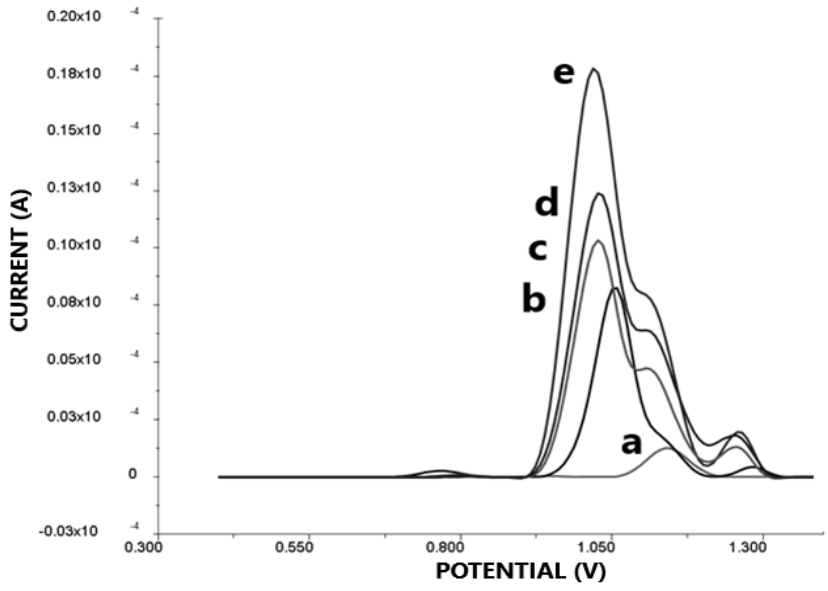


Fig.5 shows the result of the interaction time study between TAM and dsDNA in solution phase (Potential range between +0.60 V and +1.5 V, pulse amplitude 50 mV and scan rate 16 mV/s). According to the data obtained, as the interaction time increases, the signal increases in the guanine oxidation region and the oxidation signal of TAM. To our best knowledge, the increase of DNA signal overtime was interpreted as the possibility of opening of the double helix with the TAM effect. ssDNA and dsDNA were measured respectively to investigate the interaction mechanism of the TAM with DNA. Two different methodologies are surface phase and solution phase interaction also compared to the clarification of the reaction mechanism. Figure-6 shows solution phase interaction and Figure-7 shows the surface phase interaction results.

Figure-6. DPV voltammograms of solution phase interactions results; A) dsDNA interaction; a: dsDNA, b: TAM, c: Interaction, and B) ssDNA interaction; a: ssDNA, b: TAM, c: Interaction (other conditions are as in Fig. 5).

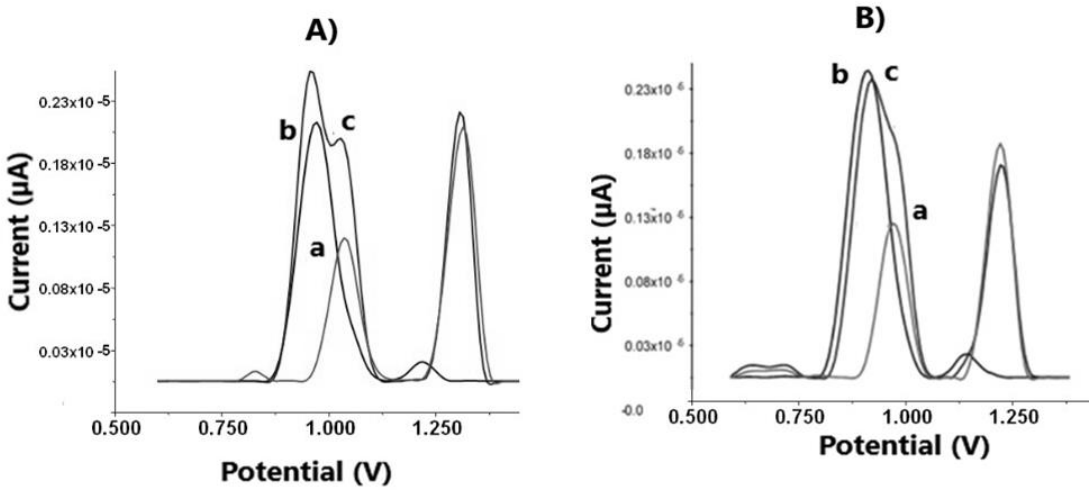
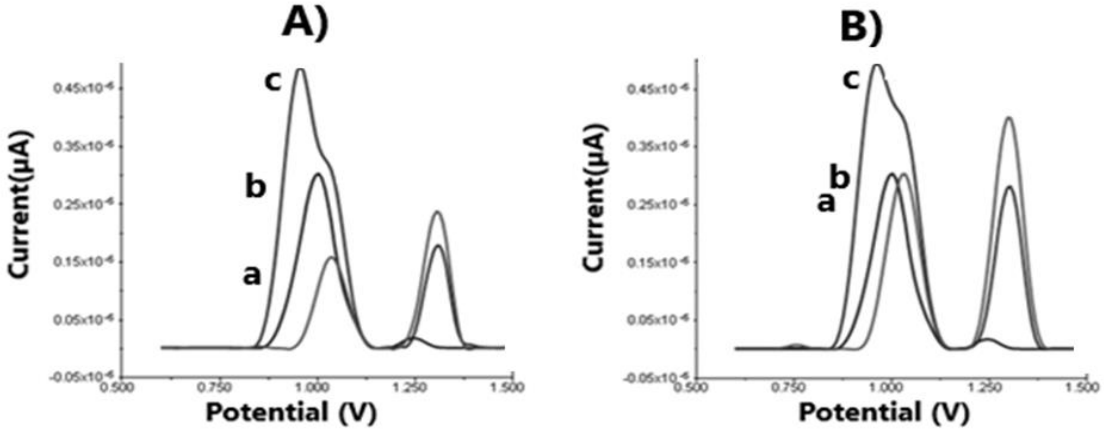


Figure-7. DPV voltammograms of surface phase interactions results;
A) dsDNA interaction; a: dsDNA, b: TAM, c:Interaction;
B) ssDNA interaction; a: ssDNA, b: TAM, c: Interaction (other conditions are as in Fig. 5).



In Fig.6 and Fig.7, the interaction of the A) dsDNA- TAM and B) ssDNA-TAM were showed the solution phase interaction results (Fig.6) and surface phase interaction results (Fig.7) respectively.

In Fig.6-A DPV voltammograms of solution phase interactions results for dsDNA interaction respectively a: dsDNA= 1.30µA, b: TAM=2.11µA, c: Interaction=2.05 µA (n=5).

In Fig.6-B interaction results for ssDNA is a: dsDNA= 1.16µA, b: TAM=2.33µA, c: Interaction=2.08 µA (n=5).

In Fig.7-A DPV voltammograms of surface phase interactions results for dsDNA interaction respectively a: dsDNA= 1.90µA, b: TAM=2.72µA, c: Interaction=3.18 µA (n=5). In Fig.7-B interaction results for ssDNA is a: dsDNA= 2.80 µA, b: TAM=3.15 µA, c: Interaction=3.40 µA (n=5).

In the solution interaction phase, the dsDNA signal in Fig-6A-a about 1.34µA and ssDNA Fig-6B-a is 1.16 µA. Similarly in surface phase reaction signal in dsDNA Fig-6A-a is 1.90µA and ssDNA Fig-7B-a is 2.80 µA. In Figures, 6A/B and 7A/B concluded that the individual ssDNA signal and the individual dsDNA signal were compared, that the ssDNA gave higher signal because of its open helix structure.

In the solution interaction phase, the TAM signal in Fig-6A-a about 2,11 µA and ssDNA Fig-6B-a is 2,33µA. Similarly in surface phase reaction signal in dsDNA Fig-7A-a is 2,72 µA and ssDNA Fig-7B-a is 3,15 µA. In the solution phase, TAM interacted with ssDNA and dsDNA for an hour and immobilized to the electrode surface for 20 minutes. On the other hand, ssDNA and dsDNA immobilized to the electrodes selectively and interacted with TAM for an hour. The difference between the time of the period resulted in the signal difference.

After the interaction, there was an increase in guanine oxidation peak currents 65% in dsDNA Fig-6A-c and ssDNA Fig-6B-c signal about 55% in the solution phase. Also, there was an increase in guanine oxidation peak currents about 60% in dsDNA Fig-7A-c and ssDNA Fig-7B-c signal about 82% in surface phase interaction. TAM does not interact with the adenine base even though it has make signal changes with guanine.

As a result, the signals of dsDNA and ssDNA in the surface and solution phase gave parallel results. In the surface phase, procedure DNA materials selectively immobilized to the electrode surface and then rinsed with buffer solution to remove unbound materials in all the stages. In the solution phase, there is only one stage to remove unbound materials.

The interaction of TAM with dsDNA might be attributed to its intercalation into the base stacking domain of DNA double helix. The interaction of TAM with ssDNA indicated that the backbone of ssDNA is negatively charged phosphate on the exterior and can easily attract to the investigated cationic TAM via electrostatic attraction.

As a result, the interaction of the TAM molecule with both ssDNA and dsDNA was determined by the newly developed biosensor.

4. CONCLUSIONS

Tamoxifen, an anticancer drug, has been developed by using voltammetric techniques and new and sensitive methods can be used to quantify this drug. Besides, CV and DPV techniques were used to elucidate the mechanism of interaction of the molecule with the double stranded DNA (dsDNA) and single strand DNA (ssDNA).

Electrochemical reactions of TAM were investigated using a pencil graphite electrode. Measurements at various pH values show that TAM has adsorption controlled process. The peak of TAM was recorded using the DPV technique using PGE. To understand the adsorption controlled properties of the current type, various buffer solutions were tested and B-R Buffer pH=4.50 was found to be the most suitable buffer solution. The equations between log ip and logv are examined. Scan rate values of 10-60mV/s at 5ppm concentration at pH=4.50 B-R

buffer were examined and it was concluded that TAM active ingredient was adsorption controlled.

Oxidation signals of the active ingredient in the range of +0.6/+1.5 V were obtained by depositing the active ingredient TAM by AdsDPV technique for 60 sec at +0.75 V. Linearity was obtained in the pencil graphite electrode in the concentration range of 2.50-70μM. The obtained validation parameters are given in Table1.

The interaction mechanism of the TAM was evaluated by using PGEs transducers with the DPV method. Reference articles and results show that TAM and DNA interact electrostatically and/or intercalatively (Synder and Brown, 2002). The findings of these studies suggest that the TAM does not interact with the adenine base even though it has make signal changes with guanine.

TAM tablet was obtained from a local commercial source and prepared in the same amount as the active ingredient. The tablet solution was taken and analyzed directly after preparation. Recovery studies were carried out by adding a known amount of pure active ingredient to a known amount of the analyzed solution. As a result, the % recovery value indicates that tablet TAM additives do not significantly affect our results. Interaction studies with DNA materials were performed and the behavior of tamoxifen active ingredient was investigated electrochemically on PGE. The interaction path in the solution phase and then the interaction path on the electrode surface are examined respectively. The interaction between the active ingredient and DNA materials was evaluated by using guanine oxidation peak current. The intra-day and inter-day reproducibility of guanine signals was 0.98% and 1.58% respectively.

In this article, the interaction of tamoxifen with dsDNA/ssDNA was investigated by voltammetric studies. The study of the interaction between the anticancer drug Tamoxifen and dsDNA/ssDNA 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 DPV 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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USE OF AGRICULTURAL WASTE ASHES AS ADDITIVES MATERIAL IN BIOACTIVE GLASS PRODUCTION

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ABSTRACT

Demand for renewable energy sources is increasing day by day. The rapidly increasing population and energy needs on a global scale have increased the number of studies on renewable energy sources. In addition to energy needs, the search for disposal and storage of wastes harmful to the natural environment is of great importance. Disposal and storage requires high costs and labor. The need for energy and the necessity of protecting the natural environment provided the solution to convert wastes into energy or value added products without damaging the environment. The wastes of the crops produced as a result of agricultural production can be converted into energy or value added products. Some of the plant wastes meet the energy needs and some of them can be used in the production of value-added biomaterials. Biomaterials are materials which are used in the field of health and show bioactivity with parts such as organs and tissues. Bioactive glasses, which are one of these materials, are used in the health sector especially in dental filling, prosthesis and composite applications. The most important feature of raw materials to be used in the production of bioactive glasses is SiO2 (silica). Plants receive silica from soil during development and this silica is also present in plant waste. In this study, a wide literature review was conducted and the percentage of mass ash after burning of rice stalks, corn stalks, hazelnut shells, corn stalks which were firstly burned for heating and energy purposes were determined. Then, the composition of the ash obtained SiO2 (silica) and other substances were determined and all the results obtained in the light of this information was compiled to provide information about the possibilities of use in bioactive glass production.

Keywords: *Agricultural waste ashes, Biomaterial, Bioactive glass, Agricultural wastes, Ash..*

1. INTRODUCTİON

Leaves, straw, stalks, fertilizers that occur in all stages of production, processing and post-harvest of agricultural production are defined as agricultural waste (Palabıyık and Altunbaş 2004). The basis of plant and living organisms is called biomass. Plants collect organic matter in their bodies as a result of photosynthesis using solar energy. Biomass formed by plant wastes has become an important energy source as it is among renewable energy sources (TÜGİAD, 2004).

Biomass sources vary widely. Resources such as agricultural, vegetable, animal wastes, forest products wastes, aquatic plant wastes constitute biomass. In terms of agricultural activity, there are approximately 26 million hectares of land in our country. The results of one study there are approximately 60 million tons of agricultural waste in Turkey. The highest share among the wastes constituting this amount is caused by vegetable wastes after corn, cotton and wheat cultivation (Başçetinçelik et al, 2004). Annual potential amounts of biomass species in Turkey are given in the table below (Demirbaş, 2008).

Table.1. Annual biomass potential in Turkey (Demirbaş, 2008)

Biomass type	Annual potential (million tons)
Annual plant	6,30
Multiple year plant	6,14
Forest waste	4,46
Agricultural ındustry wastes	7,12
Tree ındustry wastes	3,57
Animal products wastes atıkları	1,5
Other	1,3
Total	32

Agricultural wastes are less hazardous than other solid wastes and have more rapid recycling. Leaving agricultural wastes in the environment should be avoided and used as raw materials. Re-evaluation and conversion to value-added products should be prepared by experts in certain technical stages and carried out in accordance with the legislation. Environmental pollution will be reduced with the utilization of agricultural wastes, economic recovery and the variety of raw materials will be increased. The ways of evaluating agricultural wastes differ according to their types like other wastes (Akırmak 2010).

Agricultural wastes are divided into 3 groups. These are plant production and subsequent wastes, animal production and subsequent wastes and agricultural products production and subsequent wastes (Akırmak 2010).

When the agricultural waste potential is compared as product in our country, the most waste is wheat, barley, corn and sunflower stalks. The potential of some green vegetable waste products in Turkey is given in the table below (Saracoglu, 2010).

Table.2. Annual waste production of some products grown in Turkey (Saraçoğlu, 2010)

Agricultural waste	Annual production (million tons)
Wheat stalk	26,4
Barley stalk	13,5
Corn stalk	4,2
Cotton stalk	2,9
Sunflower stalk	2,7
Sugar beet stalk	2,3
Nut shell	0,8
Rice stalk	0,4

All kinds of materials created for use in a wide range of medical applications are called biomedical materials (biomaterials). Biomaterials are materials that replace natural or artificial based organs, tissues, or any other region in the body of non-drug substances. In particular, the concept of biocompatibility is a term that determines the biological performance of biomaterials. Biocompatible materials are used as biomaterials and the ability of the material to respond appropriately to the body system ensures compliance. (Hulbert. et al. 1987; TÜBİTAK, 2008).

Biomaterials; metals, polymers, composites and ceramics. Ceramics used to replace parts of the body that lose their function are defined as bioceramics. Bioceramics can be formed as polycrystalline ceramics, bioactive glass, bioactive glass ceramics and bioactive composites (MEDİCİNECUBE, 2008).

Bioglass is silicate based glass and consists of sodium, calcium and phosphate. Bioactive glasses and bioactive hydroxyapatite ceramics are generally used in dental and middle ear treatments. Bioactive glass ceramics are used in overloaded basins and backbone areas (Kükürtçü, 2008).

These are biomaterials in which chemical bonding occurs between tissue and implants as a result of the displacement of silica groups in bioactive glasses with calcium and phosphorus (Er, 2005). The main constituents of many bioactive glasses were determined as SiO_2, Na_2O, CaO, P2O5. The best bioactive glass composition is 45S5. According to the composition opening, 45% by weight SiO_2 and CaO / P2O5 should be 5: 1. Bone binding as a biomaterial is not possible in bioactive glasses with a CaO / P2O5 ratio lower than this ratio (Hench, 1998).

