

Selective Voltammetric Determination of Dopamine Using a Palladium Particle-Modified Electrode with Molecularly Imprinted Nicotinamide Polymer

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Abstract—A method was developed to fabricate a glassy carbon electrode with electrodeposited palladium particles and a molecularly imprinted polymer derived from nicotinamide. This approach enables the determination of dopamine in the presence of structurally related compounds. The incorporation of a polymer featuring specific recognition sites tailored to the template molecule significantly enhanced the sensitivity and selectivity of dopamine detection. The immobilization of palladium particles on the electrode surface further improved the selectivity of voltammetric dopamine determination, even in the presence of adrenaline and noradrenaline, which exhibited a 200 mV difference in oxidation peak potentials. The analytical signal showed a linear bilogarithmic dependence on dopamine concentration within the range 5.0×10^{-9} to 5.0×10^{-3} M. This method was successfully applied to an analysis of urine samples, demonstrating its practical utility in real-world applications.

Keywords: chemically modified electrodes, molecularly imprinted polymer, nicotinamide, palladium particles, glassy-carbon electrode, voltammetric determination of dopamine

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Dopamine (2-(3,4-dihydroxyphenyl)ethylamine, DA) serves as both a hormone and a neurotransmitter of the sympathoadrenal system, regulating a spectrum of physiological functions in a human body, including locomotor, cognitive, and endocrine functions [1]. Impaired synthesis of dopamine is implicated in Parkinson's disease [2]. Dopamine deficiency can also manifest itself as mood disorders, depression, tremors, loss of coordination, muscle spasms, restless leg syndrome, or even gastrointestinal issues [3]. Furthermore, elevated levels of this hormone are observed in kidney diseases associated with impaired blood circulation within them [4].

The difficulty in measuring dopamine in blood plasma and other biological fluids stems from its relatively low concentrations in biological samples [5]. Dopamine also undergoes rapid oxidation by platelet monoamine oxidases in blood, necessitating rapid (15–30 min) analysis in human biological samples [6]. The determination of dopamine is often complicated by the presence of other catecholamines, such as adrenaline (AD) and noradrenaline (NAD), their derivatives, and interfering components (urea, amino acids, ascorbic and uric acids, etc.) [7]. Therefore, there is a critical need in instrumental methods with high selectivity and sensitivity for detecting markers of

diseases associated with disrupted neurotransmitter metabolism.

Voltammetry with chemically modified electrodes (CMEs) significantly enhances the analytical capabilities of the method for determining organic compounds [8, 9]. The electrochemical determination of dopamine in the presence of adrenaline or noradrenaline is conducted using CMEs coated with films based on Nafion [10], gold nanoparticles [11], and carbon materials [12], as well as their composites [13, 14].

For the selective voltammetric determination of dopamine, the development of electrodes modified with molecularly imprinted polymers (MIPs), possessing molecular memory, is highly relevant. These electrodes combine high mechanical and chemical strength, typical of crosslinked materials with high selectivity towards target biomolecules due to the formation of complexes between the template molecule and functional groups of the polymer [15, 16].

In recent decades, electrochemical analysis has demonstrated extensive development through the application of novel materials in the construction of various sensors. These materials enable the creation of sensors with high selectivity and sensitivity, capable of recognizing organic substances in biological fluids in the presence of interfering components. Monomers

used in molecularly imprinted polymer synthesis can exhibit high toxicity and mutagenicity [17], highlighting the importance of environmentally friendly materials such as nicotinamide (NA). Nicotinamide is a key component of the vitamin B group complex and a reactive moiety of nicotinamide adenine dinucleotide, making it a promising material for MIP synthesis [18].

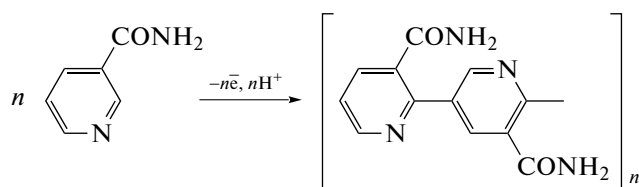
The present study explores the feasibility of using a glassy-carbon electrode (GCE) modified with a composite of electrodeposited palladium nanoparticles and a molecularly imprinted polymer based on nicotinamide (MIP-GCE) for the selective voltammetric determination of dopamine in the presence of adrenaline and noradrenaline.

