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Selective Determination of Dopamine in the Presence of Ascorbic Acid and Uric Acid at Neutral *pH* Using a Silver Nanoparticles-modified Carbon Paste Electrode

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ABSTRACT

Developing simple, sensitive and selective sensing systems for dopamine is important due to its biological significance. In this work, a silver nanoparticles-modified carbon paste electrode (AgNPs-CPE) has been constructed and used to detect of dopamine (DA) in the simultaneous presence of ascorbic acid (AA) and uric acid (UA) at neutral pH 7.0 by cyclic voltammetry. The modified electrode showed good performance toward the oxidation and determination of DA in the presence of AA and UA. In a mixture of the three compounds, DA showed a pair of redox peaks at about 182.0 and 116.0 mV for anodic and cathodic peaks potential, respectively, while AA and UA exhibited an oxidation peak at about 320.0 mV. Under these circumstances DA more easily oxidized than AA and UA at the surface of modified electrode and precisely determined by differential pulse voltammetry. A sensitivity of 0.074 μA/μM with a wide linear range of 12.5-300.0 μM and detection limit of 0.61 μM were obtained. The modified electrode was applied successfully for DA quantification in dopamine hydrochloride injection sample in the presence of AA and UA (100 μM).

Keywords: Dopamine, Ascorbic acid, Uric acid, Silver nanoparticles, Carbon paste electrode

INTRODUCTION

Dopamine (DA) is one of the more interesting neurotransmitters which play important roles in the function of the central nervous, renal, hormonal and cardiovascular systems [1,2]. Therefore, there is intense investigation in the development of methods for DA quantification in blood and biological fluids [3-6]. Electrochemical methods have proven to be rapid, simple, and sensitive in the determination of neurotransmitters such as DA [7-9]. However, an overlapping voltammetric response has been observed because the oxidation of DA at bare electrodes occurs with the oxidation of ascorbic acid (AA) and uric acid (UA) at a potential close to that of DA, which is always present with AA and UA in biological tissues [10]. Thus, it is an interesting to determine DA in the presence of AA and UA in electrochemical analysis. For this purpose, various modified electrodes have been constructed in order to improve the sensitivity and selectivity of the working electrode toward DA [11-16]. For example, the metal nanoparticle-modified electrodes with excellent chemical, electrical and mechanical properties are good candidates for the development of electrochemical detection of DA [17-23]. Among the metal nanoparticles, silver nanoparticles have gained considerable attention due to their interesting optical, catalytical, thermal conductivity and electrical properties [24-26].

The present study, deals with the fabrication of a silver nanoparticles-modified carbon paste electrode (AgNPs-CPE) and its application for determination of DA in the presence of AA and UA at pH 7.0. The electrochemical behavior of these species at the modified electrode was investigated using cyclic voltammetry and differential pulse voltammetry techniques. To the best of our knowledge, almost no attention has been paid to the detection of DA in the presence of AA and UA in physiological pH at AgNPs-CPE. The detection of DA in dopamine hydrochloride injection sample was finally demonstrated as real sample application.

EXPERIMENTAL

Apparatus

All electrochemical experiments were performed with a

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Metrohom model 797 VA. A conventional three electrode system was used for all electrochemical experiments, which consisted of a platinum wire as auxiliary electrode, an Ag/AgCl/saturated KCl as reference electrode, and a bare or modified carbon paste electrode (CPE) as working electrode. All experiments were conducted at room temperature. Measurements of pH were made with a Denver Instrument Model 270 pH meter equipped with a Metrohom glass electrode.

An Analytik Jena SPECORD 250 (Jena, Germany) spectrophotometer, equipped with a peltier temperature controller, was used for recording absorption spectrum of silver nanoparticles. Dynamic light scattering (DLS) measurement was performed on a Malvern Instrument (Brookhaven Instruments Corporation, USA). Scanning electron microscopy (SEM) image of silver nanoparticles was obtained with a Ziess EM 902A Scanning Electron Microscope (Germany).

Chemicals and Solutions

All chemicals were of analytical reagent grade and used without further purification. Graphite powder, high purity Nujol oil, silver nitrate, poly(vinyl pyrrolidone) (PVP) and sodium borohydride ($NaBH_4$) were purchased from Fluka. Dopamine, Ascorbic and Uric acid were purchased from Acros Organics. Doubly distilled water was used throughout the experiments.

