Voltammetric performance of nanofiber structured over-oxidized poly(3,4-ethylenedioxythiophene) modified pencil graphite electrodes for dobutamine sensing

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Abstract: A modified electrode was developed for the electrochemical sensing of dobutamine (DBT), one of the catecholamines. For this modification process, pencil graphite electrodes (PGE) were modified with over-oxidized nanofiber structured poly (3,4-ethylenedioxythiophene) (PGE/OPEDOTNF) by electropolymerization. The electrochemical performance of PGE/OPEDOTNF was evaluated by cyclic and differential pulse voltammetry. In addition, the performances of non-nanofiber PEDOT-modified PGE electrodes were also examined for comparison. The characterization of the modified electrodes was carried out by scanning electron microscopy and electrochemical methods. The signal of the modified electrodes was observed in a linear range of 0.1-2.0 μM against DBT using the differential pulse voltammetry method. The limit of detection and quantification are calculated as 0.026 μM and 0.086 μM, respectively. The effect of the interfering species was examined. It has been shown that DBT can be detected sensitively and selectively using pencil graphite electrodes modified with nanofiber-structured poly(3,4-ethylenedioxythiophene). The repeatability of PGE/OPEDOTNF electrodes was found to be 5.2%. PGE/OPEDOTNF electrodes remained stable for 15 days without losing their electrochemical activity.

Keywords: Dobutamine, electrochemical sensing, modified electrode, poly(3,4-ethylenedioxythiophene), nanofiber structure, pencil graphite electrode.

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1. INTRODUCTION

Organic compounds that regulate physiology as both disease markers and pharmaceuticals are called catecholamines (1). Catecholamines are essential because they are involved in many physiological and biochemical processes in humans. Dobutamine (DBT), one of the synthetic catecholamines, is widely used in clinical practice (2). DBT plays an essential role in the functioning of the central nervous, renal, hormonal, and cardiovascular systems. It transmits messages to parts of the brain for proper coordination of body movements. Abnormalities in the DBT level in the brain are associated with various neurological diseases such as Parkinson's, schizophrenia and psychiatric disorders such as epilepsy (3). DBT increases contraction, increases heart rhythm, improves blood flow, relieves signs (symptoms) of coronary heart disease, and stimulates the heart's ß1-adrenoceptors. There is no significant increase in heart rate or systemic blood pressure following injection of DBT due to vasodilation caused by ß2-receptors. Therefore, DBT is widely used in medical treatment as an anticarcinogen, cardiostimulator, antioxidant, and antimutagen (4). DBT is an electroactive compound with catechol, secondary amine, and phenol functional groups (5). Since catecholamines such as DBT play an essential role in many physiological and biochemical processes, it is crucial to determine such compounds in body fluids or in environments where they dissolve in terms of clinical applications.

Various electrodes are used in the electrochemical determination of biologically important substances. In some cases where high voltages must be applied, the current values may not be at the desired levels. Similar problems can be overcome by using modified electrodes. Surface modification of the electrodes leads to some improvements in sensitive determinations and reproducibility, and in some cases, the selectivity increases by immobilization of functional groups on the relevant surface (5).

Conductive polymers with superior chemical, electronic, and mechanical properties are used to prepare electrochemical sensors for analytical purposes (6-10). Poly(3,4-ethylenedioxythiophene) (PEDOT), one of the conductive polymers, has many properties that make it a superior electrode material in sensor and biosensor production (11-13). These features include its high electronic and ionic conductivity, transparency, mechanical flexibility, and the presence of functional groups for chemical modification (14). In addition, the high electrocatalytic activity and environmental stability of PEDOT are why it is widely used to improve the performance of electrochemical sensors (15-16). PEDOT-modified electrodes have been used in the electrochemical determination of biologically important compounds such as ascorbic acid (AA), dopamine (DA), uric acid, serotonin, and glutathione (10, 12, 17, 18). There are a limited number of studies in the literature on the electrochemical determination of DBT (2, 4, 5, 19-26). Pencil graphite electrode (PGE) modified with nanofiber-structured PEDOT has not been studied for DBT determination.

Pencil graphite is widely used in electrochemical determinations due to its low cost (27-31). There are usually two types: larger-diameter wood tips or finer-diameter mechanical tips. The graphite tip used in wooden body ones is obtained by heating a mixture of graphite and clay in different proportions to achieve the desired hardness. Then, the product is impregnated with oil and wax for smooth and noiseless writing (32). PGE has high electrochemical activity, mechanical strength, low cost, and is easy to modify, miniaturize and regenerate. In addition, thanks to its graphite structure, PGE increases the electrochemical signal and reduces the overvoltage. Also, there are no time-consuming electrode surface cleaning and polishing steps. A highly effective trace analysis method occurs when used in sensitive and high-accuracy electrochemical techniques such as differential pulse, square wave, and stripping voltammetry. The reproducible signals of PGEs with well-defined voltammetric peaks have shown that the electrodes provide good sensitivity and reproducibility as a viable, renewable, and economical tool (33-35). Tavares and Barbeira determined that most pencil tips have an electrical resistance of fewer than 5 ohms, regardless of the manufacturer and hardness, and therefore are suitable for use as electrode material (36). Due to the mentioned advantages, PGE was preferred and modified with nanofiber-structured PEDOT in our study.

2. EXPERIMENTAL SECTION

2.1. Chemicals and electrodes

3,4-ethylenedioxythiophene, lithium perchlorate, sodium carbonate, potassium chloride, sodium chloride, epinephrine, phenylalanine, glucose, and paracetamol were purchased from Sigma-Aldrich. DBT, uric acid, and dopamine were purchased from Alfa Aesar. Ascorbic acid was purchased from Roth. Ultra-pure water used for the preparation of stock and buffer solutions was obtained from the TKA Smart 2 Pure ultra-pure water device.

Pencil graphite electrode (PGE, 0.7 mm diameter, 2B, Faber Castell) and modified PGE were used as the working electrode, Pt foil (1x1 cm, Aldrich 99.9%) was used as the counter electrode and Ag/AgCl (3.0 M KCl) as the reference electrode. To attach the PGE used in electrochemical measurements, the system prepared from mechanical pencils with a metal tip was used. Experimental studies were conducted using a three-electrode system in a five-necked electrochemical cell.

2.2. Apparatus

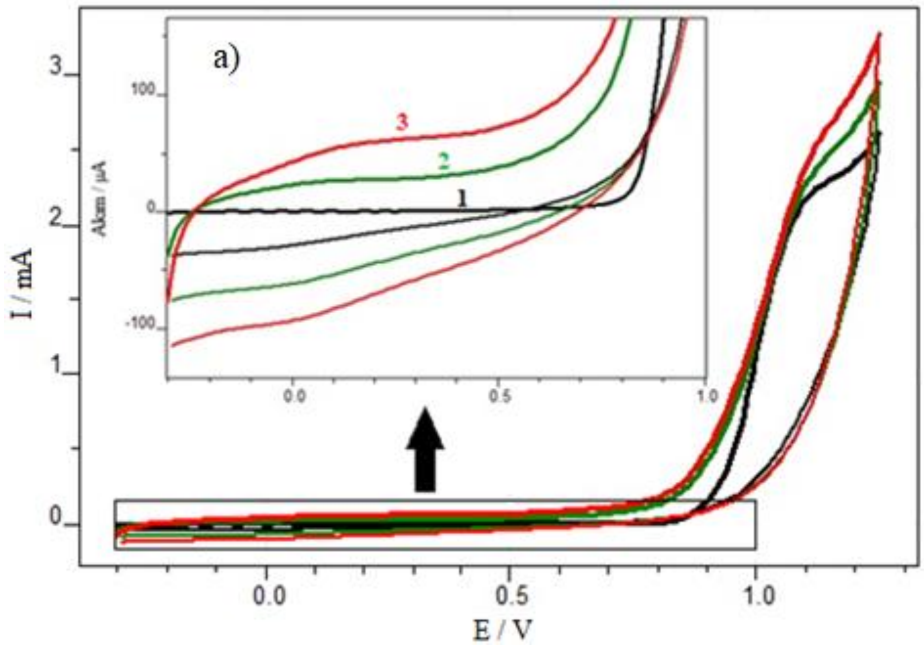
Voltammetric measurements were performed by the IVIUM (CompactStat model) potentiostat-galvanostat system. The analysis and evaluation of the data were carried out with Ivium Soft software. pH measurements were carried out using a Thermo Scientific (Orion 3 Star model) pH meter. Scanning electron microscope (SEM) images were obtained using a NanoSEM 650 model (FEI Microscope) device.

