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Simultaneous determination of dopamine, uric acid, and tryptophan using an MWCNT modified carbon paste electrode by square wave voltammetry

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A highly sensitive method was investigated for the simultaneous determination of dopamine (DA), uric acid (UA), and tryptophan (TRP) using a multiwall carbon nanotubes/5-amino-3',4'-dimethoxy-biphenyl-2-ol modified carbon paste electrode (5ADMBCNPE). The 5ADMBCNPE displayed excellent electrochemical catalytic activities towards the oxidation of DA, UA, and TRP. The electrochemical profile of the proposed modified electrode was analyzed by cyclic voltammetry (CV), which showed a shift in the oxidation peak potential of DA at 160 mV to a less positive value compared with an unmodified carbon paste electrode. Square wave voltammetry (SWV) in 0.1 M phosphate buffer solution (PBS) at pH 7.0 was performed to determine DA in the range from 1.2 to 800.0 μM, with a detection limit of 0.16 μM. The present method was applied to the determination of DA in some real samples.

Key Words: Dopamine, uric acid, tryptophan, carbon nanotube paste electrode, modified electrode

Introduction

Electrode surface modification is a field of paramount importance in modern electrochemistry especially due to the various application possibilities of modified electrodes.[1−4] In recent years, chemically modified carbon

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paste electrodes have received increasing attention due to their potential applications in various analyses and also due to the relative ease of electrode preparation and regeneration.[5−9]

Carbon nanotubes (CNTs) are one of the most important nanomaterials due to their high chemical stability; high surface area; high mechanical properties; unique electrical conductivity, metallic, and structural characteristics; and mechanical strength and elasticity. Carbon-based electrodes are currently in widespread use in electroanalytical chemistry, because of their broad potential window, low cost, rich surface chemistry, low background current, and chemical inertness.[10−18]

Dopamine (DA) is a biological molecule formed as a result of the decarboxylation of 3,4-dihydroxy phenylalanine. It acts as a neurotransmitter in both the central and peripheral nervous system. Some diseases found to be associated with either low concentration or abnormal metabolisms of DA are Parkinson's disease, epilepsy, senile dementia, and HIV infection.[19,20]

Several analytical techniques have been used in the past for DA detection but each with one disadvantage or another. For example, chromatographic and electrophoretic methods are very expensive, complex, and cumbersome even though they are selective and specific. The fluorometric method requires a lot of sample and it is time consuming. Electrochemical methods have also been employed with the use of different modified electrodes.[21−24]

Uric acid (UA) is the end product of catabolism of the purine nucleosides.[25] Most of the UA produced from the catabolism is reabsorbed into the blood circulation system after primary filtration and partial secretion by the kidney. UA levels in physiological fluids such as plasma and urine serve as valuable indicators for certain clinical conditions. For example, an elevated level of UA in blood is associated with gout, renal failure, leukemia, and lymphoma as well as other pathological conditions.[26] Therefore, a simple, sensitive, and accurate analytical method for the quantitation of UA would be useful for physiological investigations as well as disease diagnosis. As an electrochemical device, DA can also be studied via electrochemical techniques.[27−30]

Tryptophan (TRP) is well known as an essential amino acid in humans and herbivores, and it is a precursor to the neurotransmitter serotonin. Serotonin availability in the brain depends on blood TRP levels, which can modulate the psychoneural control of spontaneous alternation through presynaptic inhibition of hippocampal cholinergic terminals. Therefore, simple, sensitive and inexpensive detection of TRP is of great interest. Currently, HPLC[31] and capillary electrophoresis techniques[32] have most frequently been used for such applications owing to their high performance in separation efficiency. However, these methods are often complex, tedious, and time-consuming. For simple, sensitive, and inexpensive detection of TRP, methods based on electroanalysis might have substantially more utility by their electrochemical activity. In the literature, several electrochemical methods to determine TRP have been developed.[33−36]

In the present work, we describe the preparation of a new electrode composed of CNPE modified with 5-amino-2',4'-dimethoxy-biphenyl-2-ol (5ADMBCNPE) and investigate its performance for the electrocatalytic determination of DA in aqueous solutions. We also evaluate the analytical performance of the modified electrode for quantification of DA in the presence of UA and TRP.