Silica (SiO_2) is a raw material that can be used in the production of electronics, ceramics, polymer materials and bioactive glass. It is possible to produce silica with initiators such as tetraethylorthosilicate - TEOS under laboratory conditions and it is not economical because of high cost (Konukoğlu, 2013). During the growth process, plants pick up various minerals and silicas from the soil (Mo et al., 2016). Since silica production with tetraethylorthosilicate - TEOS is not economical, the use of agricultural wastes containing SiO_2 has become an alternative (Konukoğlu, 2013).

2. MATERİAL AND METOD

The material of this study is to determine the mass percentages and mineralogical contents of the agricultural waste ashes according to the results obtained in the domestic and international researches. The percentages of masses of agricultural wastes after incineration were determined and the highest and lowest ash amount were explained by graphs. Then, it was tried to determine which waste is more suitable than the basic mineralogical composition sought in the additives that can be used in the production of bioactive glass. In the light of the data obtained, it was tried to put forth the ratio of agricultural wastes to meet the composition of bioactive glass and the possibilities of converting them to value added products by preventing the damages to the environment of ashes which are waste of plant wastes.

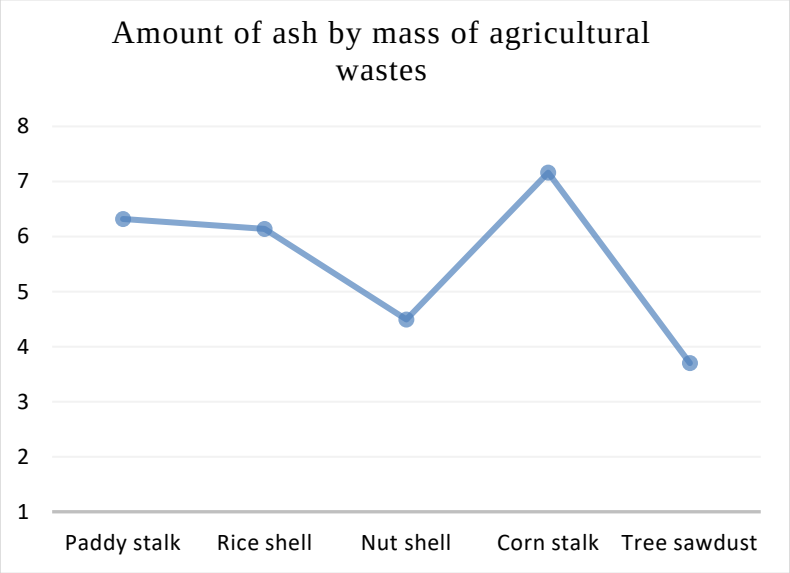
Figure.1. Agricultural wastes of used in research

3. RESULT AND DİSCUSSION

3.1. Determination of Ash Amount by Mass of Plant Wastes

Agricultural waste ash determined in the research is burned in houses, greenhouse and animal husbandry enterprises or kilns for heating and energy purposes and the resulting ashes are left directly to the environment. The associated ashes are left to the environment as waste at no cost to the environment. The highest amount of ash was obtained from corn stalk and the lowest was obtained from hazelnut shell (Er and Özdemir, 2018). The mass percentages of agricultural waste ashes are given in Figure.2.

Figure 2. Amount of ash by mass of plant wastes



3.2. Chemical Composition of Bioactive Glass

According to the studies, the highest substance in the chemical composition of bioglass is SiO_2 followed by CaO, Na_2O and P2O5 (Hench, 1982). Available in bioactive glasses containing Al_2O_3 and MgO. The amount of bioactive glass components in the plant wastes determined in the study was determined.

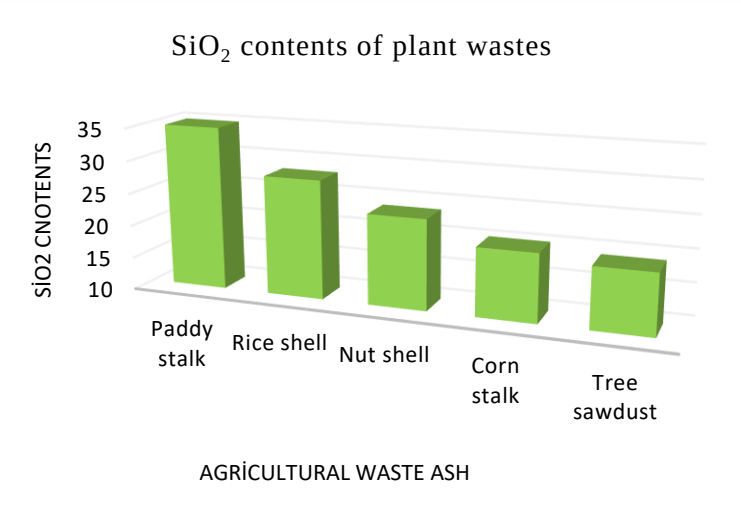
Table 3. Chemical composition of bioactive glass (Hench, 1982)

Component	SiO_2	CaO	Na_2O	P2O5
Percent (%)	45.0	24.5	24.5	6.0

3.3. Determination of Chemical Contents of Plant Wastes

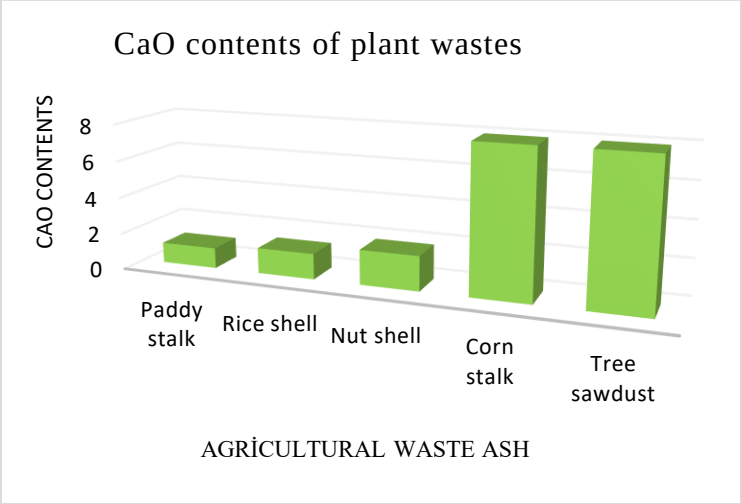
While determining the chemical composition of plant wastes, the most important SiO_2 contents of the bioactive glass components were determined. In terms of SiO_2 contents, the best ratio was found in paddy stem and rice husk and hazelnut husk showed similar values (Er and Özdemir, 2018). SiO_2 contents of all waste ash are given as follows.

Figure 3. SiO_2 contents of plant wastes



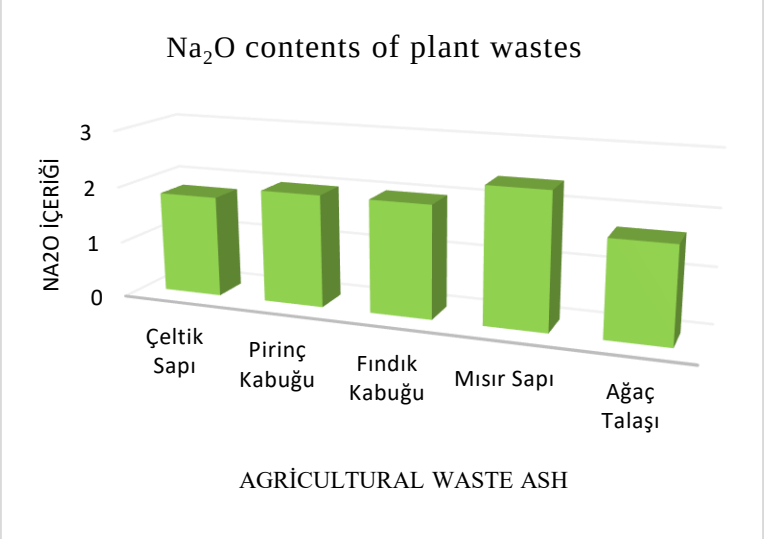
The second component sought in bioactive glasses is the CaO content. As a result of the analysis, the best CaO value is the wood waste ash (Er and Özdemir, 2018). The value of this waste is followed by hazelnut shell ash. Other wastes are less valuable and very close to each other. The CaO contents of the plant wastes are given as follows.

Figure 4. CaO contents of plant wastes



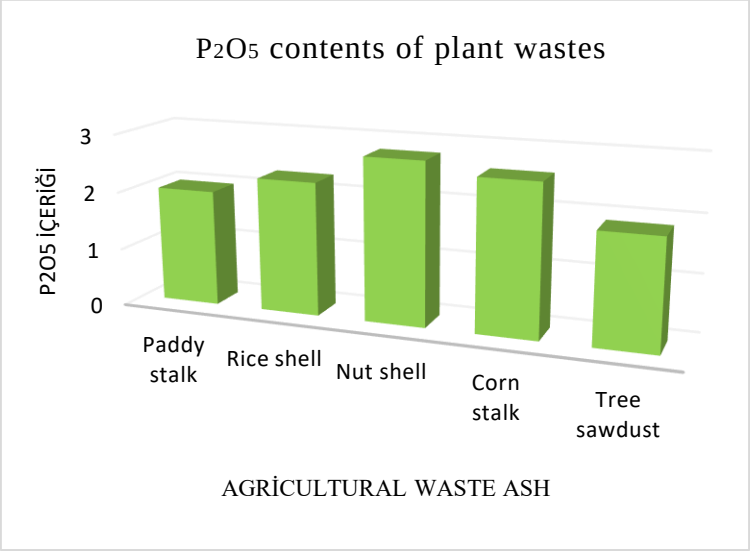
When the Na_2O values, which should be close to the CaO content in the bioactive glass composition, are considered, it is observed that the corn stalk ash gives the best result even if there is a small difference that all the wastes take close values (Er and Özdemir, 2018). When compared in terms of Na_2O content, it can be said that all plant wastes in the study have almost the same characteristics. Na_2O contents of plant wastes are given as follows.

Figure 5. Na_2O contents of plant wastes



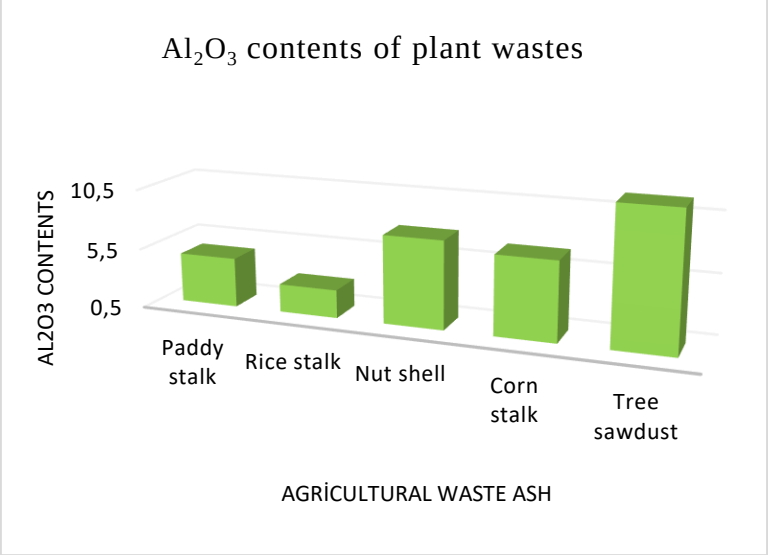
The P2O5 contents of plant wastes were close to each other and hazelnut husks and corn stalk ash gave the best results (Er and Özdemir, 2018). In terms of bioactive glasses, all wastes showed usable quality results. P2O5 contents of plant wastes are given as follows.

Figure 6. P2O5 contents of plant wastes



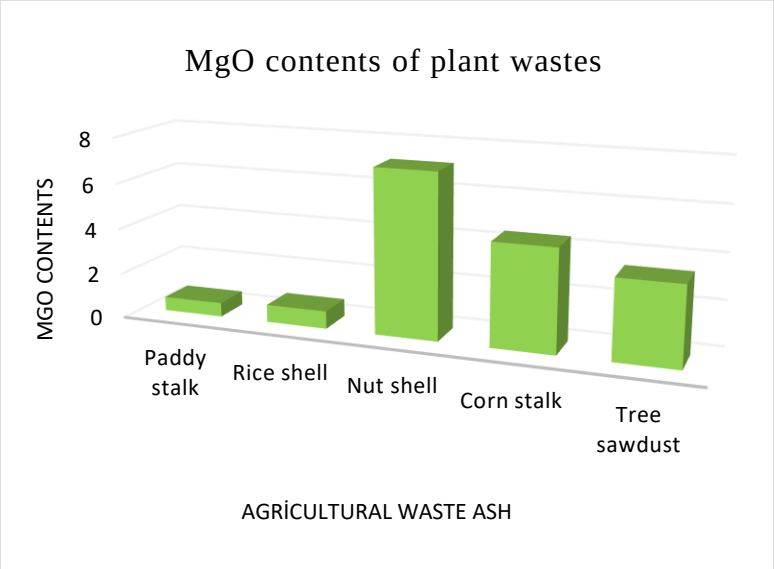
Another substance used in the production of bioactive glass is Al_2O_3. When the Al_2O_3 contents of the plant waste ashes were compared, the best results were found in wood shavings ash and the best values were found in hazelnut shells and corn stalk ashes (Er and Özdemir, 2018). Al_2O_3 contents of plant wastes are given as follows.

Figure 7. Al_2O_3 contents of plant wastes



MgO is the last substance determined in plant wastes. As a result of the analysis, all wastes were taken close values and the best value was found in hazelnut shell ash. MgO contents of plant wastes are given as follows (Er and Özdemir, 2018).

Figure 8. MgO contents of plant wastes



4. RESULTS

SiO_2, which has the highest share in bioactive glass content, was found to be the highest in the paddy stalk and rice husk ash of the plant wastes studied and hazelnut shell ash gave the best result after these wastes. It is possible to say that corn stalk and wood shavings ashes come after other wastes but there is not much difference between them as a percentage of SiO_2. It has been observed that other chemical compositions of bioactive glass are present in varying proportions in all of the plant wastes within the scope of the research and can be used in making bioactive glass.

As a result of the chemical contents of the plant wastes determined within the scope of the research, it was concluded that there are substances in the composition of bioactive glass. The plant wastes in the study have no economic value since they are the waste material of the plant wastes burned for heating and energy purposes. Since these wastes are seen in the group of materials that are harmful to the environment, the assessment of these wastes will also prevent the damages to nature caused by plant waste. In this way, it can be mentioned that the production of bioactive glasses which can be used in high value added health field can be realized.

As a result, in this article, where national and international studies are compiled, it is seen that it is possible to use ashes of environmentally harmful agricultural wastes as biomaterials. It is recommended that researches in this area be increased and supported in terms of the availability of value-added bioactive glass production as well as prevention of environmental damage. In this way, besides contributing to the economy, agricultural wastes will be removed from being a problem.

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Research Article

ELECTROCHEMICAL BIOSENSOR FOR BRCA1 GENE AND TAMOXIFEN INTERACTION

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ABSTRACT

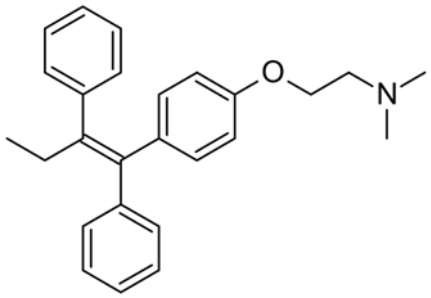
The electrochemical nanobiosensor was designed for the determination of specific DNA sequences related to breast cancer 1 (BRCA1) gene and interaction between Anticancer Drug Tamoxifen (TAM) and related DNA sequences by using pencil graphite electrode (PGE), bare and multi-walled carbon nanotube (MWCNT) 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.*

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

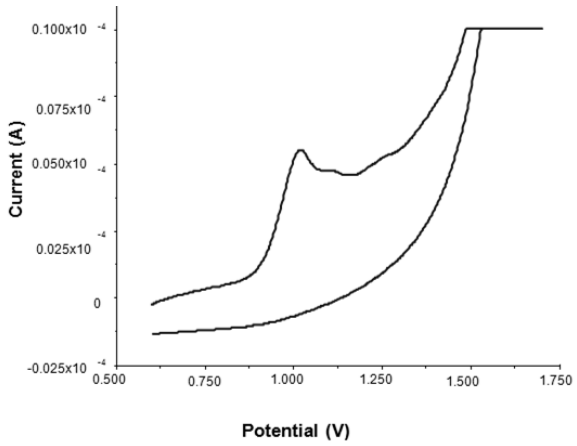
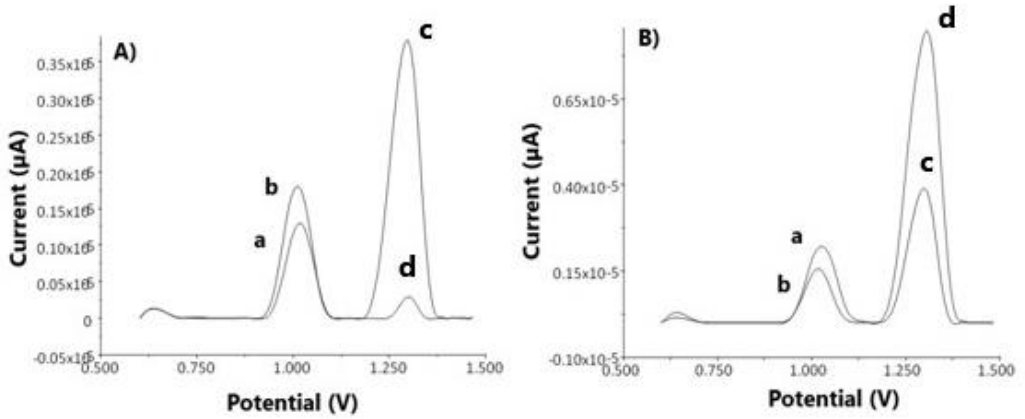


Figure-2 represented the oxidation peak current values obtained from CV voltammograms of 100µM TAM at pH=4.50 in 0.04 M B-R buffers at PGE (In experimental conditions: Potential range between +0.50 V and +1.6 V, pulse amplitude 50 mV and scan rate 16 mV/s) and shows that 100µM TAM gives a single anodic 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.