Synthesis of Silver Nanoparticles

Silver nanoparticles were prepared by chemical reduction method in aqueous solution in the presence of PVP as a stabilizing agent [27]. In this method PVP (0.08 g) was dissolved in deionized water (95 ml) and treated with solid $AgNO_3$ (0.04 g). The mixture was allowed to dissolve with stirring. The reducing solution was prepared by dissolving 0.08 g of $NaBH_4$ in 5 ml deionized water. Then, $NaBH_4$ solution was added drop by drop to the dissolved mixture at room temperature. After 15 min, the color of the solution became light brown indicating the formation of silver nanoparticles embedded in the polymer matrix. The prepared AgNPs were stored in dark glass bottles at 4 °C. Concentration of the silver nanoparticles solution was calculated using the Eq. (1).

$$C = \frac{N_{total}}{NVN_A} \quad (1)$$

Where, N_{total} is the total number of silver atoms added to the reaction solution, N is the number of silver atoms in each silver nanoparticle that can be calculated from the equation $N = 31 \times d^3$ (d is the size of the nanoparticle in nm [28]), V is the volume of the reaction solution (in liters) and N_A is the Avogadro's constant [29].

Preparation of the Working Electrode

Unmodified carbon paste was prepared by hand mixing of 65% of graphite powder and 35% of Nujol oil with a mortar and pestle. A modified paste was prepared in a similar fashion, except that the graphite powder was mixed with a desired volume of silver nanoparticles. After mixing appropriate amounts of suspension solution of silver nanoparticles with graphite powder, the mixture was dried at room temperature. Finally Nujol oil was added to the mixture and the paste was used for construction of modified carbon paste electrode. Both unmodified and modified carbon paste electrodes were constructed by packing the paste into the end of a teflon tube (2.0 mm inner diameter and 5 mm deep cavity) in contact with a steel spacer for electrical wiring, fused to a plastic tube. The electrode surface was smoothed by polishing on a piece of paper.

To identify the effective surface area of the modified electrode, $K_3[Fe(CN)_6]$ was used as a probe [30]. Cyclic voltammograms of 1×10^{-3} M, $K_3[Fe(CN)_6]$ in 1 M KCl at bareand modified electrode were recorded at various scan rates and the Randles-Sevcik equation was applied (Eq. (2)).

$$I_p = 2.69 \times 10^5 n^{3/2} A D^{1/2} C v^{1/2} \quad (2)$$

Where, n is the number of moles of electrons transferred in the reaction (n = 1), A is the effective surface area of the electrode (cm^2), D is diffusion coefficient of $K_3[Fe(CN)_6]$ in 1 M KCl (0.76×10^{-5} cm^2 s^{-1}), and C $K_3[Fe(CN)_6]$ concentration (M). According to this equation, and the known parameters the effective surface area of the modified electrode is calculated to be 2.0×10^{-4} cm^2. The effective surface area is larger than the effective surface area of the bare electrode (6.0×10^{-5} cm^2), which is due to the large surface area of the silver nanoparticles. This large active