2.3. Preparation of poly(3,4-ethylenedioxythiophene) modified electrodes

First, more electrodes were provided by dividing the PGEs into two in the middle. Then, these PGEs were ultrasonically washed with ultrapure water and ethanol for five minutes and then dried in the oven at 50° C for 1 hour. Thus, the PGEs are both cleaned of surface contamination and made ready for modification. The PGEs prepared were kept closed in a 10 mL beaker (Figure S1).

Electropolymerization was carried out in an aqueous solution containing 10 mM 3,4-ethylenedioxythiophene (EDOT), 0.10 M $LiClO_4$, 0.10 M Na_2CO_3 and 5% acetonitrile by volume (17). A certain number of voltage sweeps between –0.30 V and +1.25 V were applied to PGE, 1.0 cm of which was immersed in this solution, and a nanofiber-structured PEDOT (PEDOTNF) was obtained on the PGE surface (17). Then, the over-oxidation process of PEDOT was performed by applying 2.0 V voltage for 120 seconds in phosphate buffer (PB) solution at pH 7.0. Over-oxidation is a process that has also been applied in previous studies (7, 12, 37) so that the electrochemical signals of conductive polymers do not interfere with the electroactivity of analytes. Later, the over-oxidized PEDOT (OPEDOTNF) was conditioned by performing a differential pulse voltammetric process between 0.0 V and +0.90 V in PB solution at pH 7.0. It has been determined that the conditioning process with DPV contributes to the reproducibility of the signals obtained with the modified electrode and is a process that helps a more effective over-oxidation. This process has also been applied in previous sensor work based on conductive polymers (38). The parameters of DPV are 20 mV/s scan rate, 50 ms pulse time, and 50 mV pulse amplitude. This process improves the base signal of differential pulse voltammetric measurements in the medium containing the analyte. After each process, the electrodes were cleaned by washing with ultrapure water. The process steps performed on the PGE surface are shown schematically in Figure S2.

The voltammograms in Figure 1a belong to obtaining the nanofiber-structured PEDOT on the PGE surface by voltage sweeps with three cycles. Figure 1b shows the voltammograms obtained during the cyclic voltammetric polymerization of non-nanofiber PEDOT in three cycles. Electroactivity wasn't observed until the voltage changed from -0.30 V to 0.80 V in the first cycle, and there was no increase in the current value. As the voltage increases towards higher voltage values than 0.80 V, EDOT starts to be oxidized, and a sudden increase is observed in the current values. The peak of EDOT oxidation at 1.05 V is clearly seen. This oxidization provides the electropolymerization of EDOT molecules and PEDOT coating on the PGE surface in the form of a thin film. In each cycle, the electrochemical behavior of the PGE surface where electropolymerization takes place also changes. Therefore, in the second cycle, the current values arising from the PEDOT's own electroactivity were observed between -0.20 and 0.70 V. It is also seen that the EDOT monomer present in the medium starts to be oxidized at a lower voltage value (0.70 V) to electropolymerize. It is seen that as the voltage value increases, the oxidation current values of the EDOT are higher than in the first cycle. In the back cycle, the reduction current values of the polymer structure itself are more pronounced. Similar electroactivity was also observed for the third cycle. In addition, it is noteworthy that the oxidation peak currents of EDOT on both the PGE surface and the PEDOT-coated PGE surface in the voltammograms obtained in the solution without Na_2CO_3 (Figure 1b) are lower than those obtained in the solution containing Na_2CO_3 (Figure 1a). Apart from this, it is seen that the current values between -0.30 V and 0.70 V, in which PEDOT's own electroactivity is observed, are higher in the presence of Na_2CO_3 (Figure 1a) than in the absence of Na_2CO_3 (Figure 1b).