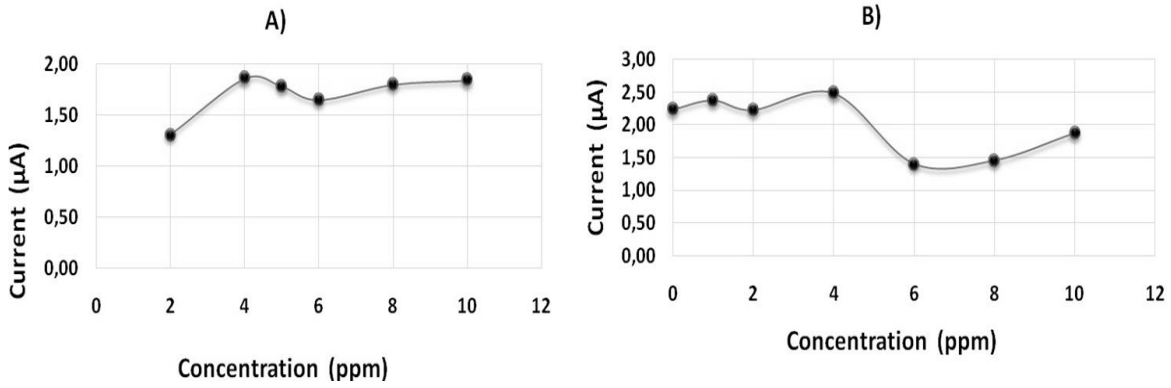
Figure-3. Voltamograms that show the hybridization result with BRCA-1 A) probe sequence to target hybridization; (a) only the probe signal of guanine, (b) hybridization signal of guanine (c) hybridization signal of adenine, (d) only probe signal of adenine; B) target sequence to probe hybridization;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

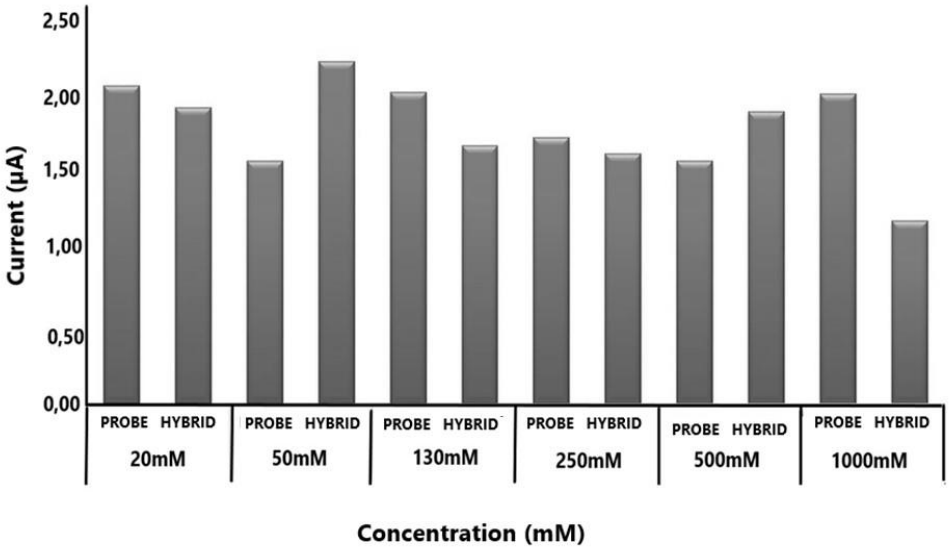


Figure-5. shows the effect of salt concentration on hybridization formed between BRCA-1 probe and target sequences (In experimental conditions: potential range between +0.50 V and +1.6 V, pulse amplitude 50 mV and scan rate 16 mV/s). The highest difference in the guanine signal between the probe and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

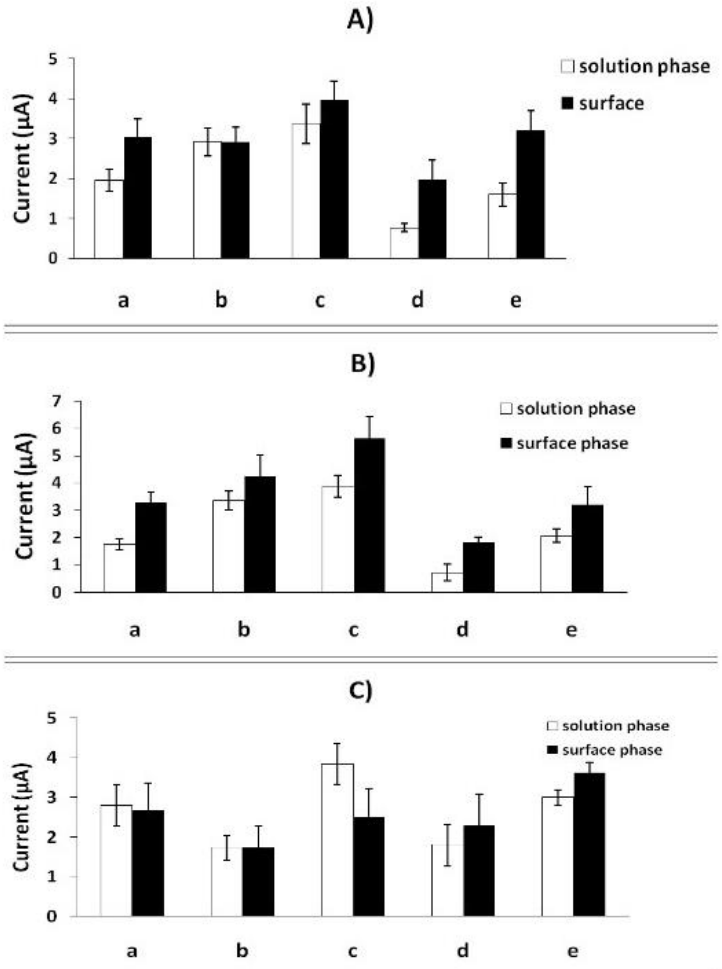


Figure-6 shows the comparison of TAM/DNA interaction in solution phase and surface phase with three different oligonucleotides by using PGE as sensor surfaces (In experimental conditions: potential range between +0.50 V and +1.6 V, pulse amplitude 50 mV and scan rate 16 mV/s). Figure-5A shows the guanine 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.

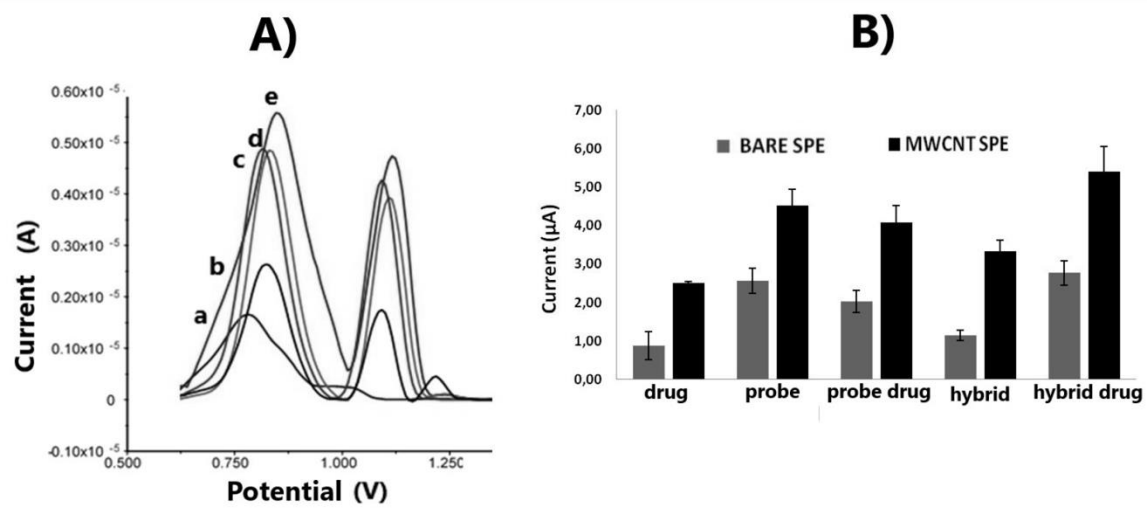


Figure-7 shows the interaction between DNA and TAM resulted in an increase of the guanine oxidation signal with SPE and MWCNT SPE (In experimental conditions: potential range between +0.50 V and +1.6 V, pulse amplitude 50 mV and scan rate 16 mV/s). MWCNT

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

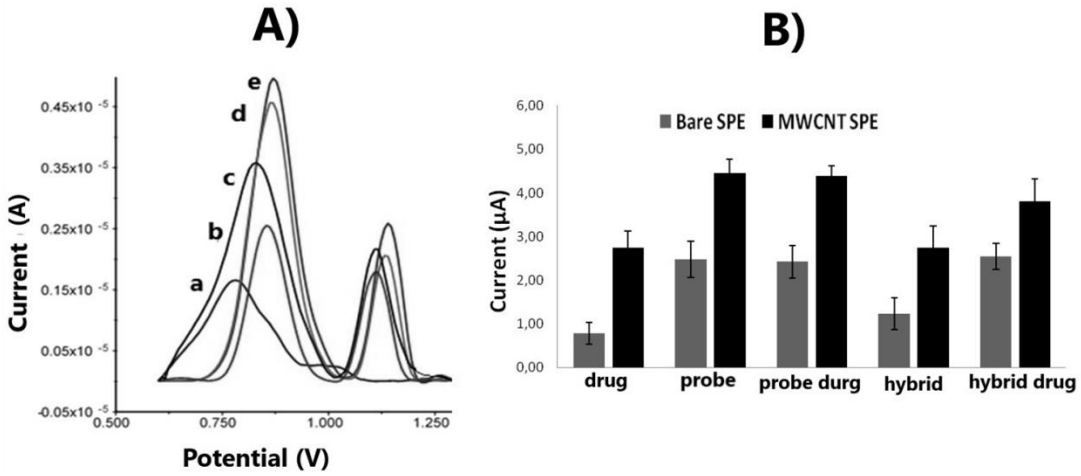


Figure-8 shows that interaction resulted in an increase of the guanine oxidation signal with SPE and MWCNT SPE (In experimental conditions: potential range between +0.50 V and +1.6 V, pulse amplitude 50 mV and scan rate 16 mV/s). Figure-8-B shows the interaction resulted in an increase of the guanine 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

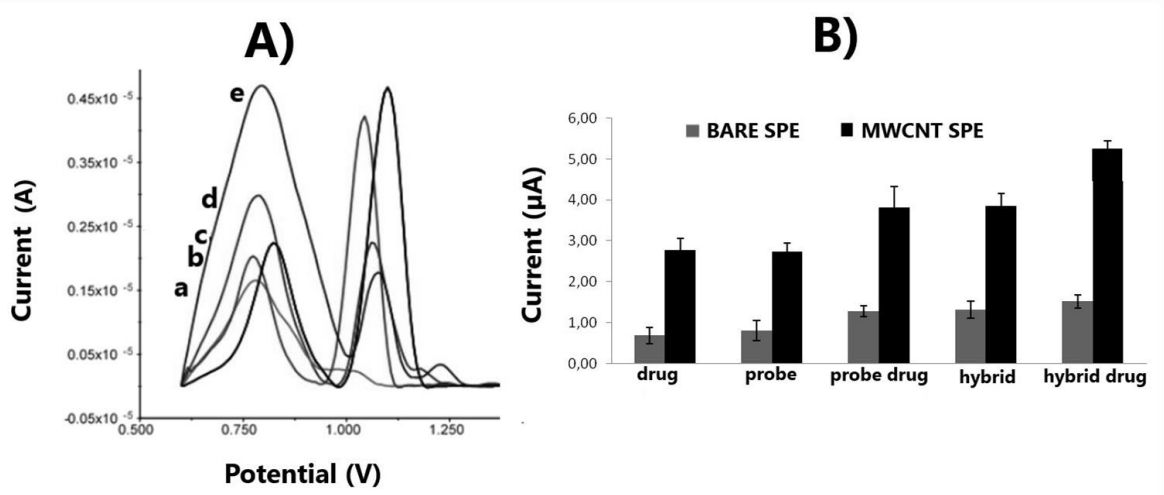


Figure-9 represents that interaction between BRCA1 seguences and TAM resulted in an increase of the guanine oxidation signal with SPE and MWCNT SPE (In experimental conditions: potential range between +0.50 V and +1.6 V, pulse amplitude 50 mV and scan rate 16 mV/s). MWCNT SPEs gave higher guanine signal 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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Research Article

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ON GENERALIZED SİXTH-ORDER PELL SEQUENCES

Abstract. In this paper, we investigate the generalized sixth order Pell sequences and we deal with, in detail, three special cases which we call them as sixth order Pell, sixth order Pell-Lucas and modified sixth order Pell sequences.

2010 Mathematics Subject Classification. 11B39, 11B83, 05A15.

Keywords. Pell numbers, sixth order Pell numbers, sixth order Pell-Lucas numbers, Hexanacci numbers.

1. Introduction

In this paper, we introduce the generalized sixth order Pell sequences and we investigate, in detail, three special cases which we call them sixth order Pell, sixth order Pell-Lucas and modified sixth order Pell sequences. First we recall the definition of a generalized Hexanacci sequence.

A generalized Hexanacci sequence $\{W_n\}_{n\geq 0} = \{W_n(W_0, W_1, W_2, W_3, W_4, W_5; r_1, r_2, r_3, r_4, r_5, r_6)\}_{n\geq 0}$ is defined by the sixth-order recurrence relations

$$\begin{aligned} W_n &= r_1W_{n-1} + r_2W_{n-2} + r_3W_{n-3} + r_4W_{n-4} + r_5W_{n-5} + r_6W_{n-6}, \\ W_0 &= a, W_1 = b, W_2 = c, W_3 = d, W_4 = e, W_5 = f \end{aligned} \tag{1.1}$$

where the initial values $W_0, W_1, W_2, W_3, W_4, W_5$ are arbitrary complex (or real) numbers and $r_1, r_2, r_3, r_4, r_5, r_6$ are real numbers.

The sequence $\{W_n\}_{n\geq 0}$ can be extended to negative subscripts by defining

$$W_{-n} = -\frac{r_5}{r_6}W_{-(n-1)} - \frac{r_4}{r_6}W_{-(n-2)} - \frac{r_3}{r_6}W_{-(n-3)} - \frac{r_2}{r_6}W_{-(n-4)} - \frac{r_1}{r_6}W_{-(n-5)} + \frac{1}{r_6}W_{-(n-6)}$$

for $n = 1, 2, 3, ...$ Therefore, recurrence (1.1) holds for all integer n.

It is well-known that the Pell sequence (OEIS: A000129, [13]) $\{P_n\}$ is defined recursively by the equation, for $n \geq 0$

$$P_{n+2} = 2P_{n+1} + P_n$$

in which $P_0 = 0$ and $P_1 = 1$. Next, we present the first few values of Pell numbers with positive and negative subscripts:

Table 1. The first few values of the Pell numbers with positive and negative subscripts.

n	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
P_n	0	1	2	5	12	29	70	169	408	985	2378	5741	13860	33461	80782
P_{-n}	0	1	−2	5	−12	29	−70	169	−408	985	−2378	5741	−13860	33461	−80782

Pell sequence has been studied by many authors and more detail can be found in the extensive literature dedicated to these sequences, see for example, [1,2,3,4,6,8,11,12,19]. For higher order Pell sequences, see [9,10,16,17,18].

In this paper we consider the case $r_1 = 2$, $r_2 = r_3 = r_4 = r_5 = r_6 = 1$ and in this case we write $V_n = W_n$. A generalized sixth order Pell sequence $\{V_n\}_{n\geq 0} = \{V_n(V_0, V_1, V_2, V_3, V_4, V_5)\}_{n\geq 0}$ is defined by the sixth-order recurrence relations

$$V_n = 2V_{n-1} + V_{n-2} + V_{n-3} + V_{n-4} + V_{n-5} + V_{n-6} \tag{1.2}$$

with the initial values $V_0 = c_0, V_1 = c_1, V_2 = c_2$, $V_3 = c_3$, $V_4 = c_4$, $V_5 = c_5$ not all being zero.