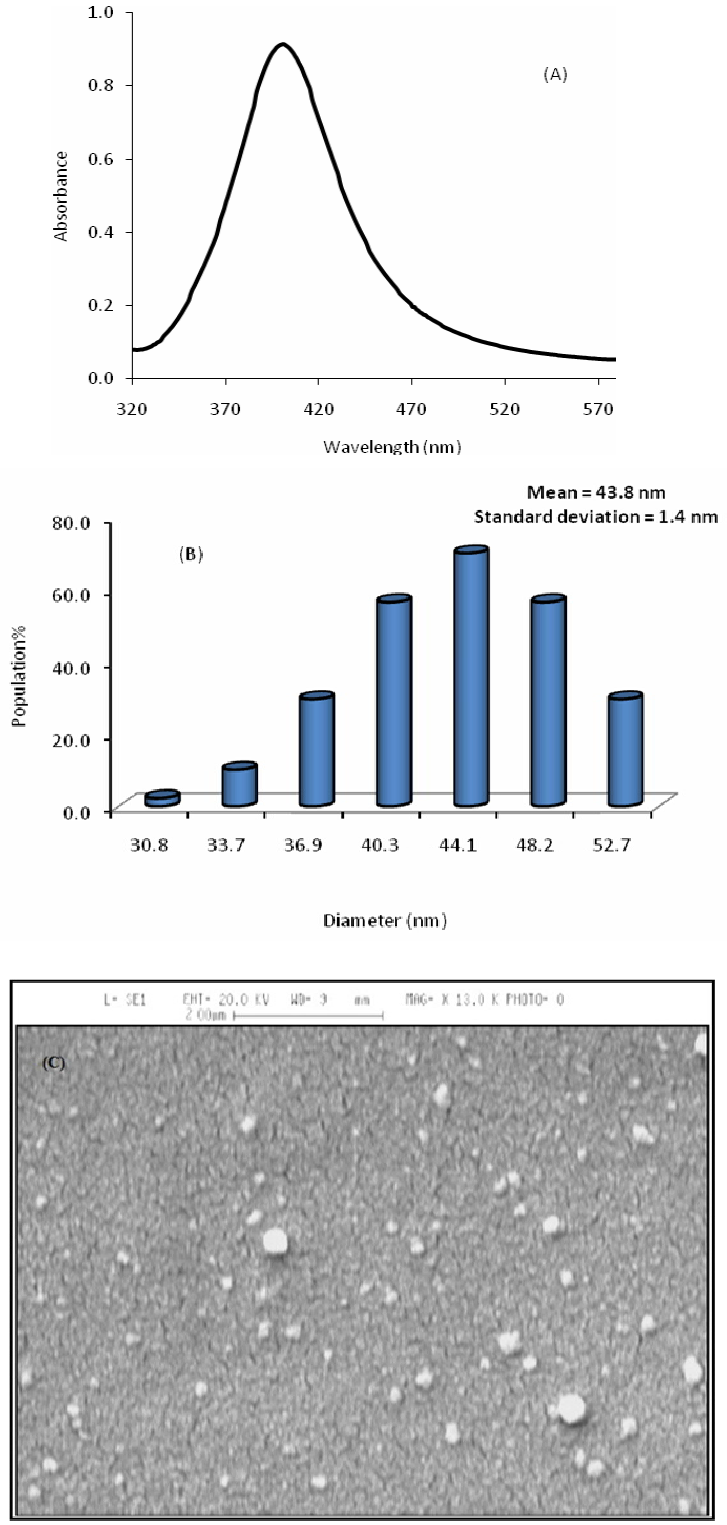

Fig. 1. UV-Vis absorption spectrum (A), particle size distributions (B) and SEM image (C) of the colloidal silver solution.

surface area of the electrode improves the redox behavior of DA.

General Procedure

Stock solutions of DA, AA and UA were freshly prepared as required in a 0.10 M phosphate buffer at pH 7.0. Voltammetric experiments were carried out in buffered solutions containing 0.05 M KCl (as supporting electrolyte) and DA, AA and UA. For all voltammetric measurments, the modified carbon-paste electrode was immersed in a 10 ml of the above-mentioned solution and the voltammograms were recorded from -0.3-0.8 V *vs.* Ag/AgCl, with scan rate of 0.1 V s^{-1}. Differential pulse voltammograms were recorded with pulse potential 50 mV, pulse duration 50 ms and pulse period 0.2 s. All measurements were carried out at room temperature (25 ± 1 °C) and pH 7.0.

RESULTS AND DISCUSSION

Characterization of the Silver Nanoparticles

UV-Vis absorption spectrum of the colloidal silver solution (Fig. 1A) shows an absorption peak at about 400 nm which indicates the formation of silver nanoparticles (AgNPs) [27]. Particle size distributions have been made using dynamic light scattering where He-Ne laser light (633 nm) is passed through diluted nanoparticles solutions. The size distributions of silver are shown in Fig. 1B. AgNPs sizes ranging from 8-150 nm with an average particle size of 43.8 nm were obtained. According to the Eq. (1) and considering the average particle size equal to 43.8 nm, the concentration of silver nanoparticles was calculated to be 9.04×10^{-10} M. Furtheremore, the SEM image of the colloidal silver solution confirms the existence of small and uniformly spherical nanoparticles (Fig. 1C).

Influence of Ag NPs/carbon Paste Ratio for Preparation of the Modified Electrode

Figure 2 shows the cyclic voltammograms of the modified electrode, prepared with different ratios of AgNPs/carbon paste, in 0.1 M phosphate buffer, 0.05 M KCl (pH 7.0) containing 500 μM DA and scan rate of 0.1 V s^{-1}. As seen the maximum reduction and oxidation peak current attained with AgNPs/carbon paste (30 pg:1 g). This optimized ratio was used in all the experiments.

