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ARISTON PUBLICATIONS
Energy & Environment Advances
ISSN: 3066-5728 https://aristonpubs.com/energy-environment-advances https://doi.org/10.69626/eea.2025.0021

RESEARCH ARTICLE

Electrochemical Simultaneous Determination of Levodopa and Benserazide in Aniline and Multi–Walled Carbon Nanotube Modified Sensor

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ABSTRACT: The present study reports the development of a highly sensitive electrochemical sensor for the simultaneous determination of levodopa and benserazide, two pharmacologically active substances used in the treatment of Parkinson's disease. The sensor was fabricated by electropolymerization of aniline on a glassy carbon electrode (GCE) in a nonaqueous medium, followed by modification with multi-walled carbon nanotubes (MWCNTs). The synergistic effect of polyaniline and MWCNTs resulted in enhanced electrochemical performance, providing high selectivity and sensitivity toward the target analytes. The modified electrode exhibited well-defined differential pulse voltammetry (DPV) responses, allowing for quantitative determination of levodopa in the linear range of 500-1000 µM and benserazide in the range of 100-500 µM, with detection limits of 67.01 µM and 163.60 µM, respectively. The electrochemical behavior of the sensor was evaluated under various conditions, including film thickness, supporting electrolyte type, and pH variations, to optimize its performance. The sensor demonstrated excellent repeatability, stability, and resistance to interference from ascorbic acid, a common interfering species. Additionally, the method was successfully applied to the determination of levodopa and benserazide in real urine samples and pharmaceutical formulations, achieving satisfactory recovery rates. These findings highlight the potential of the developed sensor as a reliable and cost-effective tool for clinical and pharmaceutical applications.

Keywords: Levodopa, Benserazide, Aniline, Multi-walled carbon nanotube

Received: 25 September 2024; Revised: 30 October 2024; Accepted: 24 December 2024; Published Online: 15 January 2025

1. INTRODUCTION

There is a certain balance between acetylcholine, which increases the excitability of nerve cells in the brain, and dopamine, which does the opposite. In Parkinson's disease, this balance is disrupted in favor of acetylcholine, and the dopamine deficit must be replaced in the treatment. Synthetic dopamine cannot cross the barrier between the blood and the brain. This problem was solved by the discovery of Levodopa, which is converted to dopamine after crossing the blood-brain barrier [1-6].

Levodopa (L-Dopa) is an aromatic amine that is metabolized to dopamine. Levodopa is used in commercial drugs in the form of combination preparations with an aromatic amino acid decarboxylase inhibitor such as carbidopa or benserazide. Generally, these substances form quinone compounds by oxidation in aqueous solution [7-10]. Chromatographic [11], spectrophotometric [12] and chemiluminescence [13] methods have also been used for the determination of the binary drug mixture of levodopa and benserazide. The common feature of these substances is that they have a monoamine neurotransmitter substance structure that does not contain a chromophore group. Therefore, their determination by spectrophotometric methods is limited. Although the chromatographic method is more popular than other methods, the presence of interferents is a significant disadvantage for this method. In addition, the determination of these substances by the chromatographic method is also possible if an electrochemical detector is used. Alternatively, the electrochemical method has recently attracted more attention due to its advantages such as being more selective,

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more sensitive, cheaper and being prepared in a shorter time compared to other methods.

With voltammetric methods, it is possible to analyze drug active ingredients from pharmaceutical preparations and biological fluids quickly, sensitively and economically without the need for any separation method [14-18]. One of the superior aspects of voltammetry is its use in the field of molecular biology with its role in redox reactions of drug active ingredients and thus in explaining the pharmacological action mechanisms of many compounds of physiological importance. When bare electrode is used as a working electrode in electroanalytical applications, electroactive interferences such as ascorbic acid and uric acid show electroactivity on the conductive electrode surface and contribute to the amperometric response of the desired species to be detected, thus significantly changing the characteristic peak of the relevant species, and as a result, satisfactory potential differences cannot be achieved in peak separation of analytes. This disadvantage of bare electrode has made the use of modified electrodes mandatory. In addition, electrode modification offers many other advantages such as reducing overpotential, increasing reaction rate and improving sensitivity [19].

Carbon nanotubes are frequently used in electrode modification in biosensor design in electroanalytical chemistry due to their unique structures, mechanical strength and electronic properties [20]. In this way, the working potential is reduced and the reaction rate of many electroactive substances is increased. Thus, carbon nanotube modified electrodes show better electrochemical performance than other known carbon electrodes [21]. The electrochemical properties of both carbon nanotubes and conductive polymers provide the modified electrode to have new and unique properties.

One of the most important problems encountered in the determination of levodopa and benserazide by electrochemical methods is the presence of electroactive ascorbic acid. The determination of ascorbic acid and catecholamines in biological fluids is important in the diagnosis and treatment of some diseases. Ascorbic acid in biological fluids can be used to reach the amount of oxidation stress in human metabolism, which is associated with cancer, diabetes and liver diseases [22]. Because ascorbic acid is found together with such catecholamines in the cerebrospinal fluid and since their peak potentials are close to each other, it creates a serious problem for the measurement of these substances [22, 23]. In order to overcome this problem, either electrocatalytic structures where the peak potentials for levodopa, benserazide and ascorbic acid can be separated or permselective coating materials that allow the passage of levodopa and benserazide while blocking electroactive ascorbic acid are needed.

In this study, it was aimed to use aniline monomer together with carbon nanotubes in the production of modified electrodes, to investigate the electrochemical properties of these electrodes and to determine the active drug substances such as levodopa and benserazide. With this modified electrode, the determination of active drug substances was carried out in the presence of interfering species such as ascorbic acid and uric acid. In addition, the selective determination of many interfering substances in biological fluids such as drug samples, blood serum and urine were successfully carried out.

2. EXPERİMENTAL DETAİLS

2.1. Chemical Substances

All chemicals used in this study were of analytical grade; levodopa standard was supplied by Alfa Aesar (A Johnson Matthey Company), benserazide standard was supplied by Sigma, multi-walled carbon nanotube (MWCNT, average diameter: 3-10 nm, length: 10-30 µm, purity: >95%, surface area: >500 m^2/g) was supplied by Grafen Inc., aniline was supplied by Acros Organics, tetrabutylammoniumtetra-fluoroborate and L-(+)-ascorbic acid were supplied by Merck. Analytical grade chemicals were used in the preparation of buffer solutions; Na_2HPO_4 (Merck), KH_2PO_4 (Carlo Erba), KCl (Merck), NaCl (Merck), H_3PO_4 (Merck), H_3BO_3 (Merck) and CH_3COOH (Merck). In the preparation of all aqueous solutions, high purity ultrapure water obtained from the Milli-Q system (Millipore, Milford, USA) was used. In addition, stock solutions of levodopa and benserazide, which were used as active ingredients in electrochemical measurements, were prepared with pure water at concentrations of 10^{-2} M. All solutions were prepared and protected from light during analysis.