EXPERIMENTAL

Cyclic voltammograms (CVAs) were recorded using a DropSens μ STAT400 bipotentiostat (Spain) equipped with a three-electrode electrochemical cell 10 mL in volume, where the GCE served as the working electrode. A silver–silver chloride electrode was used as the reference electrode, and a platinum electrode was employed as the counter electrode. Cyclic voltammograms were acquired at a potential scan rate ν ranging from 10 to 100 mV/s.

Palladium particle deposition onto the surface of the glassy-carbon electrode was performed potentiostatically from a solution containing palladium chloride PdCl_2 (Ekofarm, Russia).

The molecularly imprinted polymer was formed on the surface of the GCE through electropolymerization from a solution of nicotinamide (Aldrich, Germany) in the presence of dopamine template molecules under cyclic voltammetry conditions, as shown in Scheme 1 [19].



Scheme 1. Electrochemical polymerization of nicotinamide.

Before modification, the GCE was immersed for 20 min at room temperature in a phosphate buffer solution (standard titer; Uralkhiminvest, Russia) containing 10 μM of noradrenaline and 6.5 μM of dopamine to form a prepolymerization complex between NAD and DA molecules [20]. Subsequently, the electrode potential was scanned from -1.0 to 2.0 V over 20 cycles at a scan rate ν of 0.1 V/s to create the MIP film. Dopamine molecules embedded within the MIP were removed electrochemically by potentiodynamic electrolysis, scanning the potential between -0.2 and

0.8 V in a phosphate buffer solution for several cycles until the oxidation peak of dopamine disappeared.

The composite of the MIP and palladium particles on the electrode surface was prepared using two methods: (1) the MIP was formed on the surface of the glassy carbon electrode, followed by electrodeposition of palladium particles (Pd-MIP-GCE); (2) palladium particles were electrodeposited on the working electrode surface first, followed by the formation of the MIP (MIP-Pd-GCE).

Dopamine, adrenaline, and noradrenaline solutions were prepared by dissolving their accurately weighed portions (Aldrich, Germany). Solutions of lower concentrations were obtained by diluting the stock solution immediately before measurements. A 0.1 M H_2SO_4 solution (standard titer; Uralkhiminvest, Russia) was used as a supporting electrolyte.

Impedance measurements were conducted using an AUTOLAB PGSTAT 204N potentiostat-galvanostat (Netherlands) equipped with the built-in FRA32M module. Electrochemical impedance spectroscopy was applied to a model system consisting of a 1 mM potassium hexacyanoferrate(II)/(III) mixture (Aldrich, Germany) in 0.1 M KCl solution (Aldrich, Germany), over a frequency range from 100 kHz to 0.01 Hz with an amplitude of 5 mV, at a potential of 0.24 V. The potential was calculated as the half-sum of the oxidation and reduction peak potentials of the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox couple.

Urine was used in the study as a biological sample. Urine was collected under fasting conditions using a commercially available sterile container. The biological material was stored for no longer than 2 h at room temperature or 24 h at 4°C after collection.

Before urine analysis, proteins were removed because their presence in the sample can block the electrode surface and affect the sensitivity and accuracy of the detection. The solution was centrifuged for 3 min at 3500 rpm to separate insoluble components. Subsequently, 5 mL of the resulting sample was diluted to 10 mL with the supporting electrolyte. Dopamine was determined using cyclic voltammetry (CVA) using the modified electrode after these preparation steps.