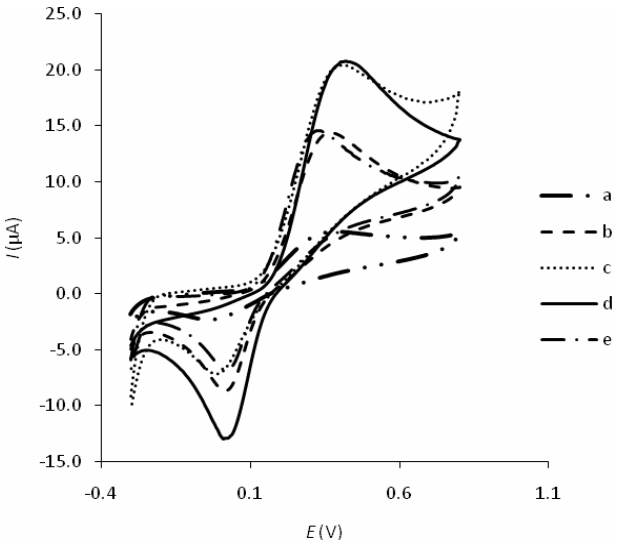

Fig. 2. Cyclic voltammograms of the AgNPs-CPE, in the presence of different ratios of AgNPs (pg)/carbon paste (g) (0.0 (a), 15.0 (b), 25.0 (c), 30.0 (d), 45.0 (e)) in 0.1 M phosphate buffer, 0.05 M KCl (pH 7.0) containing 500 μM DA and scan rate of 0.1 V s^{-1}.

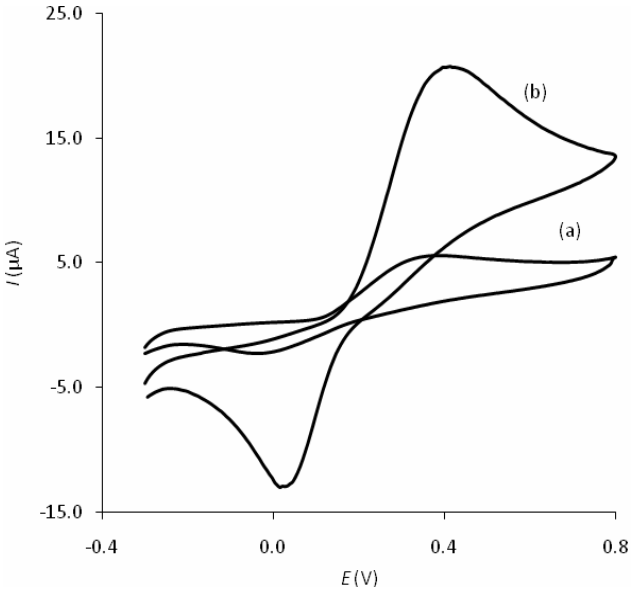

Fig. 3. Cyclic voltammograms of DA (500 μM) at the bare (a) and the AgNPs-CPE in 0.1 M phosphate buffer, 0.05 M KCl (pH 7.0) and scan rate of 0.1 V s^{-1}.

Electrochemical Behavior of DA at Modified Electrode

In the first attempt to evaluate the ability of the modified carbon paste electrode for the quantification of DA determination, the electrochemical behavior of DA was investigated at the surface of the electrode. Cyclic voltammograms of DA (500 μM) at the bare and the modified carbon paste electrode are shown in Fig. 3. As seen the redox wave of DA is very broad with a ΔE_p of 434 mV (E_{pa} = 390 mV, E_{pc} = -44 mV). Moreover, the anodic to cathodic peak current ratio is far from unity (I_a/I_c = 3.8). A pair of well-defined, sharp redox peaks with an increased current response was obtained for DA at the modified electrode. The positively shifted E_{pa} (402 mV) and E_{pc} (27 mV) results in a much smaller ΔE_p (375 mV) value. The I_a/I_c = 1.1 is much smaller than its value for DA at the bare electrode. These observations suggest faster electron transfer kinetics for DA at the modified electrode. The effect of scan rate on the electrochemical response of dopamine was investigated in the 0.02-0.90 V s^{-1} range (Fig. 4). there is a linear relationship between the I_{pa} and I_{pc} and the square root of the scan rate ($v^{1/2}$) over the range of 0.02-0.90 Vs^{-1} after subtracting the baseline current from the data (inset of Fig. 4), suggesting that the electron transfer for DA at the modified electrode is a diffusion-controlled process.

Due to simultaneous presence of AA and UA with DA in biological fluids and the proximity of their oxidation peak potential values, we have also investigated the electrochemical behavior of AA and UA at the modified electrode surface. Figure 5 (curves a and b) shows irreversible oxidation peaks at about 414 and 426 mV for AA and UA, respectively. As is clear the oxidation peak potentials of DA, AA and UA at pH 7.0 are very close to each other and the anodic peak potential of AA (E_{pa} = 414 mV) is more positive than DA (E_{pa} = 402 mV) which is