The sequence $\{V_n\}_{n\geq 0}$ can be extended to negative subscripts by defining

$$V_{-n} = -V_{-(n-1)} - V_{-(n-2)} - V_{-(n-3)} - V_{-(n-4)} - 2V_{-(n-5)} + V_{-(n-6)}$$

for $n = 1, 2, 3, ...$ Therefore, recurrence (1.2) holds for all integer n.

As $\{V_n\}$ is a sixth order recurrence sequence (difference equation), it's characteristic equation is

$$x^6 - 2x^5 - x^4 - x^3 - x^2 - x - 1 = 0. \tag{1.3}$$

The approximate value of the roots $\theta_1, \theta_2, \theta_3, \theta_4, \theta_5$ and θ_6 of Equation (1.3) are given by

$$\begin{aligned}
\theta_1 &= 2.6143662721144504208 \\
\theta_2 &= -0.76286141326240044899 \\
\theta_3 &= 0.45907924801189877223 - 0.76572377800211887372i \\
\theta_4 &= 0.45907924801189877223 + 0.76572377800211887372i \\
\theta_5 &= -0.38483167743792375813 - 0.69350597836224613636i \\
\theta_6 &= -0.38483167743792375813 + 0.69350597836224613636i
\end{aligned}$$

Note that we have the following identities:

$$\begin{aligned}
\theta_1 + \theta_2 + \theta_3 + \theta_4 + \theta_5 + \theta_6 &= 2, \\
\theta_1\theta_2\theta_3\theta_4\theta_5\theta_6 &= -1.
\end{aligned}$$

The first few generalized sixth order Pell numbers with positive subscript and negative subscript are given in the following Table 2.

Table 2. A few generalized sixth order Pell numbers

n	V_n	V_{-n}
0	V_0	V_0
1	V_1	$-V_0 - V_1 - V_2 - V_3 - 2 \times V_4 + V_5$
2	V_2	$-V_5 + 3V_4 - V_3$
3	V_3	$-V_4 + 3V_3 - V_2$
4	V_4	$-V_3 + 3V_2 - V_1$
5	V_5	$-V_2 + 3V_1 - V_0$
6	$2V_5 + V_4 + V_3 + V_2 + V_1 + V_0)$	$-V_5 + 2V_4 + V_3 + V_2 + 4V_0$
7	$5V_5 + 3V_4 + 3V_3 + 3V_2 + 3V_1 + 2V_0$	$4V_5 - 9V_4 - 2V_3 - 3V_2 - 3V_1 - 4V_0$
8	$13V_5 + 8V_4 + 8V_3 + 8V_2 + 7V_1 + 5V_0$	$-4V_5 + 12V_4 - 5V_3 + 2V_2 + V_1 + V_0$
9	$34V_5 + 21V_4 + 21V_3 + 20V_2 + 18V_1 + 13V_0$	$V_5 \ -6V_4 + 11V_3 - 6V_2 + V_1$
10	$89V_5 + 55V_4 + 54V_3 + 52V_2 + 47V_1 + 34V_0$	$V_4 - 6V_3 + 11V_2 - 6V_1 + V_0$

Now we define three special case of the sequence $\{V_n\}$. Sixth-order Pell sequence $\{P_n^{(6)}\}_{n\geq 0}$, sixth-order Pell-Lucas sequence $\{Q_n^{(6)}\}_{n\geq 0}$ and modified sixth-order Pell sequence $\{E_n^{(6)}\}_{n\geq 0}$ are defined, respectively, by the sixth-order recurrence relations

(1.4)

$$P_{n+6}^{(6)} = P_{n+5}^{(6)}+2P_{n+4}^{(6)}+P_{n+3}^{(6)}+P_{n+2}^{(6)}+P_{n+1}^{(6)}+P_n^{(6)},\ P_0^{(6)} = 0, P_1^{(6)} = 1, P_2^{(6)} = 2, P_3^{(6)} = 5, P_4^{(6)} = 13, P_5^{(6)} = 34$$

and

(1.5)

$$Q_{n+6}^{(6)} = Q_{n+5}^{(6)}+2Q_{n+4}^{(6)}+Q_{n+3}^{(6)}+Q_{n+2}^{(6)}+Q_{n+1}^{(6)}+Q_n^{(6)}, Q_0^{(6)} = 4, Q_1^{(6)} = 2, Q_2^{(6)} = 6, Q_3^{(6)} = 17, Q_4^{(6)} = 46, Q_5^{(6)} = 122$$

and

(1.6)

$$E_{n+6}^{(6)} = E_{n+5}^{(6)}+2E_{n+4}^{(6)}+E_{n+3}^{(6)}+E_{n+2}^{(6)}+E_{n+1}^{(6)}+E_n^{(6)},\quad E_0^{(6)} = 0, E_1^{(6)} = 1, E_2^{(6)} = 1, E_3^{(6)} = 3, E_4^{(6)} = 8, E_5^{(6)} = 21.$$

The sequences $\{P_n^{(6)}\}_{n\geq 0}$, $\{Q_n^{(6)}\}_{n\geq 0}$ and $\{E_n^{(6)}\}_{n\geq 0}$ can be extended to negative subscripts by defining

$$P_{-n}^{(6)} = -P_{-(n-1)}^{(6)} - P_{-(n-2)}^{(6)} - P_{-(n-3)}^{(6)} - P_{-(n-4)}^{(6)} - 2P_{-(n-5)}^{(6)} + P_{-(n-6)}^{(6)} \tag{1.7}$$

and

$$Q_{-n}^{(6)} = -Q_{-(n-1)}^{(6)} - Q_{-(n-2)}^{(6)} - Q_{-(n-3)}^{(6)} - Q_{-(n-4)}^{(6)} - 2Q_{-(n-5)}^{(6)} + Q_{-(n-6)}^{(6)} \tag{1.8}$$

and

$$E_{-n}^{(6)} = -E_{-(n-1)}^{(6)} - E_{-(n-2)}^{(6)} - E_{-(n-3)}^{(6)} - E_{-(n-4)}^{(6)} - 2E_{-(n-5)}^{(6)} + E_{-(n-6)}^{(6)} \tag{1.9}$$

for $n = 1, 2, 3, ...$ respectively. Therefore, recurrences (1.7), (1.8) and (1.9) hold for all integer n.

In the rest of the paper, for easy writing, we drop the superscripts and write P_n, Q_n and E_n for $P_n^{(6)}, Q_n^{(6)}$ and $E_n^{(6)}$, respectively.

Note that P_n, Q_n and E_n sequences are't in the database of http://oeis.org [13], yet.

Next, we present the first few values of the sixth-order Pell, sixth-order Pell-Lucas and modified sixth-order Pell numbers with positive and negative subscripts:

Table 3. The first few values of the special sixth-order numbers with positive and negative subscripts.

n	0	1	2	3	4	5	6	7	8	9	10	11	12	13
P_n	0	1	2	5	13	34	89	233	609	1592	4162	10881	28447	74371
P_{-n}	0	0	0	0	0	1	−1	0	0	0	−1	4	−4	1
Q_n	6	2	6	17	46	122	321	835	2182	5705	14916	38997	1 01953	266541
Q_{-n}	6	−1	−1	−1	−1	−6	17	−8	−1	−1	4	−34	65	−40
E_n	0	1	1	3	8	21	55	144	376	983	2570	6719	17566	45924
E_{-n}	0	0	0	0	−1	2	−1	0	0	1	−5	8	−5	1

2. Generating Functions

Next, we give the ordinary generating function $\sum_{n=0}^{\infty} V_n x^n$ of the sequence V_n.

LEMMA 1. *Suppose that $f_{V_n}(x) = \sum_{n=0}^{\infty} V_n x^n$ is the ordinary generating function of the generalized sixth-order Pell sequence $\{V_n\}_{n\geq 0}$. Then, $\sum_{n=0}^{\infty} V_n x^n$ is given by*

$$\sum_{n=0}^{\infty} V_n x^n = \frac{\begin{array}{c} V_0 + (V_1 - 2V_0)x + (V_2 - 2V_1 - V_0)x^2 + (V_3 - 2V_2 - V_1 - V_0)x^3 \\ +(V_4 - 2V_3 - V_2 - V_1 - V_0)x^4 + (V_5 - 2V_4 - V_3 - V_2 - V_1 - V_0)x^5 \end{array}}{1 - 2x - x^2 - x^3 - x^4 - x^5 - x^6}. \tag{2.1}$$

Proof.

Using the definition of generalized sixth-order Pell numbers and substracting $xf(x), x^2f(x), x^3f(x), x^4f(x), x^5f(x)$ and $x^6f(x)$ from $f(x)$ we obtain (note the shift in the index n in the third line)

$$\begin{aligned}
& (1 - 2x - x^2 - x^3 - x^4 - x^5 - x^6) f_{V_n}(x) \\
= & \sum_{n=0}^{\infty} V_n x^n - 2x \sum_{n=0}^{\infty} V_n x^n - x^2 \sum_{n=0}^{\infty} V_n x^n - x^3 \sum_{n=0}^{\infty} V_n x^n - x^4 \sum_{n=0}^{\infty} V_n x^n - x^5 \sum_{n=0}^{\infty} V_n x^n - x^6 \sum_{n=0}^{\infty} V_n x^n \\
= & \sum_{n=0}^{\infty} V_n x^n - 2 \sum_{n=0}^{\infty} V_n x^{n+1} - \sum_{n=0}^{\infty} V_n x^{n+2} - \sum_{n=0}^{\infty} V_n x^{n+3} - \sum_{n=0}^{\infty} V_n x^{n+4} - \sum_{n=0}^{\infty} V_n x^{n+5} - \sum_{n=0}^{\infty} V_n x^{n+6} \\
= & \sum_{n=0}^{\infty} V_n x^n - 2 \sum_{n=1}^{\infty} V_{n-1} x^n - \sum_{n=2}^{\infty} V_{n-2} x^n - \sum_{n=3}^{\infty} V_{n-3} x^n - \sum_{n=4}^{\infty} V_{n-4} x^n - \sum_{n=5}^{\infty} V_{n-5} x^n - \sum_{n=6}^{\infty} V_{n-6} x^n
\end{aligned}$$

and then

$$\begin{aligned}
& (1-2x-x^2-x^3-x^4-x^5-x^6)f_{V_n}(x) \\
= \; & (V_0+V_1x+V_2x^2+V_3x^3+V_4x^4+V_5x^5)-2(V_0x+V_1x^2+V_2x^3+V_3x^4+V_4x^5) \\
& -(V_0x^2+V_1x^3+V_2x^4+V_3x^5)-(V_0x^3+V_1x^4+V_2x^5)-(V_0x^4+V_1x^5)-V_0x^5 \\
& +\sum_{n=6}^{\infty}(V_n-V_{n-1}-V_{n-2}-V_{n-3}-V_{n-4}-V_{n-5}-V_{n-6})x^n \\
= \; & V_0+(V_1-2V_0)x+(V_2-2V_1-V_0)x^2+(V_3-2V_2-V_1-V_0)x^3 \\
& +(V_4-2V_3-V_2-V_1-V_0)x^4+(V_5-2V_4-V_3-V_2-V_1-V_0)x^5.
\end{aligned}$$

Rearranging the above equation, we get (2.1).

The previous Lemma gives the following results as particular examples.

COROLLARY 2. *Generated functions of sixth-order Pell, Pell-Lucas and modified Pell numbers are*

$$\sum_{n=0}^{\infty}P_nx^n=\frac{x}{1-2x-x^2-x^3-x^4-x^5-x^6},$$

and

$$\sum_{n=0}^{\infty}Q_nx^n=\frac{6-10x-4x^2-3x^3-2x^4-x^5}{1-2x-x^2-x^3-x^4-x^5-x^6},$$

and

$$\sum_{n=0}^{\infty}E_nx^n=\frac{x-x^2}{1-2x-x^2-x^3-x^4-x^5-x^6},$$

respectively.

3. Obtaning Binet Formula From Generating Function

We next find Binet formula of generalized sixth order Pell numbers $\{V_n\}$ by the use of generating function for V_n.

THEOREM 3. *(Binet formula of generalized sixth order Pell numbers)*

$$V_n=\sum_{k=1}^{6}\frac{d_k\theta_k^n}{\prod\limits_{\substack{j=1\\k\neq j}}^{6}(\theta_k-\theta_j)} \tag{3.1}$$

where

$$\begin{aligned}
d_k \; = \; & V_0\theta_k^5+(V_1-2V_0)\theta_k^4+(V_2-2V_1-V_0)\theta_k^3+(V_3-2V_2-V_1-V_0)\theta_k^2 \\
& +(V_4-2V_3-V_2-V_1-V_0)\theta_k+(V_5-2V_4-V_3-V_2-V_1-V_0)
\end{aligned}$$

for each $1\leq k\leq 6$.

Proof. Let

$$h(x) = 1 - 2x - x^2 - x^3 - x^4 - x^5 - x^6.$$

Then for some $\theta_1, \theta_2, \theta_3, \theta_4, \theta_5$ and θ_6 we write

$$h(x) = (1 - \theta_1 x)(1 - \theta_2 x)(1 - \theta_3 x)(1 - \theta_4 x)(1 - \theta_5 x)(1 - \theta_6 x)$$

i.e.,

$$1 - 2x - x^2 - x^3 - x^4 - x^5 - x^6 = (1 - \theta_1 x)(1 - \theta_2 x)(1 - \theta_3 x)(1 - \theta_4 x)(1 - \theta_5 x)(1 - \theta_6 x) \tag{3.2}$$

Hence $\frac{1}{\theta_1}, \frac{1}{\theta_2}, \frac{1}{\theta_3}, \frac{1}{\theta_4}, \frac{1}{\theta_5}$ ve $\frac{1}{\theta_6}$ are the roots of $h(x)$. This gives $\theta_1, \theta_2, \theta_3, \theta_4, \theta_5$ and θ_6 as the roots of

$$h(\frac{1}{x}) = 1 - \frac{2}{x} - \frac{1}{x^2} - \frac{1}{x^3} - \frac{1}{x^4} - \frac{1}{x^5} - \frac{1}{x^6} = 0.$$

This implies $x^6 - x^5 - x^4 - x^3 - 2x^2 - x - 1 = 0$. Now, by (2.1) and (3.2), it follows that

$$\sum_{n=0}^{\infty} V_n x^n = \frac{\begin{array}{c} V_0 + (V_1 - 2V_0)x + (V_2 - 2V_1 - V_0)x^2 + (V_3 - 2V_2 - V_1 - V_0)x^3 \\ +(V_4 - 2V_3 - V_2 - V_1 - V_0)x^4 + (V_5 - 2V_4 - V_3 - V_2 - V_1 - V_0)x^5 \end{array}}{(1 - \theta_1 x)(1 - \theta_2 x)(1 - \theta_3 x)(1 - \theta_4 x)(1 - \theta_5 x)(1 - \theta_6 x)}.$$

Then we write

$$\sum_{n=0}^{\infty} V_n x^n = \frac{A_1}{(1 - \theta_1 x)} + \frac{A_2}{(1 - \theta_2 x)} + \frac{A_3}{(1 - \theta_3 x)} + \frac{A_4}{(1 - \theta_4 x)} + \frac{A_5}{(1 - \theta_5 x)} + \frac{A_6}{(1 - \theta_6 x)}. \tag{3.3}$$

So

$$\begin{aligned} & V_0 + (V_1 - 2V_0)x + (V_2 - 2V_1 - V_0)x^2 + (V_3 - 2V_2 - V_1 - V_0)x^3 \\ & +(V_4 - 2V_3 - V_2 - V_1 - V_0)x^4 + (V_5 - 2V_4 - V_3 - V_2 - V_1 - V_0)x^5 \\ = \; & A_1(1 - \theta_2 x)(1 - \theta_3 x)(1 - \theta_4 x)(1 - \theta_5 x)(1 - \theta_6 x) + A_2(1 - \theta_1 x)(1 - \theta_3 x)(1 - \theta_4 x)(1 - \theta_5 x)(1 - \theta_6 x) \\ & +A_3(1 - \theta_1 x)(1 - \theta_2 x)(1 - \theta_4 x)(1 - \theta_5 x)(1 - \theta_6 x) + A_4(1 - \theta_1 x)(1 - \theta_2 x)(1 - \theta_3 x)(1 - \theta_5 x)(1 - \theta_6 x) \\ & +A_5(1 - \theta_1 x)(1 - \theta_2 x)(1 - \theta_3 x)(1 - \theta_4 x)(1 - \theta_6 x) + A_6(1 - \theta_1 x)(1 - \theta_2 x)(1 - \theta_3 x)(1 - \theta_4 x)(1 - \theta_5 x). \end{aligned}$$