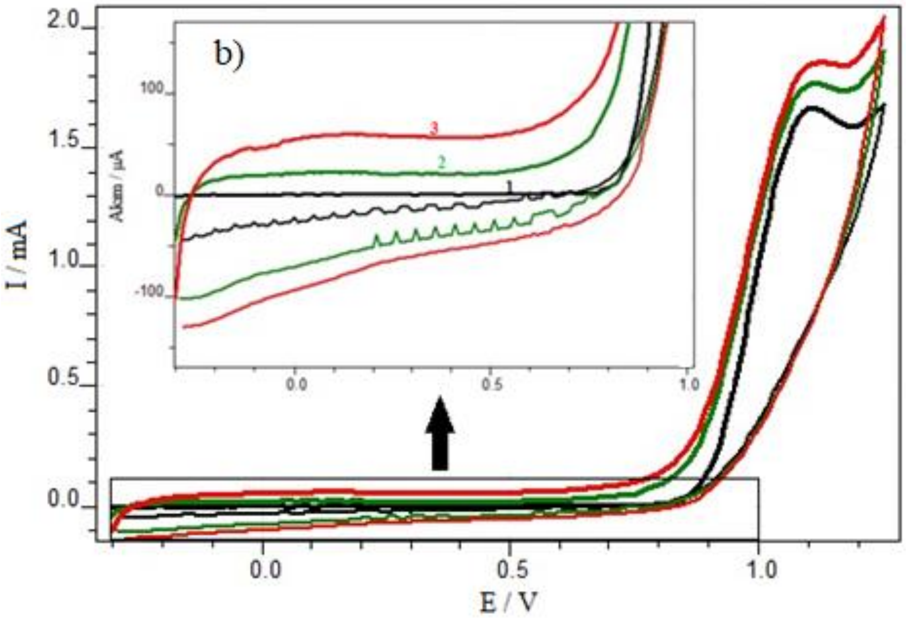


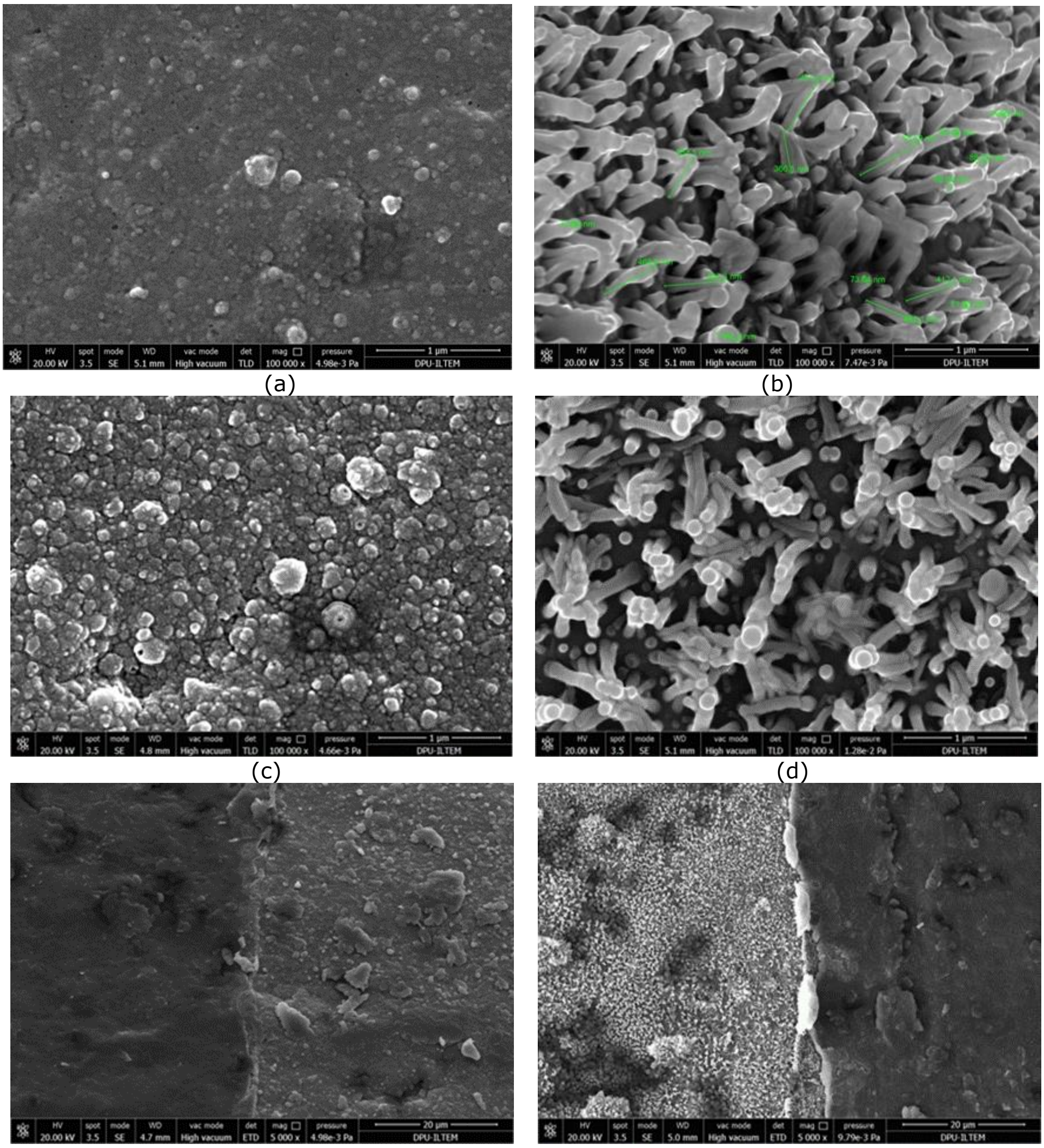
Figure 1: Voltammograms obtained on the PGE surface in a solution containing a) 10 mM EDOT + 0.10 M $LiClO_4$+ 0.10 M Na_2CO_3 b) 10 mM EDOT + 0.10 M $LiClO_4$.

3. RESULTS AND DISCUSSION

3.1. Surface morphology of PGE/OPEDOTNF electrodes

The surface morphology of PEDOT films was examined by scanning electron microscopy (SEM). The morphology of PEDOT films obtained by electrochemical methods depends on the counter ion, solvent, polymerization environment, etc., parameters used. In Figure 2, SEM images of modified electrodes obtained under optimum conditions are given. When $LiClO_4$ is used as the counter ion in the aqueous solution, the PEDOT (PEDOT) obtained on the electrode surface resembles the "cauliflower" structure (Figure 2a). On the other hand, the morphology of PEDOT (OPEDOTNF) obtained when Na_2CO_3 is present with $LiClO_4$ in an aqueous solution is in a nanofiber structure. While nanofibers vary in length between 350 and 650 nm, shorter ones are also found in places. The diameters of nanofibers are between 50 and 80 nm (Figure 2b). It is also seen that the tips of some nanofiber structures are in contact with each other. SEM images show that the surface area of nanofiber structure formation is increased compared to PGE. This increase in the surface area also significantly increased the sensitivity to the DBT molecule.

The effect of overoxidation on the morphology of PEDOT and PEDOTNF can be observed in Fig 2. When the images of the overoxidized structures in Figures 2a and b are compared with the images of the unoxidized structures in Figures 2c and d, it is seen that there is no significant difference in morphology. Nevertheless, especially in the cauliflower structured PEDOT (Figure 2c) and OPEDOT (Figure 2a) images, it is seen that the over-oxidized structure has relatively less roughness. Therefore, the over-oxidation conditions used in this study didn’t cause a big difference in terms of physical appearance and durability. However, it has been determined from the studies that the over-oxidation process is effective in determining the DBT by using the voltammetric method. In Figures 2e and f, SEM images of both regions with and without electropolymerization on the PGE surface are given. The parts on the left side of the SEM images show the areas where the surface has been modified by electropolymerization. It is understood from these images that the nanofiber structure increases the electrode surface area.



(a) (b)

(c) (d)

(e) (f)

Figure 2: SEM images of (a) PGE/OPEDOT, (b) PGE/OPEDOTNF, (c) PGE/PEDOT, (d) PGE/PEDOTNF, (e) PEDOT coated (on the left) and uncoated (on the right) parts on the PGE surface and (f) PEDOTNF coated (on the left) and uncoated (on the right) on the PGE surface (Electrode preparation conditions: Number of cycles 2, over-oxidation voltage 2.0 V and time 120 s, DPV conditioning number: 1 time)

3.2. Effect of pH and nanofiber structure on DBT oxidation

To investigate the effect of modification of PGEs with nanofiber PEDOT and the effect of medium pH on electrode performance, PGEs were modified with nanofiber and non-nanofiber PEDOT. In the literature, Özcan and İlkbaş (17) used nanofiber PEDOT, which they obtained in 3 cycles, for the electrochemical determination of uric acid. Inspired by this data in the literature, 3-cycle electropolymerization was preferred at the beginning of this study. In this part of the study, overoxidation time and voltage values for PEDOT overoxidation were chosen the same as those in Özcan and İlkbaş's study (17). Then, optimization studies have also been made for these parameters. All current values in this study are the average values obtained from 5-repetitive measurements.

One of the most important parameters that will affect the electrochemical response of DBT is the pH of the medium. Britton-Robinson Buffer (BRB) solution, which allows working in a wide pH range and is widely used in electrochemical studies, was selected as the electrolyte, and voltammetric measurements were carried out in BRB at different pH values containing 400 μM DBT. The oxidation peak currents-pH graph obtained by the cyclic voltammetry (CV) technique for DBT is shown in Figure 3. As seen in Figure 3, it reached at pH 2.0 the highest current value for DBT oxidation.

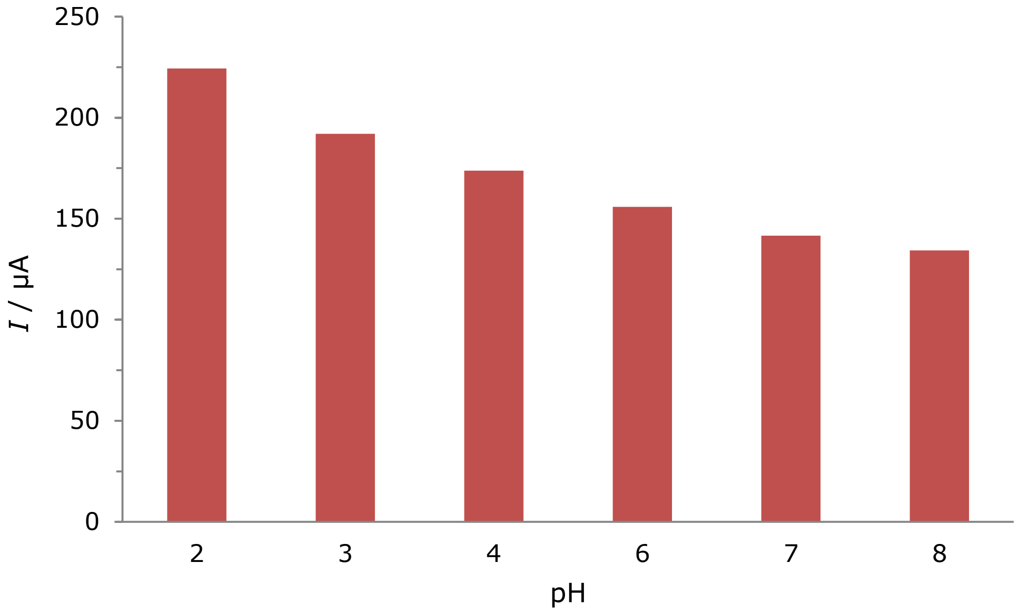


Figure 3: The peak currents obtained using CV for 400 µM DBT on PGE/OPEDOTNF-3cyc electrodes in BRB solutions at different pH values (Electrode overoxidation conditions: voltage: 2.0 V and time: 60 s, DPV applied between 0.0 and +0.90 V for conditioning 2 times).