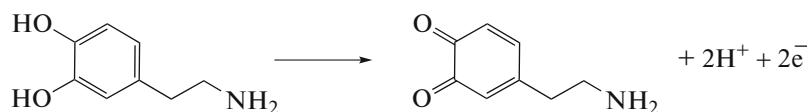
RESULTS AND DISCUSSION

Electrooxidation of dopamine on an electrode modified with a molecularly imprinted polymer based on nicotinamide. Cyclic voltammetry (CVA) profiles obtained from dopamine oxidation on an unmodified GCE in acidic media exhibit a broad anodic peak at a peak potential (E_p) of 0.75 V (Fig. 1a, curve 1). The height of the anodic peak shows a linear dependence on the concentration of dopamine in the range of 5×10^{-4} to 5×10^{-3} M.

The electrode reactions occurring on the GCE in dopamine solutions involve a two-electron process accompanied by the transfer of two protons, forming

an *o*-quinoid fragment in the oxidation product structure of this biogenic amine. The electrochem-

ical process is represented by the following equation (Scheme 2) [21]:



Scheme 2. Electrochemical oxidation of dopamine.

In the CVA anodic branch of dopamine oxidation, obtained on the MIP-GCE, there is a wide peak at $E_p = 0.55$ V (Fig. 1a, curve 2). In this case, the magnitude of the analytical signal is proportional to the concentration of dopamine in the range from 5×10^{-5} to 5×10^{-3} M. This dependence is described by the following equation:

$$\log I = (3.0 \pm 0.3) + (0.29 \pm 0.02) \log c;$$

$(I, \mu\text{A}; c, \text{M}); R = 0.994.$

Comparing the MIP-modified glassy-carbon electrode (MIP-GCE) to the unmodified GCE, an increase in the oxidation current of catecholamines by 2.4 times and a decrease in the oxidation peak potential by 200 mV are observed for the MIP-GCE. The decrease in the oxidation potential of dopamine on the MIP-GCE can be attributed to the electron-conducting properties of the formed polymer film, which consequently enhances the electron transfer rate.

As biological fluids contain not only dopamine but also other catecholamines, we evaluated the capacity of the MIP-GCE electrode for selectively detecting dopamine in the presence of adrenaline and noradrenaline. The electrode's ability to recognize the template molecule was assessed using the imprinting factor (*IF*), calculated as the ratio of the oxidation current of the organic compound on the MIP-based electrode (I_{MIP}) to that on the nonimprinted polymer-based electrode (I_{NIP}): $IF = I_{\text{MIP}}/I_{\text{NIP}}$. The imprinting factors were found to be 3.2, 3.8, and 3.1 for DA, AD, and NAD, respectively, indicating the potential of this electrode for recognizing the considered catecholamines. However, selective voltammetric determination of these catecholamines in their simultaneous presence on this MIP-GCE is challenging because the oxidation of DA, AD, and NAD occurs at similar potentials, and their respective peaks overlap with each other.

Electrocatalytic oxidation of dopamine on electrodes modified with palladium particles and a molecularly imprinted polymer based on nicotinamide. The selective determination of dopamine and adrenaline on a carbon electrode with electrodeposited palladium nanoparticles was demonstrated in [22]. This study examined the electrochemical behavior of dopamine in the absence and presence of adrenaline and noradrenaline on composite electrodes based on MIP and electrodeposited palladium particles. Figure 2a shows

voltammograms of dopamine oxidation on Pd-MIP-GCE (curve 2) and MIP-Pd-GCE (curve 3).

In the oxidation of dopamine on the investigated composite electrodes, a narrow peak is observed in the anodic branch of the cyclic voltammogram. Dopamine electrooxidation on these electrodes occurs at the same potential ($E_p = 0.55$ V). The peak height achieved is several times higher than that of the oxidation peak of the modifier and increases with increasing analyte concentration. We assume that the decrease in overpotential and the increase in current on Pd-MIP-GCE and MIP-Pd-GCE electrodes are related to the catalytic properties of the metallic modifier [22]. The highest peak current for dopamine oxidation (I_p) was obtained when using the MIP-Pd-GCE electrode (Table 1; Fig. 2a, curve 3).

