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New voltammetric strategy for determination of dopamine in the presence of high concentrations of acetaminophen, folic acid and N-acetylcysteine

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ABSTRACT

A new electrochemical sensor for the simultaneous determination of dopamine (DA), acetaminophen (AC), folic acid (FA) and N-acetylcysteine (NAC) is described. The sensor is based on carbon-paste electrode (CPE) modified with 5-amino-3′,4′-dimethyl -biphenyl-2-ol (5ADB) and takes the advantages of carbon nanotubes (CNTs), which makes the modified electrode highly sensitive for the electrochemical detection of these compounds. Under the optimum pH of 7.0, the oxidation of DA occurs at a potential about 170 mV less positive than that of the unmodified CPE. Also, square wave voltammetry was used for the simultaneous determination of DA, AC, FA and NAC at the modified electrode.

1. Introduction

Since the electrochemical properties of carbon nanotubes (CNTs) have been unveiled, their application in electrochemical sensors and biosensors has gained much attention [1,2]. Recent studies demonstrated that CNTs have a high electrocatalytic effect and a fast electron-transfer rate [3–6]. CNTs-modified electrodes can accumulate important biomolecules and alleviate surface fouling effects.

Dopamine (DA) is one of the most important catecholamine neurotransmitters in the mammalian central nervous system. Abnormalities in DA concentrations have been linked with several neurological disorders such as the debilitating ailment Parkinson's disease and the mental disorder schizophrenia [7]. DA is also believed to play a central role in Huntington's disease, a fatal genetic neurodegenerative movement disorder and has also been associated with drug addiction and attention disorders [8]. Therefore, DA has been given tremendous consideration in biomedical investigation and there is a strong need to establish sensitive, selective and reliable methods for the direct measurement of DA. Since DA is an electroactive compound and can be easily oxidized, the electroanalysis of DA based on the electro-oxidation had been reported widely with the advantages of simplicity, high speed and sensitivity [9–13].

Acetaminophen (AC) is a widely used anti-pyretic and analgesic drug with actions similar to aspirin. It is an effective and safe agent for the relief of mild to moderate pain associated with headache, arthritis and postoperative pain. Its ready access has resulted in its increased use in attempted suicide [14]. Among different methods for determination of AC, electrochemical methods maybe the most widely applied because of high sensitivity, simplicity and reproducibility of this approach [15–19].

Several chronic diseases (for example, gigantocytic anemia, leucopoenia, mentality devolution, psychosis, heart attack, and stroke), especially those concerned with malformation during pregnancy and carcinogenic processes, are related to the deficiency of folic acid (FA) [20] which is a water-soluble vitamin. Since FA is detected in biological fluids at low concentration, a highly specific and sensitive assay is called for. As FA is an electroactive component, some electrochemical methods have been reported for its determination [21–25].

N-acetylcysteine (NAC) is an endogenous antioxidant acting as a free radical scavenger and precursor of glutathione synthesis. This reactivity makes NAC a powerful antioxidant and a potentially therapeutic agent in the treatment of cancer, cardiovascular and respiratory diseases, human immunodeficiency virus (HIV) infection, acetaminophen toxicity, neurodegenerative disorder and other

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diseases characterized by free radicals production and oxidative damage [26–30].

AC electrophysiological [31] effects support the idea that this potent analgesic drug can act in the central nervous system (CNS). Animal model studies have shown that AC might protect neurons from degeneration. For example, AC can protect primary rat embryonic DA neurons from glutamate toxicity [32]. Also, AC administration at antinociceptive doses affects serotonin (5-HT) and DA levels in various brain areas and the spinal cord in rats [33]. Additionally, important drugs such as AC will interfere with DA measurements in biological samples [34]. Thus, it is necessary to develop low-cost, simple, reproducible and reliable methods for simultaneous determination of DA and AC, which is a major goal of electroanalytical research.