Figure 4 shows the cyclic voltammograms obtained with PGE/OPEDOT-3cyc and PGE/OPEDOTNF-3cyc in BRB solution at pH 2.0 containing 400 µM DBT. When the cyclic voltammograms in Figure 4 are examined, it is seen that the oxidation peak current obtained for the DBT with PGE/OPEDOTNF-3cyc is approximately two times higher than that of PGE/OPEDOT-3cyc. The adsorption of more analytes may cause this significant increase in oxidation current obtained on the electrode surface as the nanofiber structure increases the surface area. It has been determined that the nanofiber structure provides significant improvement in the determination of the DBT by voltammetric technique due to the increase of the oxidation current of DBT.

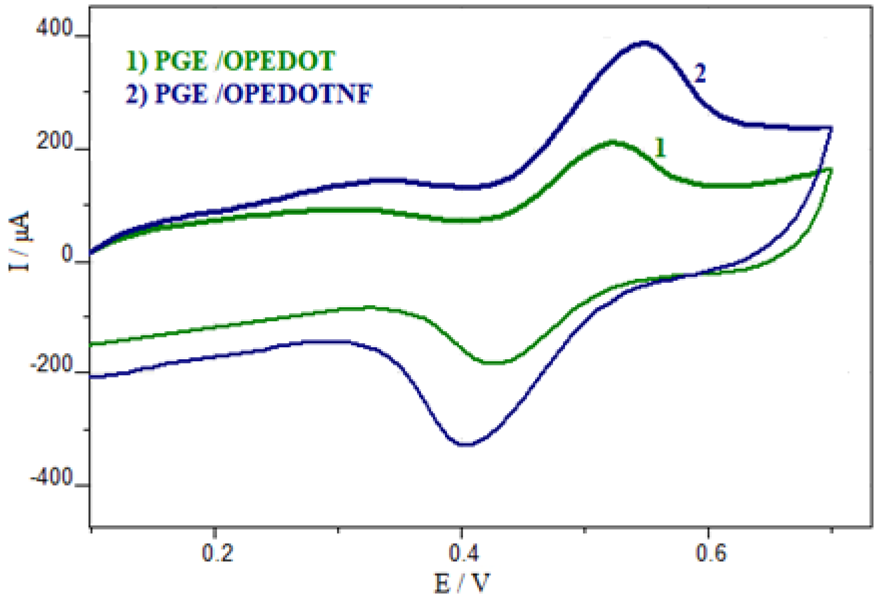


Figure 4: Cyclic voltammograms obtained with (1) non-nanofiber and (2) nanofiber structured PEDOT in BRB solution at pH 2.0 containing 400 µM DBT (Electrode over-oxidation conditions: voltage: 2.0 V and time: 60 s, DPV applied between 0.0 and +0.90 V for conditioning 2 times).

3.3. Determination of optimum conditions for electrode modification

In order to determine the optimum conditions, various parameters related to PGE/OPEDOTNF preparation were studied. It should be remembered once again that modified electrodes are prepared in two stages (see Figure S2). In the first step, PEDOT is electropolymerized on the PGE surface, and in the second step, PEDOT is over-oxidized. The over-oxidation process of the nanofiber-structured PEDOT was carried out to reduce the oxidation peak of PEDOT as much as possible. The observed peak due to the electroactivity of the PEDOT on the modified electrode surface is prevented from hindering the oxidation peaks of DBT.

3.3.1. Effect of the number of cycles used for electropolymerization

The number of cycles appears as the first parameter that will affect the performance in obtaining PEDOT by CV. Properties such as film thickness and length of nanofiber structures on the PGE surface are parameters that change with the number of cycles and affect the electrochemical signal obtained for DBT. For this reason, it was determined how the electrode performance varies with the number of cycles. The graph of the peak current values obtained by CV for BRB solution at pH 2.0 containing 400 µM DBT using PGE/OPEDOTNF electrodes produced in cycles between 1 and 10 is given in Figure S3. The current value given for the number of zero cycles is the peak current of the unmodified PGE. When the data in Figure S3 are examined, it is seen that the current value of the modified electrode obtained in one cycle is high compared to the one obtained with PGE. The modified electrode obtained with two cycles reached the highest peak current. Therefore, in the later stages of the study, the electropolymerization of OPEDOTNF on the surface of PGEs was carried out by taking two cycles. The abbreviation of the modified electrode produced in two cycles is PGE/OPEDOTNF-2cyc.

Other parameters that may affect the peak current value are the parameters of PEDOT over-oxidation after electropolymerization. The electrochemical characteristics of the over-oxidized structure depend on the voltage applied for the over-oxidation and how long this voltage is applied. Therefore, optimization studies have been carried out for these parameters in the following stages.

3.3.2. Determination of over-oxidation parameters.

After the optimum number of cycles was determined as 2, the change of DBT peak current with the variation of applied voltages for the over-oxidation of the PGE/OPEDOTNF-2cyc was investigated. The peak current values obtained using the CV technique of 400 µM DBT in BRB solution at pH 2.0 with OPEDOTNF-2cyc by applying the voltage values between 1.4-2.2 V for 60 seconds are given in Figure S4. Since the highest peak current (257 µA) is reached at a voltage of 2.0 V, it was chosen as the optimum voltage value for the over-oxidation of the PGE/PEDOTNF. The abbreviation of the modified electrode prepared under these conditions is PGE/OPEDOTNF-2cyc-2V.

Then, PGE/OPEDOTNF-2cyc-2V was over-oxidized by applying 2.0 V for different times (40-220 seconds) in PB solution at pH 7.0. The peak current values obtained using the CV technique for 400 µM DBT are shown in Figure S5. The highest peak current was obtained with the over-oxidized electrode at 2.0 V for 120 seconds (PGE/OPEDOTNF-2cyc-2V-120s). For this reason, it was concluded that the over-oxidation process for the electrode modification should be applied for 120 seconds at 2.0 V.

In addition, the effect of the number of DPV applied for conditioning was investigated by keeping the optimum voltage and time value constant (2.0 V and 120 seconds) for the over-oxidation of PGE/OPEDOTNF-2cyc-2V-120s electrodes. Until this stage, the DPV process was applied two times in the voltage range of 0.0 to +0.90 V for conditioning. In addition, to see whether there is a significant improvement as a result of the change in the number of repetitions performed for this process, the effect of the conditioning with DPV between 1 to 7 times on the cyclic voltammetric response of 400 µM DBT in BRB solution at pH 2.0 was investigated. The results are given in Figure S6. The result obtained without DPV conditioning is indicated with zero in the graph. When the figure is examined, the peak current, which increases until the number of applications of DPV is four, was then observed to decrease. To avoid spending too much time in the conditioning process, we preferred two times DPV conditioning. The abbreviation of the modified electrode obtained at these conditions is PGE/OPEDOTNF-2cyc-2V-120s-2DP.

