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ARTICLES

Carbon Paste Electrode Modified with ZrO_2 Nanoparticles and Ionic Liquid for Sensing of Dopamine in the Presence of Uric Acid[1]

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Abstract—A novel carbon paste electrode modified with ZrO_2 nanoparticles and an ionic liquid (*n*-hexyl-3-methylimidazolium hexafluorophosphate) was fabricated. The electrochemical study of the modified electrode, as well as its efficiency for simultaneous voltammetric oxidation of dopamine and uric acid is described. The electrode was also employed to study the electrochemical oxidation of dopamine and uric acid, using cyclic voltammetry, chronoamperometry and square wave voltammetry as diagnostic techniques. Square wave voltammetry exhibits linear dynamic range from 1.0×10^{-6} to 9.0×10^{-4} M for dopamine. Also, square wave voltammetry exhibits linear dynamic range from 9.0×10^{-6}–1.0×10^{-3} M for uric acid. The modified electrode displayed strong function for resolving the overlapping voltammetric responses of dopamine and uric acid into two well-defined voltammetric peaks. In the mixture containing dopamine and uric acid, the two compounds can be well separated from each other with potential difference of 155 mV, which is large enough to determine dopamine and uric acid individually and simultaneously. Finally, the modified electrode was used for determination of dopamine and uric acid in real samples.

Keywords: dopamine, uric acid, ionic liquids, carbon paste electrode, ZrO_2 nanoparticles

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Much attention has been paid to research on brain science, among which neurotransmitters are widely studied and involve a lot of neuropathies. Dopamine, or 2-(3,4-dihydroxyphenyl)ethylamine, is a member of the catecholamine neurotransmitter family with a variety of functions [1, 2]. It has a strong influence on the brain's control of learning, feeding and neurocognition [3]. The temporal fluctuation of the dopamine concentrations in the human brain has a critical effect on several neurological disorders such as Harrington's disease and Parkinson's disease [4]. Thus, dopamine detection is one of the main objectives to keep in mind since it would be of great help for monitoring patients with impaired release of this neurotransmitter in vivo.

Conventional methods to detect dopamine include capillary electrophoresis [5], fluorometry [6], chromatography [7], spectrophotometry [8] and chemiluminescence [9]. Despite their many benefits, these methods generally demand extreme experimental conditions and complicated equipment. Over the last few decades, electroanalytical sensors for determining biomolecules have gained considerable attention because of the electroactive nature of dopamine and their merits of easy operation, low cost, instant response, and high sensitivity [10–15]. Now, a number of materials, such as metal complexes, nanoparticles, organics, and self-assembled molecular films have been utilized as modifiers to fabricate highly selective dopamine sensors.

However, a major obstacle usually encountered in the detection of dopamine is the coexistence of uric acid in relatively high concentrations.

Uric acid is the end product of purine metabolism in humans and its unusual concentration levels may lead to several diseases such as hyperuricemia, gout and pneumonia [16]. Therefore, these two molecules are oxidized at almost the same potential at the traditional electrodes resulting in the overlap of voltammetric responses. To solve this problem, various chemically modified electrodes using carbon nanomaterials, metal nanoparticles, metal complexes and conducting polymers have been used for the simultaneous determination of dopamine and uric acid. Although these modified electrodes have shown improved sensitivity and selectivity compared with the traditional electrodes, they suffer from some impediment. For example, those do not exhibit a reproducible peak area and a stable behavior. Thus, it is still extremely desired to explore novel electrodes for

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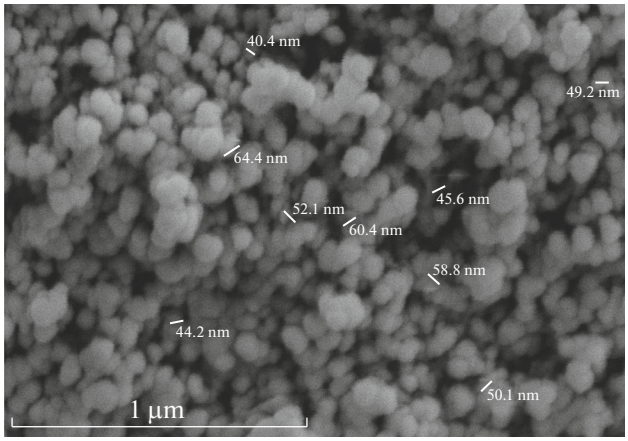

Fig. 1. Scanning electron microscope image of ZrO_2 nanoparticles.

simultaneous determination of dopamine and uric acid [17].