Experimental

Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 12, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. A conventional 3-electrode cell was used at 25 ± 1 °C. A Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and the 5ADMBCNPE were used as the reference, auxiliary, and working electrodes, respectively. A Metrohm 827 pH/Ion Meter was used for pH measurements.

All solutions were freshly prepared with double distilled water. DA, UA, TRP, and all other reagents were of analytical grade from Merck (Darmstadt, Germany). Graphite powder and paraffin oil (DC 350, density = 0.88 g cm^{-3}) as the binding agent (both from Merck) were used for preparing the pastes. Multiwalled carbon nanotubes (purity more than 95%) with o.d. between 10 and 20 nm, i.d. between 5 and 10 nm, and tube length from 0.5 to 200 μm were prepared from Nanostructured & Amorphous Materials, Inc. The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 3.0-11.0.

Synthesis of 5-amino-2',4'-dimethoxy-biphenyl-2-ol

For preparation of the title compound, 2.18 g (20 mmol) of 4-aminophenol, 4.34 g (20 mmol) of 4-bromo-1,3-dimethoxybenzene, and 4.62 g of $Pd(PPh_3)_4$ were placed into a 50 mL conical vial and 20 mL of dimethylacetamide (DMA) was added. Then a magnetic spin vane was added to the conical vial and attached to a water-cooled condenser. It was heated at about 90 °C for at least 12 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the resulting mixture was allowed to cool for a few minutes, and $Pd(PPh_3)_4$ was collected by vacuum filtration using a Hirsch funnel. Then chloroform was added to the mixture and filtered to recover the catalyst. The crude product was recrystallized from iso-propanol and chloroform (20:80) to afford pure 5-amino-2',4'- dimethoxy.

Preparation of the electrode

The 5ADMBCNPEs were prepared by hand mixing 0.01 g of 5ADMB with 0.89 g of graphite powder and 0.1 g of CNTs with a mortar and pestle. Then ~0.7 mL of paraffin oil was added to the above mixture and mixed for 20 min until a uniformly wetted paste was obtained. The paste was then packed into the end of a glass tube (ca. 3.4 mm i.d. and 15 cm long). A copper wire inserted into the carbon paste provided the electrical contact. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing with a weighing paper.

For comparison, 5ADMB modified CPE electrode (5ADMB-CPE) without CNTs, CNTs paste electrode (CNPE) without 5ADMB, and unmodified CPE in the absence of both 5ADMB and CNTs were also prepared in the same way.

Results and discussion

Electrochemical properties of 5ADMBCNPE

To the best of our knowledge there is no prior report on the electrochemical properties and, in particular, the electrocatalytic activity of 5ADMB in aqueous media. Therefore, we prepared 5ADMBCNPE and studied its electrochemical properties in a buffered aqueous solution (pH 7.0) using CV (Figure 1). It should be noted that one of the advantages of 5ADMB as an electrode modifier is its insolubility in aqueous media. Experimental results showed reproducible, well-defined, anodic, and cathodic peaks with E_{pa}, E_{pc}, and $E^{\circ\prime}$ of 0.29, 0.17, and 0.23 V vs. Ag/AgCl/KCl (3.0 M), respectively. The observed peak separation potential, $\Delta E_p = (E_{pa} - E_{pc})$ of 120 mV, was greater than the value of 59/n mV expected for a reversible system,[37] suggesting that the redox couple of 5ADMB in 5ADMBCNPE has quasi-reversible behavior in aqueous medium. The effect of the potential scan rate (ν) on the electrochemical properties of the 5ADMBCNPE was also studied by CV. Plots of both the anodic and cathodic peak currents (I_p) were linearly dependent on ν in the range of 10 to 800 mV s^{-1} (Figure 1A), indicating that the redox process of 5ADMB at the modified electrode is diffusionless in nature.[37]