DA oxidation products such as H_2O_2 and reactive quinones have been held responsible for various toxic actions of DA, which have implications in the aetiopathogenesis of Parkinson's disease. Studies have shown that NAC is a potent scavenger of both H_2O_2 and toxic quinones derived from DA and it further prevents DA mediated inhibition of Na+,K+-ATPase activity and mitochondrial respiratory chain function. The quinone scavenging ability of NAC is presumably related to its protective effect against DA mediated inhibition of mitochondrial respiratory chain activity. However, both H_2O_2 scavenging and quinone scavenging properties of NAC probably account for its protective effect against Na+,K+-ATPase inhibition induced by DA. The results have important implications in the neuroprotective therapy of sporadic Parkinson's disease since inactivation of mitochondrial respiratory activity and Na+,K+-ATPase may trigger intracellular damage pathways leading to the death of nigral dopaminergic neurons [35].

Mouse experiments suggest that FA deficiency could increase the brain's susceptibility to Parkinson's disease, according to scientists at the National Institute on Aging. In the finding, published in the January 2002 issue of the Journal of Neurochemistry, the investigators fed one group of mice a diet that included folate, while a second group was fed a diet lacking this vitamin. They then gave the mice moderate amounts of MPTP, a chemical that can cause Parkinson-like symptoms. In the mice fed folate, MPTP caused only mild symptoms of disease. But mice fed the folate-deficient diet developed severe Parkinson-like symptoms [36].

The scientists found that mice with low amounts of dietary FA had elevated levels of homocysteine in the blood and brain. They suspect that increased levels of homocysteine in the brain caused damage to the DNA of nerve cells in the substantia nigra, an important brain structure that produces DA. Loss of DA causes the nerve cells to dysfunction, leaving patients unable to direct or control their movement in a normal manner [37].

People who have Parkinson's disease often have low levels of FA in their blood, but it is not clear whether this result of the disease process or if they are simply malnourished due to their illness. But based on this study, Dr. Mattson speculates that consuming adequate amounts of FA-either in the diet or by supplementation-could help protect the aging brain against Parkinson's and other neurodegenerative diseases[37].

Therefore, simultaneous determination of DA, AC, FA and NAC is very important, but there is not any electrochemical report about simultaneous determination of these four compounds.

Electroanalytical methods have attracted more attention in recent years for environmental and biological compounds determination due to their sensitivity, accuracy, lower cost, and simplicity [38–85].

In the present work, we describe the preparation of a new electrode composed of CNPE modified with 5-amino-3′,4′-dimethyl -biphenyl-2-ol (5ADBCNPE) and investigate its performance for the electrocatalytic determination of DA in aqueous solutions. We also evaluate the analytical performance of the modified electrode for the quantification of DA in the presence of AC, FA and NAC for the first time.

2. Experimental

2.1. 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 three electrode cell was used at 25 ± 1 °C. An Ag/AgCl/KCl (3.0 M) electrode (Metrohm), a platinum wire (Metrohm), and the (5ADBCNPE) 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, AC, FA and NAC 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 2.0–9.0.

2.2. Synthesis of 5-amino-3′,4′-dimethyl-biphenyl-2-ol

For preparing of the title compound, 3.02 g (20 mmol) of N-(2-Hydroxy-phenyl)-acetamide, 3.70 g (20 mmol) of 4-bromo-o-xylene and 2.81 g of PdCl2(PPh3)2 (bis(triphenylphosphine)palladium(II) chloride) were placed in a 50 mL conical vial and add 20 ml of dimethylacetamide (DMA) and 6.72 g t-BuOK. 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 reaction was monitored by TLC. After completion of reaction, the resulting mixture was allowed to be cooled for a few minutes, and Pd(PPh3)4 was collected by vacuum filtration using a Hirsch funnel. Then, chloroform was added to the mixture and filtered to recover the catalyst. Acetamides are readily cleaved by barium hydroxide with >90 % yields. The crude product was recrystallized from iso-propanol and chloroform (20:80) to afford pure 5-amino-3',4'- dimethyl -biphenyl-2-ol with 83 % yields.