Electrochemical sensors and biosensors for pharmaceutical, food, agricultural and environmental analyses have been growing rapidly, due to advances in electrochemical measuring systems [18–29]. Among the different mercury-free solid electrodes, the carbon paste electrode (CPE) has obtained increasing attention. The CPE, invented by Adams at the end of the 1950s, is a mixture of an electrically conducting graphite powder and a pasting liquid. To date, the CPE has been widely used in electrochemistry and electroanalytical chemistry as a working electrode because of the following advantages: wide potential range, easy preparation, convenient surface renewal, low residual current, porous surface and low cost [30–35]. The application of chemically modified electrodes has been widely considered for sensitive and selective analytical electrochemical determinations, such as for the detection of trace amounts of biologically important compounds [35–37].

The application of nanomaterials in various fields of science and technology has been extensively developed due to the unique properties of these materials [38–41]. Metal and metal oxide nanoparticles are the most widely employed nanomaterials owing to excellent physical and catalytic properties of these materials. Therefore, there is a requirement for the development of these nanoparticles with tuned properties. These materials are being employed in electrochemistry to improve the performance of electrochemical techniques due to excellent electrocatalytic properties. There is currently an intense interest in the use of nanoparticles for the fabrication of modified electrodes and a wide range of bioscience applications. The fabrication of electrodes modified with nanoparticles has been the focus of recent attention owing to enhancement of the response signal, increased sensitivity and better reproducibility [42–45].

Ionic liquids (ILs) have been generating increasing interest over the last decade. Ionic liquids have a great potential for possible electrochemical applications because these compounds possess high thermal stability, no volatility, high polarity, large viscosity, high intrinsic conductivity, and wide electrochemical window [46–49].

In the present work, we describe the preparation of a new carbon paste electrode modified with an ionic liquid and ZrO_2 nanoparticles (IL–ZrO_2–CPE) and investigate its performance for the determination of dopamine in the presence of uric acid in aqueous solutions. Finally the modified electrode was used for determination of dopamine and uric acid in real samples.

EXPERIMENTAL

Apparatus and chemicals. The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System software. A conventional three electrodes cell was used at 25 ± 1°C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and IL–ZrO_2–CPE were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 710 pH meter was used for pH measurements.

Dopamine, uric acid and all the other reagents were of analytical grade and were obtained from Merck (Darmstadt, Germany). The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0–9.0. Ionic liquid (*n*-hexyl-3-methylimidazolium hexafluoro phosphate) was purchased from Sigma–Aldrich.

Preparation of the electrode. IL–ZrO_2–CPEs were prepared by mixing 0.04 g of ZrO_2 nanoparticles (Fig. 1) with 0.96 g graphite powder and approximately, 0.8 mL of ILs with a mortar and pestle. The

paste was then packed into the end of a glass tube (ca. 3.4 mm i.d. and 15 cm long). Acopper wire inserted the carbon paste provided the electrical contact.

For comparsion, ionic liquid–carbon paste in the absence of ZrO_2 nanoparticles (IL–CPE), ZrO_2 nanoparticles carbon paste electrode (ZrO_2–CPE) consist of ZrO_2 nanoparticles powder and paraffin oil, and bare CPE consisting of graphit powder and paraffin oil were also prepared in the same way.

RESULTS AND DISCUSSION

Electrochemical behavior of dopamine at the surface of various electrodes. Figure 2 displays cyclic voltammetric responses from the electrochemical oxidation of 600 μM dopamine at the surface of IL–ZrO_2–CPE (curve *5*), IL–CPE (curve *4*), ZrO_2–CPE (curve *3*) and bare CPE (curve *2*). The results showed that the oxidation of dopamine is weak at the surface of the bare CPE, but the presence of ILs in CPE could enhance the peak current and decrease the oxidation potential (decreasing the overpotential). A substantial negative shift of the potential starting from oxidation potential for dopamine and dramatic increase of the current indicates the catalytic ability of IL–ZrO_2–CPE (curve *5*) and IL–CPE (curve *4*) to dopamine oxidation. The results showed that the combination of ZrO_2 nanoparticles and the ionic liquid (curve *5*) definitely improved the characteristics of dopamine oxidation. However, IL–ZrO_2–CPE shows much higher anodic peak current for the oxidation of dopamine compared to IL–CPE, indicating that the combination of ZrO_2 nanoparticles and ionic liquids has significantly improved the performance of the electrode toward dopamine oxidation. Table 1 shows the electrochemical characteristics of dopamine oxidation on the various electrode surfaces at pH 7.0.

Effect of scan rate. The effect of potential scan rates on the oxidation current of dopamine (Fig. 3) have been studied using cyclic voltammetry (CV). The results showed that increasing in the potential scan rate induced an increase in the peak current. In addition, the oxidation process is diffusion controlled, as deduced from the linear dependence of the anodic peak current (I_p) on the square root of the potential scan rate ($\nu^{1/2}$) [50].

Figure 4 shows the Tafel plot for the sharp rising part of the voltammogram at the scan rate of 20 mV/s. If deprotonation of dopamine is a sufficiently fast step, the Tafel plot can be used to estimate the number of electrons involved in the rate determining step. A Tafel slope of 0.134 V was obtained which agrees well with the involvement of one electron in the rate determining step of the electrode process [50], assuming a charge transfer coefficient, α of 0.56.