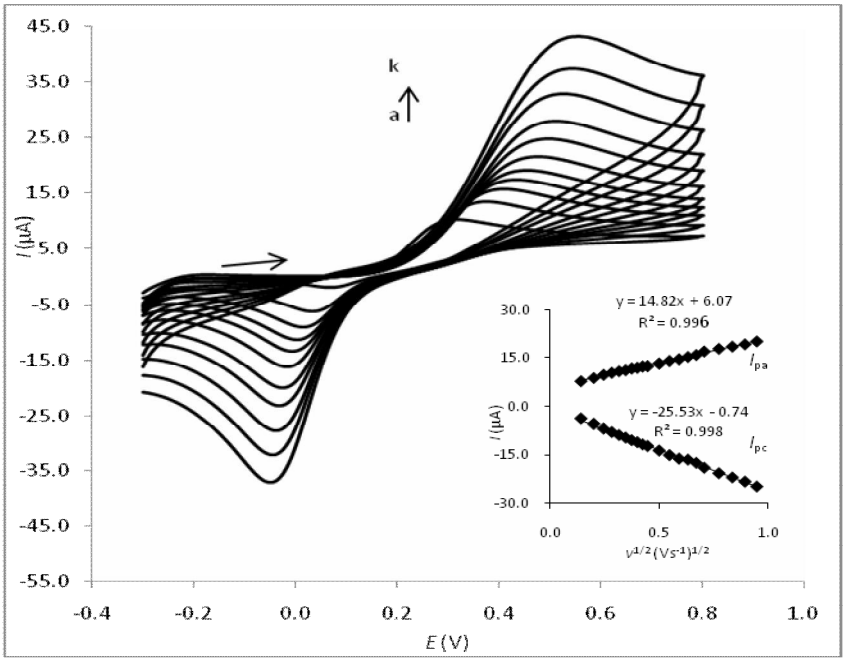

Fig. 4. Cyclic voltammograms of DA (500 μM) at the AgNPs-CPE in 0.1 M phosphate buffer solution (pH 7.0) over a range of scan rates (a-k: 0.02, 0.06, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80 and 0.90 V s^{-1}). Inset: anodic and cathodic peak currents *vs.* the square root of scan rate ($v^{1/2}$). The arrow indicates the scan direction.

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different from literature that the oxidation potential of DA is located between those of AA and UA [12,13,25]. In other words, in these conditions the oxidation tendency of DA is higher than the oxidation tendency of AA and UA. In order to describe this very unique feature of the present results obtained by the modified electrode, the cyclic voltammograms of DA, AA and UA were recorded at different pHs. Figure 6 shows the relationship between anodic peak potential and solution pH of the three mentioned species. As seen at pH values ranging from 5.0-8.0 the anodic peak potential of AA has no considerable change, whereas the anodic peak potentials for DA and UA are shifted to less positive potential with increasing pH. However, in the case of dopamine, the shift to the less positive potentials with increasing pH is more than the case of uric acid so that, at pHs ≥ 7.0 the position of the oxidation peak potential of AA changes and locates between DA and UA. This result could be explained from the fact that, at pH values lower than pK_{a1} of dopamine (8.57), the protonated form of dopamine predominates than unprotonated form and the positively charged molecule needs higher polarization potential for oxidation. This is because the average electronic charge on the molecule is lowered, therefore it is more difficult to withdraw electrons at such a lower potential, while at higher pH values the molecule is either neutral or negatively charged which increases its electro-oxidation [27-29,31]. Therefore, at pHs ≥ 7.0 the oxidation potential for dopamine is located before those of AA and UA. It is noteworthy that AA and UA have pK_a values of 4.10 and 5.75, respectively, and therefore in pH range of 5.0-8.0 are predominantly in anionic forms and by varying the pH in this range, the shift in their anodic potentials is very lower than the shift in anodic potential of dopamine. The effect of scan rate on the cyclic voltammograms of AA and UA were also recorded. Examination of the results showed that the electrochemical reaction for AA and UA at the modified electrode surface is also diffusion- controlled (data not shown).

Cyclic voltammogram of a 100 µM mixture of AA and UA (Fig. 7 (curve a)) shows only an oxidation peak at about 396 mV whereas, the cyclic voltammogram of a 100 µM mixture of DA, AA and UA at the modified electrode surface (Fig. 7 (curve b)) shows two oxidation peaks. The voltammetric peaks at about 182 mV and 116 mV are