2.2. Instruments and materials

All electrochemical operations were performed by BAS (Bioanalytical Systems, Inc.) 100W electrochemical analyzer in a triple-electrode cell. The C2 Faraday cell cage of the same company was used as the electrochemical cell. The pH of the solutions was measured with a Hanna Instruments pH 211 Microprocessor pH meter. A platinum electrode prepared in the form of a spiral disk was used as an auxiliary electrode, an Ag/AgCl electrode in 3 M KCl (CHI111) was used as a reference electrode for aqueous media, an Ag/Ag+ reference electrode (CHI112) was used for non-aqueous media, and a glassy carbon electrode (CHI104) was used as a working electrode. Glassy carbon electrodes (GCE) were cleaned on alumina powder and distilled water velvet disk (BAS, MF-1040) cleaning pads before experimental studies. Then, the GCE was activated by applying the cyclic voltammetry (CV) technique with 20 cycles in the range of –0.5 to 2.0 V in 0.1 M H_2SO_4 solution at a scan rate of 100 mV/s. All experiments were carried out under argon gas atmosphere.

2.3. Preparation of buffer solutions

To prepare phosphate buffer solutions (PBS), 1.9052 g

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KH_2PO_4, 1.9579 g Na_2HPO_4, 36.5250 g NaCl and 1.0066 g KCl were weighed and dissolved, then completed to 500 mL with deionized water in a volumetric flask. The pH of this solution was measured to be approximately 6.3. PBS buffers with pHs of 2.0, 3.0, 4.0; 5.0, 7.0, 9.0 and 11.0 were prepared by adding 0.2 M NaOH and 0.1 M H_3PO_4. To prepare Britton-Robinson buffer solutions (BRBS), 2.4700 g of H_3BO_3, 2.7 mL of H_3PO_4 and 2.3 mL of CH_3COOH were mixed and dissolved and completed to 1000 mL with deionized water in a volumetric flask. The pH of this solution was measured to be approximately 2.02. The pHs of the buffer solutions prepared with the help of 0.2 M NaOH and 0.1 M H_3PO_4 were adjusted to 2.0, 3.0, 5.0, 7.0, 9.0 and 11.0. To prepare acetate buffer solutions (ABS), 500 mL of 0.1 M CH_3COOH solution was prepared. The pH of this solution was measured to be approximately 2.7. Using 0.1 M CH_3COOH solution, buffer solutions with pH 3.0, 5.0, 7.0, 9.0, 11.0 were prepared with the help of 5.0 M NaOH and concentrated CH_3COOH solutions.

2.4. Functionalization of MWCNT

Carboxylic acid groups were attached to the surface of MWCNTs to obtain uniform dispersions of carbon nanotubes and to ensure strong interaction. First, the oxidation of MWCNT was carried out using a mixture of sulfuric acid and nitric acid at a ratio of 3:1. Thus, the raw material adsorbs carboxylic groups on the surface of the catalyst. For this purpose, 100 mg MWCNT was added to 12 mL H_2SO_4:HNO_3 (1:1) mixture, heated with stirring in a silicone oil bath at 70 °C for 12 hours, the product was filtered through a 0.22 µm porous Teflon membrane and washed with pure water until the pH reached neutral. Carboxylated MWCNTs (MWCNT-COOH) were dried in a vacuum oven at room temperature for 48 hours [24].

2.5. Modification of glassy carbon electrodes with MWCNT and polyaniline

To obtain the polyaniline polymer electrodes used in the study electrochemically, a solution of 500 mM aniline in 0.1 M H_2SO_4 was prepared. In order to determine the oxidation potential of aniline, CV technique was applied at a scanning speed of 50 mV/s between (-200) and (+1800) mV in GCE. As seen in the voltammograms in Figure 1, the first oxidation peak was obtained at 1250 mV. Therefore, the electropolymerization of aniline was carried out on the GCE surface by applying the bulk electrolysis (BE) method at 1300 mV for 10 seconds. It was observed that the color of the polymer formed on the electrode surface was light yellow. MWCNT solutions at concentrations of 0.2%- 0.5%- 1.0% (mg/µL) were prepared. In order to functionalize the MWCNT-COOH, it was kept in an ultrasonic bath for 4 hours until a homogeneous mixture was obtained in N, N-dimethylformamide (DMF). The modified electrodes were modified with two different applications. The first modification procedure

3. RESULTS AND DİSCUSSİON

3.1. Film thickness effect

Aniline monomer was grown on the GCE surface by the CV method, but the responses of the active substances could not be obtained on these film surfaces. Therefore, the BE method was tried. After the voltammogram of 500 mM aniline in 0.1 M H_2SO_4 in Figure 1 was obtained, 10 s films were created in the range of 1200-1700 mV where the oxidation peak was observed and their responses to the active substances were examined. The responses for levodopa are seen in Figure 2. When these voltammograms were examined, it was decided to grow the films at this potential since the highest peak current was obtained with the film surface grown at 1300 mV. Various film thicknesses (10, 15, 20, 25, 30, 35, and 40 seconds) of polyaniline electrodes grown at 1300 mV by bulk electrolysis were studied and the responses of these electrodes in PBS pH 7.0 medium containing 1.0 mM levodopa were investigated. The best response was obtained with a film thickness of 10 s formed with aniline, and 10 s film surfaces were used in the following studies.

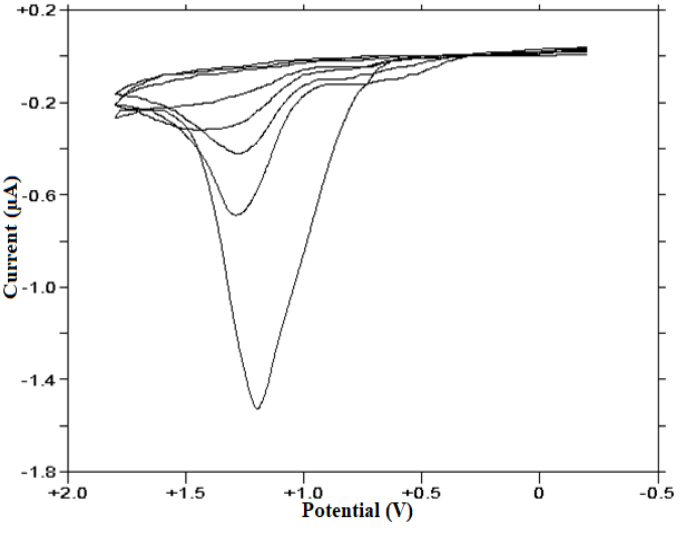

Fig. 1. CV response of 500 mM Aniline versus Ag/AgCl reference electrode in GCE, scan rate: 50 mV/s, 5 cycles.