2.3. Preparation of the electrode

The 5ADBCNPEs were prepared by hand mixing 0.01 g of 5ADB with 0.89 g graphite powder and 0.1 g 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, 5ADB modified CPE electrode (5ADB-CPE) without CNTs, CNTs paste electrode (CNPE) without 5ADB, and unmodified CPE in the absence of both 5ADB and CNTs were also prepared in the same way.

3. Results and discussion

3.1. Electrochemical properties of 5ADBCNPE

To the best of our knowledge there is no prior report on the electrochemical properties and, in particular, the electrocatalytic activity of 5ADB in aqueous media. Therefore, we prepared 5ADBCNPE and studied its electrochemical properties in a buffered aqueous solution (pH 7.0) using CV (Fig.1). It should be noted that one of the advantages of 5ADB

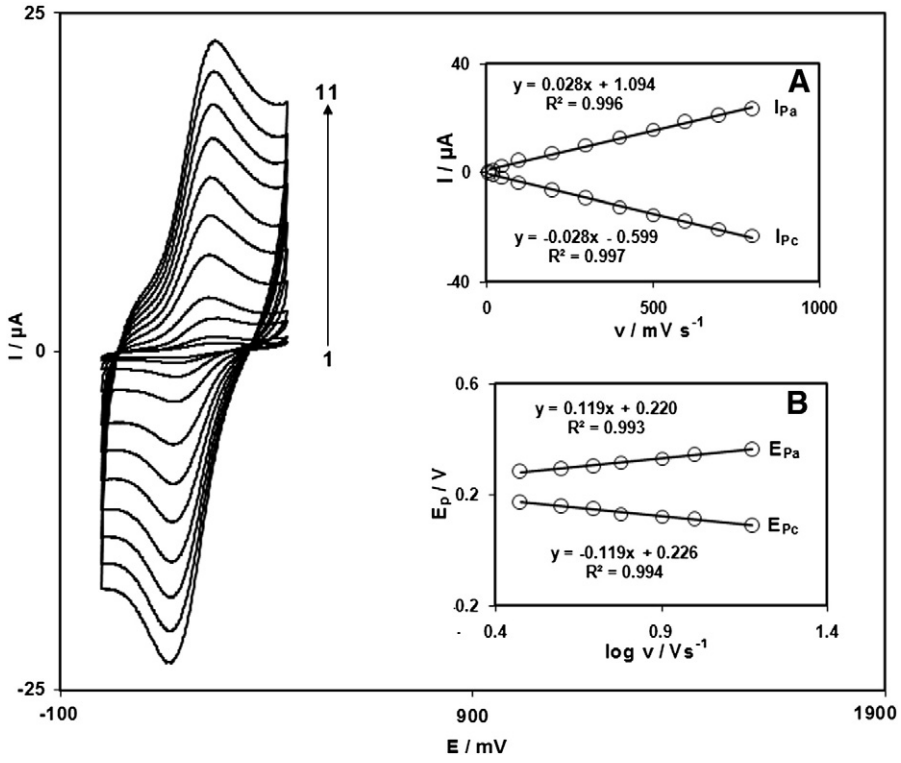

Fig. 1. CVs of 5ADBCNPE in 0.1 M PBS (pH 7.0), at various scan rates, numbers 1–11 correspond to 10, 20, 50, 100, 200, 300, 400, 500, 600, 700 and 800 mV s^{-1}. Insets: variation of (A) I_p vs. scan rate; (B) variation of E_p versus the logarithm of the high scan rates.

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 0.28, 0.18 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 100 mV, was greater than the value of 59/n mV expected for a reversible system [86], suggesting that the redox couple of 5ADB in 5ADBCNPE has a quasi-reversible behavior in aqueous medium. The effect of the potential scan rate (ν) on electrochemical properties of the 5ADBCNPE was also studied by CV. Plots of the both anodic and cathodic peak currents (I_p) were linearly dependent on ν in the range of 10 to 800 mV s^{-1} (Fig. 1A), indicating that the redox process of 5ADB at the modified electrode is diffusionless in nature.