If we consider $x = \frac{1}{\theta_1}$, we get

$$\begin{aligned} & V_0 + (V_1 - 2V_0)\frac{1}{\theta_1} + (V_2 - 2V_1 - V_0)\frac{1}{\theta_1^2} + (V_3 - 2V_2 - V_1 - V_0)\frac{1}{\theta_1^3} \\ & +(V_4 - 2V_3 - V_2 - V_1 - V_0)\frac{1}{\theta_1^4} + (V_5 - 2V_4 - V_3 - V_2 - V_1 - V_0)\frac{1}{\theta_1^5} \\ = \; & A_1(1 - \frac{\theta_2}{\theta_1})(1 - \frac{\theta_3}{\theta_1})(1 - \frac{\theta_4}{\theta_1})(1 - \frac{\theta_5}{\theta_1})(1 - \frac{\theta_6}{\theta_1}). \end{aligned}$$

This gives

$$\begin{aligned} A_1 &= \frac{\theta_1^5(V_0+(V_1-2V_0)\frac{1}{\theta_1}+(V_2-2V_1-V_0)\frac{1}{\theta_1^2}+(V_3-2V_2-V_1-V_0)\frac{1}{\theta_1^3} \\ &\quad +(V_4-2V_3-V_2-V_1-V_0)\frac{1}{\theta_1^4}+(V_5-2V_4-V_3-V_2-V_1-V_0)\frac{1}{\theta_1^5})}{(\theta_1-\theta_2)(\theta_1-\theta_3)(\theta_1-\theta_4)(\theta_1-\theta_5)(\theta_1-\theta_6)} \\ &= \frac{d_1}{(\theta_1-\theta_2)(\theta_1-\theta_3)(\theta_1-\theta_4)(\theta_1-\theta_5)(\theta_1-\theta_6)} \end{aligned}$$

Similarly, we obtain

$$\begin{aligned} A_2 &= \frac{d_2}{(\theta_2-\theta_1)(\theta_2-\theta_3)(\theta_2-\theta_4)(\theta_2-\theta_5)(\theta_2-\theta_6)}, \\ A_3 &= \frac{d_3}{(\theta_3-\theta_1)(\theta_3-\theta_2)(\theta_3-\theta_4)(\theta_3-\theta_5)(\theta_3-\theta_6)} \\ A_4 &= \frac{d_4}{(\theta_4-\theta_1)(\theta_4-\theta_2)(\theta_4-\theta_3)(\theta_4-\theta_5)(\theta_4-\theta_6)} \\ A_5 &= \frac{d_5}{(\theta_5-\theta_1)(\theta_5-\theta_2)(\theta_5-\theta_3)(\theta_5-\theta_4)(\theta_5-\theta_6)} \\ A_5 &= \frac{d_6}{(\theta_6-\theta_1)(\theta_6-\theta_2)(\theta_6-\theta_3)(\theta_6-\theta_4)(\theta_6-\theta_5)}. \end{aligned}$$

Thus (3.3) can be written as

$$\sum_{n=0}^{\infty} V_n x^n = A_1(1-\theta_1 x)^{-1}+A_2(1-\theta_2 x)^{-1}+A_3(1-\theta_3 x)^{-1}+A_4(1-\theta_4 x)^{-1}+A_5(1-\theta_5 x)^{-1}+A_6(1-\theta_6 x)^{-1}.$$

This gives

$$\begin{aligned} \sum_{n=0}^{\infty} V_n x^n &= A_1\sum_{n=0}^{\infty}\theta_1^n x^n + A_2\sum_{n=0}^{\infty}\theta_2^n x^n + A_3\sum_{n=0}^{\infty}\theta_3^n x^n + A_4\sum_{n=0}^{\infty}\theta_4^n x^n + A_5\sum_{n=0}^{\infty}\theta_5^n x^n + A_6\sum_{n=0}^{\infty}\theta_6^n x^n \\ &= \sum_{n=0}^{\infty}(A_1\theta_1^n + A_2\theta_2^n + A_3\theta_3^n + A_4\theta_4^n + A_5\theta_5^n + A_6\theta_6^n)x^n. \end{aligned}$$

Therefore, comparing coefficients on both sides of the above equality, we obtain

$$V_n = A_1\theta_1^n + A_2\theta_2^n + A_3\theta_3^n + A_4\theta_4^n + A_5\theta_5^n + A_6\theta_6^n$$

and then we get (3.1).

Next, using Theorem 3, we present the Binet formulas of sixth-order Pell, Pell-Lucas and modified Pell sequences.

COROLLARY 4. *Binet formulas of sixth-order Pell, Pell-Lucas and modified Pell sequences are*

$$P_n = \sum_{k=1}^{6} \frac{\theta_k^{n+4}}{\prod\limits_{\substack{j=1 \\ k\neq j}}^{6}(\theta_k-\theta_j)}$$

and

$$Q_n = \sum_{k=1}^{6}\theta_k^n = \theta_1^n+\theta_2^n+\theta_3^n+\theta_4^n+\theta_5^n+\theta_6^n,$$

and

$$E_n = \sum_{k=1}^{6} \frac{(\theta_k - 1)\theta_k^{n+3}}{\prod\limits_{\substack{j=1 \\ k \neq j}}^{6} (\theta_k - \theta_j)}.$$

respectively.

Note that Binet formula of generalized sixth order Pell numbers can be represented as

$$V_n = \sum_{k=1}^{6} \frac{\theta_k d_k \theta_k^n}{(2\theta_k^5 + 2\theta_k^4 + 3\theta_k^3 + 4\theta_k^2 + 5\theta_k + 6)} \tag{3.4}$$

which can be derived from a result ((4.20) in page 25) of Hanusa [5]. When we compare (3.1) and (3.4), we see the following identities:

$$\begin{aligned}
\frac{1}{(\theta_1-\theta_2)(\theta_1-\theta_3)(\theta_1-\theta_4)(\theta_1-\theta_5)(\theta_1-\theta_6)} &= \frac{\theta_1}{2\theta_1^5 + 2\theta_1^4 + 3\theta_1^3 + 4\theta_1^2 + 5\theta_1 + 6} \\
\frac{1}{(\theta_2-\theta_1)(\theta_2-\theta_3)(\theta_2-\theta_4)(\theta_2-\theta_5)(\theta_2-\theta_6)} &= \frac{\theta_2}{2\theta_2^5 + 2\theta_2^4 + 3\theta_2^3 + 4\theta_2^2 + 5\theta_2 + 6} \\
\frac{1}{(\theta_3-\theta_1)(\theta_3-\theta_2)(\theta_3-\theta_4)(\theta_3-\theta_5)(\theta_3-\theta_6)} &= \frac{\theta_3}{2\theta_3^5 + 2\theta_3^4 + 3\theta_3^3 + 4\theta_3^2 + 5\theta_3 + 6} \\
\frac{1}{(\theta_4-\theta_1)(\theta_4-\theta_2)(\theta_4-\theta_3)(\theta_4-\theta_5)(\theta_4-\theta_6)} &= \frac{\theta_4}{2\theta_4^5 + 2\theta_4^4 + 3\theta_4^3 + 4\theta_4^2 + 5\theta_4 + 6} \\
\frac{1}{(\theta_5-\theta_1)(\theta_5-\theta_2)(\theta_5-\theta_3)(\theta_5-\theta_4)(\theta_5-\theta_6)} &= \frac{\theta_5}{2\theta_5^5 + 2\theta_5^4 + 3\theta_5^3 + 4\theta_5^2 + 5\theta_5 + 6} \\
\frac{1}{(\theta_6-\theta_1)(\theta_6-\theta_2)(\theta_6-\theta_3)(\theta_6-\theta_4)(\theta_6-\theta_5)} &= \frac{\theta_6}{2\theta_6^5 + 2\theta_6^4 + 3\theta_6^3 + 4\theta_6^2 + 5\theta_6 + 6}
\end{aligned}$$

Using the above identities, we can give the Binet formulas of sixth-order Pell, Pell-Lucas and modified Pell sequences in the following form: Binet formulas of sixth-order Pell, Pell-Lucas and modified Pell sequences are

$$P_n = \sum_{k=1}^{6} \frac{\theta_k^{n+5}}{(2\theta_k^5 + 2\theta_k^4 + 3\theta_k^3 + 4\theta_k^2 + 5\theta_k + 6)}$$

and

$$Q_n = \theta_1^n + \theta_2^n + \theta_3^n + \theta_4^n + \theta_5^n + \theta_6^n,$$

and

$$E_n = \sum_{k=1}^{6} \frac{(\theta_k - 1)\theta_k^{n+4}}{(2\theta_k^5 + 2\theta_k^4 + 3\theta_k^3 + 4\theta_k^2 + 5\theta_k + 6)}$$

respectively.

4. Simson Formulas

There is a well-known Simson Identity (formula) for Fibonacci sequence $\{F_n\}$, namely,

$$F_{n+1}F_{n-1} - F_n^2 = (-1)^n$$

which was derived first by R. Simson in 1753 and it is now called as Cassini Identity (formula) as well. This can be written in the form

$$\begin{vmatrix} F_{n+1} & F_n \\ F_n & F_{n-1} \end{vmatrix} = (-1)^n.$$

The following Theorem gives generalization of this result to the generalized Hexanacci sequence $\{W_n\}$.

THEOREM 5 (Simson Formula of Generalized Hexanacci Numbers). *For all integers n we have*

(4.1)
$$\begin{vmatrix} W_{n+5} & W_{n+4} & W_{n+3} & W_{n+2} & W_{n+1} & W_n \\ W_{n+4} & W_{n+3} & W_{n+2} & W_{n+1} & W_n & W_{n-1} \\ W_{n+3} & W_{n+2} & W_{n+1} & W_n & W_{n-1} & W_{n-2} \\ W_{n+2} & W_{n+1} & W_n & W_{n-1} & W_{n-2} & W_{n-3} \\ W_{n+1} & W_n & W_{n-1} & W_{n-2} & W_{n-3} & W_{n-4} \\ W_n & W_{n-1} & W_{n-2} & W_{n-3} & W_{n-4} & W_{n-5} \end{vmatrix} = (-1)^n r_6^n \begin{vmatrix} W_5 & W_4 & W_3 & W_2 & W_1 & W_0 \\ W_4 & W_3 & W_2 & W_1 & W_0 & W_{-1} \\ W_3 & W_2 & W_1 & W_0 & W_{-1} & W_{-2} \\ W_2 & W_1 & W_0 & W_{-1} & W_{-2} & W_{-3} \\ W_1 & W_0 & W_{-1} & W_{-2} & W_{-3} & W_{-4} \\ W_0 & W_{-1} & W_{-2} & W_{-3} & W_{-4} & W_{-5} \end{vmatrix}.$$

Proof. (4.1) is given in Soykan [14], see also [15].

A special case of the above theorem is the following Theorem which gives Simson formula of the generalized sixth-order Pell sequence $\{V_n\}$.

THEOREM 6 (Simson Formula of Generalized Sixth-Order Pell Numbers). *For all integers n we have*

$$\begin{vmatrix} V_{n+5} & V_{n+4} & V_{n+3} & V_{n+2} & V_{n+1} & V_n \\ V_{n+4} & V_{n+3} & V_{n+2} & V_{n+1} & V_n & V_{n-1} \\ V_{n+3} & V_{n+2} & V_{n+1} & V_n & V_{n-1} & V_{n-2} \\ V_{n+2} & V_{n+1} & V_n & V_{n-1} & V_{n-2} & V_{n-3} \\ V_{n+1} & V_n & V_{n-1} & V_{n-2} & V_{n-3} & V_{n-4} \\ V_n & V_{n-1} & V_{n-2} & V_{n-3} & V_{n-4} & V_{n-5} \end{vmatrix} = (-1)^n \begin{vmatrix} V_5 & V_4 & V_3 & V_2 & V_1 & V_0 \\ V_4 & V_3 & V_2 & V_1 & V_0 & V_{-1} \\ V_3 & V_2 & V_1 & V_0 & V_{-1} & V_{-2} \\ V_2 & V_1 & V_0 & V_{-1} & V_{-2} & V_{-3} \\ V_1 & V_0 & V_{-1} & V_{-2} & V_{-3} & V_{-4} \\ V_0 & V_{-1} & V_{-2} & V_{-3} & V_{-4} & V_{-5} \end{vmatrix}.$$

The previous Theorem gives the following results as particular examples.

COROLLARY 7. *Simson formula of sixth-order Pell, Pell-Lucas and modified Pell numbers are given as*

$$\begin{vmatrix} P_{n+5} & P_{n+4} & P_{n+3} & P_{n+2} & P_{n+1} & P_n \\ P_{n+4} & P_{n+3} & P_{n+2} & P_{n+1} & P_n & P_{n-1} \\ P_{n+3} & P_{n+2} & P_{n+1} & P_n & P_{n-1} & P_{n-2} \\ P_{n+2} & P_{n+1} & P_n & P_{n-1} & P_{n-2} & P_{n-3} \\ P_{n+1} & P_n & P_{n-1} & P_{n-2} & P_{n-3} & P_{n-4} \\ P_n & P_{n-1} & P_{n-2} & P_{n-3} & P_{n-4} & P_{n-5} \end{vmatrix} = (-1)^n,$$

$$\begin{vmatrix} Q_{n+5} & Q_{n+4} & Q_{n+3} & Q_{n+2} & Q_{n+1} & Q_n \\ Q_{n+4} & Q_{n+3} & Q_{n+2} & Q_{n+1} & Q_n & Q_{n-1} \\ Q_{n+3} & Q_{n+2} & Q_{n+1} & Q_n & Q_{n-1} & Q_{n-2} \\ Q_{n+2} & Q_{n+1} & Q_n & Q_{n-1} & Q_{n-2} & Q_{n-3} \\ Q_{n+1} & Q_n & Q_{n-1} & Q_{n-2} & Q_{n-3} & Q_{n-4} \\ Q_n & Q_{n-1} & Q_{n-2} & Q_{n-3} & Q_{n-4} & Q_{n-5} \end{vmatrix} = -884552(-1)^n = 884552(-1)^{n+1},$$

$$\begin{vmatrix} E_{n+5} & E_{n+4} & E_{n+3} & E_{n+2} & E_{n+1} & E_n \\ E_{n+4} & E_{n+3} & E_{n+2} & E_{n+1} & E_n & E_{n-1} \\ E_{n+3} & E_{n+2} & E_{n+1} & E_n & E_{n-1} & E_{n-2} \\ E_{n+2} & E_{n+1} & E_n & E_{n-1} & E_{n-2} & E_{n-3} \\ E_{n+1} & E_n & E_{n-1} & E_{n-2} & E_{n-3} & E_{n-4} \\ E_n & E_{n-1} & E_{n-2} & E_{n-3} & E_{n-4} & E_{n-5} \end{vmatrix} = 6(-1)^n.$$

5. Some Identities

In this section, we obtain some identities of sixth order Pell, sixth order Pell-Lucas and modified sixth order Pell numbers. First, we can give a few basic relations between $\{P_n\}$ and $\{Q_n\}$.

LEMMA 8. *The following equalities are true:*

$$\begin{aligned} Q_n &= -6P_{n+6} + 11P_{n+5} + 7P_{n+4} + 8P_{n+3} + 9P_{n+2} + 17P_{n+1}, \\ Q_n &= -P_{n+5} + P_{n+4} + 2P_{n+3} + 3P_{n+2} + 11P_{n+1} - 6P_n, \\ Q_n &= -P_{n+4} + P_{n+3} + 2P_{n+2} + 10P_{n+1} - 7P_n - P_{n-1}, \\ Q_n &= -P_{n+3} + P_{n+2} + 9P_{n+1} - 8P_n - 2P_{n-1} - P_{n-2}, \\ Q_n &= -P_{n+2} + 8P_{n+1} - 9P_n - 3P_{n-1} - 2P_{n-2} - P_{n-3}, \end{aligned} \tag{5.1}$$

and

$$\begin{aligned}
442276P_n &= -39853Q_{n+6} + 96245Q_{n+5} + 14588Q_{n+4} + 15965Q_{n+3} + 14650Q_{n+2} + 10285Q_{n+1},\\
442276P_n &= 16539Q_{n+5} - 25265Q_{n+4} - 23888Q_{n+3} - 25203Q_{n+2} - 29568Q_{n+1} - 39853Q_n,\\
442276P_n &= 7813Q_{n+4} - 7349Q_{n+3} - 8664Q_{n+2} - 13029Q_{n+1} - 23314Q_n + 16539Q_{n-1},\\
442276P_n &= 8277Q_{n+3} - 851Q_{n+2} - 5216Q_{n+1} - 15501Q_n + 24352Q_{n-1} + 7813Q_{n-2},\\
442276P_n &= 15703Q_{n+2} + 3061Q_{n+1} - 7224Q_n + 32629Q_{n-1} + 16090Q_{n-2} + 8277Q_{n-3}.
\end{aligned}$$

Proof. Note that all the identities hold for all integers n. We prove (5.1). To show (5.1), writing

$$Q_n = a \times P_{n+5} + b \times P_{n+4} + c \times P_{n+3} + d \times P_{n+2}$$

and solving the system of equations

$$\begin{aligned}
Q_0 &= a \times P_6 + b \times P_5 + c \times P_4 + d \times P_3 + e \times P_2 + f \times P_1\\
Q_1 &= a \times P_7 + b \times P_6 + c \times P_5 + d \times P_4 + e \times P_3 + f \times P_2\\
Q_2 &= a \times P_8 + b \times P_7 + c \times P_6 + d \times P_5 + e \times P_4 + f \times P_2\\
Q_3 &= a \times P_9 + b \times P_8 + c \times P_7 + d \times P_6 + e \times P_5 + f \times P_4\\
Q_4 &= a \times P_{10} + b \times P_9 + c \times P_8 + d \times P_7 + e \times P_6 + f \times P_5\\
Q_5 &= a \times P_{11} + b \times P_{10} + c \times P_9 + d \times P_8 + e \times P_7 + f \times P_6
\end{aligned}$$

we find that $a = -6, b = 11, c = 7, d = 8, e = 9, f = 17$. The other equalities can be proved similarly.