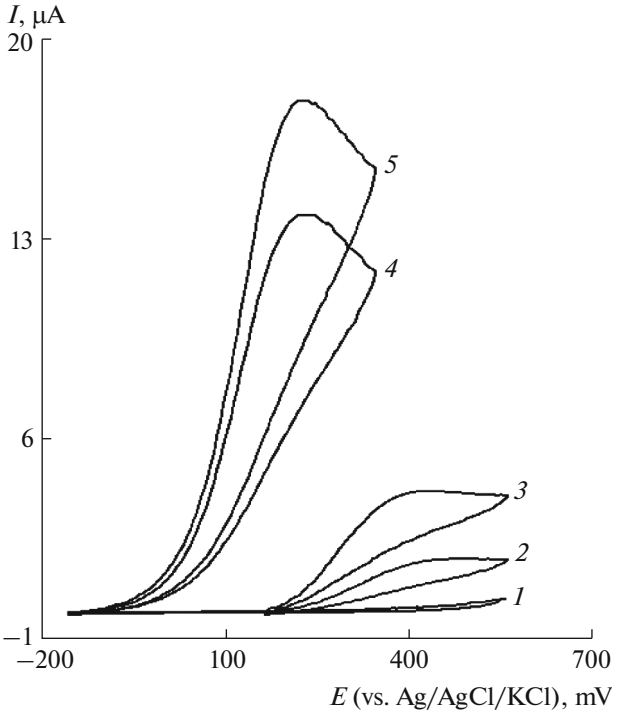

Fig. 2. Cyclic voltammograms of CPE in 0.1 M PBS (pH 7.0) (*1*), CPE (*2*), ZrO_2–CPE (*3*), IL–CPE (*4*), and IL–ZrO_2–CPE (*5*), in the presence of 600 μM dopamine in 0.1 M PBS (pH 7.0) respectively. In all cases the scan rate was 50 mV/s.

Chronoamperometric measurements. Chronoamperometric measurements of dopamine (Fig. 5) at IL–ZrO_2–CPE were carried out by setting the working electrode potential at 0.3 V vs. Ag/AgCl/KCl (3.0 M) for the various concentrations of dopamine in phosphate buffer solution (PBS) (pH 7.0). For electroactive materials (dopamine in this case) with a diffusion coefficient of D, the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [50]:

$$I = nFAD^{1/2}c_b\pi^{-1/2}t^{-1/2}, \quad (1)$$

Table 1. Oxidation of dopamine on various electrodes at pH 7.0

Electrode	Anodic peak potential, mV	Anodic peak current, μA
CPE	450	1.9
ZrO_2–CPE	400	4.3
IL–CPE	230	14
IL–ZrO_2–CPE	230	18