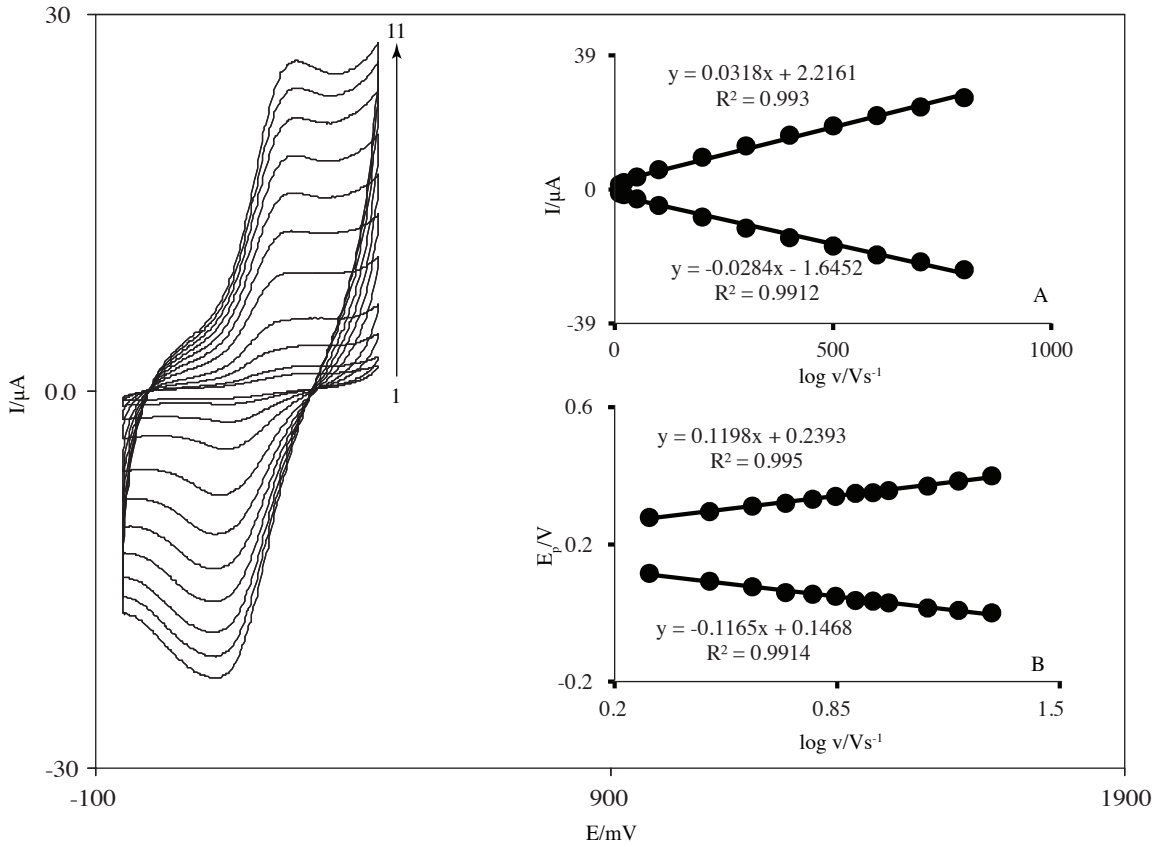

Figure 1. CVs of 5ADMBCNPE in 0.1 M PBS (pH 7.0), at various scan rates, numbers 1-11 correspond to 10, 20, 50, 100, 200, 300, 400, 500, 600, 700, and 800 mV s^{-1}. Insets: variation of (A) I_p vs. scan rate; (B) Variation of E_p versus the logarithm of the high scan rates.

The apparent charge transfer rate constant, k_s, and the charge transfer coefficient, α, of a surface-confined redox couple can be evaluated from CV experiments by using the variation of anodic and cathodic peak potentials with logarithm of scan rate, according to the procedure of Laviron.[38] Figure 1B shows such plots, indicating that the E_p values are proportional to the logarithm of scan rate for ν values higher than 2 V s^{-1} (Figure 1B). The slopes of the plots in Figure 1B can be used to extract the kinetic parameters α_c and α_a (cathodic and anodic transfer coefficients, respectively). The slopes of the linear segments are equal to $-2.303RT/\alpha nF$ and $2.303RT/(1-\alpha)nF$ for the cathodic and anodic peaks, respectively. The evaluated value for the α is 0.5.

Also, Eq. (1) can be used to determine the electron transfer rate constant between modifier (5ADMB) and CNPE:

$$\log k_s = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log(RT/nF\nu) - \alpha(1-\alpha)nF\Delta E_p/2.3RT \quad (1)$$

where $(1-\alpha)n_\alpha = 0.5$, ν is the sweep rate, and all other symbols have their conventional meanings. The value of k_s was evaluated to be 7.94 s^{-1} using Eq. (1).