3.2. Support electrolyte and pH effect

The most suitable support electrolyte solution medium that can increase the response of active substances on the polyaniline modified electrode was investigated. For this purpose, phosphate buffer, britton-robinson buffer and acetate buffer were used as electrolyte solutions. The DPV responses of the solutions of active substances in the pH range of 2-11 for PBS and BR buffer and in the pH range of 3.0-11.0 for AT were investigated on the modified electrode.

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The highest current response and the best peak resolution were measured in PBS pH 7.0 medium.

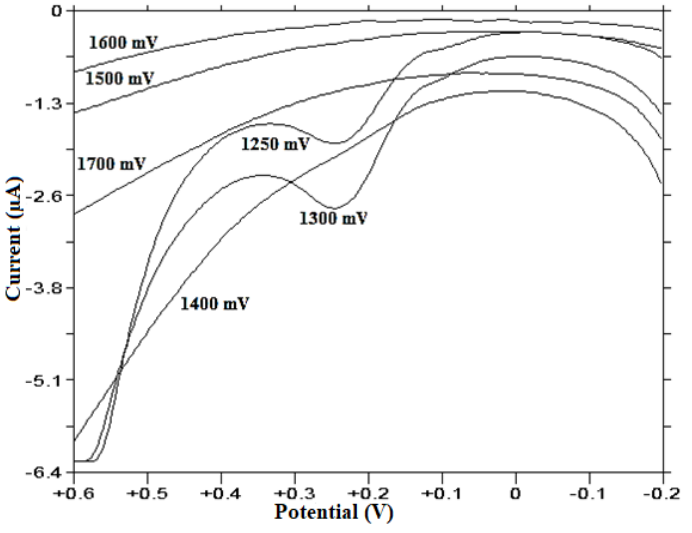

Fig. 2. DPV responses to 1.0 mM levodopa of polyaniline electrodes grown at various potentials by the 10 s BE method.

3.3. Determination of benserazide and levodopa in modified sensors

Solutions of 1.0 mM benserazide and 1.0 mM levodopa in PBS pH 7.0 buffer were prepared and the DPV responses in the prepared modified sensors were investigated. As a result of comparing the DPV responses of 1.0 mM benserazide and 1.0 Mm levodopa in modified sensors, the voltammograms in Figure 3 were obtained. When the differential pulse voltammograms were examined, the best separation was achieved in the modified sensor (MWCNT/AN/GCE) obtained by coating polyaniline (AN) on GCE and dropping 20 µL of 1.0% MWCNT on it. The current values obtained were also more satisfactory. A peak current of 4.256 x 10^{-4} A was obtained at 164 mV for benserazide and 1.976 x 10^{-4} A was obtained at 320 mV for levodopa.

3.4. Validation of the analytical method

DPV is an effective, selective and sensitive method for the determination of organic drug compounds at low detection limits. Therefore, DPV was used in the quantitative evaluation of benserazide and levodopa. Validation of the studied method was evaluated by performing repeat analyses of standard solutions in electrolyte solution considering precision and accuracy. The best response was decided considering the peak shape, peak current sensitivity and repeatability. All solutions used in analytical experiments were freshly prepared to ensure the stability of the analyte in solution. Within the scope of method validation, regression equations were obtained from the calibration graphs of peak current plotted against benserazide and levodopa concentrations with DPV technique, detection limits were found, recovery studies were performed in tablet dosage forms and urine samples, and interference effects were investigated besides ascorbic acid (AA).

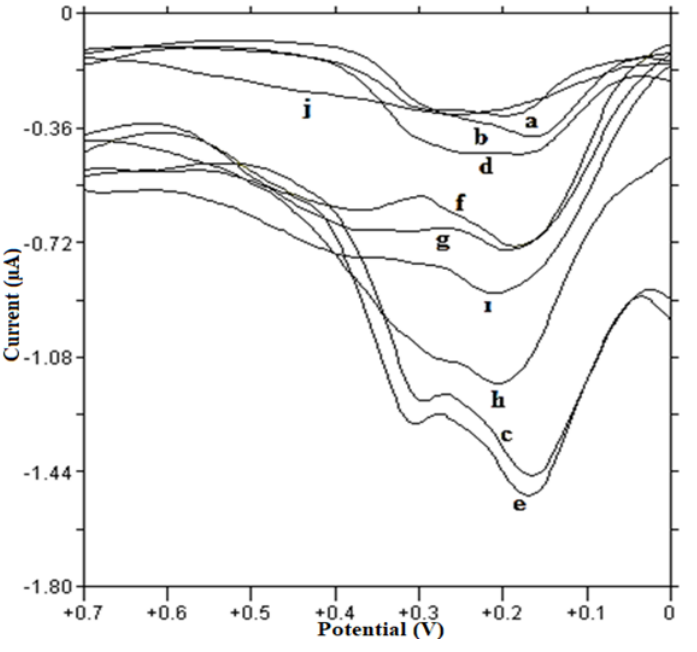

Fig. 3. DPV responses of a mixture of 1.0 mM levedopa and 1.0 mM benserazide on modified sensors. After polyaniline was coated on the GCE surface, a) 20 µL of 0.2% MWCNT was dropped, b) 10 µL of 0.5% MWCNT was dropped, c) 20 µL of 0.5% MWCNT was dropped, d) 10 µL of 1.0% MWCNT was dropped, e) 20 µL of 1.0% MWCNT was dropped on the sensors, and f) 20 µL of 0.2% MWCNT was dropped, g) 10 µL of 0.5% MWCNT was dropped, h) 20 µL of 0.5% MWCNT was dropped, i) 10 µL of 1.0% MWCNT was dropped, j) 20 µL of 1.0% MWCNT was dropped on the GCE surface and then coated with polyaniline. DPV responses on covered sensors.

Calibration curve studies are based on the linear correlation between anodic oxidation peak currents and concentration. The measurement limits were calculated from the formulas limit of detection (LOD) = 3 × s/m and lower limit of detection (LOQ) = 10 × s/m. In these formulas, s is the standard deviation of repeated peak currents (with 10 repetitions) at a certain concentration in the calibration range, and m is the slope value of the relevant calibration curve [25].

The precision of the method was calculated by using the DPV technique from 10 independent repetitions of 1.0 mM benserazide (BS) and 1.0 mM levodopa (LD) solutions on the same day (within-day repeatability) and by taking measurements for 5 consecutive days (within-day repeatability). In between-day measurements, 3 repetitions

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were made and the average was taken into account. Precision and accuracy, evaluated as relative standard deviation (% RSD), were calculated by dividing the within-day and between-day standard deviation by the average of the measurement values and multiplying by 100.