To characterize electron transfer on the investigated electrodes, we used electrochemical impedance spectroscopy (EIS). Figure 3 presents the corresponding Nyquist plots. The semicircle in the high-frequency region corresponds to the charge transfer-lim-

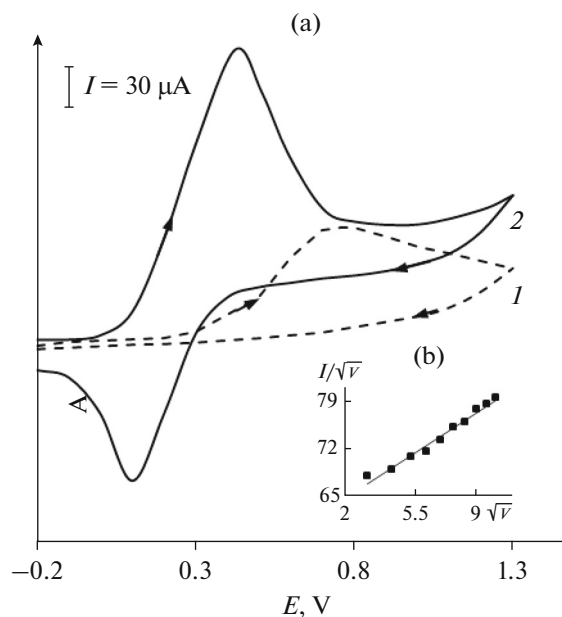


Fig. 1. (a) Cyclic voltammograms obtained on (1) an unmodified GCE and (2) MIP-GC in the presence of dopamine ($c = 5 \times 10^{-3}$ M) in a 0.1 M H_2SO_4 supporting electrolyte solution. (b) Dependence of I/\sqrt{v} on \sqrt{v} during dopamine electrooxidation on the MIP-GCE.

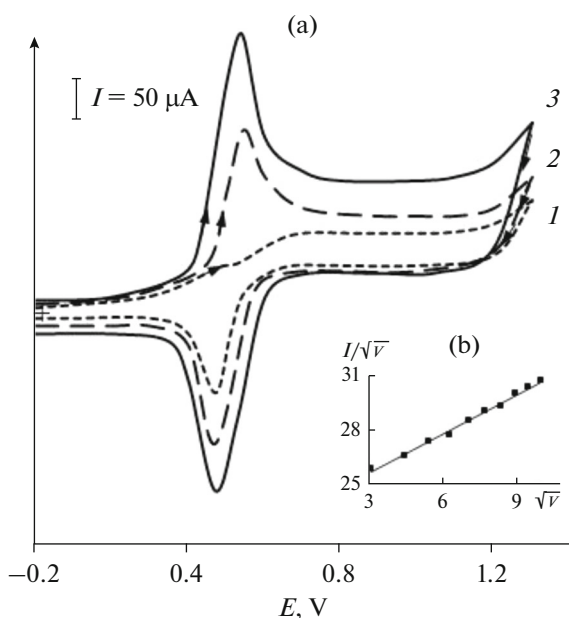


Fig. 2. Cyclic voltammograms obtained on (1, 3) the MIP-Pd-GCE and (2) Pd-MIP-GCE in (1) the absence and (2, 3) and presence of dopamine ($c = 5 \times 10^{-3}$ M) in a 0.1 M H_2SO_4 supporting electrolyte solution. (b) Dependence of I/\sqrt{v} on \sqrt{v} during dopamine electrooxidation on the MIP-Pd-GCE.

iting step. The linear portion at lower frequencies describes the diffusion component of charge transfer for the modified electrodes. For the modified electrodes, the diameter of the semicircle is significantly smaller than that of the bare GCE, indicating an enhancement in the charge transfer rate. As shown in Table 1, the electron transfer resistance (R_{et}) markedly decreases for the modified electrodes compared to the GCE (by 5 times for Pd-GCE, 32 times for Pd-MIP-GCE, and 236 times for MIP-Pd-GCE). Hence, electrodes modified the MIP and palladium particles exhibit high electron transfer rates, with the MIP-Pd-GCE demonstrating the best performance in terms of these characteristics.