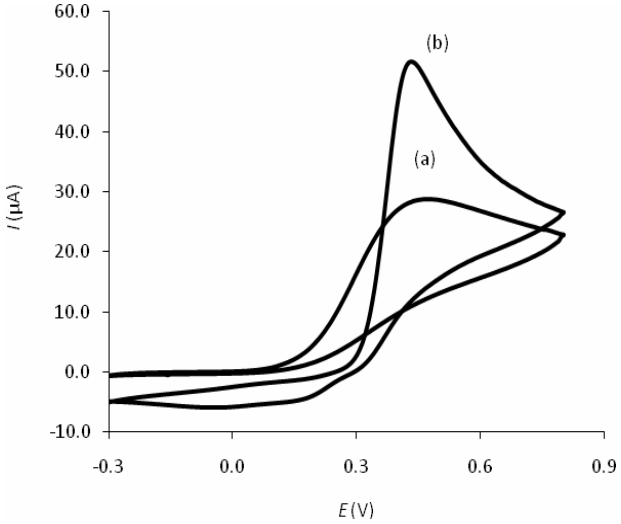

Fig. 5. Cyclic voltammograms of AA (a) and UA (b) (0.1 M) at the AgNPs-CPE in 0.1 M phosphate buffer solution, 0.05 M KCl (pH 7.0) and scan rate of 0.1 V s^{-1}.

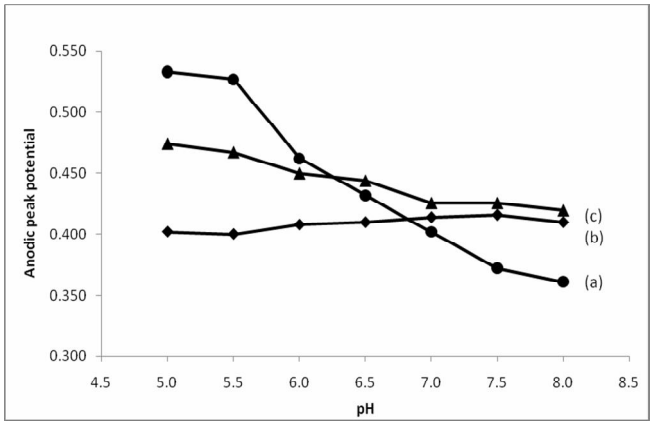

Fig. 6. Plot of anodic peak potential *vs*. pH. (a) DA, (b) AA, (c) UA.

attributed to the redox pair for DA, which is shifted considerably in comparison to the response of DA, while the oxidation peak at about 320 mV is attributed to the overlapping voltammetric response of AA and UA. The voltammetric signal of DA is slightly changed, while the oxidation peak current at about 320 mV increases for a

mixture solution containing 100 μM DA, 200 μM AA and 200 μM UA (Fig. 7 (curve c)). However, in the presence of AA and UA (100 μM) the oxidation peak current of dopamine was found to increase with increasing dopamine concentration (Fig. 8). These observations indicate that it is possible to determine DA without interference of AA and UA. Figure 9A depicts differential pulse voltammograms of a solution containing AA and UA (100 μM) with varying concentration of dopamine at the modified electrode surface in the potential range of -0.3-0.8 V. As seen, in the absence of DA there is only one oxidation peak at about 300 mV due to the voltammetric response of AA and UA. In the presence of dopamine another oxidation peak at about 152 mV is appeared that increases with increasing concentration of DA. The calibration curve was obtained by plotting the I_{pa} at 152 mV *vs.* DA concentration (Fig. 9B). The linear part was over the concentration range 12.5-300.0 μM DA with a regression equation of I_{pa} (μ*A*) = 0.074 *C* + 2.19 (*C* in μM, R^2 = 0.995) .The sensitivity of the electrode response to DA (the slope of the calibration curve) is 0.074 μA/μM. The detection limit, C_m, has been calculated by using the equation $C_m = 3S_{bl}/m$, where S_{bl} is the standard deviation of 10 replicate measurements of the oxidation peak current in the absence of DA (S_{bl} = 1.51 × 10^{-2} μA) and m is the slope of the calibration plot (0.074 μA/μM). From the analysis of these data the detection limit for dopamine under these experimental conditions is estimated to be on the order of 0.61 μM. Comparison of the performance of the modified electrode towards DA oxidation with some previous results is listed in Table 1. The results strongly suggest that DA can be selectively determined at the modified carbon paste electrode in the presence of AA and UA.

Analytical Application

The modified electrode was applied to determination of *DA* in dopamine hydrochloride injection samples. The DA injection sample provided from Caspian tamin pharmaceutical *co.* Rasht-Iran with a specified content of 40 mg ml^{-1}. The sample was used after dilution using phosphate buffer (0.1 M, pH 7.0). Differential pulse voltammetry was applied for quantitative measurement of dopamine in buffered- solution (0.1 M, pH 7.0) containing AA (100 μM), UA (100 μM) and KCl (0.05 M). The results presented in Table 2 seem to be satisfactory. Therefore, the

