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RESEARCH ARTICLE

Highly Selective and Sensitive Electrochemical Detection of Levodopa and Benserazide Using SWCNT–Modified Polyaniline–Based Sensor

Ebru Kuyumcu Savan [1,*], Gamze Erdoğdu [2]

ABSTRACT: This study presents the development of a highly selective and sensitive electrochemical sensor for the simultaneous determination of levodopa (LD) and benserazide (BS), essential pharmaceutical compounds in Parkinson's disease treatment. The sensor was fabricated through electropolymerization of aniline on a glassy carbon electrode (GCE) in a nonaqueous medium, followed by modification with single-walled carbon nanotubes (SWCNTs). The incorporation of SWCNTs enhanced the electrode's conductivity, surface area, and electrocatalytic properties, leading to improved detection performance. Various experimental parameters, including pH, polymerization conditions, and SWCNT concentration, were systematically optimized to achieve maximum sensitivity and selectivity. The developed sensor exhibited a linear response range of 500–1000 µM for LD and 100–500 µM for BS, with detection limits of 183.2 µM and 44.5 µM, respectively. The sensor's robustness was demonstrated by its stability in the presence of ascorbic acid, a common interfering species, ensuring accurate determination of target analytes. Furthermore, real sample analysis was conducted using pharmaceutical formulations and human urine, confirming the practical applicability of the proposed sensor. The results indicate that the SWCNT-modified polyaniline-based electrochemical sensor offers a rapid, reliable, and cost-effective approach for the simultaneous quantification of LD and BS, making it a promising tool for pharmaceutical and clinical applications.

Keywords: Electrochemical sensor, Levodopa, Benserazide, Polyaniline, Single-walled Carbon nanotubes.

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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].

[1] Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, İnönü University, 44280, Malatya, Turkey
[2] Department of Chemistry, Faculty of Arts and Sciences, İnönü University, Malatya, Turkey

*Author to whom correspondence should be addressed:
ebru.savan@inonu.edu.tr (Ebru Kuyumcu Savan)

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, more sensitive, and cheaper are 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 was 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 (SWCNT, diameter: <2 nm, EC: >100 S/cm, length: 5-30 µm, purity: >95%, surface area: >380 m^2/g) was supplied by Grafen Inc., aniline was supplied by Acros Organics, tetrabutylammonium tetrafluoroborate 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 an argon gas atmosphere.

2.3. 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. To determine the oxidation potential of aniline, the 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. SWCNT solutions at concentrations of 0.2%- 0.5%- 1.0% (mg/µL) were prepared. To functionalize the SWCNT, 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. In the first modification procedure, polyaniline was electrochemically coated on the GCE surface with the BE technique at 1300 mV for 10 seconds, and 10 µL or 20 µL of SWCNT dispersions were dropped on it. In the second modification procedure, 10 µL or 20 µL of SWCNT dispersions were dropped on the GCE surface and dried at room temperature for 1 day. And then the surface of these electrodes was electrochemically coated with polyaniline.

3. RESULTS AND DISCUSSION

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 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 1. 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 BE were studied. 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.

3.2. Supporting 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 solution (PBS), Britton-Robinson buffer solution (BRBS), and acetate buffer solution (ABS) 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 ABS were investigated on the modified electrode. The highest current response and the best peak resolution were measured in a PBS pH 7.0 medium.