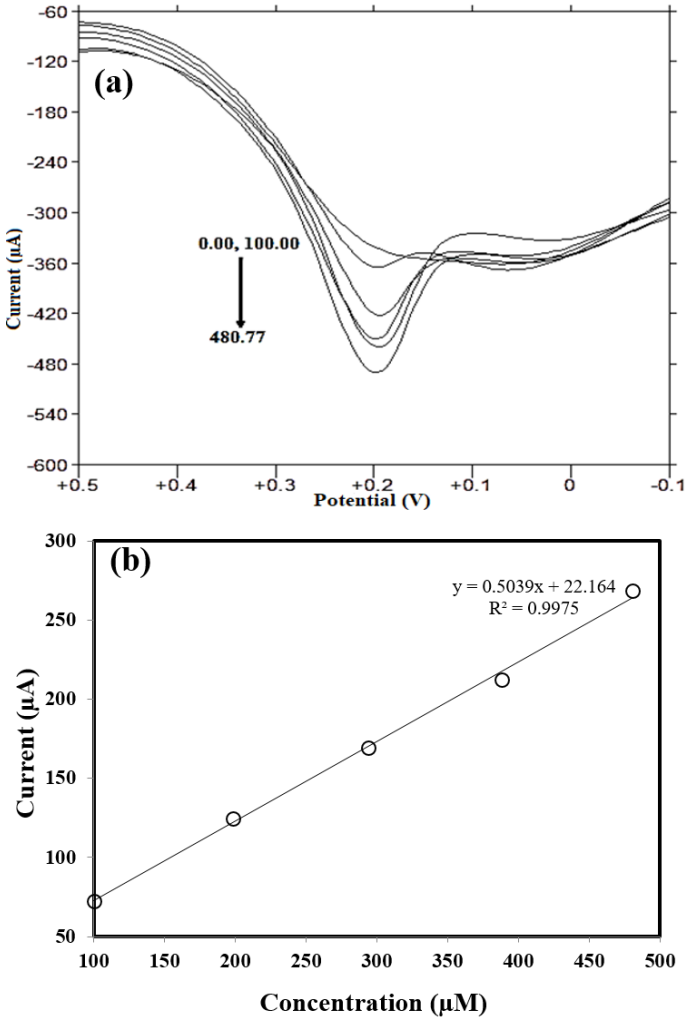

Fig. 4. (a) DPV responses of benserazide in the modified MWCNT/AN/GCE sensor in the concentration range of 0.0, 100.0 - 480.77 µM. (b) Calibration graph of benserazide in the concentration range of 100.00 - 480.77 µM.

1.0 mM stock solutions of the active ingredients were prepared in ultrapure water and the necessary concentrations for calibration studies were diluted from these stock solutions and used. In the detection limit studies, standard solutions with the lowest concentration of 1.0 µM were used and certain volumes of the 1.0 mM stock solution were added to the pH 7.0 PBS electrolyte medium. The concentrations were recalculated considering the dilution. Three replicates were made for each concentration and the average of the results was taken. The differential pulse voltammograms and calibration graph of increasing concentrations of benserazide (0.00, 100.00, 198.02, 294.12, 388.35, 480.77 µM) in 0.1 M PBS with pH 7.0 in the modified modified MWCNT/AN/GCE sensor are shown in Figure 4. In addition, ten consecutive DPV responses of 388.35 µM benserazide for the stability test of the modified MWCNT/AN/GCE sensor are shown in Figure 5. The differential pulse voltammograms of the modified MWCNT/AN/GCE sensor for increasing concentrations of levodopa (0.00, 10.00, 19.98, 29.94, 39.88, 49.80, 99.11, 196.27, 291.54, 384.99, 476.64, 566.57, 654.82, 741.43, 826.45, 909.92 µM) in 0.1 M PBS, pH 7.0, and the calibration graph of the responses in the concentration range of 10-50 µM are shown in Figure 6. Ten consecutive DPV responses of 826.45 µM levodopa for the stability test of the modified MWCNT/AN/GCE sensor are shown in Figure 7. The characteristics of the calibration curves and related validation parameters using the data obtained by DPV technique of benserazide and levodopa in the modified MWCNT/AN/GCE sensor are summarized in Table 1.

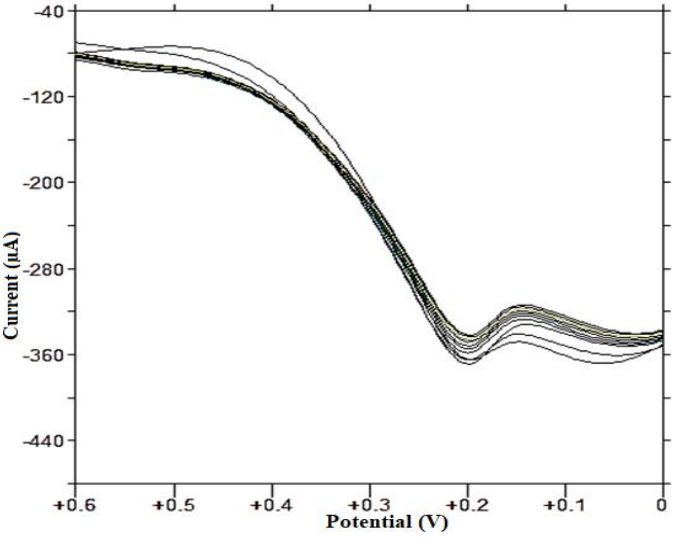

Fig. 5. Ten-repeat DPV responses of 388.35 µM benserazide in the modified MWCNT/AN/GCE sensor.

3.5. Effect of interference in the analysis of levodopa and benserazide binary mixture

The effect of ascorbic acid interference in the 0.1 M PBS electrolyte medium with pH 7.0 of the binary mixture of levodopa and benserazide was studied. For this purpose, the concentration of ascorbic acid and benserazide was kept constant and the concentration of levodopa was increased. When Figure 8(a) is examined, the peaks of ascorbic acid and benserazide remained the same, while the peaks of levodopa increased linearly. In addition, the concentration of the binary mixture of levodopa and benserazide was kept constant and the concentration of ascorbic acid was increased (Figure 8(b)). While the peak current heights remained constant for levodopa and benserazide, the peak current heights increased proportionally with the increase in the concentration of ascorbic acid.