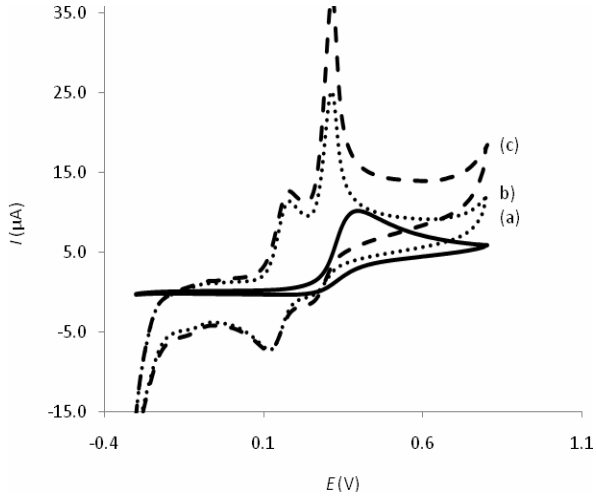

Fig. 7. Cyclic voltammograms of a mixture of AA-UA (100 μM) (a), DA-AA-UA (100 μM) (b) and DA (100 μM) - AA (200 μM) - UA (200 μM) (c) at the AgNPs-CPE in 0.1 M phosphate buffer solution, 0.05 M KCl (pH 7.0) and scan rate of 0.1 V s^{-1}.

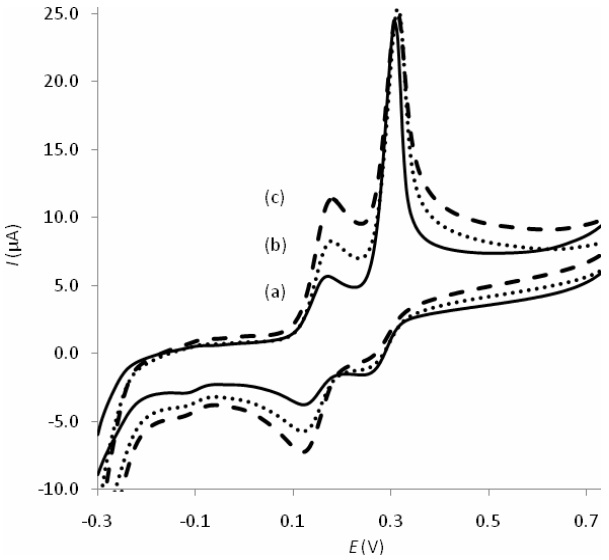

Fig. 8. Cyclic voltammograms of a mixture of AA (100 μM), UA (100 μM) and DA (50 μM (a), 75 μM (b), 100 μM (c)) at the AgNPs-CPE in 0.1 M phosphate buffer solution, 0.05 M KCl (pH 7.0) and scan rate of 0.1 V s^{-1}.

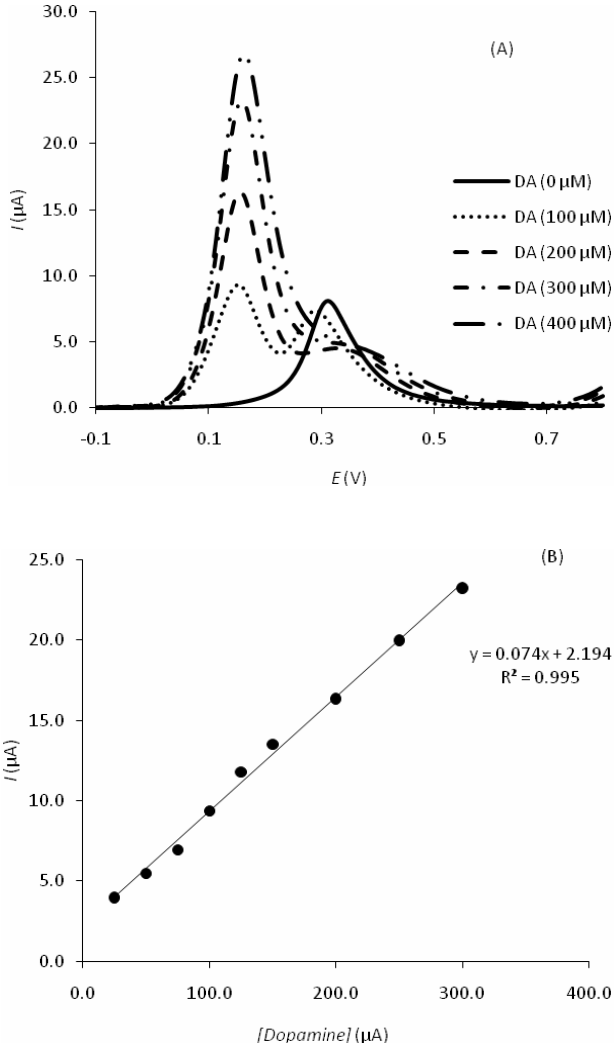

Fig. 9. (A) Differential pulse voltammograms of a mixture of AA (100 µM), UA (100 µM) and DA (0.0 µM (—), 100 µM (…), 200 µM (---), 300 µM (–•–), 400 µM (—••) at the AgNPs-CPE in 0.1 M phosphate buffer solution, 0.05 M KCl (pH 7.0). (B) Calibration curve of DA in the presence of AA and UA.

modified electrode provides a possible and simple method for determining of DA with good precision and accuracy.