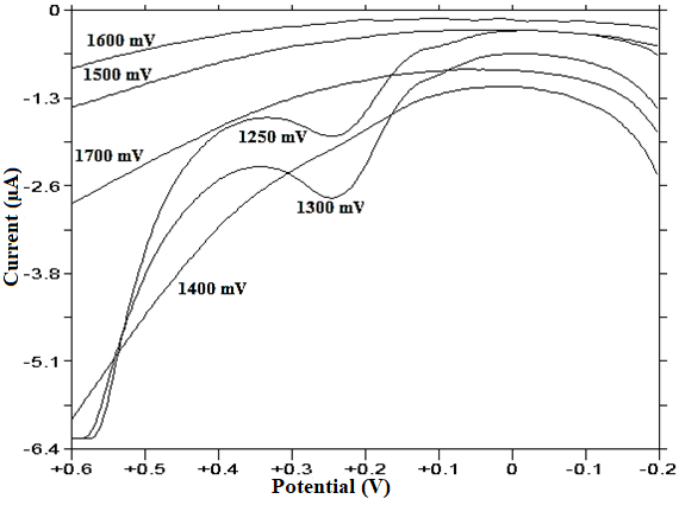

Fig. 1. DPV responses to 1.0 mM levodopa of polyaniline electrodes grown at various potentials by the 10 s BE method.

3.3. Determination of benserazide and levodopa at 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 at the prepared modified sensors were investigated. As a result of comparing the DPV responses of 1.0 mM benserazide and 1.0 Mm levodopa at modified sensors, the voltammograms in Figure 2 were obtained. When the differential pulse voltammograms were examined, the best separation was achieved at the modified sensor (SWCNT/AN/GCE) obtained by coating polyaniline (AN) on GCE and dropping 20 µL of 1.0% SWCNT on it. The current values obtained were also more satisfactory. A peak current of 4.139 x 10^{-4} A was obtained at 180 mV for benserazide and 9.737 x 10^{-4} A was obtained at 324 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 the 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).

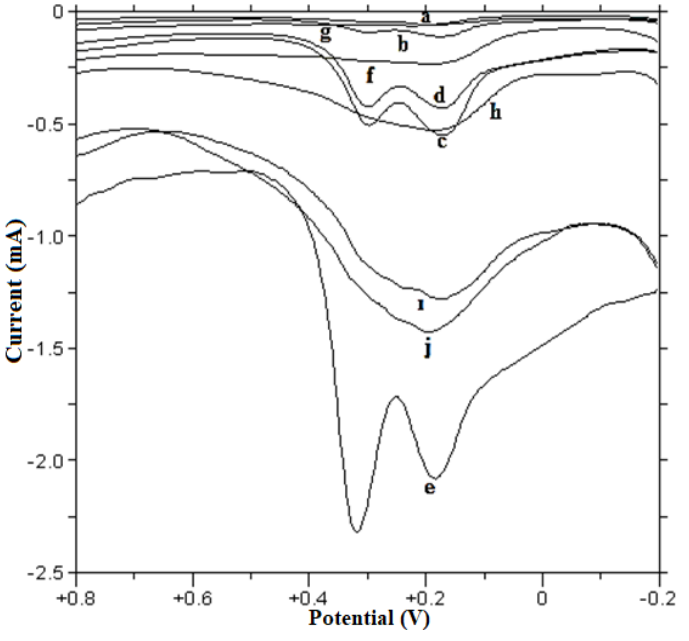

Fig. 2. DPV responses of a mixture of 1.0 mM levedopa and 1.0 mM benserazide at modified sensors, after polyaniline was coated on the GCE surface, (a) 20 µL of 0.2% SWCNT was dropped, (b) 10 µL of 0.5% SWCNT was dropped, (c) 20 µL of 0.5% SWCNT was dropped, (d) 10 µL of 1.0% SWCNT was dropped, (e) 20 µL of 1.0% SWCNT was dropped on the sensors, and (f) 20 µL of 0.2% SWCNT was dropped, (g) 10 µL of 0.5% SWCNT was dropped, (h) 20 µL of 0.5% SWCNT was dropped, (i) 10 µL of 1.0% SWCNT was dropped, (j) 20 µL of 1.0% SWCNT was dropped on the GCE surface and then coated with polyaniline.

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 [24]. 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 and by taking measurements with 3 repetitions for 5 consecutive days.

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 at the modified SWCNT/AN/GCE sensor are shown in Figure 3. The differential pulse voltammograms at the modified SWCNT/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 4. The characteristics of the calibration curves and related validation parameters using the data obtained by the DPV technique of benserazide and levodopa in the modified SWCNT/AN/GCE sensor are summarized in Table 1.