Influence of pH

The electrochemistry of 5ADMB molecule is generally pH dependent. Thus, the electrochemical behavior of 5ADMBCNPE was studied at different pHs using SWV. It was observed that the anodic and cathodic peak potentials of 5ADMBCNPE shift to less positive values with increasing pH. Potential-pH diagrams were constructed by plotting the anodic potential values as the function of pH and a slope of 50.2 mV/pH for E_p was obtained, indicating that the system obeys the Nernst equation for an equal electron and proton transfer reaction.[37]

Electrocatalytic oxidation of DA at a 5ADMBCNPE

Figure 2 depicts the CV responses for the electrochemical oxidation of 0.1 mM DA at unmodified CPE (curve b), CNPE (curve d), 5ADMBCPE (curve e), and 5ADMBCNPE (curve f). While the anodic peak potential for DA oxidation at the CNPE and unmodified CPE are 400 and 450 mV, respectively, the corresponding potential at 5ADMBCNPE and 5ADMBCPE is ~290 mV. These results indicate that the peak potential for DA oxidation at the 5ADMBCNPE and 5ADMBCPE electrodes shift by ~110 and 160 mV toward negative values compared to CNPE and unmodified CPE, respectively. However, 5ADMBCNPE shows much higher anodic peak current for the oxidation of DA compared to 5ADMBCPE, indicating that the combination of CNTs and the mediator (5ADMB) significantly improved the performance of the electrode toward DA oxidation. In fact, 5ADMBCNPE in the absence of DA exhibited a well-behaved redox reaction (Figure 2, curve c) in 0.1 M PBS (pH 7.0). However, there was a drastic increase in the anodic peak current in the presence of 0.1 mM DA (curve f), which can be related to the strong electrocatalytic effect of the 5ADMBCNPE towards this compound.[37]

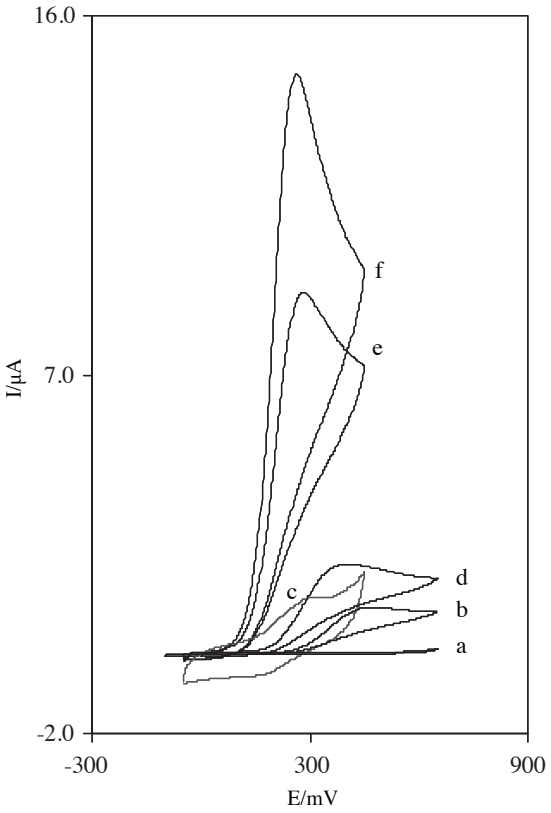

Figure 2. CVs of (a) unmodified CPE in 0.1 M PBS (pH 7.0), (b) unmodified CPE in 0.1 mM DA, (c) 5ADMBCNPE in 0.1 M PBS, (d) CNPE in 0.1 mM DA, (e) 5ADMBCPE in 0.1 mM DA, and (f) 5ADMBCNPE in 0.1 mM DA. In all cases the scan rate was 10 mV s^{-1}.

The effect of scan rate on the electrocatalytic oxidation of DA at the 5ADMBCNPE was investigated by linear sweep voltammetry (LSV) (Figure 3). The oxidation peak potential shifted to more positive potentials with increasing scan rate, confirming the kinetic limitation in the electrochemical reaction. Moreover, a plot of peak height (I_p) vs. the square root of scan rate ($\nu^{1/2}$) was found to be linear in the range of 2-10 mV s^{-1}, suggesting that, at sufficient overpotential, the process is diffusion rather than surface controlled (Figure 3A). The Tafel slope (b) can be obtained from the slope of E_p vs. log v using Eq. (2):[39]

$$Ep = b/2 \log v + \text{ constant.} \tag{2}$$

The Tafel slope was found to be 111.6 mV (Figure 3, inset B), which indicates that a one-electron transfer process is the rate limiting step assuming a transfer coefficient (α) of about 0.47.