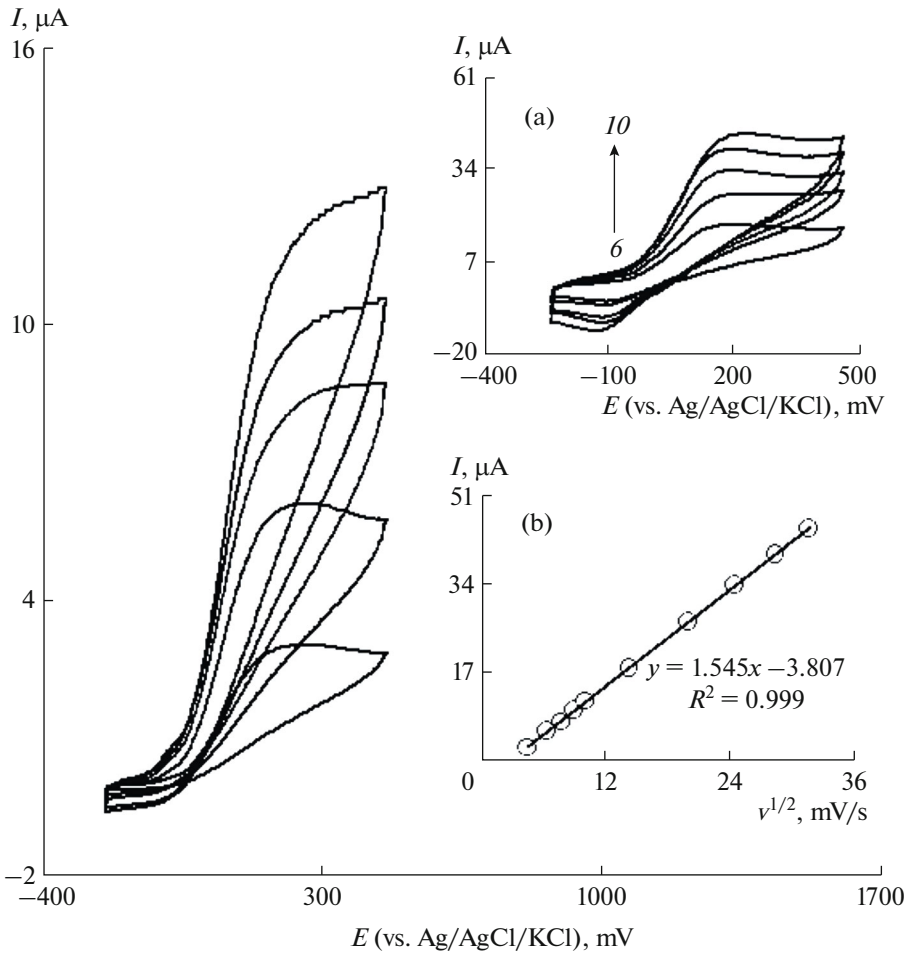

Fig. 3. Main figure and inset (a): cyclic voltammograms of IL–ZrO_2–CPE in 0.1 M PBS (pH 7.0) containing 200 μM dopamine at various scan rates; numbers *1–10* correspond to 20, 40, 60, 80, 100, 200, 400, 600, 800 and 1000 mV/s, respectively. Inset (b): variation of anodic peak current vs. square root of scan rate.

Table 2. Some modified electrodes used in the determination of dopamine

Electrode	Modifier	Method	LOD, M	LDR*, M	Reference
Carbon paste	ZnO nanorods and 3-(4'-amino-3'-hydroxy-biphenyl-4-yl)-acrylic acid	Square wave voltammetry	5.6×10^{-8}	3.0×10^{-7}–1.0×10^{-4}	[11]
Gold	2-(2,3-Dihydroxy phenyl)-1,3-dithiane	Differential pulse voltammetry	5.1×10^{-7}	7.0×10^{-7}–5.0×10^{-4}	[51]
Glassy carbon electrode	Graphene/SnO_2 nanocomposite	Differential pulse voltammetry	1.0×10^{-6}	1.0×10^{-6}–5.0×10^{-5}	[52]
Carbon paste	IL–ZrO_2–CPE	Square wave voltammetry	5.0×10^{-7}	1.0×10^{-6}–9.0×10^{-4}	This work

* Linear dynamic range.

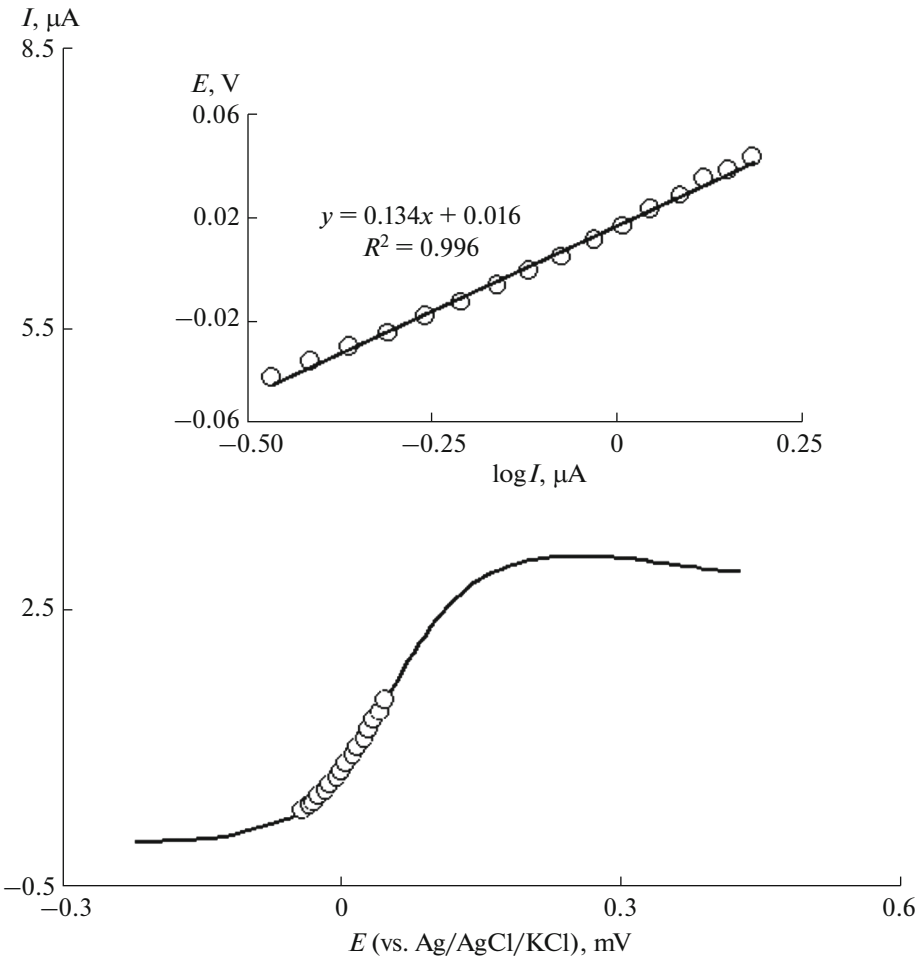

Fig. 4. Linear sweep voltammetry (LSV) (at 20 mV/s) of an IL–ZrO_2–CPE in 0.1 M PBS (pH 7.0) containing 200 μM dopamine. The points are the data used in the Tafel plot. The inset shows the Tafel plot derived from the LSV.