The positive slope of the dependence of I/\sqrt{v} on \sqrt{v} , obtained during dopamine oxidation on the MIP-Pd-GCE (Fig. 2b), as for MIP-GCE (Fig. 1b), indicates an adsorption contribution to the current due to the

Table 1. Voltammetric characteristics of dopamine oxidation and parameters of the electrochemical impedance of electrodes ($n = 5$, $P = 0.95$)

Electrode	I_p , μA	R_{et} , kOhm
GCE	56.1 ± 1.7	26.2 ± 0.8
GCE	125 ± 4	4.9 ± 0.2
Pd-MIP-GCE	210 ± 6	0.81 ± 0.03
MIP-Pd-GCE	332 ± 9	0.110 ± 0.006

presence of sites (imprints) in the polymer film structure capable of specific interactions with substrate molecules.

The magnitude of the analytical signal on the MIP-Pd-GCE is proportional to the concentration of dopamine in the range from 5×10^{-9} to 5×10^{-3} M. This dependence is described by the following equation:

$$\log I = (3.3 \pm 0.2) + (0.24 \pm 0.01) \log c;$$

$$(I, \mu\text{A}; c, \text{M}); R = 0.991.$$

The accuracy of the procedure for determining the test compounds was assessed using the standard addition method. The relative standard deviation (RSD) did not exceed 4.0% across the entire concentration range (Table 2). Accuracy was characterized by the recovery (R): $R = (\text{Found}/\text{Added}) \times 100\%$. The obtained values of R , ranging from 99 to 101% (Table 2), confirm the high accuracy of the developed procedure.

We investigated the electrochemical behavior of dopamine, adrenaline, and noradrenaline in their simultaneous presence on the MIP-Pd-GCE. Similar to the electrode modified with electrochemically deposited palladium nanoparticles [22], two oxidation peaks were observed in the anodic branch of the cyclic voltammograms for DA and AD on the MIP-Pd-GCE. Dopamine oxidized at $E_p = 0.55$ V, and adrenaline at $E_p = 0.75$ V. A similar pattern was observed during the oxidation of dopamine in the presence of noradrenaline. The difference in oxidation potentials (peak potential difference is 200 mV) allows for the selective determination of dopamine in the presence of adrenaline and noradrenaline.

To account for the mutual effect of these compounds on the analytical signal recorded during the electrooxidation of dopamine on the MIP-Pd-GCE, we employed the standard addition method. This involved varying the concentrations of interfering components in the mixture while maintaining a constant concentration of dopamine. In determining DA in the DA/AD or DA/NAD mixtures, cyclic voltammograms were sequentially recorded in solutions with a constant dopamine concentration ($c = 5 \times 10^{-3}$ M) and increasing concentrations of adrenaline or noradrenaline ranging from 5×10^{-5} to 5×10^{-3} M. Figure 4 shows the cyclic voltammograms of dopamine oxidation on the MIP-Pd-GCE in the presence of adrenaline or noradrenaline. The presence of these catecholamines in the solution within the investigated concentration range does not interfere with the voltammetric characteristics of dopamine oxidation.

Thus, the voltammetric method for dopamine determination on the MIP-GCE demonstrates high selectivity and sensitivity due to the properties of the molecularly imprinted polymer, the recognition sites of which have shapes and sizes matching the imprinted dopamine template molecules. However, on the sur-