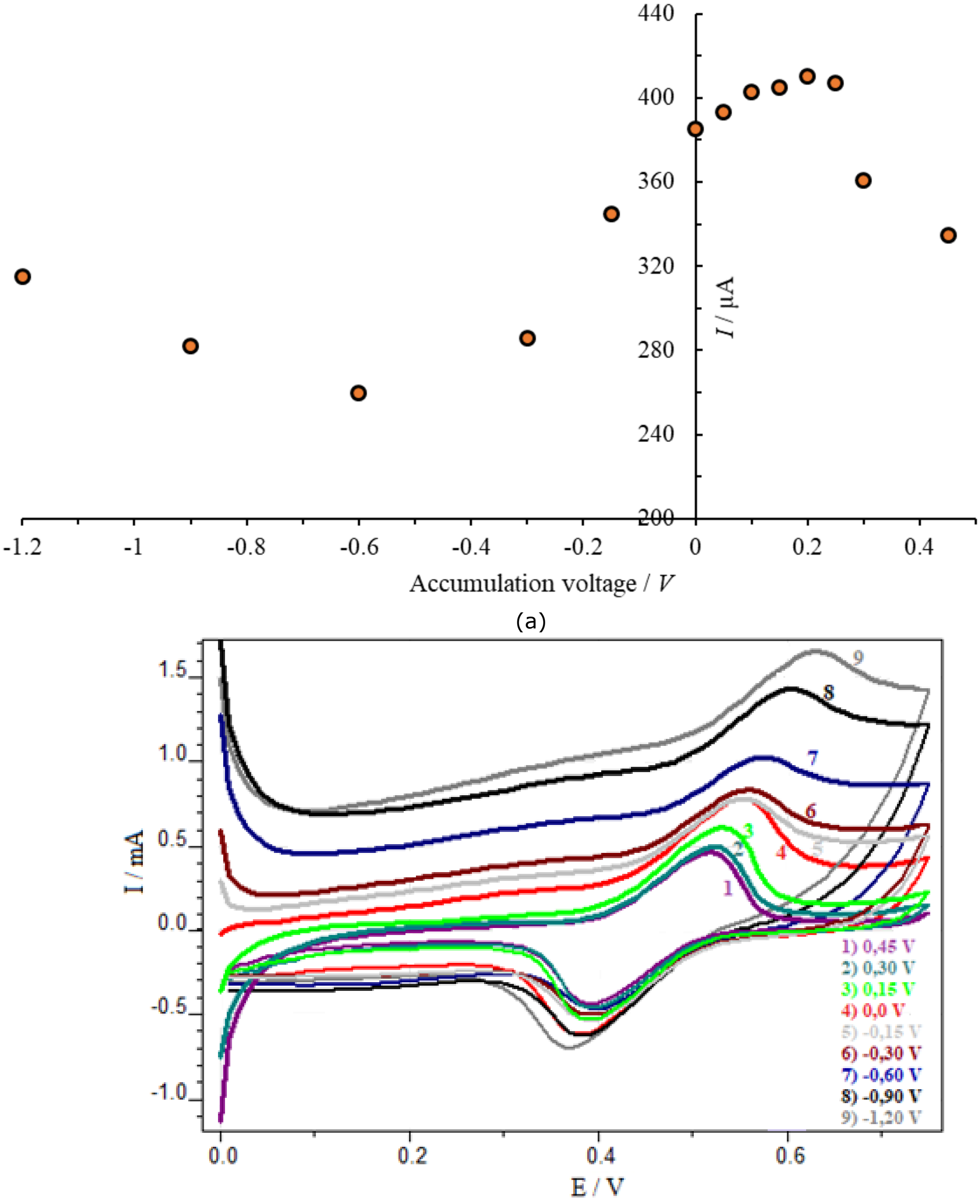
3.3.3. Determination of DBT accumulation conditions on the modified electrode

In determining the trace amount of electroactive analytes, a pre-concentration can be made by applying a constant voltage to the electrode. This pre-concentration will further increase the sensitivity of voltammetric measurements. A lower DBT concentration (100 µM) was preferred to test the effect of pre-concentration in DBT determination.

PGE/OPEDOTNF-2cyc-2V-120s-2DP were immersed in BRB solution at pH 2.0 containing 100 µM DBT. Different voltage values were applied for an accumulation time of 250 seconds to ensure DBT accumulation on the modified electrode. The oxidation current values obtained for different deposition voltages are shown in Figure 5a. The highest current value for DBT with PGE/OPEDOTNF-2cyc-2V-120s-2DP was obtained as 410 µA at 0.20 V accumulation voltage. If the accumulation process is not performed, the current value was found as 105 µA with PGE/OPEDOTNF-2cyc-2V-120s-2DP and 10.8 µA with unmodified PGE. The results show that the accumulation process is important in amplifying the DBT signal, especially at low concentrations.

The cyclic voltammograms from which these data were obtained are given in Figure 5b. The base signal values obtained when the accumulation process is applied at different voltages change. To determine the current value originating from DBT only, the "Analysis/PeakfindAdvanced/Baseline Subtract/Automatic/Subtract Baseline" command was applied on the page where cyclic voltammograms were opened in IviumSoft software. The current values in the graph in Figure

5a are obtained by applying this process. This baseline correction was also applied to subsequent voltammetric measurements using the accumulative process.



(a)

(b)

Figure 5: a) The effect of the accumulation voltage on the DBT oxidation current obtained by CV using PGE/OPEDOTNF-2cyc-2V-120s-2DP b) The corresponding cyclic voltammograms (in the presence of 100 µM DBT in BRB solution at pH 2.0, applied for 250 s accumulation time).

Cyclic voltammograms showing that accumulation increases the sensitivity of the PGE/OPEDOTNF-2cyc-2V-120s-2DP electrode is shown in Figure S7. The voltammogram obtained without accumulation with PGE is also included in the figure for comparison purposes. In order to investigate, the effect of the accumulation time, different accumulation times (seconds) were applied at 0.20 V to PGE/OPEDOTNF-2cyc-2V-120s-2DP in BRB solution at pH 2.0 containing 100 µM DBT. The relationship between peak current obtained for DBT oxidation versus accumulation time is shown in Figure S8. The results show that the longer the accumulation time, the higher the peak current. However, a deposition time of 350 seconds (5.8 minutes) was preferred, considering that long deposition times would prolong the determination time more than necessary. In this preference, the time required for repeated measurements, which must be made analytically, is also considered. The calibration curve obtained for DBT by CV using PGE/OPEDOTNF-2cyc-2V-120s-2DP is given in Figure 6. The results show that the CV technique can be used for sensitive determination of DBT.

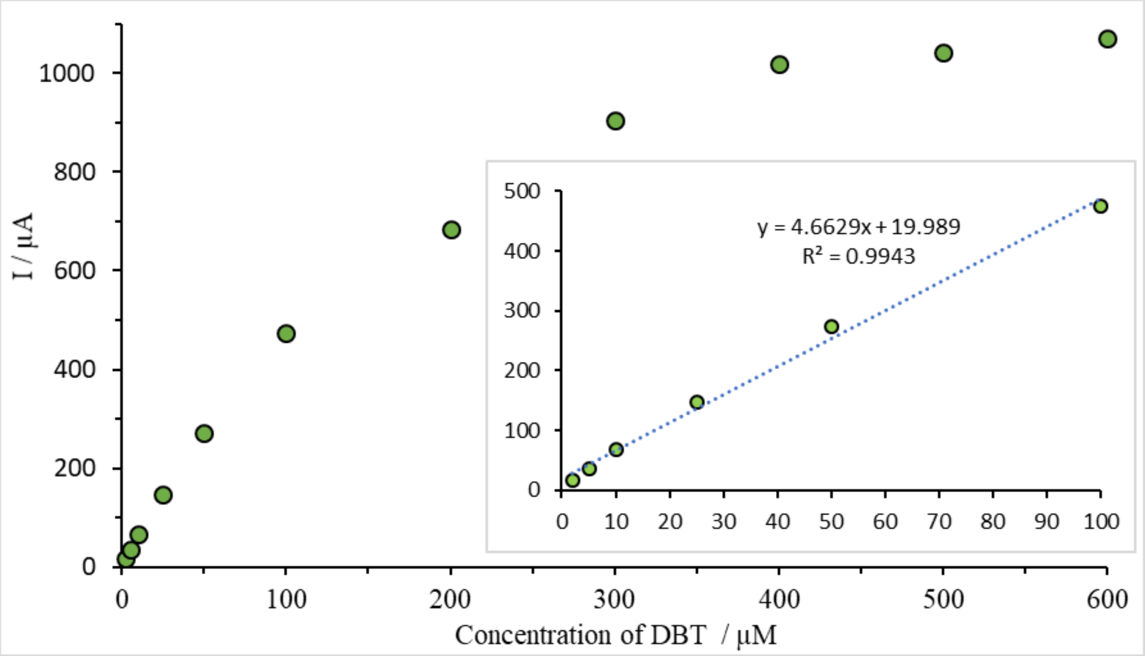


Figure 6: Calibration graph of PGE/OPEDOTNF-2cyc-2V-120s-2DP electrode obtained by CV at different DBT concentrations in BRB solution at pH 2.0 (Accumulation voltage: 2.0 V; accumulation time: 350 seconds, unstirred solution, data with baseline processing).