where D and c_b are the diffusion coefficient (cm^2/s) and the bulk concentration (mM), respectively. Experimental plots of I vs. $t^{-1/2}$ were employed, with the best fits for different concentrations of dopamine. The slopes of the resulting straight lines were then plotted vs. dopamine concentrations. From the resulting slope and Cottrell equation the mean value of the D for dopamine was found to be 1.18×10^{-6} cm^2/s.

Calibration plot and limit of detection. The peak currents of dopamine oxidation at the surface of the modified electrode (Fig. 6) can be used for determination of dopamine in solution. Therefore, square wave voltammetry (SWV) experiments were done for different concentrations of dopamine. The oxidation peak currents of dopamine at the surface of the modified electrode were proportional to the concentration of the dopamine within the range 1.0×10^{-6} to 9.0×10^{-4} M with detection limit (3σ) of 5.0×10^{-7} M for dopamine. These values are comparable with values reported by other research groups for electro oxidation of dopamine (see Table 2).

The electrode developed is characterized by wide linear dynamic range and short time of the procedure avoiding application of electron transfer mediator. Despite better LODs, other voltammetric methods suffer from extensive and expensive pre-treatment steps and time-consuming analysis.

In the case of uric acid, peak currents of its oxidation at the surface of modified electrode were linearly dependent on its concentrations, over the range of 9.0×10^{-6}–1.0×10^{-3} M, and the detection limit (3σ) obtained was 5.0×10^{-6} M.

Simultaneous determination of dopamine and uric acid. To our knowledge, no paper has used the ZrO_2 nanoparticles-modified electrode for simultaneous determination of dopamine and uric acid and this is

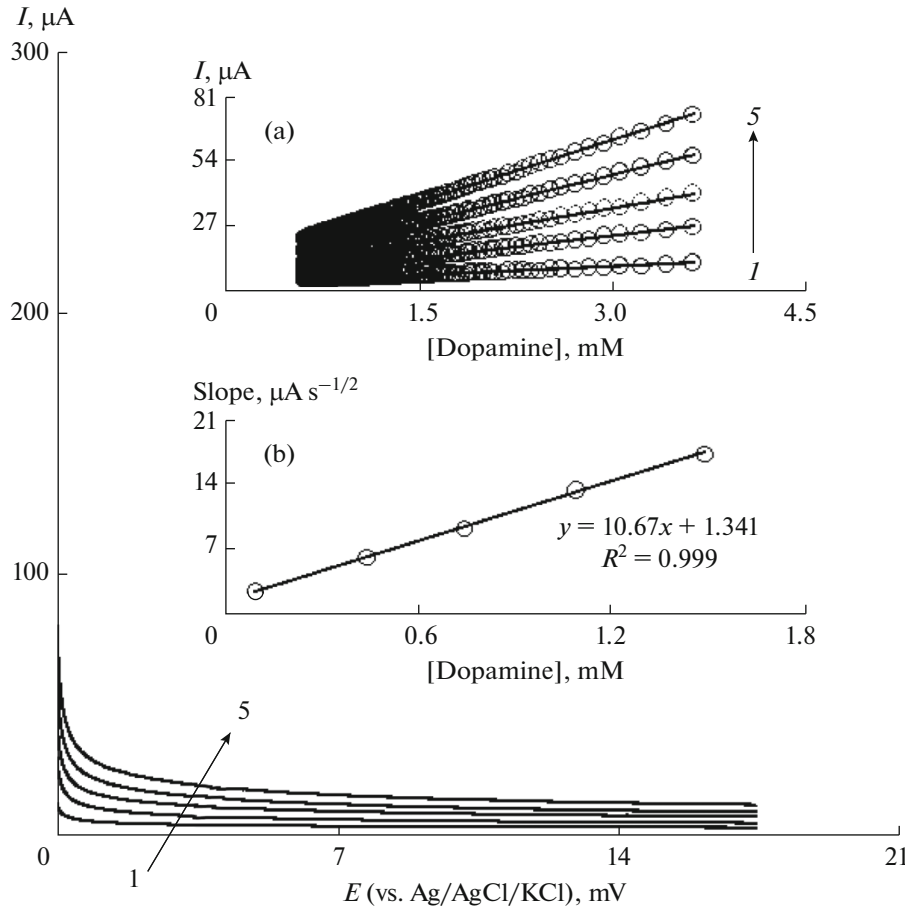

Fig. 5. Chronoamperograms obtained at IL–ZrO_2–CPE in 0.1 M PBS (pH 7.0) for different concentrations of dopamine. Numbers *1–5* correspond to 0.1, 0.45, 0.75, 1.1 and 1.5 mM of dopamine. Inset (a): plots of *I* vs. $t^{-1/2}$ obtained from chronoamperograms *1–5*. Inset (b): plot of the slope of the straight lines against dopamine concentration.