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 [87]. Fig. 1B shows such plots, indicating that the E_p values are proportional to the logarithm of scan rate for ν values higher than 3 V s^{-1} (Fig. 1B). The slopes of the plots in Fig. 1B can be used to extract the kinetic parameters α_c and α_a (cathodic and anodic transfer coefficients, respectively). The slope of the linear segments is equal to $-2.303RT/\alpha nF$ and $2.303RT/(1-\alpha)nF$ for the cathodic and anodic peaks, respectively. The evaluated value for α is 0.5.

Also, Eq. (1) can be used to determine the electron transfer rate constant between the modifier (5ADB) 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 having their conventional meanings. The value of k_s was evaluated to be 23.9 s^{-1} using Eq. (1).

3.2. Influence of pH

The electrochemistry of 5ADB molecule is generally pH dependent. Thus, the electrochemical behavior of 5ADBCNPE was studied at different pHs using CV (Fig. 2). It was observed that the anodic and cathodic peak potentials of 5ADBCNPE shift to less positive values with increasing pH. Inset of Fig. 2 shows potential-pH diagrams constructed by plotting the anodic, cathodic and half-wave potential values as the function of pH. As can be seen the slopes are 47.845, 49.262 and 48.298 mV/pH

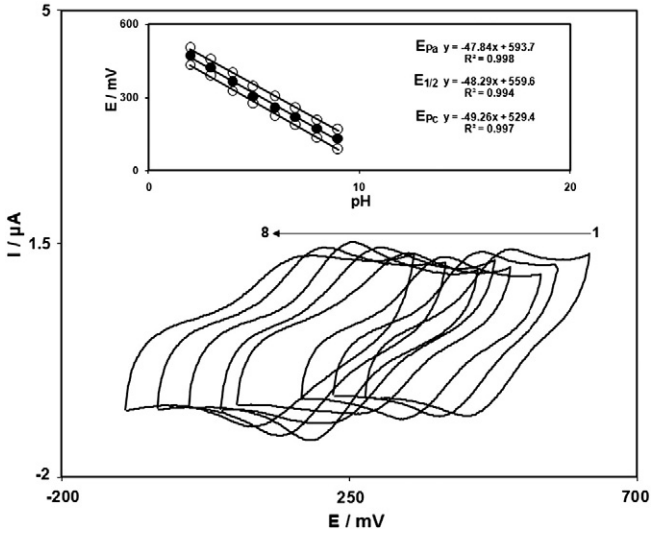

Fig. 2. CVs (at 20 mV s^{-1}) of 5ADBCNPE at various buffered pHs. The numbers 1–8 correspond to 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 pHs, respectively. Inset: Plot of E_{pa}, E_{pc} and $E_{1/2}$ vs. pH.

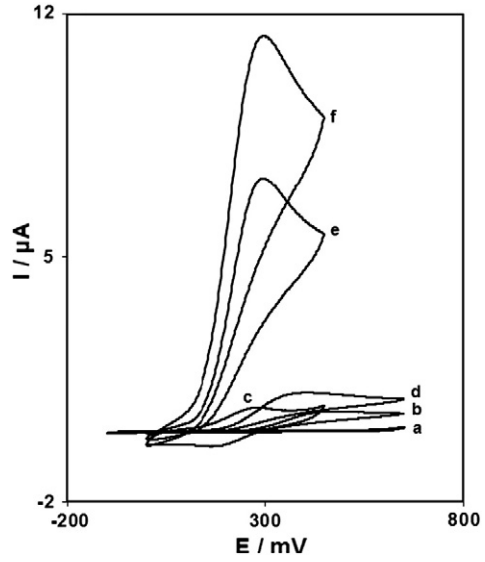

Fig. 3. CVs of (a) unmodified CPE in 0.1 M PBS (pH 7.0), (b) unmodified CPE in 50.0 μM DA, (c) 5ADBCNPE in 0.1 M PBS, (d) CNPE in 0.1 mM DA, (e) 5ADBCPE in 0.1 mM DA, and (f) 5ADBCNPE in 50.0 μM DA. In all cases the scan rate was 10 mV s^{-1}.

for E_{pa}, E_{pc} and $E_{1/2}$ respectively, indicating that the system obeys the Nernst equation for an equal electron and proton transfer reaction [86].