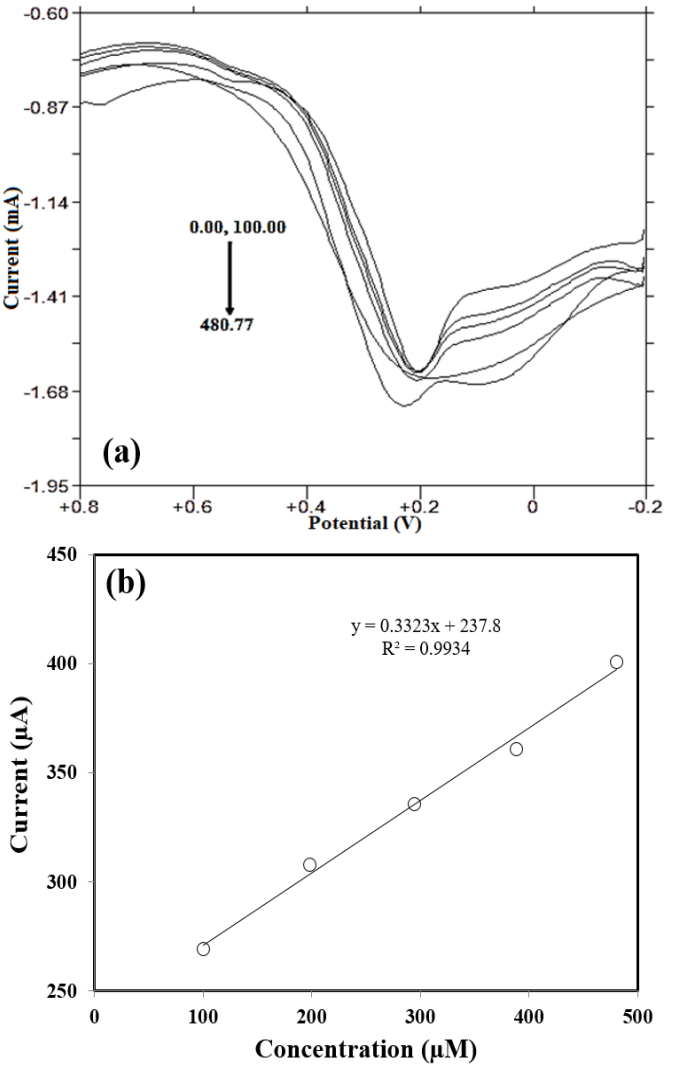

Fig. 3. (a) DPV responses of benserazide at the modified SWCNT/AN/GCE sensor in the concentration range of 0.0, 100.0 - 480.77 µM. (b) Calibration graph of benserazide in the concentration range of 100.00 - 480.77 µM.

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

	Benserazide	Levodopa
The equation of the calibration curve	I (µA) = 0.3323C(µM) + 237.8	I (µA) = 1.2583C(µM) - 202.06
Measured Potential (mV)	248	308
Linearity Range (M)	1.0×10^{-4} - 5.0×10^{-4}	5.0×10^{-4} - 10.0×10^{-4}
Slope (µA/µM)	0.332	1.2583
Intercept (µA)	237.80	-202.059
Correlation Coefficient	0.993393	0.99147
Standard Deviation of Slope	0.15644	0.058351
Standard Deviation of Intercept	5.0337	41.52
LOD (µM)	44.486	183.15
LOQ (µM)	148.29	610.498
Intraday repeatability of potential (RSD%)	2.2936	0.6825
Inter-day repeatability of potential (RSD%)	2.5415	0.5500
Intraday repeatability of current (RSD%)	17.299	4.190
Inter-day repeatability of current (RSD%)	27.592	12.23