the first report for simultaneous determination of dopamine and uric acid using IL–ZrO_2–CPE.

The determination of dopamine and uric acid in mixtures was performed at the IL–ZrO_2–CPE using SWV. The concentration of uric acid was varied, while keeping the dopamine concentration constant. The results are shown in Fig. 7. When the concentration of dopamine is kept constant at 400 μM, the peak current of uric acid is proportional to its concentration. No changes in the peak current and potential of dopamine can be found.

Also, determination of two compounds was performed by simultaneously changing the concentrations of dopamine and uric acid, and recording the SWVs (Fig. 8). The voltammetric results showed well-defined anodic peaks at potentials of 175 and 330 mV, corresponding to the oxidation of dopamine and uric acid, respectively, indicating that simultaneous determination of these compounds is feasible at the IL–ZrO_2–CPE, as shown in Fig. 8.

The repeatability and stability of IL–ZrO_2–CPE. The long-term stability of the IL–ZrO_2–CPE was tested over a 3-week period. When CVs were recorded after the modified electrode was stored in atmosphere at room temperature, the peak potential for dopamine oxidation was unchanged and the current signals showed less than 2.6% decrease relative to the initial response. The antifouling properties of the modified electrode toward dopamine oxidation and its oxidation products were investigated by recording the CVs of the modified electrode before and after use in the presence of dopamine. CVs were recorded in the presence of dopamine after having cycled the potential 20 times at a scan rate of 50 mV/s. The peak potentials were unchanged and the currents decreased by less than 2.1%. Therefore, at the surface of IL–ZrO_2–CPE, not only the sensitivity increase, but

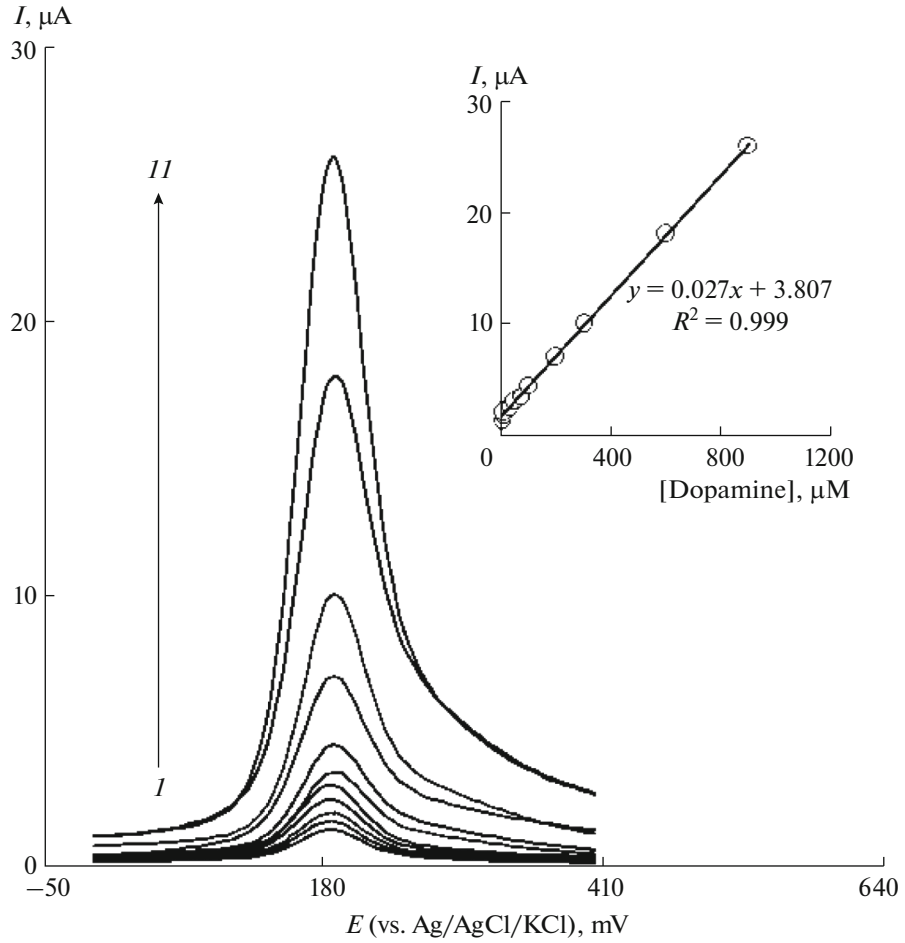

Fig. 6. Square wave voltammograms of IL–ZrO_2–CPE in 0.1 M PBS (pH 7.0) containing different concentrations of dopamine. Numbers *1–11* correspond to 1.0, 5.0, 10, 30, 50, 70, 100, 200, 300, 600 and 900 μM of dopamine. Inset shows the plot of the peak current as a function of dopamine concentration in the range of 1.0–900 μM.