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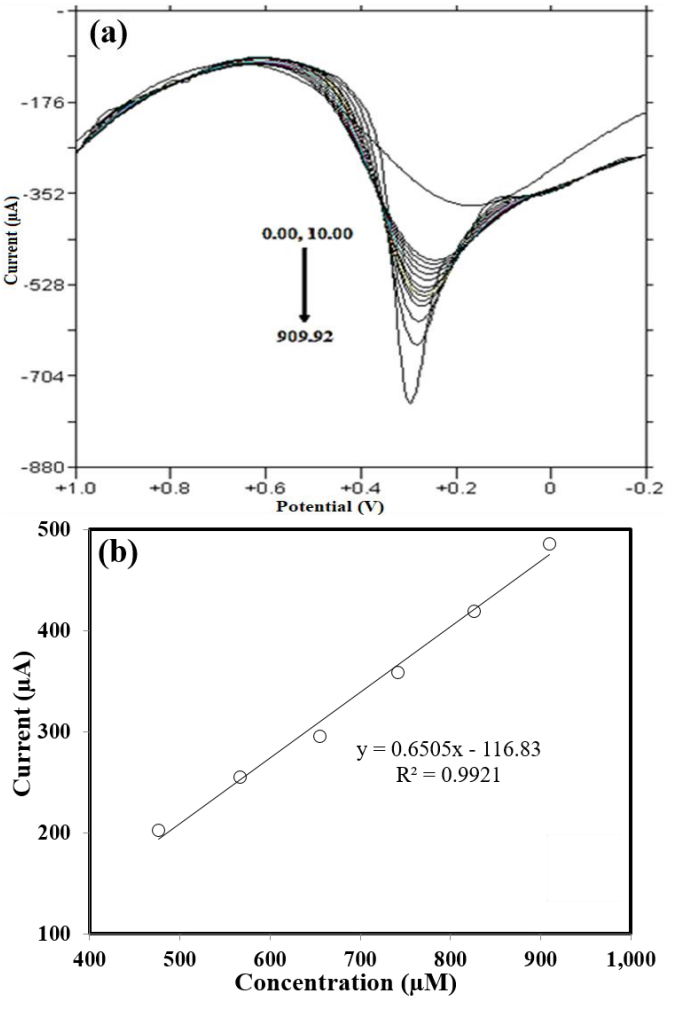

Fig. 6. (a) DPV responses of levodopa in the modified MWCNT/AN/GCE sensor in the concentration range of 0.0, 10.0 - 990.92 µM. (b) Calibration graph for levodopa in the concentration range of 476.64 - 909.92 µM.

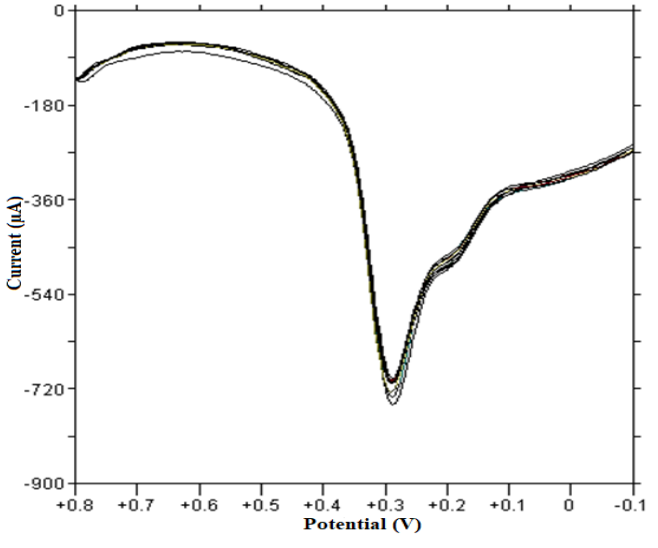

Fig. 7. Ten DPV responses of 826.45 µM concentration of levodopa on the modified MWCNT/AN/GCE sensor.

In the determination of the binary mixture in the presence of ascorbic acid, ten consecutive measurements were taken at five-second intervals in order to see the stability of the measurement and the voltammograms obtained are shown in Figure 8(c).

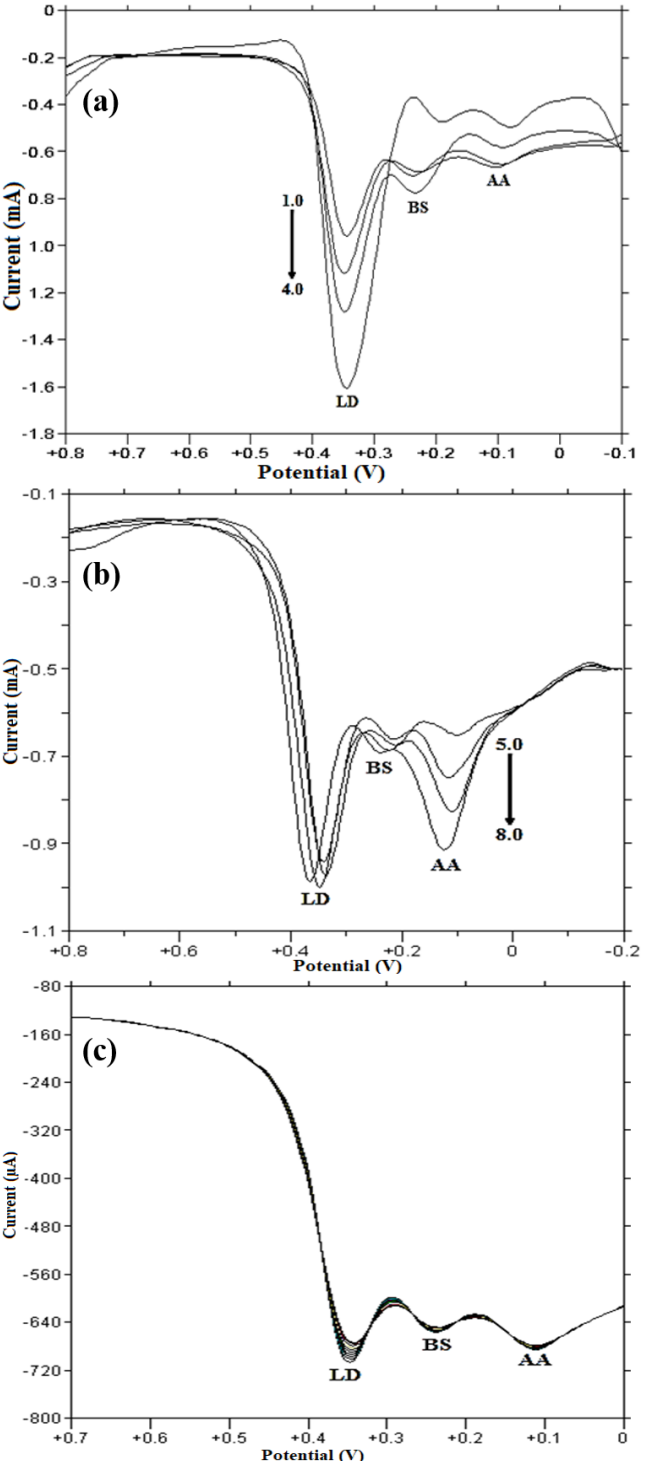

Fig. 8. DPV responses of the modified MWCNT/AN/GCE sensor in PBS buffer in the presence of, (a) 5 mM ascorbic acid (AA) and 0.1 mM benserazide (BS) with levodopa (LD) (1.0, 2.0, 3.0, 4.0 mM), (b) 0.1 mM LD, 0.1 mM BS and AA (5.0, 6.0, 7.0, 8.0 mM), and (c) 0.1 mM BS and 1.0 mM LD in the presence of 6.0 mM AA.