While preparing PGE/OPEDOTNF-2cyc-2V-120s-2DP, the over-oxidation process, which has been carried out so far and whose optimum conditions were determined as 120 seconds at 2.0 V, was carried out in phosphate buffer at pH 7.0. In order to determine the effect of the pH of the over-oxidation solution on DBT determinations, it was performed in PB solution at different pH values (6, 7, and 10). The current values obtained by CV technique in BRB solution at pH 2.0 containing 100 µM DBT using over-oxidized electrodes at different pH values are given in Figure S9. The values obtained in pH 7.0 PB solution were higher; this value was chosen as optimum. In addition, data obtained when deposition was performed by mixing is also given in Figure S9. In DBT determinations, a significant increase was observed in the oxidation peak current, as the mixing of the solution during deposition made it easier for the analyte to reach the electrode surface.

However, it is an effective strategy to use other electroanalytical methods, such as differential pulse voltammetry (DPV) or square wave voltammetry, to improve the sensitivity in determining electroactive compounds. Since DPV is widely used in detecting analyte signals with lower concentrations, it aims to increase the measurement sensitivity and lower the detection limit too much lower values. The DPV method was preferred to determine the analyte in the following parts. The necessary optimizations for DPV measurements were also worked.

3.4. Determination of dobutamine using the DPV technique

In the previous section, the parameters that will affect the DBT determinations were investigated by using the data obtained using the CV technique. Based on these data, the determined values for the number of cycles, over-oxidation voltage and time were used to prepare modified electrodes. In the measurements made using the DPV method at lower concentration values, the optimization of the parameters that will affect the accumulation of DBT on the electrode surface has been studied. Section 3.4 is related to these studies. Scan rate: 20 mV/s, pulse time:5 0 ms, and pulse amplitude: 50 mV were used in DPV measurements.

3.4.1. Effect of accumulation time and number of DPV processes used in conditioning

To determine the effect of accumulation time, 0.20 V for different times was applied to the PGE/OPEDOTNF-2cyc-2V-120s-2DP electrode immersed in BRB solution at pH 2.0 containing 20.0 µM DBT, and then DPV measurements were performed. To compare the current values obtained from the differential pulse voltammograms accurately, the peak current values obtained after baseline correction on the voltammograms were used. Baseline corrected, and uncorrected DP voltammograms obtained both with and without accumulation are presented in Figure S10. The results of the experiments carried out to determine the relationship between the current values and the accumulation time are shown in the graph in Figure S11. While there is a remarkable increase until the 100th second, the increase in the current values after the 100th second is at lower levels. Therefore, it was decided that 100 seconds was a reasonable value for the accumulation time in this stage.

The potential of the number of DPV processes applied for conditioning the PGE/OPEDOTNF-2cyc-2V-120s electrode to affect the measurements in the new situation was also investigated. It was observed that the highest current value was reached with the modified electrode (PGE/OPEDOTNF-2cyc-2V-120s-1DP) obtained by conditioning once with the DP process (See Figure S12). Following these results, studies on the accumulation time were performed again using PGE/OPEDOTNF-2cyc-2V-120s-1DP. Since the sensitive DPV method and accumulation are used together, the DBT concentration was lowered ten times and selected as 2.0 mM. The graph of DBT oxidation current-accumulation time is shown in Figure S13. The data of Figure S13 were evaluated in terms of both high current values and a reasonable time. It was decided to apply the accumulation time of 250 seconds with a voltage value of 0.20 V on the surface of the PGE/OPEDOTNF-2cyc-2V-120s-1DP electrode. This accumulation time was used in interference, stability, and reproducibility studies.

3.4.2. Variation of oxidation current of DBT with concentration

Using PGE/OPEDOTNF-2cyc-2V-120s-1DP electrodes, the variation of DBT concentration and oxidation peak current values of DBT was measured using the DPV method. The graph obtained for three different deposition times (100, 250, and 400 s) is given in Figure 7. The linear range is between 0.1-4.0 μM for 100 seconds accumulation time, while the linear range is between 0.1-2.0 μM for 250 seconds accumulation time. As the accumulation time increases, the slope value of the linear concentration range increases, in which case more sensitive measurements can be made. For the 400-second accumulation time, the linear range narrowed while the slope value increased in accordance with the behavior mentioned above. The small graph at the bottom right of Figure 7 shows the linear range obtained with a correlation coefficient of 0.997 for a 250-seconds accumulation time. As a result of the calculations, the limit of detection (LOD) was found to be 0.026 μM, and the limit of quantification (LOQ) was 0.086 μM for the accumulation time of 250 seconds. LOD and LOQ values were calculated with the help of the following equations: LOD = 3 s / m and LOQ = 10 s / m (m is the slope of the linear equation obtained from the calibration graph in Figure 7., and s is the standard deviation of the current values obtained in BRT solution at pH 2 absence of DBT). Figure 8 shows the DP voltammograms obtained with PGE/OPEDOTNF-2cyc-2V-120s-1DP electrode for concentration values in the range of 0.1-8.0 μM in 250 seconds accumulation time.