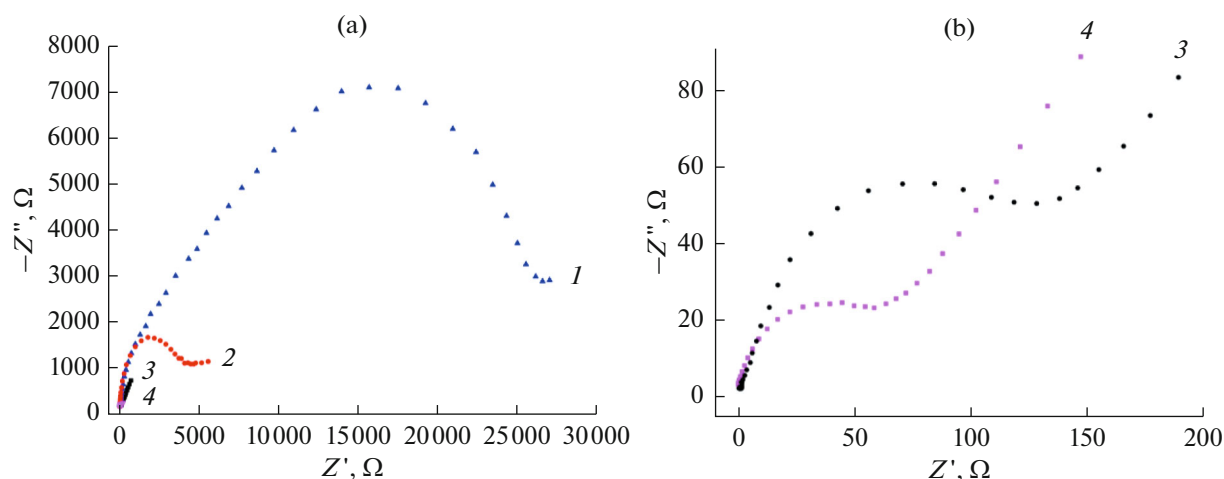


Fig. 3. Nyquist plots obtained on (a) (1) an unmodified GCE and (2) Pd-GCE (2), (b) (3) Pd-MIP-GCE and (4) MIP-Pd-GCE in the presence of 1.0 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ in 0.1 M KCl over a frequency range from 0.01 Hz to 10 kHz with an amplitude of 5 mV at a potential of 0.24 V.

face of the MIP-modified electrode, structurally similar compounds may also accumulate alongside dopamine. To separate the oxidation peaks of catecholamines, we used the catalytic properties of palladium within the MIP-Pd-GCE. The immobilization of micro- and nanoparticles of palladium on the GCE with varying electrochemical activity leads to the separation of the oxidation peaks of dopamine, adrenaline, and noradrenaline. A decrease in the overpotential and an increase in the oxidation currents of these compounds are also observed on the Pd-MIP-GCE, albeit to a lesser extent, likely due to the constraint on template molecule accumulation within the cavities of the MIP caused by the electrodeposited palladium particles. Therefore, for dopamine determination in urine samples, the MIP-Pd-GCE composite electrode was employed.

Voltammetric determination of dopamine in urine using an electrode modified with palladium nanoparticles and a molecularly imprinted polymer of nicotinamide. The proposed voltammetric procedure enables the selective and highly sensitive determination of dopamine in urine. The recovery of dopamine deter-

mination in urine samples is 98–99% (Table 3), confirming the absence of interference from matrix components (noradrenaline, adrenaline, uric acid, glucose, urea, amino acids, etc.) and high accuracy in dopamine determination on the MIP-Pd-GCE. These findings indicate the potential applicability of the proposed electrode for voltammetric determination of DA in real samples.

CONCLUSIONS

Thus, a modified electrode has been developed using a composite of electrochemically deposited palladium nanoparticles and a molecularly imprinted polymer derived from the environmentally friendly monomer nicotinamide. This composite electrode enables the selective voltammetric determination of dopamine in the presence of adrenaline and noradrenaline with high sensitivity. The proposed method has been successfully tested in an analysis of urine samples.

FUNDING

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants involved in the study.