Table 3. Determination of dopamine and uric acid in real samples using IL–ZrO_2–CPE ($n = 5$, $P = 0.95$)

Sample	Spiked, μM		Found, μM		Recovery, %		RSD, %	
	dopamine	uric acid	dopamine	uric acid	dopamine	uric acid	dopamine	uric acid
Dopamine injection	0	0	15.0	ND	–	–	3.2	–
	2.5	20.0	17.4	20.5	99.4	102.5	1.9	3.1
	5.0	30.0	20.6	29.8	103.0	99.3	1.7	2.2
	7.5	40.0	22.9	39.1	101.8	97.7	2.9	2.6
	10.0	50.0	24.3	50.5	97.2	101.0	2.4	2.1
Urine	0	0	ND*	10.0**	–	–	–	2.7
	5.0	20.0	4.9	31.1	98.0	103.7	3.3	1.6
	10.0	30.0	10.3	39.5	103.0	98.7	1.8	2.9
	15.0	40.0	14.9	51.5	99.3	103.0	1.9	2.4
	20.0	50.0	20.3	59.8	101.5	99.7	2.8	3.2

* Not detected.

** Interference from ascorbic acid can be minimized by using ascorbic acid oxidase enzyme.

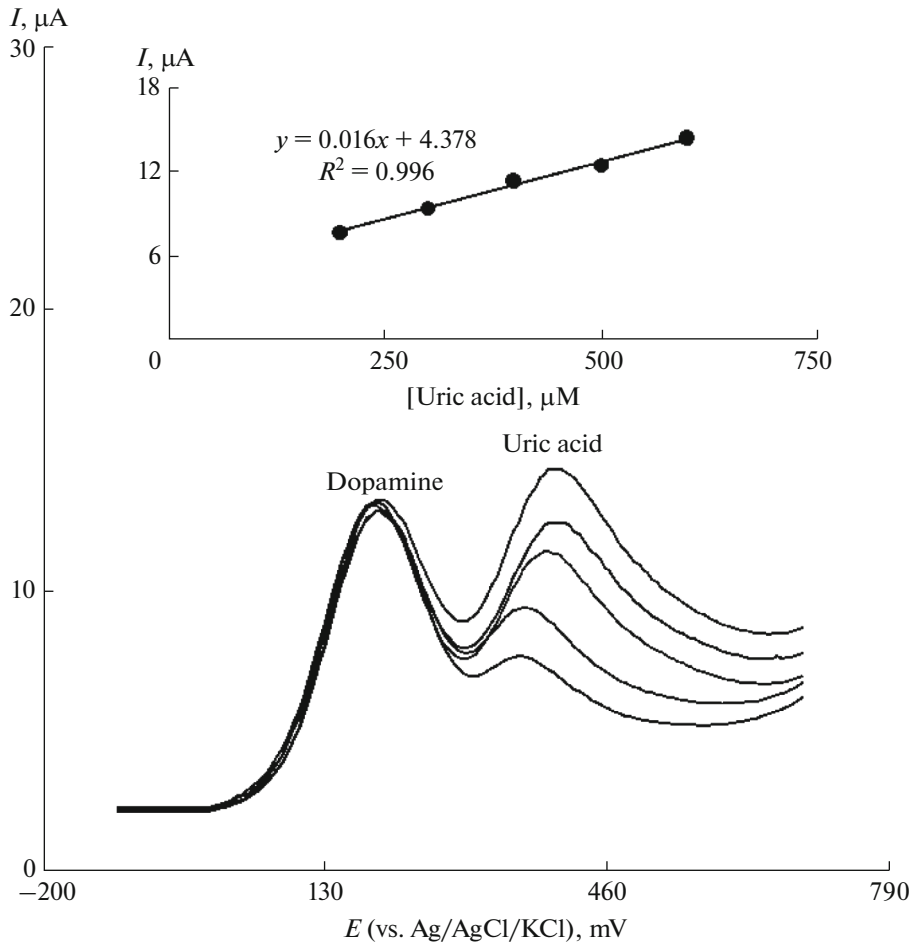

Fig. 7. Square wave voltammograms of IL–ZrO_2–CPE in 0.1 M PBS (pH 7.0) containing 400 μM dopamine and different concentrations of uric acid. Curves from inner to outer correspond to 200, 300, 400, 500 and 600 mM of uric acid. Inset: plot of the electrocatalytic peak current as a function of uric acid concentration.

the fouling effect of the analyte and its oxidation product also decreases.