Chronoamperometric measurements

Chronoamperometric measurements of DA at 5ADMBCNPE were obtained for the various concentration of DA in PBS (pH 7.0) (Figure 4). For an electroactive material (DA 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.[37] Experimental plots of I vs. $t^{-1/2}$ were employed, with the best fits for different concentrations of DA (Figure 4A). The slopes of the resulting straight lines were then plotted vs. DA concentration (Figure 4B). From the resulting slope and Cottrell equation the mean value of the D was found to be 6.55×10^{-6} cm^2/s.

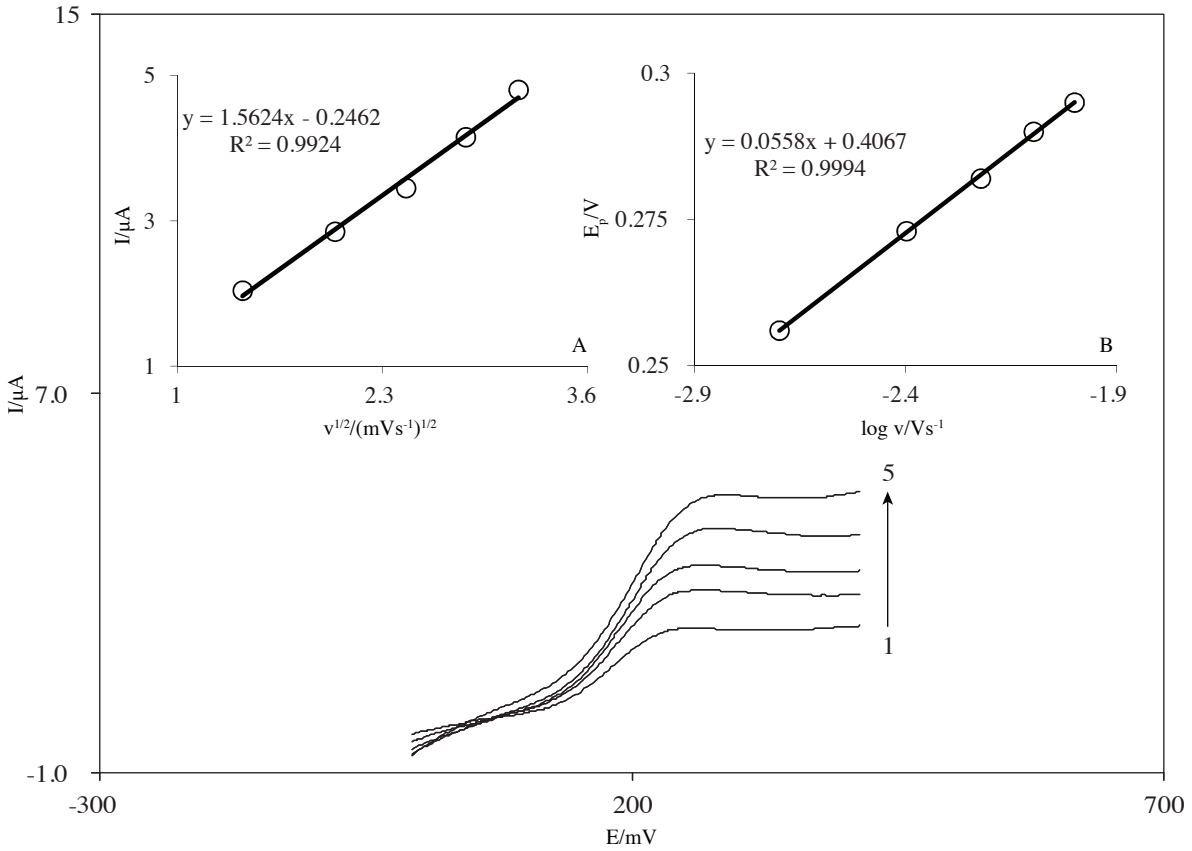

Figure 3. Linear sweep voltammograms of 5ADMBCNPE in 0.1 M PBS (pH 7.0) containing 10.0 μM DA at various scan rates; numbers 1-5 correspond to 2, 4, 6, 8, and 10 mV s^{-1}, respectively. Insets: Variation of (A) anodic peak current vs. $\nu^{1/2}$; (B) anodic peak potential vs. log v.