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Table 1. Validation data obtained from modified MWCNT/AN/GCE sensor in the quantitative determination of benserazide and levodopa.

	Benserazide	Levodopa
The equation of the calibration curve	I(μA) = 0.5039C(μM) + 22.164	I(μA) = 0.6505C(μM) -116.83
Measured Potential (mV)	212	290
Linearity Range (M)	1.0×10^{-4} - 5.0×10^{-4}	5.0×10^{-4} - 10.0×10^{-4}
Slope (μA.μM-1)	0.504	0.650
Intercept (μA)	22.164	-116.830
Correlation Coefficient	0.99750	0.99208
Standard Deviation of Slope	0.015	0.029
Standard Deviation of Intercept	4.69	20.68
Limit of Determination (μM) (LOD)	163.60	67.01
Lower Limit of Determination (μM) (LOQ)	545.35	223.36
Intraday repeatability of potential (%RSD)	0.77	0.51
Inter-day repeatability of potential (%RSD)	2.23	0.97
Intraday repeatability of current (%RSD)	17.42	3.30
Inter-day repeatability of current (%RSD)	88.14	12.23

No shift was observed in the peak potentials of all analytes, while the peak current heights remained constant. The fact that this result was given in the presence of ascorbic acid at the physiological pH value of pH 7.0 of the binary mixture is promising that the binary mixture can be directly determined by this method in real samples.

The interference effect of the binary mixture of levodopa-benserazide on ascorbic acid in urine samples was studied and 1 mL of urine sample was taken and 9 mL of buffer was added while preparing the samples. For this purpose, the concentration of levodopa and benserazide was kept constant and the concentration of ascorbic acid was increased (Figure 9 (a)), and the concentration of ascorbic acid and benserazide was kept constant and the concentration of levodopa was increased (Figure 9(b)). In addition, in the determination of the binary mixture in the presence of ascorbic acid in urine medium, 10 consecutive measurements were taken at 5 s intervals in order to see the stability of the measurement and the obtained voltammograms are shown in Figure 9(c). The interference effect studies in urine samples prove that these three species can be selectively separated and determined very well in real samples.

3.6. Real sample applications and recovery studies

To evaluate the practical applicability and reliability of the developed sensor, real sample analysis and recovery studies were performed using pharmaceutical tablet formulations and biological samples. The sensor's effectiveness was assessed through the determination of levodopa and benserazide in commercially available Madopar tablets and human urine samples, ensuring its feasibility for real-world applications.

For pharmaceutical sample analysis, Madopar tablets, which contain levodopa and benserazide as active ingredients, were prepared at appropriate concentrations. A 1.0 mL aliquot of the prepared solution was diluted to a final volume of 10 mL using 0.1 M phosphate-buffered saline (PBS) at pH 7.0. To ensure the reproducibility and accuracy of the method, five replicate samples were prepared and analyzed using differential pulse voltammetry (DPV) on the modified multi-walled carbon nanotube/aniline/glassy carbon electrode (MWCNT/AN/GCE) sensor. The electrochemical responses obtained were used to quantify the active substances by referencing the pre-established calibration curves, and the corresponding recovery values were calculated.

In addition to pharmaceutical formulations, the sensor's performance was further validated in complex biological matrices by analyzing urine samples spiked with known amounts of the target analytes. Since urine contains various endogenous compounds that could potentially interfere with the electrochemical response, a sample pretreatment step was employed to minimize matrix effects. Urine samples were diluted in a 1:10 ratio with 0.1 M PBS buffer (pH 7.0), after which precise amounts of levodopa and benserazide standard solutions were introduced to achieve the desired analyte concentrations. The prepared samples were then subjected to DPV analysis using the modified sensor, and the obtained recovery values were calculated to assess the sensor's accuracy in detecting the target compounds within a complex biological environment. The results of these recovery studies for the Madopar tablet solutions are summarized in Table 2, while Table 3 presents the recovery values obtained for urine sample analysis.

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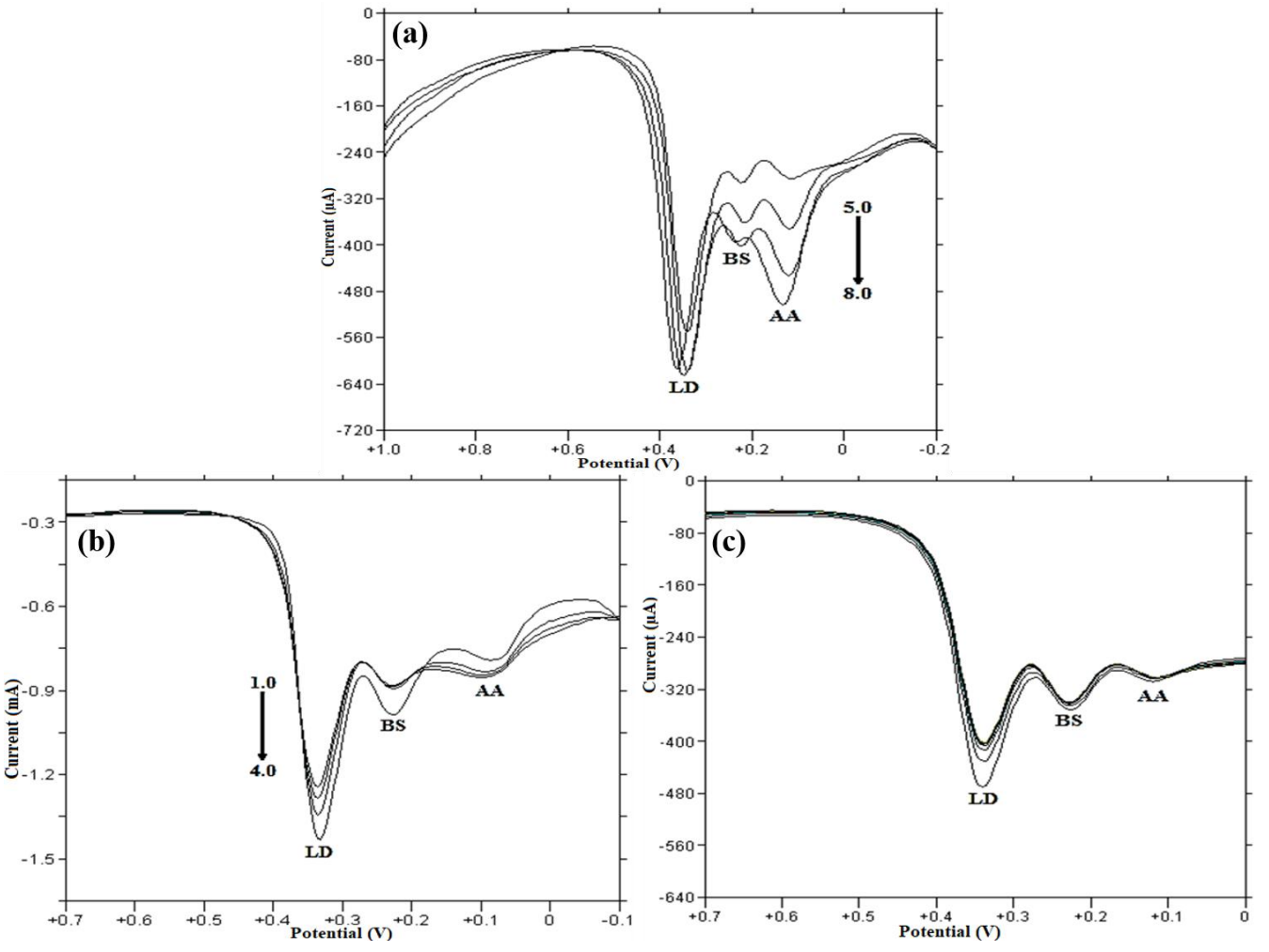