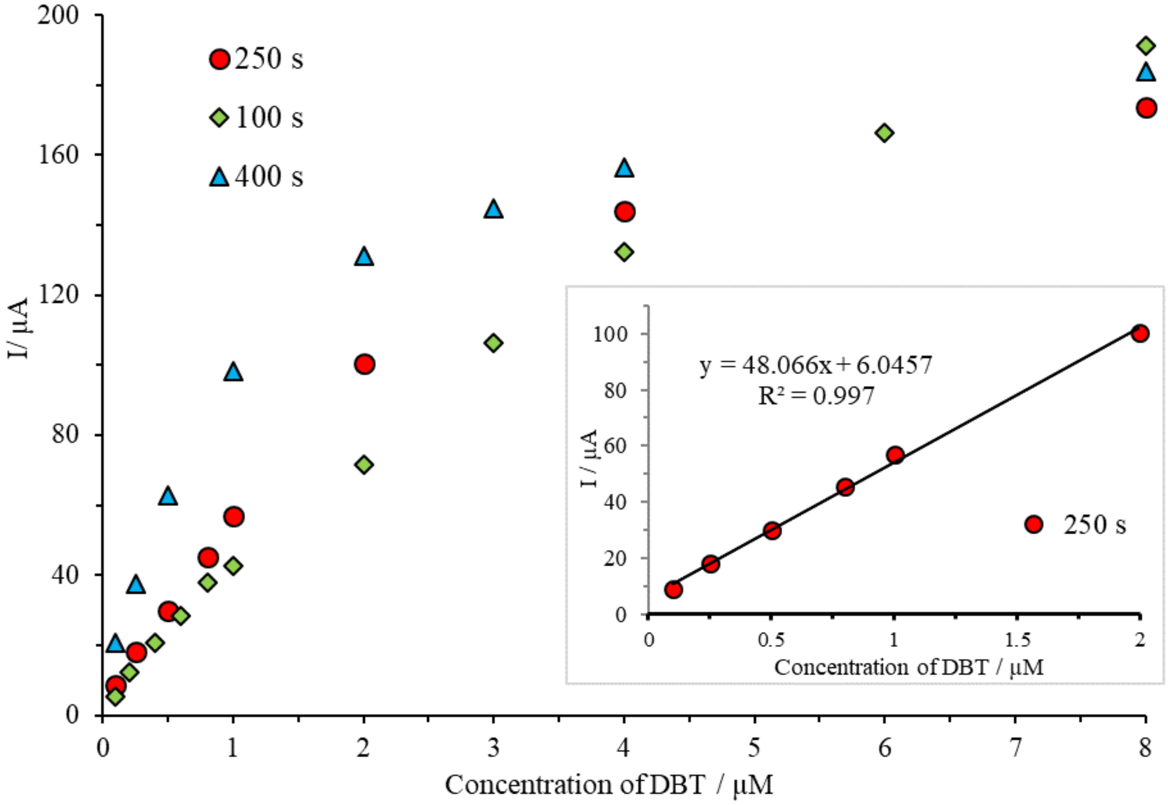


Figure 7: The effect of accumulation time (100 s, 250 s, and 400 s) on the calibration graph of PGE/OPEDOTNF-2cyc-2V-120s-1DP electrode obtained by DPV technique in BRB solution at pH 2.0. (Accumulation voltage: 0.20 V, solution stirring rate: 250 rpm).

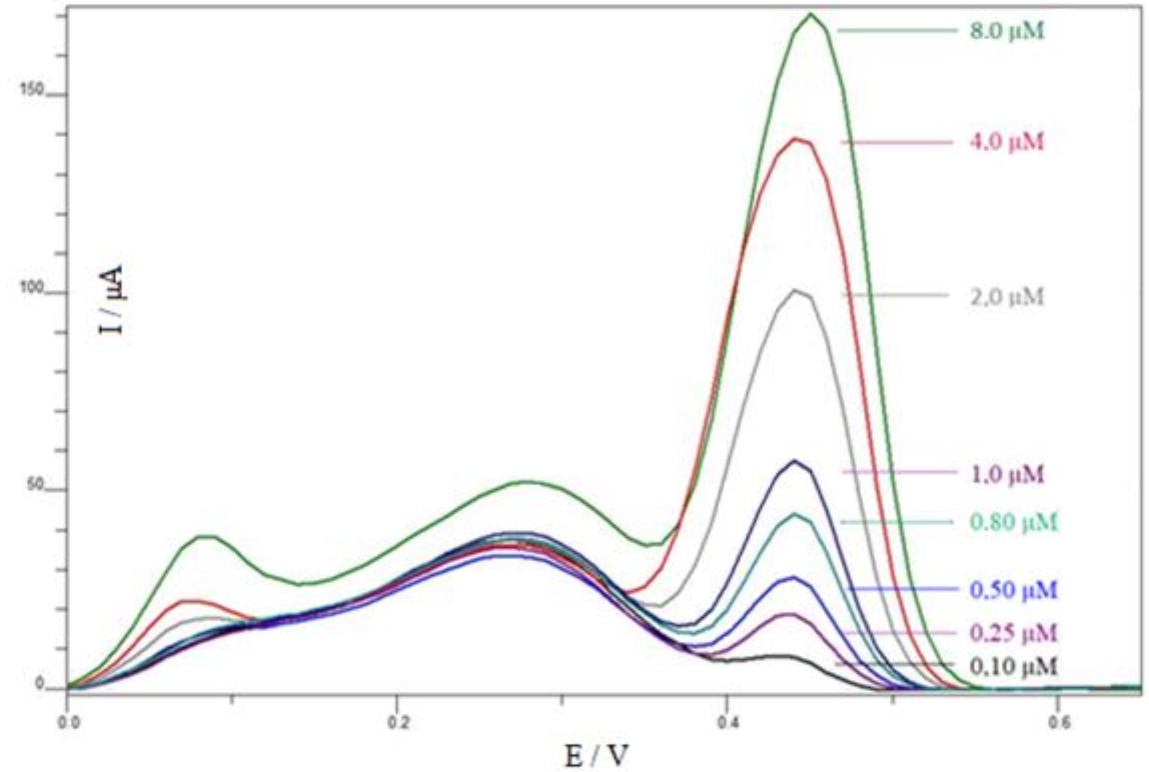


Figure 8: DP voltammograms obtained for DBT in BRB solution at pH 2.0 using the PGE/OPEDOTNF-2cyc-2V-120s-1DP electrode (Accumulation voltage: 0.20 V, accumulation time: 250 seconds, solution stirring rate: 250 rpm).

Linear range and LOD data are presented in Table 1 in comparison with the literature. LOD values are at lower concentrations than in some studies in the literature (3, 20, 22, 40).

Table 1: Comparison of the linear range and LOD of PGE/OPEDOTNF-2cyc-2V-120s-1DP in DBT determinations with the literature.

Modified Electrode	**Linear Range (µM)**	**Limit of Detection, LOD (µM)**	**Reference**
GQDs/NiMnO$_3$/CPE[a]	0.08–40	0.02	[5]
BNPs–MWCNTs/GCE[b]	0.005–1	0.009	[39]
PAO/GCE[c]	0.05–100	0.002	[2]
IL/GPE[d]	0.17–100	0.053	[20]
MgO-N/GCE[e]	1–30	0.092	[22]
AuCoPcCPE[f]	6.0–200	0.084	[3]
GC/CNT/ILC/RGO/CW[g]	0.02–40	0.000497	[25]
CPE/MWCNTPE[h]	18-1100	3.1	[40]
PGE/OPEDOTNF[i]	0.1-2.0	0.026	This study

[a] Graphene Quantum Dot-NiMnO$_3$ nanoparticle-Carbon paste electrode.
[b] Boehmite nanoparticle-Multi walled carbon nanotube-Glassy carbon electrode.
[c] Poly (Acridine Orange) film-Glassy carbon electrode.
[d] Ionic Liquid-Graphite paste electrode.
[e] MgO microflowers / Nafion-Glassy carbon electrode.
[f] Gold nanoparticle-Cobalt phthalocyanine modified carbon paste electrode.
[g] Glassy carbon electrode-Multi walled carbon nanotube-Ionic liquid crystal-Graphene-18-Crown-6.
[h] Carbon paste electrode-Multi walled carbon nanotube.
[i] Pencil graphite electrode-Over oxidized poly (3,4-ethylenedioxythiophene) nanofiber.

3.5. Effect of scanning rate on the current

Using the PGE/OPEDOTNF-2cyc-2V-120s-1DP electrode, cyclic voltammograms were recorded for 25 μM DBT (in BRB solution at pH 2.0) at scan rates in the range of 10-500 mV/s. The graph of the current values and cyclic voltammograms obtained for the oxidation of DBT against the scan rates can be seen in Figure S14. Figure 15a shows a plot of peak current values versus the square root of scan rates. Figure S15b shows the graph of the logarithms of the peak current values versus the logarithms of the scan rates, and the slope of the graph is 1.057. The linearity of the graphs in Figures S14 and S15b shows that the electrochemical oxidation of DBT is adsorption controlled. In addition, the fact that the slope of the graph in Figure S15b is close to 1.0 and the graph in Figure S15a is not linear supports that the oxidation current is adsorption controlled.

3.6. Interference study

Many different types of biological molecules coexist in body fluids. Knowing whether interfering species affect the analyte signal is important when working with real samples. Therefore, PGE/OPEDOTNF-2cyc-2V-120s-1DP electrodes were tested in the presence of 1.0 μM DBT and different concentrations of interference molecules. Electrolyte species found in physiological fluids such as KCl and NaCl and electroactive species such as uric acid, ascorbic acid, paracetamol, and phenylalanine found in body fluids were tested in the interference study. Neurotransmitter substances such as epinephrine and dopamine were also tested. The results obtained are given in Table 2 in comparison.