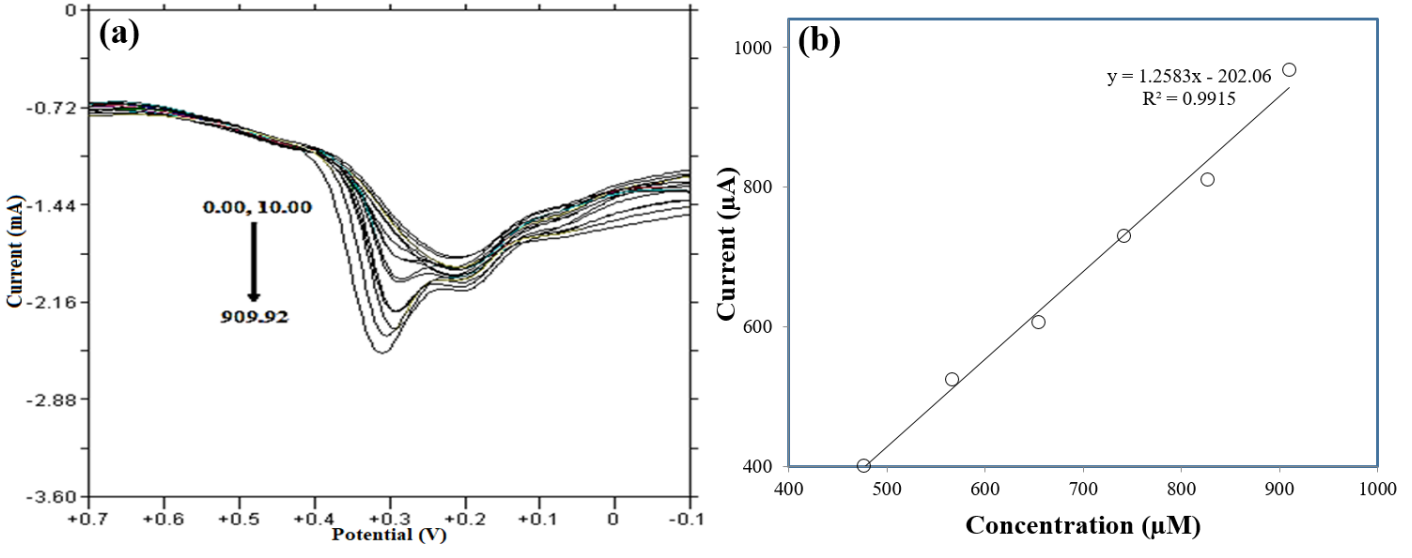

Fig. 4. (a) DPV responses of levodopa at the modified SWCNT/AN/GCE sensor in the concentration range of 0.0, 10.0 - 990.92 µM. (b) Calibration graph for levodopa in the concentration range of 476.64 - 909.92 µM.

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 5(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 5(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. In the determination of the binary mixture in the presence of ascorbic acid, ten consecutive measurements were taken at five-second intervals to see the stability of the measurement, and the voltammograms obtained are shown in Figure 5(c). No shift was observed in the peak potentials of all analytes, while the peak current heights remained constant.