Table 2. Results of the voltammetric determination of dopamine on the MIP-Pd-GCE in a 0.1 M H_2SO_4 supporting electrolyte solution ($n = 6$, $P = 0.95$)

Added, μM	Found, μM	SE, %	RSD, %
0.50	0.500 ± 0.014	100	3
1.50	1.49 ± 0.06	99	4
2.50	2.52 ± 0.08	101	3

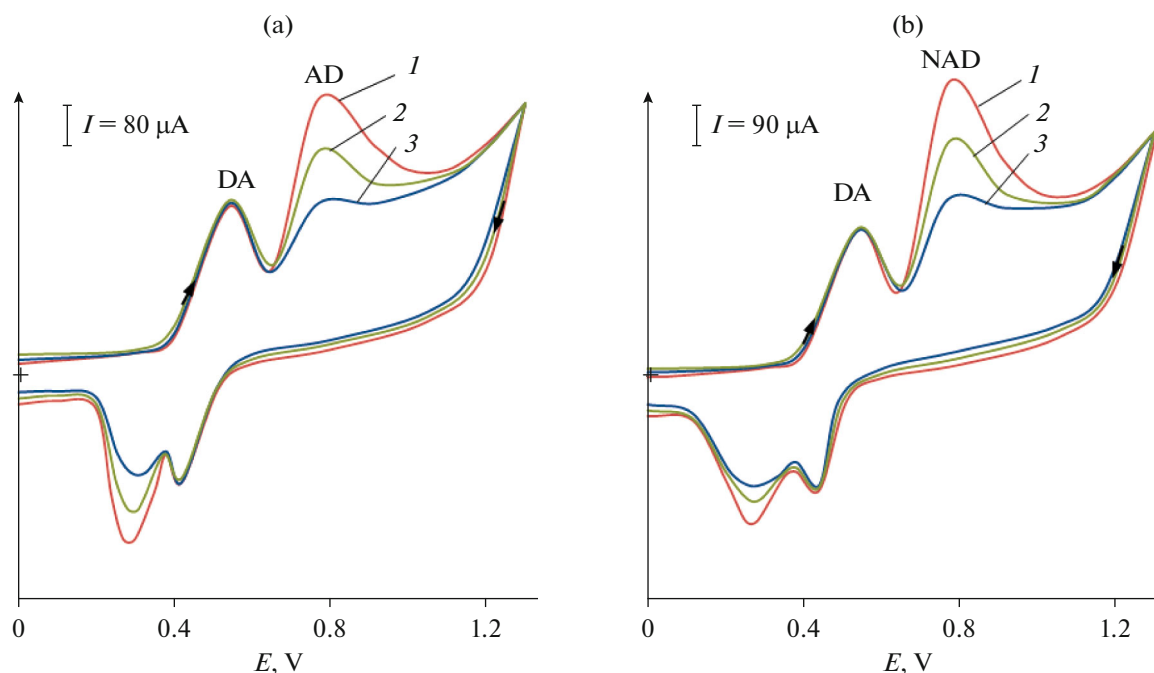


Fig. 4. Cyclic voltammograms obtained on the MIP-Pd-GC electrode during dopamine electrooxidation ($c = 5 \times 10^{-3}$ M) in the presence of (a) adrenaline at concentrations of (1) 5×10^{-5} , (2) 5×10^{-4} , and (3) 5×10^{-3} M and (b) noradrenaline at concentrations of (1) 5×10^{-5} , (2) 5×10^{-4} , and (3) 5×10^{-3} M in a 0.1 M H_2SO_4 supporting electrolyte solution.

Table 3. Results of the voltammetric determination of dopamine in urine samples on the MIP-Pd-GCE in a 0.1 M H_2SO_4 supporting electrolyte solution ($n = 6$, $P = 0.95$)

Sample no.	Norm, μM [23]	Added, μM	Calculated, μM	Found, μM	SE, %	RSD, %
1	0.34–2.6	–	–	2.50 ± 0.08	–	2
		0.5	3.0	2.95 ± 0.09	98	3
		1.0	3.5	3.47 ± 0.06	99	2
		5.0	7.5	7.42 ± 0.22	99	4
2	0.34–2.6	–	–	1.81 ± 0.05	–	3
		0.5	2.3	2.26 ± 0.09	98	4
		1.0	2.8	2.77 ± 0.08	99	3
		5.0	6.8	6.7 ± 0.3	98	5

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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