3.3. Electrocatalytic oxidation of DA at a 5ADBCNPE

Fig. 3 depicts the CV responses for the electrochemical oxidation of 50.0 μM DA at unmodified CPE (curve b), CNPE (curve d), 5ADBCPE (curve e) and 5ADBCNPE (curve f). As it is seen, while the anodic peak potential for DA oxidation at the CNPE, and unmodified CPE are 400 and 450 mV, respectively, the corresponding potential at 5ADBCNPE and 5ADBCPE is ~280 mV. These results indicate that the peak potential for DA oxidation at the 5ADBCNPE and 5ADBCPE electrodes shift by ~120 and 170 mV toward negative values compared to CNPE and unmodified CPE, respectively. However, 5ADBCNPE shows much higher anodic peak current for the oxidation of DA compared to 5ADBCPE, indicating that the combination of CNTs and the mediator (5ADB) has significantly improved the performance of the electrode toward DA oxidation. In fact, 5ADBCNPE in the absence of DA exhibited a well-behaved redox reaction (Fig. 3, 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 50.0 μM DA (curve f), which can be related to the strong electrocatalytic effect of the 5ADBCNPE towards this compound [86].

The effect of scan rate on the electrocatalytic oxidation of DA at the 5ADBCNPE was investigated by CV (Fig. 4). Results showed, the oxidation peak potential shifted to more positive potentials with increasing scan rate, confirming the kinetic limitation in the electrochemical reaction. Also, 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 4–20 mV s^{-1} (Fig. 4A), suggesting that, at sufficient overpotential, the process is diffusion rather than surface controlled [86].

The Tafel slope (b) can be obtained from the slope of E_p vs. log v using Eq. (2) [86]:

$$Ep = b/2 \log v + \text{constant} \quad (2)$$

The Tafel slope was found to be 108.0 mV (Fig. 4, inset B), which indicates that a one-electron transfer process is the rate limiting step assuming a transfer coefficient (α) is about 0.45.