Fig. 9. (a) DPV responses of modified MWCNT/AN/GCE sensor in urine sample in the presence of 0.1 mM LD, 0.1 mM BS and AA (5.0, 6.0, 7.0, 8.0 mM), (b) LD in the presence of 5 mM AA and 0.1 mM BS (1.0, 2.0, 3.0, 4.0 mM), and (c) 0.1 mM LD, 0.1 mM BS and 5.0 mM AA.

Table 2. Calculated results for Madopar tablet sample.

	Amount in tablet (mg)		Measured Amount (mg)		% Recovery	
Sample	**LD**	**BS**	**LD**	**BS**	**LD**	**BS**
1	1.97	0.99	1.96	0.98	99.36	99.60
2	1.97	0.99	1.97	0.98	99.77	99.18
3	1.97	0.99	1.96	0.93	99.28	93.84
4	1.97	0.99	1.94	0.92	98.22	92.85
5	1.97	0.99	1.93	0.91	97.65	91.99
				X[a]	98.85	95.49
				s[b]	0.79	3.24
				%RSD[c]	0.80	3.4
				% RE[d]	1.15	4.51

[a] X represents the mean of the results, [b] s represents the standard deviation, [c] RSD% represents the coefficient of variation, [d] RE% represents the percent relative error.

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Table 3. Results calculated from DPV responses with the addition of standard active ingredients to Madopar tablet sample and urine sample.

	Measured Amount (mg)						% Recovery			
	Amount added (mg)		Tablet		Urine		Tablet		Urine	
Sample	LD	BS	LD	BS	LD	BS	LD	BS	LD	BS
1	0.197	0.294	0.197	0.299	0.184	0.271	99.92	101.7	93.12	92.22
2	0.197	0.294	0.189	0.271	0.185	0.277	96.07	92.22	93.74	94.48
3	0.197	0.294	0.193	0.293	0.183	0.281	97.65	99.83	92.57	95.64
4	0.197	0.294	0.201	0.281	0.182	0.279	101.9	95.64	92.16	95.10
5	0.197	0.294	0.197	0.277	0.175	0.269	99.82	94.48	88.66	91.49
						X	99.07	96.77	92.05	93.79
						s	2.020	3.478	1.776	1.635
						RSD%	2.039	3.595	1.930	1.744
						RE%	0.928	3.232	7.951	6.214

The sensor demonstrated high accuracy and precision, with recovery values falling within acceptable analytical limits, indicating its robustness and reliability. The successful application of the sensor for pharmaceutical and urine sample analysis highlights its potential for use in routine quality control processes within the pharmaceutical industry, as well as for clinical diagnostics and therapeutic drug monitoring. The demonstrated sensitivity and specificity of the sensor in real sample analysis further establish its suitability for rapid and cost-effective detection of levodopa and benserazide in practical applications.

4. CONCLUSION

In this study, an advanced electrochemical sensor based on polyaniline and MWCNT-modified glassy carbon electrode was successfully designed for the simultaneous determination of levodopa and benserazide. The unique combination of polyaniline and MWCNTs provided remarkable improvements in electrocatalytic activity, selectivity, and sensitivity. Systematic investigations into the effect of film thickness, supporting electrolytes, and pH conditions confirmed that a polyaniline film grown at 1300 mV for 10 s in PBS (pH 7.0) offered the optimal sensor response. The sensor demonstrated robust analytical performance, exhibiting clear and distinct oxidation peaks for levodopa and benserazide with well-defined peak separation. The developed method provided low detection limits and a wide linear dynamic range, making it suitable for trace-level detection of these compounds. Additionally, validation studies confirmed excellent repeatability, stability, and precision in both standard solutions and complex biological matrices such as urine samples. The presence of ascorbic acid, a potential interfering species, did not significantly affect sensor performance, further reinforcing its specificity. A key advantage of the developed sensor lies in its real-world applicability. The method was successfully employed for the quantitative determination of levodopa and benserazide in pharmaceutical formulations, achieving satisfactory recovery values. These results suggest that the sensor could be utilized for routine quality control in pharmaceutical industries and clinical diagnostics, offering a rapid, cost-effective, and reliable alternative to conventional analytical techniques. Future research could explore further modifications to enhance sensor performance, including the incorporation of other conductive nanomaterials and polymeric matrices. Additionally, miniaturization of the sensor for point-of-care applications and integration into portable diagnostic devices could expand its practical usability in medical and pharmaceutical settings. Overall, the developed polyaniline/MWCNT/GCE sensor represents a promising advancement in electrochemical sensing technology for biomedical and pharmaceutical analysis.

DECLARATIONS

Ethical Approval

We affirm that this manuscript is an original work, has not been previously published, and is not currently under consideration for publication in any other journal or conference proceedings. All authors have reviewed and approved the manuscript, and the order of authorship has been mutually agreed upon.

Funding

Not applicable

Availability of data and material

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All of the data obtained or analyzed during this study is included in the report that was submitted.

Conflicts of Interest

The authors declare that they have no financial or personal interests that could have influenced the research and findings presented in this paper. The authors alone are responsible for the content and writing of this article.

Authors' contributions

All authors contributed equally in the preparation of this manuscript.

ACKNOWLEDGEMENTS

This study was supported by the Inonu University Scientific Research Projects Unit with Project number 2013-54.

REFERENCES

[1] Goodman, G., Rall, T.W., Nıes, A.S, Taylor, P. **1990**. The Pharmacological Basis of Therapeutics, 18th Ed, Pergamon Pres, New York.