Note that all the identities in the above Lemma can be proved by induction as well.

Secondly, we present a few basic relations between $\{P_n\}$ and $\{E_n\}$.

Lemma 9. *The following equalities are true:*

$$\begin{aligned}
E_n &= 2P_{n+6} - 5P_{n+5} - P_{n+3} - P_{n+2} - P_{n+1},\\
E_n &= -P_{n+5} + 2P_{n+4} + P_{n+3} + P_{n+2} + P_{n+1} + 2P_n,\\
E_n &= P_n - P_{n-1},
\end{aligned}$$

and

$$\begin{aligned}
6P_n &= E_{n+6} - E_{n+5} - 2E_{n+4} - 3E_{n+3} - 4E_{n+2} - 5E_{n+1},\\
6P_n &= E_{n+5} - E_{n+4} - 2E_{n+3} - 3E_{n+2} - 4E_{n+1} + E_n,\\
6P_n &= E_{n+4} - E_{n+3} - 2E_{n+2} - 3E_{n+1} + 2E_n + E_{n-1},\\
6P_n &= E_{n+3} - E_{n+2} - 2E_{n+1} + 3E_n + 2E_{n-1} + E_{n-2},\\
6P_n &= E_{n+2} - E_{n+1} + 4E_n + 3E_{n-1} + 2E_{n-2} + E_{n-3}.
\end{aligned}$$

Thirdly, we give a few basic relations between $\{Q_n\}$ and $\{E_n\}$.

LEMMA 10. *The following equalities are true:*

$$\begin{aligned}
3Q_n &= 5E_{n+6} - 8E_{n+5} - 10E_{n+4} - 9E_{n+3} - 5E_{n+2} + 23E_{n+1}, \\
3Q_n &= 2E_{n+5} - 5E_{n+4} - 4E_{n+3} + 28 \times E_{n+1} + 5E_n, \\
3Q_n &= -E_{n+4} - 2E_{n+3} + 2E_{n+2} + 30E_{n+1} + 7E_n + 2E_{n-1}, \\
3Q_n &= -4E_{n+3} + E_{n+2} + 29E_{n+1} + 6E_n + E_{n-1} - E_{n-2}, \\
3Q_n &= -7E_{n+2} + 25E_{n+1} + 2E_n - 3E_{n-1} - 5E_{n-2} - 4E_{n-3},
\end{aligned}$$

and

$$\begin{aligned}
221138E_n &= -25069Q_{n+6} + 78334Q_{n+5} - 35686Q_{n+4} + 5831Q_{n+3} + 4485Q_{n+2} + 2960Q_{n+1}, \\
221138E_n &= 28196Q_{n+5} - 60755Q_{n+4} - 19238Q_{n+3} - 20584Q_{n+2} - 22109Q_{n+1} - 25069Q_n, \\
221138E_n &= -4363Q_{n+4} + 8958Q_{n+3} + 7612Q_{n+2} + 6087Q_{n+1} + 3127Q_n + 28196Q_{n-1}, \\
221138E_n &= 232Q_{n+3} + 3249Q_{n+2} + 1724Q_{n+1} - 1236Q_n + 23833Q_{n-1} - 4363Q_{n-2}, \\
221138E_n &= 3713Q_{n+2} + 1956Q_{n+1} - 1004Q_n + 24065Q_{n-1} - 4131Q_{n-2} + 232Q_{n-3}.
\end{aligned}$$

We now present a few special identities for the modified sixth order Pell sequence $\{E_n\}$.

THEOREM 11. *(Catalan's identity) For all natural numbers n and m, the following identity holds*

$$E_{n+m}E_{n-m} - E_n^2 = (P_{n+m} - P_{n+m-1})(P_{n-m} - P_{n-m-1}) - (P_n - P_{n-1})^2$$

Proof. We use the identity

$$E_n = P_n - P_{n-1}.$$

Note that for $m = 1$ in Catalan's identity, we get the Cassini identity for the modified sixth order Pell sequence.

COROLLARY 12. *(Cassini's identity) For all natural numbers n and m, the following identity holds*

$$E_{n+1}E_{n-1} - E_n^2 = (P_{n+1} - P_n)(P_{n-1} - P_{n-2}) - (P_n - P_{n-1})^2.$$

The d'Ocagne's, Gelin-Cesàro's and Melham' identities can also be obtained by using $E_n = P_n - P_{n-1}$.The next theorem presents d'Ocagne's, Gelin-Cesàro's and Melham' identities of modified sixth order Pell sequence $\{E_n\}$.

THEOREM 13. *Let n and m be any integers. Then the following identities are true:*

(a): *(d'Ocagne's identity)*

$$E_{m+1}E_n - E_mE_{n+1} = (P_{m+1} - P_m)(P_n - P_{n-1}) - (P_m - P_{m-1})(P_{n+1} - P_n).$$

(b): *(Gelin-Cesàro's identity)*

$$E_{n+2}E_{n+1}E_{n-1}E_{n-2} - E_n^4 = (P_{n+2} - P_{n+1})(P_{n+1} - P_n)(P_{n-1} - P_{n-2})(P_{n-2} - P_{n-3}) - (P_n - P_{n-1})^4$$

(c): *(Melham's identity)*

$$E_{n+1}E_{n+2}E_{n+6} - E_{n+3}^3 = (P_{n+1} - P_n)(P_{n+2} - P_{n+1})(P_{n+6} - P_{n+5}) - (P_{n+3} - P_{n+2})^3$$

Proof. Use the identity $E_n = P_n - P_{n-1}$.

6. Linear Sums

The following Theorem presents summing formulas of generalized sixth order Pell numbers.

THEOREM 14. *For $n \geq 0$ we have the following formulas:*

(a): *(Sum of the generalized sixth order Pell numbers)*

$$\sum_{k=0}^{n} V_k = \frac{1}{6}(V_{n+6} - V_{n+5} - 2V_{n+4} - 3V_{n+3} - 4V_{n+2} - 5V_{n+1} - V_5 + V_4 + 2V_3 + 3V_2 + 4V_1 + 5V_0).$$

(b):

$$\sum_{k=0}^{n} V_{2k} = \frac{1}{6}(-V_{2n+2} + 4V_{2n+1} + 2V_{2n} + 3V_{2n-1} + V_{2n-2} + 2V_{2n-3} - 2V_5 + 5V_4 - 2V_3 + 6V_2 - V_1 + 7V_0).$$

(c):

$$\sum_{k=0}^{n} V_{2k+1} = \frac{1}{6}(2V_{2n+2} + V_{2n+1} + 2V_{2n} + V_{2n-2} - V_{2n-3} + V_5 - 4V_4 + 4V_3 - 3V_2 + 5V_1 - 2V_0).$$

Proof.

(a): Using the recurrence relation

$$V_n = 2V_{n-1} + V_{n-2} + V_{n-3} + V_{n-4} + V_{n-5} + V_{n-6}$$

i.e.

$$V_{n-6} = V_n - 2V_{n-1} - V_{n-2} - V_{n-3} - V_{n-4} - V_{n-5}$$

we obtain

$$\begin{aligned}
V_0 &= V_6 - 2V_5 - V_4 - V_3 - V_2 - V_1 \\
V_1 &= V_7 - 2V_6 - V_5 - V_4 - V_3 - V_2 \\
V_2 &= V_8 - 2V_7 - V_6 - V_5 - V_4 - V_3 \\
&\vdots \\
V_{n-6} &= V_n - 2V_{n-1} - V_{n-2} - V_{n-3} - V_{n-4} - V_{n-5} \\
V_{n-5} &= V_{n+1} - 2V_n - V_{n-1} - V_{n-2} - V_{n-3} - V_{n-4} \\
V_{n-4} &= V_{n+2} - 2V_{n+1} - V_n - V_{n-1} - V_{n-2} - V_{n-3} \\
V_{n-3} &= V_{n+3} - 2V_{n+2} - V_{n+1} - V_n - V_{n-1} - V_{n-2} \\
V_{n-2} &= V_{n+4} - 2V_{n+3} - V_{n+2} - V_{n+1} - V_n - V_{n-1} \\
V_{n-1} &= V_{n+5} - 2V_{n+4} - V_{n+3} - V_{n+2} - V_{n+1} - V_n \\
V_n &= V_{n+6} - 2V_{n+5} - V_{n+4} - V_{n+3} - V_{n+2} - V_{n+1}.
\end{aligned}$$

If we add the equations by side by, we get

$$\begin{aligned}
\sum_{k=0}^{n} V_k &= (V_{n+6} + V_{n+5} + V_{n+4} + V_{n+3} + V_{n+2} + V_{n+1} - V_5 - V_4 - V_3 - V_2 - V_1 - V_0 + \sum_{k=0}^{n} V_k) \\
&\quad -2(V_{n+5} + V_{n+4} + V_{n+3} + V_{n+2} + V_{n+1} - V_4 - V_3 - V_2 - V_1 - V_0 + \sum_{k=0}^{n} V_k) \\
&\quad -(V_{n+4} + V_{n+3} + V_{n+2} + V_{n+1} - V_3 - V_2 - V_1 - V_0 + \sum_{k=0}^{n} V_k) \\
&\quad -(V_{n+3} + V_{n+2} + V_{n+1} - V_2 - V_1 - V_0 + \sum_{k=0}^{n} V_k) \\
&\quad -(V_{n+2} + V_{n+1} - V_1 - V_0 + \sum_{k=0}^{n} V_k) \\
&\quad -(V_{n+1} - V_0 + \sum_{k=0}^{n} V_k)
\end{aligned}$$

Then, solving the above equality we obtain

$$\sum_{k=0}^{n} V_k = \frac{1}{6}(V_{n+6} - V_{n+5} - 2V_{n+4} - 3V_{n+3} - 4V_{n+2} - 5V_{n+1} - V_5 + V_4 + 2V_3 + 3V_2 + 4V_1 + 5V_0).$$

(b) and (c): Using the recurrence relation

$$V_n = 2V_{n-1} + V_{n-2} + V_{n-3} + V_{n-4} + V_{n-5} + V_{n-6}$$

i.e.

$$2V_{n-1} = V_n - V_{n-2} - V_{n-3} - V_{n-4} - V_{n-5} - V_{n-6}$$

we obtain

$$\begin{aligned}
2V_3 &= V_4 - V_2 - V_1 - V_0 - V_{-1} - V_{-2} \\
2V_5 &= V_6 - V_4 - V_3 - V_2 - V_1 - V_0 \\
2V_7 &= V_8 - V_6 - V_5 - V_4 - V_3 - V_2 \\
2V_9 &= V_{10} - V_8 - V_7 - V_6 - V_5 - V_4 \\
&\vdots \\
2V_{2n-1} &= V_{2n} - V_{2n-2} - V_{2n-3} - V_{2n-4} - V_{2n-5} - V_{2n-6} \\
2V_{2n+1} &= V_{2n+2} - V_{2n} - V_{2n-1} - V_{2n-2} - V_{2n-3} - V_{2n-4} \\
2V_{2n+3} &= V_{2n+4} - V_{2n+2} - V_{2n+1} - V_{2n} - V_{2n-1} - V_{2n-2} \\
2V_{2n+5} &= V_{2n+6} - V_{2n+4} - V_{2n+3} - V_{2n+2} - V_{2n+1} - V_{2n}.
\end{aligned}$$

Now, if we add the above equations by side by, we get

$$\begin{aligned}
2(-V_1 + \sum_{k=0}^{n} V_{2k+1}) &= (V_{2n+2} - V_2 - V_0 + \sum_{k=0}^{n} V_{2k}) - (-V_0 + \sum_{k=0}^{n} V_{2k}) \\
&\quad -(-V_{2n+1} + \sum_{k=0}^{n} V_{2k+1}) - (-V_{2n} + \sum_{k=0}^{n} V_{2k}) - (-V_{2n+1} - V_{2n-1} + V_{-1} \\
&\quad + \sum_{k=0}^{n} V_{2k+1}) - (-V_{2n} - V_{2n-2} + V_{-2} + \sum_{k=0}^{n} V_{2k}).
\end{aligned} \tag{6.1}$$

Similarly, using the recurrence relation

$$V_n = 2V_{n-1} + V_{n-2} + V_{n-3} + V_{n-4} + V_{n-5} + V_{n-6}$$

i.e.

$$2V_{n-1} = V_n - V_{n-2} - V_{n-3} - V_{n-4} - V_{n-5} - V_{n-6}$$

we write the following obvious equations;

$$\begin{aligned}
2V_2 &= V_3 - V_1 - V_0 - V_{-1} - V_{-2} - V_{-3} \\
2V_4 &= V_5 - V_3 - V_2 - V_1 - V_0 - V_{-1} \\
2V_6 &= V_7 - V_5 - V_4 - V_3 - V_2 - V_1 \\
2V_8 &= V_9 - V_7 - V_6 - V_5 - V_4 - V_3 \\
&\vdots \\
2V_{2n-2} &= V_{2n-1} - V_{2n-3} - V_{2n-4} - V_{2n-5} - V_{2n-6} - V_{2n-7} \\
2V_{2n} &= V_{2n+1} - V_{2n-1} - V_{2n-2} - V_{2n-3} - V_{2n-4} - V_{2n-5} \\
2V_{2n+2} &= V_{2n+3} - V_{2n+1} - V_{2n} - V_{2n-1} - V_{2n-2} - V_{2n-3} \\
2V_{2n+4} &= V_{2n+5} - V_{2n+3} - V_{2n+2} - V_{2n+1} - V_{2n} - V_{2n-1} \\
2V_{2n+6} &= V_{2n+7} - V_{2n+5} - V_{2n+4} - V_{2n+3} - V_{2n+2} - V_{2n+1}
\end{aligned}$$

Now, if we add the above equations by side by, we obtain

$$\begin{aligned}
(6.2)\ 2(-V_0 + \sum_{k=0}^{n} V_{2k}) &= (-V_1 + \sum_{k=0}^{n} V_{2k+1}) - (-V_{2n+1} + \sum_{k=0}^{n} V_{2k+1}) - (-V_{2n} + \sum_{k=0}^{n} V_{2k}) \\
&\quad -(-V_{2n+1} - V_{2n-1} + V_{-1} + \sum_{k=0}^{n} V_{2k+1}) - (-V_{2n} - V_{2n-2} + V_{-2} + \sum_{k=0}^{n} V_{2k}) \\
&\quad -(-V_{2n+1} - V_{2n-1} - V_{2n-3} + V_{-3} + V_{-1} + \sum_{k=0}^{n} V_{2k+1}).
\end{aligned}$$

Then, solving the system (6.1)-(6.2) using

$$\begin{aligned}
V_{-1} &= (-V_0 - V_1 - V_2 - V_3 - 2V_4 + V_5) \\
V_{-2} &= (-V_5 + 3V_4 - V_3) \\
V_{-3} &= (-V_4 + 3V_3 - V_2)
\end{aligned}$$

the required result of (b) and (c) follow.

As special cases of above Theorem, we have the following three Corollaries. First one presents some summing formulas of sixth order Pell numbers.

COROLLARY 15. *For $n \geq 0$ we have the following formulas:*

(a): *(Sum of the sixth order Pell numbers)*

$$\sum_{k=0}^{n} P_k = \frac{1}{6}(P_{n+6} - P_{n+5} - 2P_{n+4} - 3P_{n+3} - 4P_{n+2} - 5P_{n+1} - 1).$$

(b): $\sum_{k=0}^{n} P_{2k} = \frac{1}{6}(-P_{2n+2} + 4P_{2n+1} + 2P_{2n} + 3P_{2n-1} + P_{2n-2} + 2P_{2n-3} - 2)$.

(c): $\sum_{k=0}^{n} P_{2k+1} = \frac{1}{6}(2P_{2n+2} + P_{2n+1} + 2P_{2n} + P_{2n-2} - P_{2n-3} + 1)$.

Second one presents some summing formulas of sixth order Pell-Lucas numbers.