Electrocatalytic determination of DA

The SWV method was used to determine the concentration of DA. The plot of peak current vs. DA concentration consisted of 2 linear segments with slopes of 0.0536 and 0.036 μA μM^{-1} in the concentration ranges of 1.2 to 40.0 μM and 40.0 to 800.0 μM, respectively. The decrease in sensitivity (slope) of the second linear segment is likely due to kinetic limitation. The detection limit (3σ) of DA was found to be 0.16 μM.

Simultaneous determination of DA, UA, and TRP

To our knowledge, there is no report on the simultaneous determination of DA, UA, and TRP using 5ADMBCNPE. Therefore, the main objective of this study was to detect DA, UA, and TRP simultaneously using

5ADMBCNPE. This was performed by simultaneously changing the concentrations of DA, UA, and TRP, and recording the SWVs. The voltammetric results showed well-defined anodic peaks at potentials of 240, 410, and 750 mV, corresponding to the oxidation of DA, UA, and TRP, respectively, indicating that simultaneous determination of these compounds is feasible at the 5ADMBCNPE as shown in Figure 5.

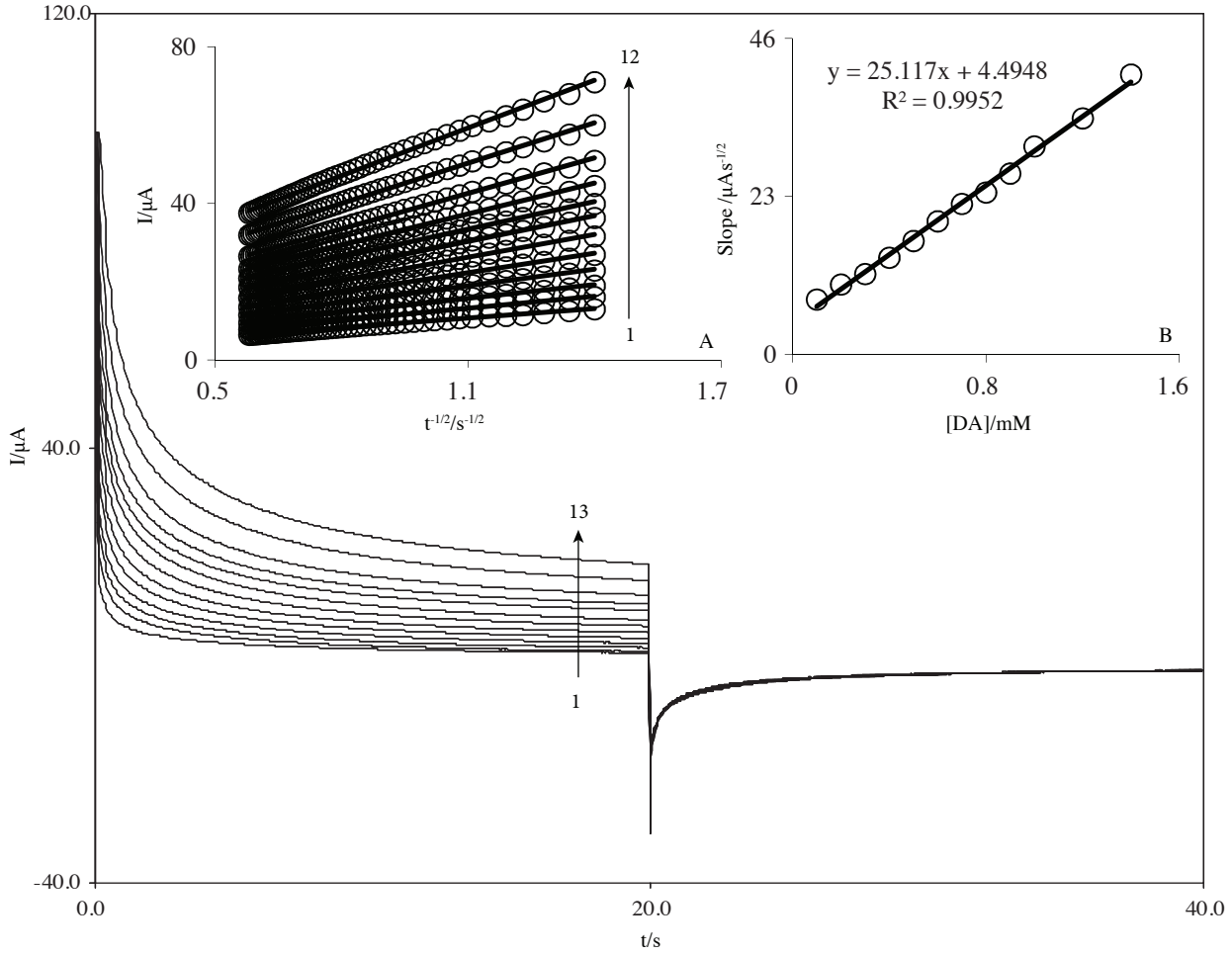

Figure 4. (A) Chronoamperograms obtained at 5ADMBCNPE in 0.1 M PBS (pH 7.0) for different concentration of DA. The numbers 1-13 correspond to 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, and 1.4 mM of DA. Insets: (A) Plots of I vs. $t^{-1/2}$ obtained from chronoamperograms 2-13. (B) Plot of the slope of the straight lines against DA concentration.

The sensitivity of the modified electrode towards the oxidation of DA was 0.054 μA μM^{-1}. This is very close to the value obtained in the absence of UA and TRP (0.053 μA μM^{-1}), indicating that the oxidation processes of these compounds at the 5ADMBCNPE are independent and, therefore, simultaneous determination of their mixtures is possible without significant interferences.