When the Table 2 is examined, the electrochemical signal is not seriously affected when the concentration of species such as glucose, NaCl, and KCl is 100 times higher than DBT. Epinephrine and dopamine, both electroactive and neurotransmitters like DBT, have interfering effects. One of the most important reasons for this may be that the modified electrode's improvement effect on the signal works for these interferent molecules. In addition, it is thought that the interfering species also accumulate on the electrode surface depending on their chemical structure and interaction degree during the accumulation process, and the interference effect reaches even higher levels than expected. Decreasing the DBT signal when non-electroactive species are at a concentration of 250, 500, or 1000 times higher than the DBT concentration may be related to the decrease in the amount of DBT accumulated on the electrode surface. Therefore, an additional modification to increase selectivity will be needed for the determination of DBT in the presence of interferent species. The molecular imprinting method could be an important alternative here. It is reported in the literature that conductive polymeric structures produced electrochemically by molecular imprinting method increase selectivity (41-43).

3.7. Stability and reproducibility of PGE/OPEDOTNF

The stability studies were carried out using PGE/OPEDOTNF-2cyc-2V-120s electrodes. Many electrodes prepared under the same conditions were kept in a closed plastic box, including desiccant material at room temperature. Stability studies were carried out using CV and DPV tecniques with disposable modified electrodes. CV measurements were performed using PGE/OPEDOTNF-2cyc-2V-120s-2DP electrode in BRB solution at pH 2.0 buffer containing 100 μM DBT. DPV measurements were performed using PGE/OPEDOTNF-2cyc-2V-120s-1DP electrode in BRB solution at pH 2.0 buffer containing 1 μM DBT. The measurement results for electrode stability is shown in Figure 9. When the PGE/OPEDOTNF-2dng-2V-120s-1DP electrode was used (Figure 9a), it was determined that the current value did not change significantly for 15 days and decreased by 4% on the 15th day. Then, the response of the electrode reduced day by day; for example, this decrease reached 36% for the 213th day. Similar behavior was observed for the PGE/OPEDOTNF-2dng-2V-120s-2DP electrode (Figure 9b).

To determine the reproducibility for PGE/OPEDOTNF-2cyc-2V-120s-1DP electrodes, the currents of the electrodes produced under the same conditions were measured by DPV in BRT solution at pH 2.0 containing 1.0 μM DBT. The standard deviation of the electrochemical response was calculated as 5.2% in 5 separate measurements performed with the modified electrodes.

Table 2: Effect of interfering species on the current response obtained by DPV technique using PGE/OPEDOTNF-2cyc-2V-120s-1DP for 1.0 μM DBT (Accumulation time: 250 s, Accumulation voltage: 0.20 V).

Interfering species	**The concentration of the interfering species / (μM)**	**Change in Current[a] for 1.0 μM DBT with PGE/OPEDOTNF / (μA)**	**Deviation in current / (%)**
Ascorbic acid	5	-0.16	0.27
	20	-3.05	5.21
Dopamine	0.5	+10.07	17.21
	1	+23.15	39.57
	3	+57.07	97.56
Epinephrine	1	+16.34	27.94
	2	+33.50	57.28
	3	+40.64	69.48
Phenylalanine	100	-1.21	2.06
Glucose	25	-1.23	2.11
	100	-4.13	7.06
	250	-5.06	8.65
	1000	-7.65	13.07
KCl	50	-1.09	1.86
	100	-4.25	7.27
	250	-7.94	13.58
	500	-17.67	30.20
NaCl	25	-1.29	2.21
	50	-1.95	3.33
	100	-5.86	10.01
	250	-8.65	14.78
	500	-17.54	29.98
Paracetamol	10	-11.96	20.45
	15	-19.82	33.89
Uric acid	2	-9.81	16.77
	4	-15.59	26.65
	8	-19.20	32.82

[a] The current without interfering species = 58.5 μA.

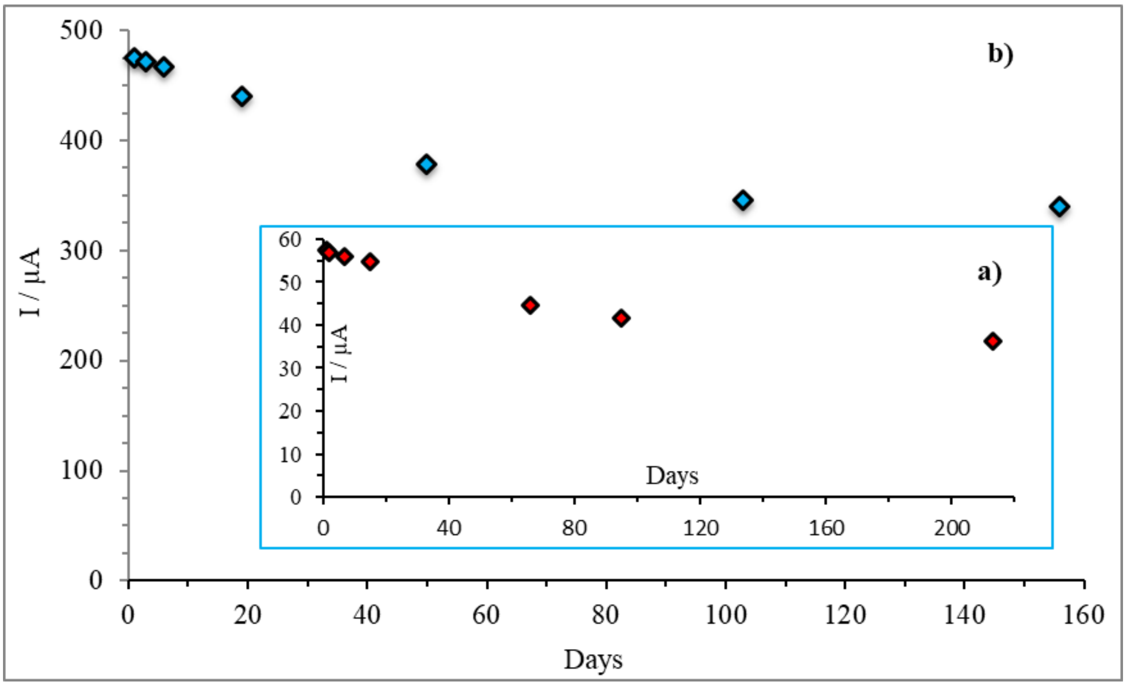


Figure 9: Life-time measurements for (a) 1 µM DBT with PGE/OPEDOTNF-2cyc-2V-120s-1DP using DPV and (b) 100 µM DBT with PGE/OPEDOTNF-2cyc-2V-120s-2DP using CV. (Accumulation voltage: 0.20 V).

4. CONCLUSION

A study was carried out on modifying the pencil graphite electrode with over-oxidized nanofiber structure PEDOT so that the DBT can be sensitively determined by voltammetric methods. It was determined that the oxidation current of DBT obtained with PGE modified with nanofiber-structured PEDOT was 2 times higher than that of non-nanofiber. Parameters that may affect the current response of modified electrodes against DBT were examined, and optimum conditions were determined. As a result of DPV measurements performed with PGE/OPEDOTNF-2cyc-2V-120s-1DP electrodes in the presence of DBT, it was observed that the response of the electrodes against DBT in the concentration range of 0.1 µM to 2.0 µM was linear. The limit of detection (LOD) was calculated as 0.026 µM, and the limit of quantification (LOQ) as 0.086 µM. In addition, interference studies have been made for DBT concentration. PGE/OPEDOTNF-2cyc-2V-120s-1DP electrodes have a reproducibility of 5.2% and can remain stable for 15 days without losing effectiveness. In addition, the modified PGE in this study provides a significant advantage in terms of cost since it is an electrode material that can be obtained very cheaply.

The data of this study, with some additional changes, will lead to future research and studies. Developing the electrochemical determination of biologically important compounds that are simple, inexpensive, and compatible with miniaturized systems has become very important. From this point of view, the study significantly contributed to determining DBT, which is one of the very important catecholamines.

5. CONFLICT OF INTEREST

There are no conflicts that need to be reported.

6. ACKNOWLEDGMENTS

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