COROLLARY 16. *For $n \geq 0$ we have the following formulas:*

(a): *(Sum of the sixth order Pell-Lucas numbers)*

$$\sum_{k=0}^{n} Q_k = \frac{1}{6}(Q_{n+6} - Q_{n+5} - 2Q_{n+4} - 3Q_{n+3} - 4Q_{n+2} - 5Q_{n+1} + 14).$$

(b): $\sum_{k=0}^{n} Q_{2k} = \frac{1}{6}(-Q_{2n+2} + 4Q_{2n+1} + 2Q_{2n} + 3Q_{2n-1} + Q_{2n-2} + 2Q_{2n-3} + 28)$.

(c): $\sum_{k=0}^{n} Q_{2k+1} = \frac{1}{6}(2Q_{2n+2} + Q_{2n+1} + 2Q_{2n} + Q_{2n-2} - Q_{2n-3} - 14)$.

Last one presents some summing formulas of modified fourth order Pell numbers.

COROLLARY 17. *For $n \geq 0$ we have the following formulas:*

(a): *(Sum of the modified sixth order Pell numbers)*

$$\sum_{k=0}^{n} E_k = \frac{1}{6}(E_{n+6} - E_{n+5} - 2E_{n+4} - 3E_{n+3} - 4E_{n+2} - 5E_{n+1}).$$

(b): $\sum_{k=0}^{n} E_{2k} = \frac{1}{6}(-E_{2n+2} + 4E_{2n+1} + 2E_{2n} + 3E_{2n-1} + E_{2n-2} + 2E_{2n-3} - 3)$.

(c): $\sum_{k=0}^{n} E_{2k+1} = \frac{1}{6}(2E_{2n+2} + E_{2n+1} + 2E_{2n} + E_{2n-2} - E_{2n-3} + 3)$.

7. Matrices Related with Generalized Sixth-Order Pell numbers

Matrix formulation of W_n can be given as

$$\begin{pmatrix} W_{n+5} \\ W_{n+4} \\ W_{n+3} \\ W_{n+2} \\ W_{n+1} \\ W_n \end{pmatrix} = \begin{pmatrix} r_1 & r_2 & r_3 & r_4 & r_5 & r_6 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \end{pmatrix}^n \begin{pmatrix} W_5 \\ W_4 \\ W_3 \\ W_2 \\ W_1 \\ W_0 \end{pmatrix} \tag{7.1}$$

For matrix formulation (7.1), see [7]. In fact, Kalman give the formula in the following form

$$\begin{pmatrix} W_n \\ W_{n+1} \\ W_{n+2} \\ W_{n+3} \\ W_{n+4} \\ W_{n+5} \end{pmatrix} = \begin{pmatrix} 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \\ r_1 & r_2 & r_3 & r_4 & r_5 & r_6 \end{pmatrix}^n \begin{pmatrix} W_0 \\ W_1 \\ W_2 \\ W_3 \\ W_4 \\ W_5 \end{pmatrix}.$$

We define the square matrix A of order 6 as:

$$A = \begin{pmatrix} 2 & 1 & 1 & 1 & 1 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \end{pmatrix}$$

such that $\det M = -1$. From (1.2) we have

$$\begin{pmatrix} V_{n+5} \\ V_{n+4} \\ V_{n+3} \\ V_{n+2} \\ V_{n+1} \\ V_n \end{pmatrix} = \begin{pmatrix} 2 & 1 & 1 & 1 & 1 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \end{pmatrix} \begin{pmatrix} V_{n+4} \\ V_{n+3} \\ V_{n+2} \\ V_{n+1} \\ V_n \\ V_{n-1} \end{pmatrix}. \tag{7.2}$$

and from (7.1) (or using (7.2) and induction) we have

$$\begin{pmatrix} V_{n+5} \\ V_{n+4} \\ V_{n+3} \\ V_{n+2} \\ V_{n+1} \\ V_n \end{pmatrix} = \begin{pmatrix} 2 & 1 & 1 & 1 & 1 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \end{pmatrix}^n \begin{pmatrix} V_5 \\ V_4 \\ V_3 \\ V_2 \\ V_1 \\ V_0 \end{pmatrix}.$$

If we take $V = P$ in (7.2) we have

$$\begin{pmatrix} P_{n+5} \\ P_{n+4} \\ P_{n+3} \\ P_{n+2} \\ P_{n+1} \\ P_n \end{pmatrix} = \begin{pmatrix} 2 & 1 & 1 & 1 & 1 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \end{pmatrix} \begin{pmatrix} P_{n+4} \\ P_{n+3} \\ P_{n+2} \\ P_{n+1} \\ P_n \\ P_{n-1} \end{pmatrix}. \tag{7.3}$$

We also define

$$B_n = \begin{pmatrix} P_{n+1} & \sum_{k=0}^{4} P_{n-k} & \sum_{k=0}^{3} P_{n-k} & \sum_{k=0}^{2} P_{n-k} & \sum_{k=0}^{1} P_{n-k} & P_n \\ P_n & \sum_{k=1}^{5} P_{n-k} & \sum_{k=1}^{4} P_{n-k} & \sum_{k=1}^{3} P_{n-k} & \sum_{k=1}^{2} P_{n-k} & P_{n-1} \\ P_{n-1} & \sum_{k=2}^{6} P_{n-k} & \sum_{k=2}^{5} P_{n-k} & \sum_{k=2}^{4} P_{n-k} & \sum_{k=2}^{3} P_{n-k} & P_{n-2} \\ P_{n-2} & \sum_{k=3}^{7} P_{n-k} & \sum_{k=3}^{6} P_{n-k} & \sum_{k=3}^{5} P_{n-k} & \sum_{k=3}^{4} P_{n-k} & P_{n-3} \\ P_{n-3} & \sum_{k=4}^{8} P_{n-k} & \sum_{k=4}^{7} P_{n-k} & \sum_{k=4}^{6} P_{n-k} & \sum_{k=4}^{5} P_{n-k} & P_{n-4} \\ P_{n-4} & \sum_{k=5}^{9} P_{n-k} & \sum_{k=5}^{8} P_{n-k} & \sum_{k=5}^{7} P_{n-k} & \sum_{k=5}^{6} P_{n-k} & P_{n-5} \end{pmatrix}$$

and

$$C_n = \begin{pmatrix} V_{n+1} & \sum_{k=0}^{4} V_{n-k} & \sum_{k=0}^{3} V_{n-k} & \sum_{k=0}^{2} V_{n-k} & \sum_{k=0}^{1} V_{n-k} & V_n \\ V_n & \sum_{k=1}^{5} V_{n-k} & \sum_{k=1}^{4} V_{n-k} & \sum_{k=1}^{3} V_{n-k} & \sum_{k=1}^{2} V_{n-k} & V_{n-1} \\ V_{n-1} & \sum_{k=2}^{6} V_{n-k} & \sum_{k=2}^{5} V_{n-k} & \sum_{k=2}^{4} V_{n-k} & \sum_{k=2}^{3} V_{n-k} & V_{n-2} \\ V_{n-2} & \sum_{k=3}^{7} V_{n-k} & \sum_{k=3}^{6} V_{n-k} & \sum_{k=3}^{5} V_{n-k} & \sum_{k=3}^{4} V_{n-k} & V_{n-3} \\ V_{n-3} & \sum_{k=4}^{8} V_{n-k} & \sum_{k=4}^{7} V_{n-k} & \sum_{k=4}^{6} V_{n-k} & \sum_{k=4}^{5} V_{n-k} & V_{n-4} \\ V_{n-4} & \sum_{k=5}^{9} V_{n-k} & \sum_{k=5}^{8} V_{n-k} & \sum_{k=5}^{7} V_{n-k} & \sum_{k=5}^{6} V_{n-k} & V_{n-5} \end{pmatrix}$$

THEOREM 18. *For all integer $m, n \geq 0$, we have*

(a): $B_n = A^n$

(b): $C_1 A^n = A^n C_1$

(c): $C_{n+m} = C_n B_m = B_m C_n$.

Proof.

(a): By expanding the vectors on the both sides of (7.3) to 6-colums and multiplying the obtained on the right-hand side by A, we get

$$B_n = AB_{n-1}.$$

By induction argument, from the last equation, we obtain

$$B_n = A^{n-1} B_1.$$

But $B_1 = A$. It follows that $B_n = A^n$.

(b): Using (a) and definition of C_1, (b) follows.

(c): We have $AC_{n-1} = C_n$, i.e. $C_n = AC_{n-1}$. From the last equation, using induction we obtain $C_n = A^{n-1} C_1$. Now

$$C_{n+m} = A^{n+m-1} C_1 = A^{n-1} A^m C_1 = A^{n-1} C_1 A^m = C_n B_m$$

and similarly

$$C_{n+m} = B_m C_n.$$

Some properties of A^n matris can be given as

$$A^n = 2A^{n-1} + A^{n-2} + A^{n-3} + A^{n-4} + A^{n-5} + A^{n-6}$$

and

$$A^{n+m} = A^n A^m = A^m A^n$$

for all integer m and n.

THEOREM 19. *For $m, n \geq 0$ we have*

$$\begin{aligned} V_{n+m} &= V_n P_{m+1} + V_{n-1}(P_m + P_{m-1} + P_{m-2} + P_{m-3} + P_{m-4}) \\ &\quad + V_{n-2}(P_m + P_{m-1} + P_{m-2} + P_{m-3}) + V_{n-3}(P_m + P_{m-1} \\ &\quad + P_{m-2}) + V_{n-4}(P_m + P_{m-1}) + V_{n-5}P_m \\ &= V_n P_{m+1} + P_{m-4}V_{n-1} + P_{m-1}\left(V_{n-1} + V_{n-2} + V_{n-3} + V_{n-4}\right) \\ &\quad + P_{m-2}\left(V_{n-1} + V_{n-2} + V_{n-3}\right) + P_{m-3}\left(V_{n-1} + V_{n-2}\right) \\ &\quad + P_m\left(V_{n-1} + V_{n-2} + V_{n-3} + V_{n-4} + V_{n-5}\right) \end{aligned} \tag{7.4}$$

Proof. From the equation $C_{n+m} = C_n B_m = B_m C_n$ we see that an element of C_{n+m} is the product of row C_n and a column B_m. From the last equation we say that an element of C_{n+m} is the product of a row C_n and column B_m.We just compare the linear combination of the 2nd row and 1st column entries of the matrices C_{n+m} and $C_n B_m$. This completes the proof.

REMARK 20. *By induction, it can be proved that for all integers $m, n \leq 0$, (7.4) holds. So, for all integers m, n (7.4) is true.*

COROLLARY 21. *For all integers m, n, we have*

$$\begin{aligned}
P_{n+m} &= P_nP_{m+1} + P_{n-1}(P_m + P_{m-1} + P_{m-2} + P_{m-3} + P_{m-4}) \\
&\quad + P_{n-2}(P_m + P_{m-1} + P_{m-2} + P_{m-3}) + P_{n-3}(P_m + P_{m-1} + P_{m-2}) \\
&\quad + P_{n-4}(P_m + P_{m-1}) + P_{n-5}P_m, \\
Q_{n+m} &= Q_nP_{m+1} + Q_{n-1}(P_m + P_{m-1} + P_{m-2} + P_{m-3} + P_{m-4}) \\
&\quad + Q_{n-2}(P_m + P_{m-1} + P_{m-2} + P_{m-3}) + Q_{n-3}(P_m + P_{m-1} + P_{m-2}) \\
&\quad + Q_{n-4}(P_m + P_{m-1}) + Q_{n-5}P_m, \\
E_{n+m} &= E_nP_{m+1} + E_{n-1}(P_m + P_{m-1} + P_{m-2} + P_{m-3} + P_{m-4}) \\
&\quad + E_{n-2}(P_m + P_{m-1} + P_{m-2} + P_{m-3}) + E_{n-3}(P_m + P_{m-1} + P_{m-2}) \\
&\quad + E_{n-4}(P_m + P_{m-1}) + E_{n-5}P_m.
\end{aligned}$$

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Research Article

GROWING POSSIBILITIES OF MEDICINAL AND AROMATIC PLANTS IN GREENHOUSES CLIMATIZED BY BIOGAS POTENTIAL IN TEKIRDAĞ REGION

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ABSTRACT

Medicinal and aromatic plants that are generally grown in natural environment and obtained by collection from these natural environments cannot meet the need.In this respect, it is possible to increase the possibility of growing medicinal and aromatic plants by providing the necessary climatic conditions especially with greenhouse systems. In the greenhouse, the highest cost is the provision of air conditioning. In this respect, greenhouse air conditioning system can be provided with renewable energy or waste materials. In this study, information on some medicinal and aromatic plants that can be grown in greenhouses conditioned by biogas which can be produced from wastes of bovine and ovine animal production structures in Tekirdağ region where agriculture and animal husbandry are carried out intensively in our country and waste evaluation can be obtained without being subject to low cost medical and aromatic plants. intended. For this purpose, biogas potential and energy production opportunities of each district of Tekirdağ were determined and medicinal and aromatic plants that can be grown by providing greenhouse air conditioning were determined. As a result of the research, Capers (Capparis spinosa) (Barbera and Lorenzo, 1984), Rosemary (Rosmarinus officinalis) (Ayanoğlu et al., 2016), Poppy (Papaver somniferum linnaeus) (Erdurmuş and Öneş, 1990), Mint (Mentha piperita) (Anonim, 2019), which can be easily provided with biogas production potential in Tekirdağ region and which have high economic returns with demand. Medicinal Sage (Salvia officinalis) (Bağdat, 2008), Gojiberry (Lycium barbarum) (Anonim, 2015a; Anonim 2015b), St. John's Wort (Hypericum perforatum) (Bayram ve ark., 2002), Lemon Balm (Melissa officinalis) (Anonim,2019) has been concluded that medicinal and aromatic plants such as can be grown.

Keywords: *Medical and aromatic plants, Greenhouses systems, Greenhouse air conditioning, Biogas.*

1.INTRODUCTION

Medicinal and aromatic plants, as well as therapeutic properties of essential oils, cosmetic products, coloring dyes, plant protection products and products produced from these products are used in many products such as intermediates (Lubbe ve Verpoorte, 2011). The annual market value of medicinal and aromatic plants is about $ 60 billion (Kumar, 2009).

When evaluated in terms of climatic characteristics and agricultural potential, our country has a large medicinal and aromatic plant growing potential. Grown plants can be used in a wide range of fields such as medicine, cosmetics, foodstuffs. In order to properly evaluate the market opportunities of medicinal and aromatic plants, the products must be of a certain quality and quantity. In particular, cultivation of suitable varieties according to ecological requirements, breeding studies, post-harvest procedures will help improve the production and marketing opportunities of medicinal and aromatic plants (Bayram ve ark., 2010).

As a result of the bond between humans and plants dating back centuries, humanity has used nature as a natural pharmacy and has grown medicinal and aromatic plants for various purposes such as spices, food, medicine and healing. Although the demand for short-term medicinal and aromatic plants has been reduced with the introduction of unnatural substances, the interest that has arisen due to the damage of artificial substances has gathered around medical and aromatic plants **(**Özçelik ve Balabanlı,2005).

The unconscious removal of the plants causing the disruption of natural vegetation leads to the destruction of rare and endemic plants (Özhatay ve Atay, 1997). The global markets and the pharmaceutical industry are evaluating products of high quality and hence the so-called standard. In today's conditions, supply of high quality products and sufficient standard conditions in our country is met by the collection of plants grown in natural environment and these plants need to be brought to the desired conditions with regular and modern culture (Faydaoğlu ve Sürücüoğlu, 2011).

Greenhouse can be defined as providing the most suitable environmental conditions and plant growing environments when the climatic conditions do not allow plant growing, and to obtain high economic yields throughout the year. In this respect, ventilation, heating, cooling and lighting in greenhouse systems are the most important factors to be considered in planning (Öneş, 1986; Arıcı, 1999). Plants in greenhouse systems have adapted to average temperatures of 17-27 °C. Optimum values are between 15-20 °C at night and 22-28 °C at daytime. (Castilla ve Hernandez, 2007).

Produced as a result of fermentation of biogas, animal husbandry and plant based organic wastes under anaerobic conditions odorless, colorless, lighter than air and a blue flame burning event occurs (Anonim,2004). Depending on the amount of methane contained in its composition, it has a heat value in the range of 17-25 MJ /m^3. 1 m^3 of biogas is equivalent to 1,46 kg of coal or 5,76 kWh of electricity (Yılmaz ve Atalay, 2004; Anonim 2005).

The highest share in biogas energy production is 85% and the rest is composed of livestock-based wastes. When animal husbandry is considered as waste sources, approximately 93% of the total is from cattle and sheep and the rest is from poultry (Balat, 2005).

2. MATERIAL AND METHOD

Tekirdağ province is located in Thrace region of our country. Its geographical coordinates are 26°40′-28°10 ′ east longitudes 40°35′ 41°35 ′ northern latitudes. Tekirdağ, which has Black Sea climatic characteristics along the Marmara Sea, has the characteristics of cold weather in summer and drier semi-continental climate in summer. (Anonim, 2007).