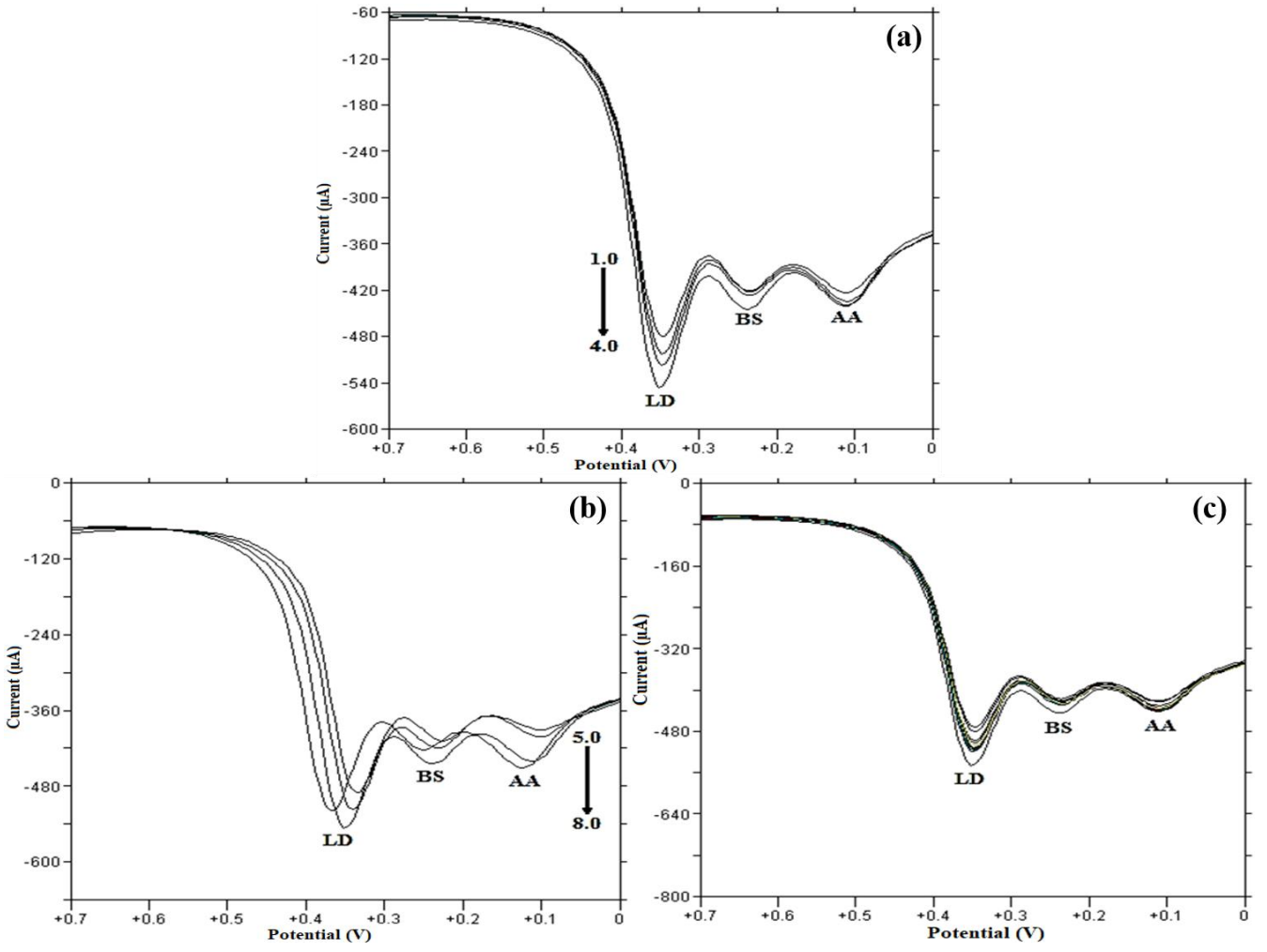

Fig. 5. DPV responses of the modified SWCNT/AN/GCE sensor in PBS buffer in the presence of (a) 5 mM AA and 0.1 mM BS with LD (1.0, 2.0, 3.0, 4.0 mM), (b) 0.1 mM LD, 0.1 mM BS and AA (5.0, 6.0, 7.0, 8.0 mM), (c) 0.1 mM BS and 1.0 mM LD in the presence of 6.0 mM AA.

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. 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 6(a)), and the concentration of ascorbic acid and benserazide was kept constant and the concentration of levodopa was increased (Figure 6(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 to see the stability of the measurement and the obtained voltammograms are shown in Figure 6(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

Solutions of tablet applications containing levodopa-benserazide (Madopar) binary mixtures were prepared to contain the active substance at appropriate concentrations. 1.0 mL of this solution was taken and completed to 10 mL with 0.1 M PBS electrolyte with a pH of 7.0, and 5 replicate samples were prepared. The analysis of these samples was carried out using the DPV technique on the modified SWCNT/AN/GCE sensor. The corresponding active substance amounts in the mixture were found from the appropriate calibration graphs and the recovery values were calculated. In addition, the same procedures were applied to the solutions prepared by adding standard substances to urine samples, and the amount of each active substance in the mixture and the recovery values were calculated. In the application performed on the urine sample, urine was diluted with 0.1 M PBS buffer (pH 7.0) at a ratio of 1:10 and a certain volume of drug samples was added to provide the desired concentration of active substances. The recovery studies of the Madopar (100 mg levodopa / 50 mg benserazide) tablet