[2] Atta, N.F., Galal, A. and El-Gohary, A.R, **2020**. Crown ether modified poly(hydroquinone)/carbon nanotubes based electrochemical sensor for simultaneous determination of levodopa, uric acid, tyrosine and ascorbic acid in biological fluids, *Journal of Electroanalytical Chemistry,* 863, p.114032.

[3] Beitollahi, H., Safaei, M. and Tajik, S., **2020.** Electrochemical deduction of levodopa by utilizing modified electrodes: A review, *Microchemical Journal*, 152; 104287.

[4] Li, Z.Y., Gao, D.Y., Wu, Z.Y. and Zhao, S., **2020.** Simultaneous electrochemical detection of levodapa, paracetamol and l-tyrosine based on multi-walled carbon nanotubes, *RSC Advances,* 10(4), p.14218-14224.

[5] Safaei, M., Beitollahi, H. and Shishehbore, M.R., **2020**. Electrochemical Sensing of Levodopa in Presence of Tryptophan Using Modified Graphite Screen Printed Electrode with Magnetic Core-Shell Fe3O4@SiO2/GR *Nanocomposite, Surface Engineering and Applied Electrochemistry*, 56(2): 184-191.

[6] Ghalehno, M.H., Mirzaei, M. and Torkzadeh-Mahani, M., **2019.** Electrochemical Determination of Levodopa on Carbon Paste Electrode Modified with Salmon Sperm DNA and Reduced Graphene Oxide-Fe3O4 Nanocomposite, *Russian Journal Of Electrochemistry*, 55(10), p.933-942.

[7] Ommaty R.**2009**. Vademecum Modern Medicine Guide, 32ed Pelikan Publishing.

[8] Savan, E.K. and Erdogdu, G. **2019**. Application of Single-Walled Carbon Nanotube and Poly (3-Methylthiophene) Modified Sensors for Electrochemical Determination of Levodopa and Benserazide, *Analytical & Bioanalytical Electrochemistry*, 11(5), p.585-597.

[9] Savan, E.K., Pasahan, A., Aksoy, B., Gungor, O., Koytepe, S. and Seckin, T., **2016**. Preparation and properties of selective polyurethane films and their use for the development of biomedical dopamine sensor, *International Journal of Polymeric Materials and Polymeric Biomaterials*, 65(8), p.402-408.

[10] Miraki M., Karimi-Maleh H., Taher, M.A., Cheraghi, S., Karimi, F., Agarwal, S. and Gupta, V.K., **2019**. Voltammetric amplified platform based on ionic liquid/NiO nanocomposite for determination of benserazide and levodopa, *Journal of Molecular Liquids*, 278, p.672-676.

[11] Pan, L., Guo, Y.and Li, Z., **2010.** Simultaneous Determination of Levodopa, Benserazide and 3-O-Methyldopa in Human Serum by LC–MS–MS, *Chromatographia*, 72(7-8), p.627-633.

[12] Dinc, E. and Ustundag O., **2002**. Chemometric resolution of a mixture containing hydrochlorothiazide and amiloride by absorption and derivative spectrophotometry, *Journal of Pharmaceutical and Biomedical Analysis,* 29(1-2), p.371-379.

[13] Marques, K.L., Santos, J.L., Lopes, J.A. and Lıma, J.L., **2008**. Simultaneous chemiluminometric determination of levodopa and benserazide in a multi-pumping flow system with multivariate calibration, Analytical Sciences, 24(8), p.985-991.

[14] Özkan, S.A. and Uslu, B. and Aboul-Enein, H.Y., **2003.**Analysis of Pharmaceuticals and Biological Fluids Using Modern Electroanalytical Techniques. *Critical Reviews in Analytical Chemistry*, 33, p.155-181.

[15] Aksoy, B. Pasahan, A., Gungor, O., Koytepe, S. and Seckin, T., **2017**. A novel electrochemical biosensor based on polyimide- boron nitride composite membranes, *International Journal of Polymeric*

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Materials and Polymeric Biomaterials, 66(4), p.203-212.

[16] Kuyumcu Savan, E. and Erdoğdu, G., **2017**. Simultaneous determination of levodopa and benserazide using poly(3-methylthiophene) and a multi-walled carbon nanotube sensor, *Journal of Solid State Electrochemistry,* 21(8), p.2209-2217.

[17] Gungor, O., Pasahan, A., Cengiz, M.A., Koytepe, S. and Seckin, T., **2015**. Fructose-Based Polyurethane Membranes: Synthesis, Characterization, and Their Use as Voltammetric pH Electrode, International Journal of Polymeric Materials and Polymeric Biomaterials, 64(11), p.563-569.

[18] Pasahan, A, Koytepe, S, Cengiz, M.A. and Seckin T., **2013.** Synthesis and characterization of polyurethanes containing glucose for selective determination of epinephrine in the presence of a high concentration of ascorbic acid, *Polymer International,* 62(2), p.246-250.

[19] Hu, C.G., Wang, W.L., Liao, K.J. and Wang, Y.T., **2005**. Simultaneous measurement of epinephrine and ascorbic acid at the carbon nanotube electrode, *Journal of Metastable and Nanocrystalline Materials*, 23, p.305-308.

[20] Ouveria-Caridade, C., Pauliukaite, R. and Brett, C.M.A., **2008.** Development of electrochemical oxidase biosensors based on carbon nanotube-modified carbon film electrodes for glucose and ethanol, *Electrochima Acta,* 53, p.6732-6739.

[21] Wang, J.X., Li, M.X., Shi, Z.J., Li, N.Q. and Gu, Z.N., **2002.** Direct electrochemistry of cytochrome c at a glassy carbon electrode modified with single-wall carbon nanotubes, *Analytical Chemistry,*74, p.1993-1997.

[22] Wu, Z., Zhao, H., Xue, Y., He, Y., Li, X. and Yuan, Z., **2010.** Poly (pyridine-3-boronic acid)/Multiwalled Carbon Nanotubes Modified Glassy Carbon Electrodes for Simultaneous, *Electroanalysis,* 22(19), p.2196-2201.

[23] Liu, A., Honma, I. and Zhou, H., **2005.** Amperometric biosensor based on tyrosinase-conjugated polysacchride hybrid film: Selective determination of nanomolar neurotransmitters metabolite of 3,4-dihydroxyphenylacetic acid (DOPAC) in biological fluid, Biosensors and Bioelectronics, 21, p.809–816.

[24] Tsang, SC., Chen, Y.K., Harrıs, P.J.F. and Green, M.L.H.**, 1994**. A simple chemical method of opening and filling carbon nanotubes, *Nature*, 372, p.159-162.

[25] Ermer, J.and Miller, J.H., **2005.** Method validation in pharmaceutical analysis, Wiley-VCH, Weinheim.

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