Interferences study. The influence of various substances as compounds potentially interfering with the determination of dopamine was studied under optimum conditions with 20.0 μM dopamine at pH 7.0. The potentially interfering substances were chosen from the group of substances commonly found with dopamine in pharmaceuticals and/or in biological fluids. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error of less than ±5% in the determination of dopamine. According to the results, glucose, sucrose, lactose, fructose, citric acid, methanol, ethanol, Mg^{2+}, SO_4^{2-}, Al^{3+}, NH_4^+, CO_3^{2-}, Cl^- or F^-, alanine, methionine, phenylalanine, glycine, folic acid (vitamin B_9), saturated starch solution and urea did not interfere with the determination of dopamine. But ascorbic acid showed serious interference in equal molar concentration. Although ascorbic acid is interference, interference from ascorbic acid can be minimized by using ascorbic acid oxidase enzyme.

Real sample analysis. In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of dopamine and uric acid in dopamine injection and urine samples (Table 3). Satisfactory recovery of the experimental results was found for dopamine and uric acid. The reproducibility of the method was demonstrated by the mean relative standard deviation (RSD).

CONCLUSIONS

In the present study, a ZrO_2 nanoparticles/ionic liquid modified carbon paste electrode was constructed. The modified electrode was applied for dopamine and uric acid determination. Excellent fea-

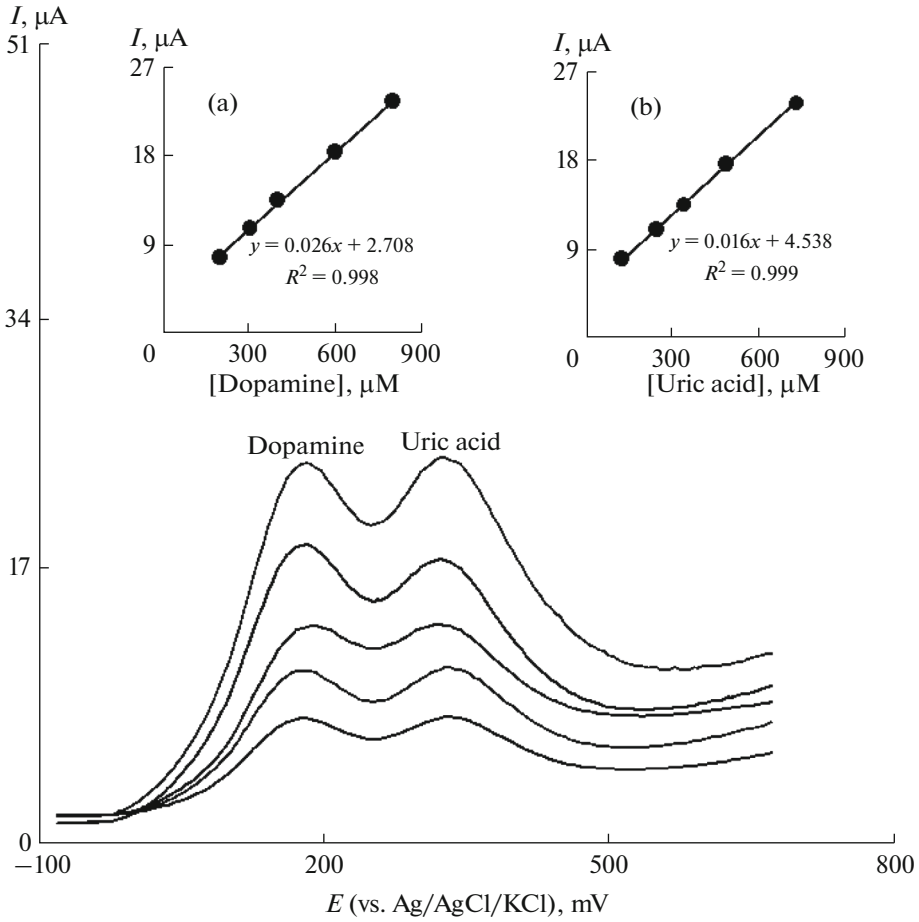

Fig. 8. Square wave voltammograms of IL–ZrO_2–CPE in 0.1 M PBS (pH 7.0) containing different concentrations of dopamine and uric acid, μM, from inner to outer: 200 + 200, 300 + 400, 400 + 550, 600 + 800 and 800 + 1200, respectively. Inset: (a): plot of I_p vs. dopamine concentration; inset (b): plot of I_p vs. uric acid concentration.

tures, like a wide linear range, low detection limit, high reproducibility and repeatability and long time stability proved the successful application of this sensor for the determinations of dopamine and uric acid.

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