The average temperature and average sunshine duration of Tekirdağ province during the 12 months between 1938 and 2018 are given in the table below (MGM, 2018). When the average values are examined, it can be said that there is no value below 0 °C in winter and this situation creates a positive value for greenhouse cultivation. As you move from the coast to the inner parts, temperatures decrease slightly with the effect of blackness and do not make a big difference.

Table 1. Average temperature and sunshine duration of Tekirdağ province (General Directorate of Meteorology 2019)

TEKİRDAĞ	1	2	3	4	5	6	7	8	9	10	11	12	ANNUAL
Average Temperature (°C)	4,7	5,4	7,3	11,8	16,8	21,3	23,8	23,8	22,0	15,4	11,0	7,1	14,0
Average insolation (hours)	2,7	3,3	4,2	5,8	7,6	8,9	9,8	8,9	7,3	4,8	3,3	2,5	69,1

Figure 1. Tekirdağ province map

In order to determine biogas potential in Tekirdağ province, the number of bovine and ovine animals was reached according to TÜİK records. For this purpose, in 2018 Çerkezköy, Çorlu, Ergene, Hayrabolu, Kapakli, Malkara, Marmara Eregli, Muratli, Saray, Suleymanpasa and Sarkoy districts in 2018, the total number of animals in cattle and ovine production structures, the amount of waste generated in accordance with these numbers and the amount of waste biogas-derived energy potentials were calculated. The highest energy potential among the districts and the energy potential of the provinces in general were calculated. With the calculated amount of energy, it was found that some medicinal and aromatic plants with high economic yield that can be grown in greenhouses where air conditioning process will be made, meet the general climate demands.

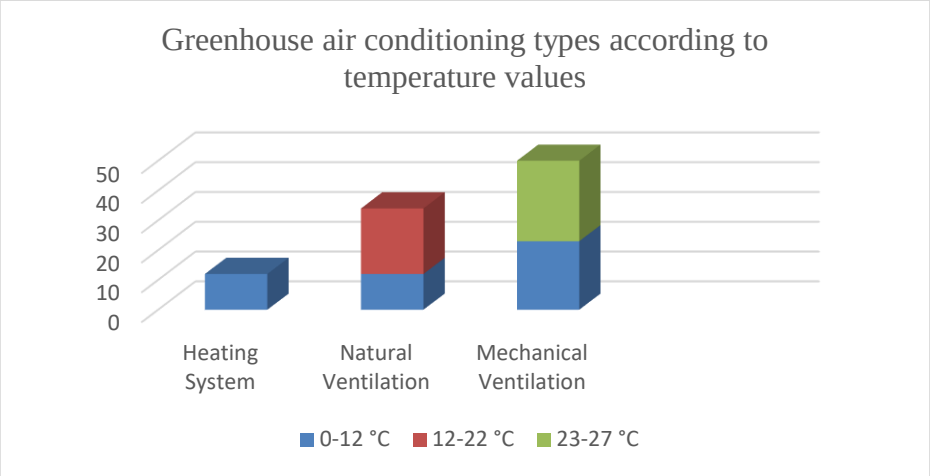
The aim of this study was to determine the biogas potential of Tekirdağ region and to determine the medicinal and aromatic plants that can be grown in greenhouses where air conditioning and energy can be produced.

3. FINDINGS AND DISCUSSION

3.1.Air Conditioning in Greenhouse Conditions According to Temperature Values

In order to achieve the desired yield in greenhouse systems, when the external temperatures fall below 12 °C, natural ventilation should be operated between 12-22 °C and cooling system should be operated between 22 and 27 °C (Zabeltitz, 2011). The average temperature in Tekirdağ is below 12 °C in November, December, January, February, March and April. In this respect, there is a need for heating in the greenhouse activities to be held in Tekirdağ in 6 months of the year. Natural ventilation is required in May, June, September and October, while natural and mechanical ventilation can be utilized in July and August as the temperature is not very high.

Figure 2. In greenhouse air conditioning requirements according to outdoor temperature



Plants grown under greenhouse conditions are generally temperate climate plants in the range of 17-27 °C. In terms of greenhouse air conditioning, the minimum value is 15 °C for heating and the maximum value is 35 °C for cooling (Alpay ve Erdem, 2018). Plants with climatic demand under these values can be grown without air conditioning according to natural climatic conditions.

3.2.Medicinal and Aromatic Plant Growing in Greenhouse Conditions

Within the scope of the research, the general climatic characteristics of some medicinal and aromatic plants that are determined according to the indoor air temperature values according to outdoor air temperature values and which can be grown by air conditioning in greenhouse conditions are summarized as headings. The selected plants were selected from medicinal and aromatic plants with high economic returns and demand in the market.

3.2.1.Capers *(Capparis spinosa)* (Barbera and Lorenzo, 1984) naturally grows in optimum conditions at 13 °C, but the best range is 13-20 °C. Outside the temperature, the appropriate humidity value changes to 6.3 - 8.3 pH (Ölmez et al., 2011; Akgül, 1996; Barbera and Lorenzo, 1984). Apart from the temperature condition of the caper plant to be grown under greenhouse conditions, it is also important to ensure proper humidity.

Figure 3. Capers (*Capparis spinosa*)

3.2.2.Rosemary *(Rosmarinus officinalis)* (Ayanoğlu et al., 2016). is a type of plant that can be considered frost resistant in the range of 20-25 °C in terms of temperature requirements. Since it does not like excess water, irrigation is not necessary. Despite this situation, it can grow in some rainy areas, but the growing soil should not be too wet (Ayanoğlu et al., 2016).

Figure 4. Rosemary (*Rosmarinus officinalis*)

3.2.3.Poppy *(Papaver somniferum)* (Erdurmuş and Öneş, 1990) seeds can germinate at 4 °C. If the soil temperature is lower, the seeds remain germinated. Plants whose output is late and caught in the frost event during the cotyledon period are damaged by frost. In the first stage, then the leaves are seen to be lost (Erdurmuş and Öneş, 1990).

Poppy plant which needs a total temperature of 2300-2700 °C during the growing period loves the sun and heat (Erdurmuş.ve Öneş, 1990). In this respect, it is of great importance to ensure sufficient temperature conditions in the poppy plant to be grown in greenhouse conditions.

Figure 5. Poppy (*Papaver somniferum*)

3.2.4.Mint *(Mentha piperita)* (Anonim, 2019) increases the amount of oil contained in hot climate conditions. The best development temperature range is 12-15 °C. Seeds can germinate in the 20-30 °C range (Anonim, 2019). Mint, which does not like to grow in shady areas, does not like high light intensity. In this respect, the shading in greenhouse conditions and the lighting systems used should be considered

Figure 6. Mint (*Mentha piperita*)

3.2.5.Medicinal Sage (*Salvia officinalis*) (Bağdat, 2008) is a heat-loving plant, and its natural growing conditions are sunny and sheltered from the wind. It becomes more efficient when watering. Aromatic properties, can be used for landscaping, cosmetic and vegetable dye sage can be found in soap formation (Bağdat, 2008).

Figure 7. Medicinal Sage (*Salvia officinalis*)

3.2.6.Gojiberry (*Lycium barbarum*) (Anonim, 2015a; Anonim 2015b) is a plant that loves sunlight and can develop between -27 °C and 39 °C. The so-called worm grape plant is resistant to dry summer conditions, the highest yield is obtained when drip irrigation (Anonim, 2015a; Anonim 2015b).

Figure 8. Gojiberry (*Lycium barbarum*)

3.2.7.St. John's Wort (*Hypericum perforatum*) (Bayram ve ark., 2002) is more light demand in germination period, but there is a demand for semi-shaded and abundant sunny climate (Bayram ve ark., 2002). When the frost resistant gantry is grown in greenhouse conditions, care should be taken not to over-operate the shading system.

Figure 9. St. John's Wort (*Hypericum perforatum)*

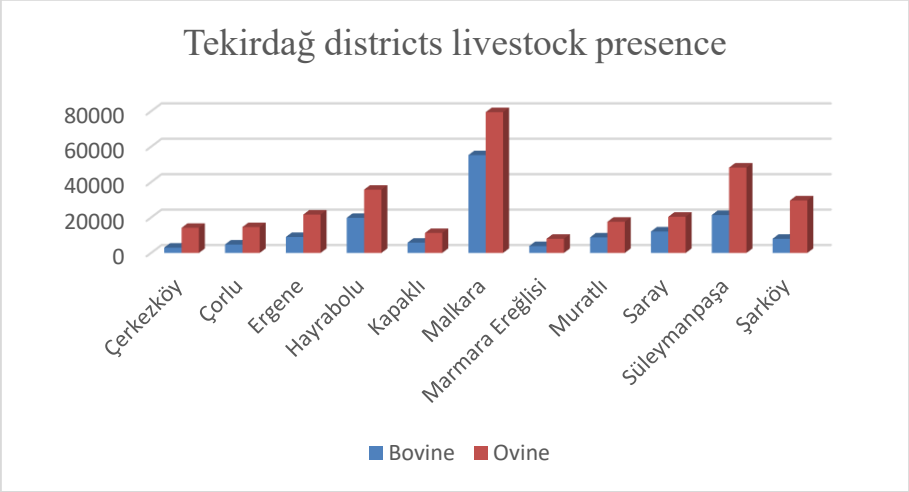
3.2.8.Lemon balm (*Melissa officinalis*) (Anonim,2019) is a shade-resistant plant that loves heat and sunny areas. Desired dorms cannot be obtained in areas with high humidity and shade (Anonim,2019). In this respect, warm conditions should be ensured for the sons to be grown in greenhouse conditions. Measures should be taken against high humidity and the shade system should not be opened because it does not like shade.

Figure 10. Lemon balm (*Melissa officinalis*)

3.3.Determination of Biogas Potential of Tekirdag Province

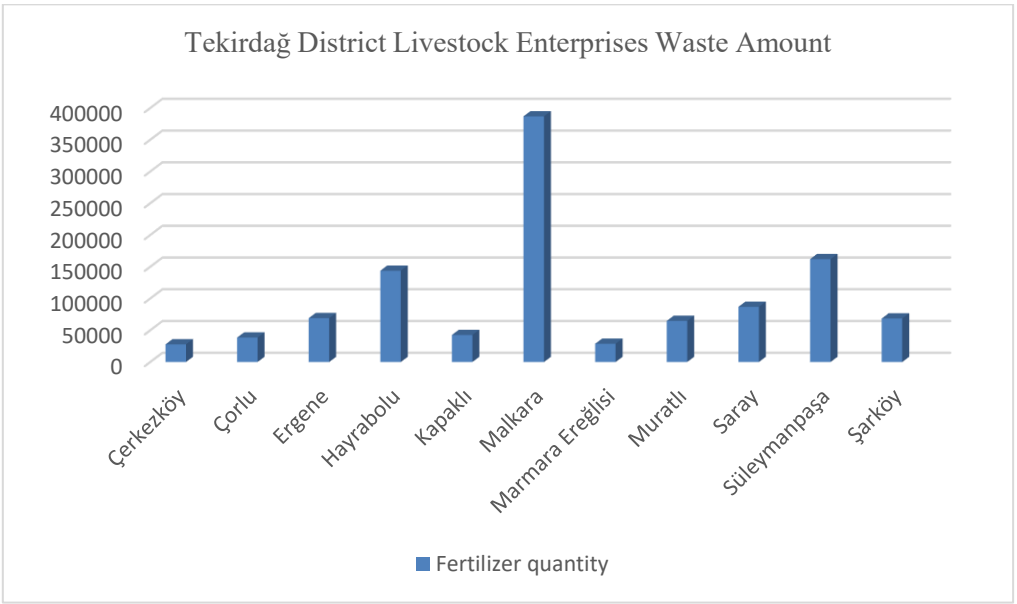
The number of bovine and ovine animals determined within the scope of the research is given below in districts. (TÜİK, 2019). Malkara is the district with the highest number of bovine animals, and Çerkezköy is the district with the lowest number of cattle. When evaluated in terms of ovine animal assets, the highest number of animals is in Malkara district and the least animal is in Marmara Ereğlisi district. The numbers of animals in the districts are given in the graph below.

Figure 11. Tekirdağ districts livestock assets



After determining the number of dovine and ovine in the province, the amount of waste generated in these animal production structures was determined daily. The amount of fertilizer for bovine animals is 6 tons / year and for sheep animals is 0.7 tons / year. According to fertilizer amounts, the amount of biogas that can be produced was 0.42-0.60 m^3 / day in cattle and 0.37-0,61 m^3 / day in sheep (Toruk and Eker, 2012). According to these ratios, the amount of fertilizer and biogas potential in terms of cattle and sheep production originating from animal production structures calculated on districts of Tekirdağ province are given in the following figures.

Figure 12. Amount of waste originating from livestock enterprises in Tekirdağ province

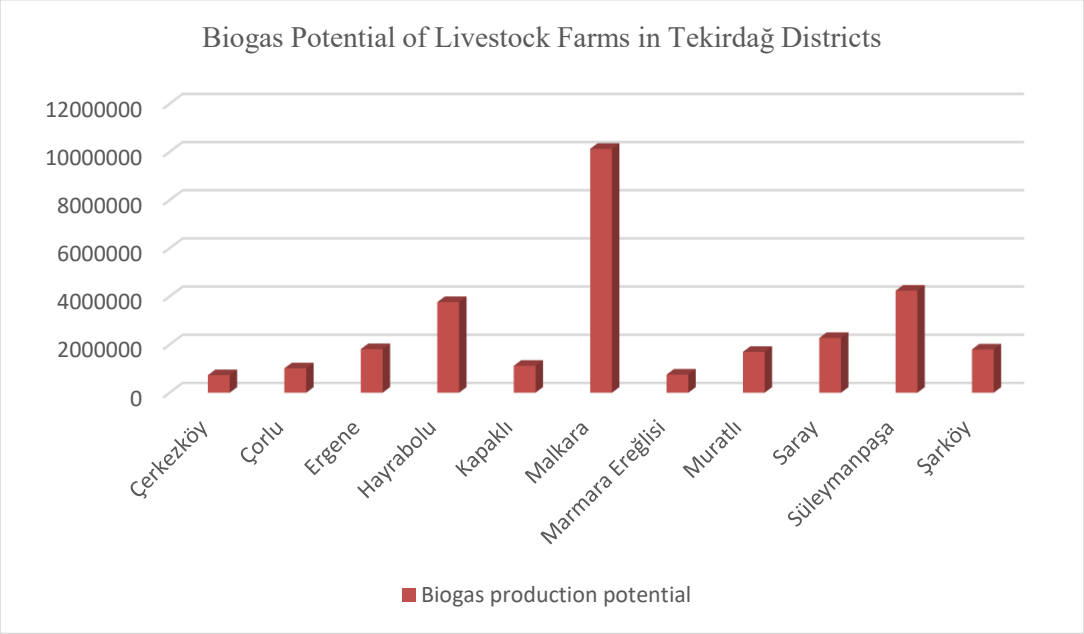


When the total wastes originating from cattle and ovine livestock enterprises are evaluated, it is seen that the highest amount is in Malkara district and Süleymanpaşa and Hayrabolu districts follow this district in quantity. The least amount of waste was found in Marmara Ereğlisi and Çerkezköy.

4. RESULTS

When evaluated in terms of energy production potential with biogas, Malkara has the highest production potential in direct proportion to the amount of waste. Merkez (Süleymanpaşa) and Hayrabolu are also districts with high biogas production potential. Despite having a certain production potential, Çerkezköy and Marmara Ereğlisi districts have the lowest settlements compared to other districts.

Figure 13. Biogas production potential originating from livestock enterprises in Tekirdağ province



There is a potential of biogas and energy production due to animal husbandry grown in all districts of Tekirdağ. In particular, enterprises with a large number of animals can produce energy along with biogas tanks as well as animal production structures. They can also use this energy in greenhouses with climate control to be established around livestock enterprises and carry out greenhouse activities. In particular, medicinal and aromatic plants supplied from the natural environment meet the demand decreasing day by day with these products can be grown in greenhouse conditions is possible to achieve high yield.

Caparis (Capparis spinosa), Rosemary (Rosmarinus officinalis), Poppy (Papaver somniferum linnaeus), Mint (Mentha piperita), Medicinal Sage (Salvia officinalis), Gojiberry (Lycium barbarum), Yellow Centaur (Hypericum perforatum) It is possible to grow medicinal and aromatic plants such as lemon balm (Melissa officinalis) in greenhouses conditioned by biogas energy in Tekirdağ conditions. In terms of medicinal and aromatic plants, it is possible to mention that many plants with medicinal and aromatic properties can be produced in the desired amount at the desired time in greenhouse conditions, since the desired temperature is generally 10-35 °C. It is of great importance to increase the studies in this field and to support the cultivation of medicinal and aromatic plants in greenhouse conditions, especially with good agricultural practices.

By providing greenhouse air conditioning with biogas energy, animal manure, which is an environmentally damaging waste, can be turned into an input for agricultural production. In this way, conservation of natural resources and energy savings can be achieved, and supply of the necessary raw materials in the field of medicine and medicine in today's conditions will be realized at the lowest costs.

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