The fabrication reproducibility of the modified electrode was examined from the response to 200 µM of DA at five

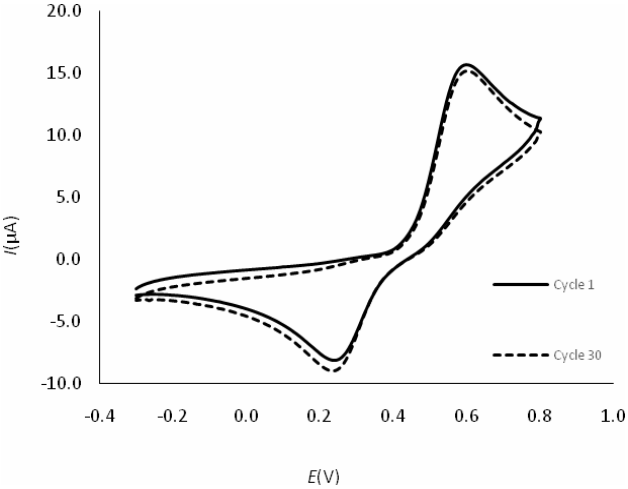

Fig. 10. The 1st and 30th recorded cyclic voltammogram of DA (200 µM) at the AgNPs-CPE in 0.1 M phosphate buffer solution, 0.05 M KCl (pH 7.0) and scan rate 0.1 V s^{-1}.

different modified electrodes. An accepted reproducibility was obtained with relative standard deviations (RSD%) of 8% and 3% for an average I_{pa} of 11.3 µA and an average I_{pc} of 11.2 µA, respectively. the stability of the modified electrode was investigated by cycling the electrode potential over the range of -0.3-0.8 V. According to Fig. 10, after 30 cycles the peak potential remained nearly unchanged while the anodic peak current decreased about 3% and cathodic peak current increased about 5%.

CONCLUSIONS

In summary, the results of the study confirmed that the silver nanoparticles-modified carbon paste electrode can be effectively used for selective and sensitive determination of DA in the presence of AA and UA at pH 7.0. The preparation procedure of the modified electrode is very simple and rapid. The modified electrode offers an excellent and wide linear calibration range, low detection limit and good reproducibility. The results showed that dopamine was effectively determined in injection samples containing AA and UA.

Table 1. Comparison of Different Modified Electrodes for DA Determination in the Presence of AA and UA

Electrode	Dynamic range (μM)	Detection limit (μM)	Method	Ref.
RNA-GCE[a]	0.37-36.00	0.20	DVP	[11]
Poly PAR-GCE[b]	5.00-30.00	0.20	DVP	[12]
Chitosan-gold electrode	0.50-19.50	0.36	SWV	[13]
Poly Calmagite-CPE[c]	10.00-35.00	0.01	DVP	[8]
HDS/SDS/CPE[d]	80.00-160.00	0.10	DVP	[14]
PA-SWNTs/Pt[e]	0.00-10.00	0.08	DVP	[15]
Poly DBF/GCE[f]	0.20-200.00	0.03	DVP	[16]
AgNPs-CPE	12.50-300.00	0.61	DVP	This work

[a]RNA-modified glassy carbon electrode. [b]Poly(4-(2-pyridyl-azo)-resorcinol) modified glassy carbon electrode. [c]Poly calmagite modified carbon pate electrode. [d]Hydroxyl double salt/surfactant film modified carbon paste electrode. [e]Platinum electrode modified by single-walled carbonnanotubes and phytic acid. [f]Glassy carbon electrode modified with dibromofluorescein.

Table 2. Determination Results of DA in Dopamine Hydrochloride Injection Samples (n = 3) in Buffered-solution (0.1 M, pH 7.0) Containing AA (100 μM), UA (100 μM) and KCl (0.05 M), Using Differential Pulse Voltammetry

DA concentration (μM)		Recovery% ± RSD%
Added	Found	
85.0	81.70	96.10 ± 4.10
95.0	91.40	96.20 ± 4.50
100.0	102.00	102.00 ± 3.30

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