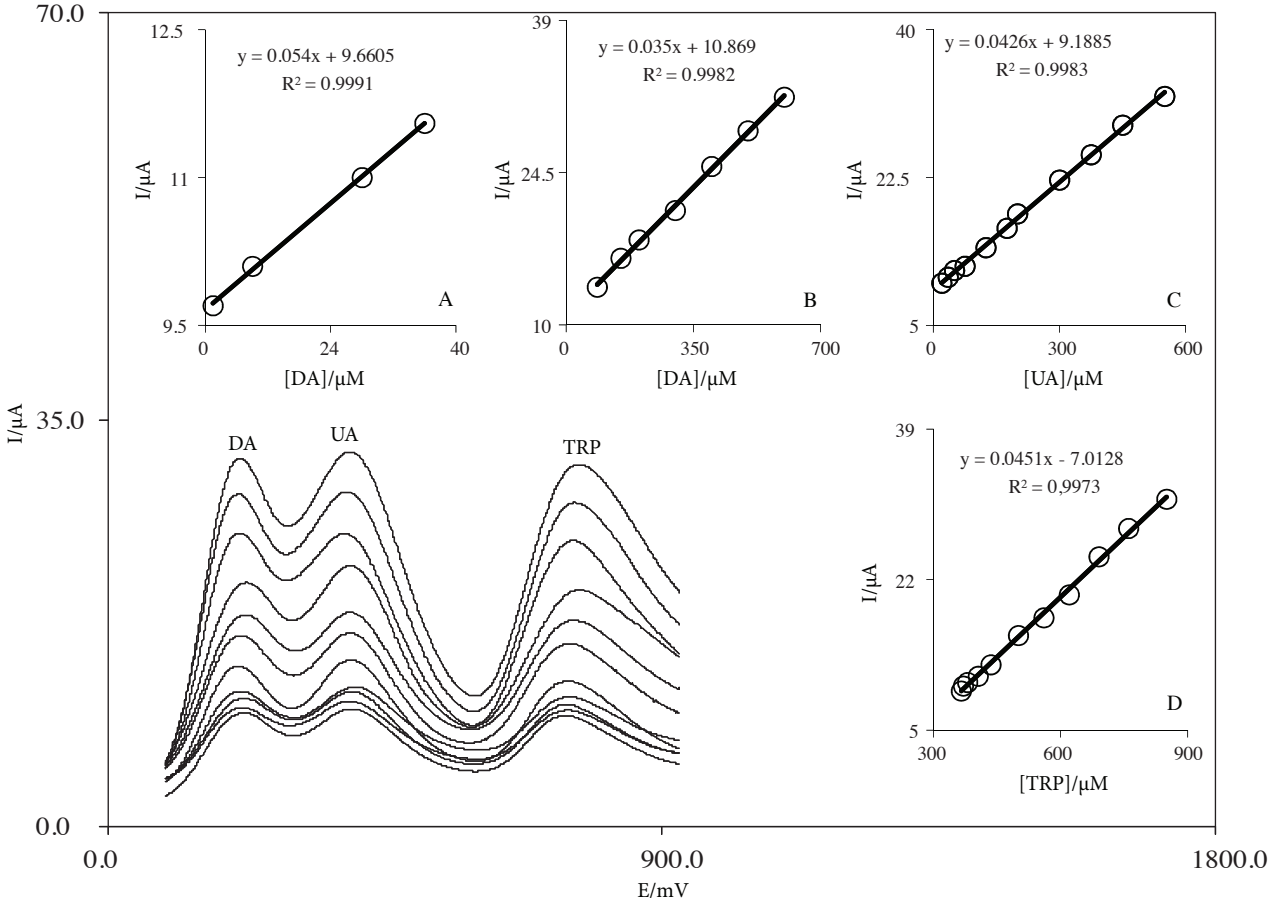

Figure 5. SWVs of 5ADMBCNPE in 0.1 M PBS (pH 7.0) containing different concentrations of DA + UA + TRP in μM, from inner to outer: 1.2 + 20.0 + 365.0, 7.5 + 35.0 + 370.0, 25.0 + 50.0 + 380.0, 35.0 + 75.0 + 405.0, 85.0 + 125.0 + 435.0, 150.0 + 175.0 + 500.0, 200.0 + 200.0 + 560.0, 300.0 + 300.0 + 620.0, 400.0 + 375.0 + 690.0, 500.0 + 450.0 + 760.0, and 600.0 + 550.0 + 850.0, respectively. Insets (A), (B), (C) and (D) are plots of I_p vs. DA, UA, and TRP concentrations, respectively.

Real sample analysis

In order to evaluate the analytical applicability of the proposed method, it was applied to the determination of DA in DA ampoule. One milliliter of a DA ampoule was diluted to 10 mL with PBS (0.1 M, pH 7.0); then different capacity of the diluted solution was transferred into each of a series of 10 mL volumetric flasks and diluted to the mark with PBS. Each sample solution was transferred into the electrochemical cell and SVW was recorded between 0.0 and 0.6 V at a scan rate of 10 mV s^{-1}. The I_{pa} was measured at the oxidation potential of DA and the concentration of this compound was obtained from the calibration plot. This procedure was repeated 3 times for each sample, and the average amount of DA in the injection was found to be 40.2 mg, a value in good agreement with the value on the ampoule label (40.0 mg). Moreover, a constructed sensor was applied to the determination of DA in urine samples (see Table). Although ascorbic acid showed interference,

its interference can be minimized, if necessary, by using ascorbic oxidase enzyme, which exhibits high selectivity for the oxidation of ascorbic acid.[40−42]

Table. Determination of DA in DA injection and urine samples. All the concentrations are in μM (n = 3).

Sample	No.	DA content	Added	Found	Recovery %	RSD %
DA injection						
	1	5.0	0	5.1	102.0	1.7
	2	5.0	5.0	9.9	99.0	3.4
	3	5.0	10.00	14.9	99.3	2.2
	4	5.0	15.0	20.3	101.5	2.9
Urine sample						
	1	ND[1]	2.5	2.4	96.0	2.6
	2	ND	5.0	4.9	98.0	3.4
	3	ND	10.0	10.1	101.0	1.8

[1]Not detected

Conclusions

A simple, fast, reproducible, and direct procedure was used for the fabrication of the multiwall carbon nanotube modified carbon paste electrode. The modified electrode greatly catalyzed the electrooxidation reactions of DA, UA, and TRP, improving their electrochemical reversibility and oxidation peak separation. Thus, the large peak separations between DA, UA, and TRP allow their simultaneous analysis through square wave voltammetry. The proposed method could be applied to the determination of DA in DA injection and urine samples with quite promising results.

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