sample in the modified SWCNT/AN/GCE sensor are summarized in Table 2. The recovery studies of the responses in the modified SWCNT/AN/GCE sensor as a result of the addition of levodopa and benserazide standard substances to the Madopar tablet sample and urine sample are summarized in Table 3. Analytical studies and the obtained recovery values showed positive results in terms of the feasibility of the determination of binary mixtures in the modified MWCNT/AN/GCE sensor with the applied method.

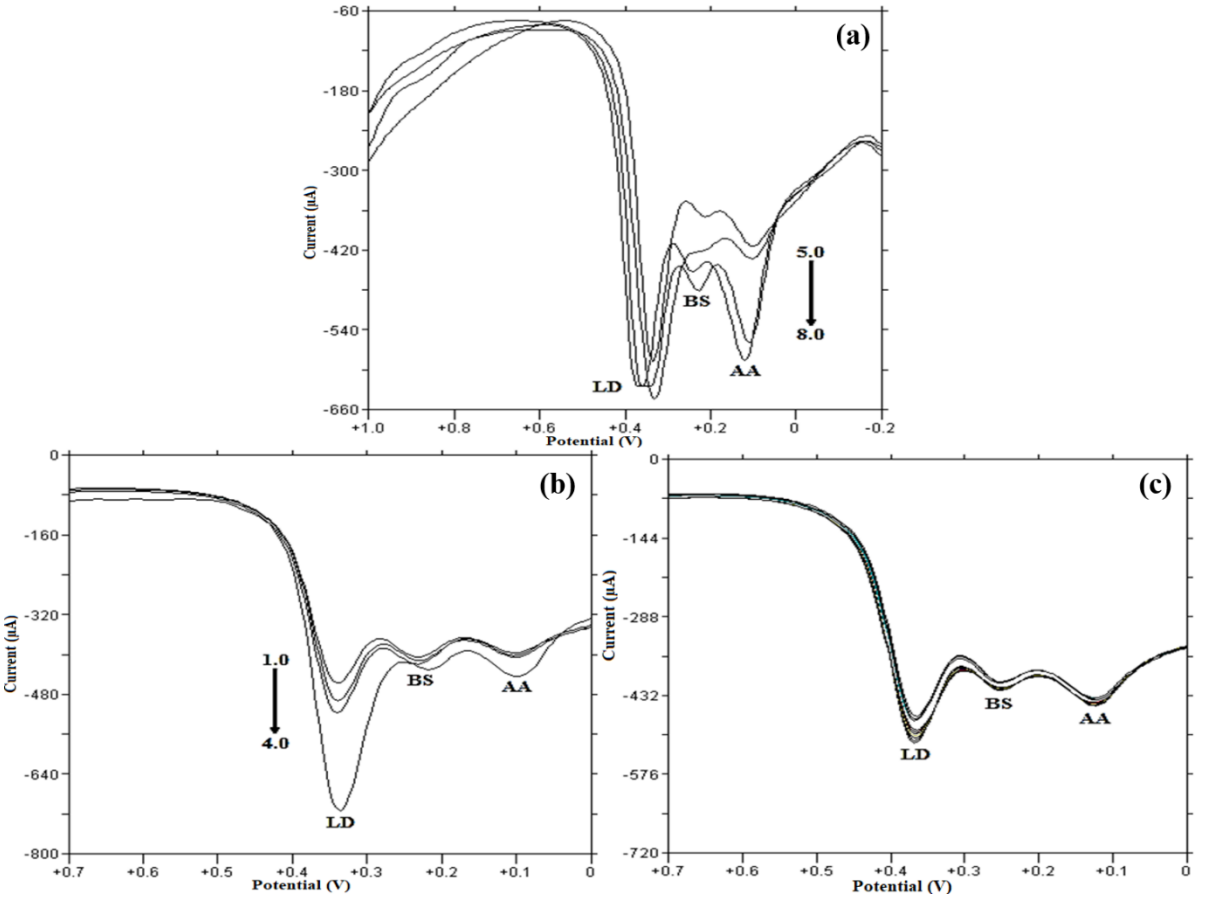

Fig. 6. (a) DPV responses of modified SWCNT/AN/GCE sensor in urine sample in the presence of 0.1 mM LD, 0.1 mM BS and AA (5.0, 6.0, 7.0, 8.0 mM), (b) LD in the presence of 5 mM AA and 0.1 mM BS (1.0, 2.0, 3.0, 4.0 mM), (c) 0.1 mM LD, 0.1 mM BS, and 5.0 mM AA.

Table 2. Calculated results for Madopar tablet sample.

	Amount in tablet (mg)		Measured Amount (mg)		Recovery %	
Sample	**LD**	**BS**	**LD**	**BS**	**LD**	**BS**
1	1.97191	0.98595	1.94874	0.98592	98.8248	99.9973
2	1.97191	0.98595	1.98045	0.94244	100.433	95.5868
3	1.97191	0.98595	1.89070	0.95234	95.8820	96.5908
4	1.97191	0.98595	1.88890	0.94315	95.7906	95.6586
5	1.97191	0.98595	1.89935	0.94995	96.3205	96.3488
				X[a]	97.4502	96.8365
				s[b]	1.85896	1.62715
				RSD%[c]	1.90760	1.68030
				RE%[d]	2.54975	3.16353

[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.

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

			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	0.198	0.293	0.188	0.281	100.6	99.3	95.34	95.76
2	0.197	0.294	0.194	0.289	0.185	0.279	98.22	98.42	93.79	94.93
3	0.197	0.294	0.192	0.287	0.178	0.273	97.21	97.82	90.11	92.91
4	0.197	0.294	0.191	0.284	0.174	0.270	96.73	96.68	87.97	91.77
5	0.197	0.294	0.200	0.282	0.171	0.268	101.2	96.19	86.86	91.35
						X	98.81	97.76	90.81	93.34
						s	1.817	1.262	3.274	1.732
						RSD%	1.839	1.291	3.605	1.856
						RE%	1.192	2.236	9.187	6.656

4. CONCLUSION

In this study, we successfully developed an electrochemical sensor utilizing polyaniline-modified glassy carbon electrodes further enhanced with SWCNTs to achieve a highly sensitive and selective detection of levodopa and benserazide. The modification process significantly improved the electrode's electrochemical response, enhancing the peak current and resolution for both analytes. The sensor demonstrated excellent performance under optimized conditions, with a pH of 7.0 in PBS medium and the application of 20 µL of 1.0% SWCNT solution, which facilitated superior electron transfer kinetics. Method validation was performed through differential pulse voltammetry (DPV), yielding well-defined calibration curves: *Ipa* (µA) = 1.2583*C*(µM) - 202.06 for LD (476.64–909.92 µM) and *Ipa* (µA) = 0.3323C(µM) + 237.8 for BS (100.00–480.77 µM). The detection limits of 183.2 µM for LD and 44.5 µM for BS affirm the high sensitivity of the fabricated sensor. Recovery studies in pharmaceutical tablet formulations and human urine samples further validated the sensor's accuracy and reliability. Importantly, the presence of ascorbic acid did not interfere with the analyte signals, demonstrating the method's selectivity and robustness. The developed sensor's ability to perform rapid, precise, and simultaneous detection without requiring complex separation techniques highlights its potential for real-world pharmaceutical and clinical applications. Its ease of fabrication, cost-effectiveness, and reproducibility make it an attractive alternative to conventional analytical methods for LD and BS detection. Future studies could explore miniaturization for point-of-care testing and further improvements in detection limits through nanomaterial enhancements. Overall, this work establishes the SWCNT-modified polyaniline-based electrochemical sensor as a powerful tool for reliable monitoring of levodopa and benserazide, contributing to better pharmaceutical quality control and clinical diagnostics.

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.

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Not applicable

Availability of data and material

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.

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