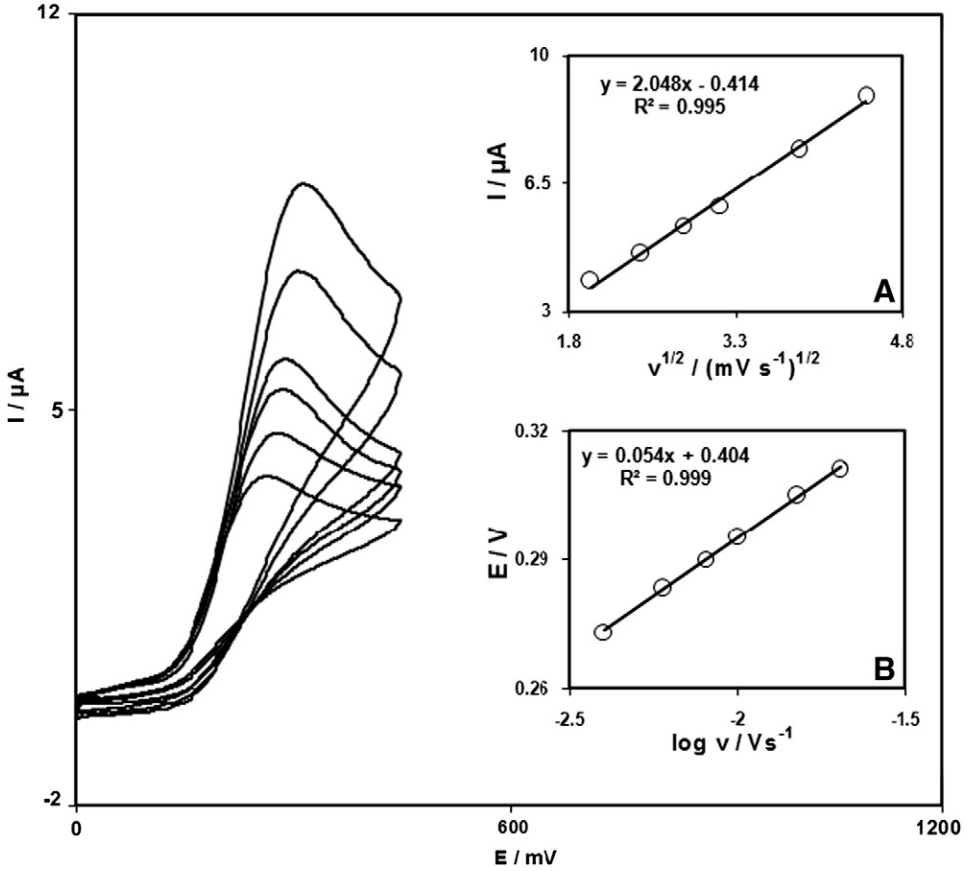

Fig. 4. CVs of 5ADBCNPE in 0.1 M PBS (pH 7.0) containing 10.0 μM DA at various scan rates; from inner to outer scan rates of 4, 6, 8, 10, 15 and 20 mV s^{-1}, respectively. Insets: Variation of (A) anodic peak current vs. $\nu^{1/2}$; (B) the variation of the anodic peak potential vs log v.

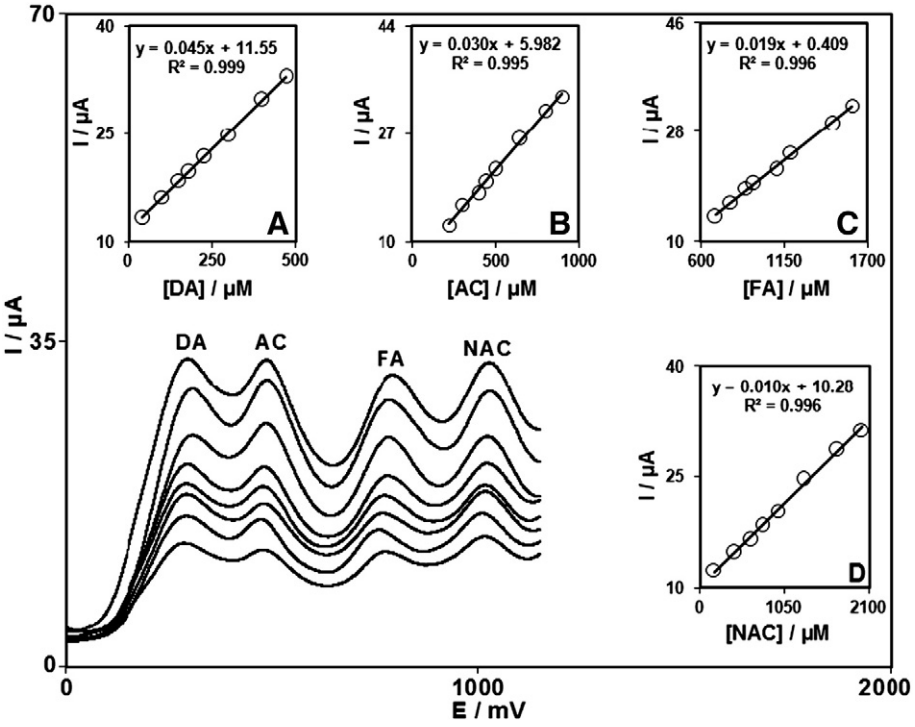

Fig. 5. SWVs of 5ADBCNPE in 0.1 M PBS (pH 7.0) containing different concentrations of DA + AC + FA + NA in μM, from inner to outer: 40.0 + 225.0 + 700.0 + 170.0, 100.0 + 300.0 + 800.0 + 425.0, 150.0 + 400.0 + 900.0 + 630.0, 180.0 + 450.0 + 950.0 + 765.0, 225.0 + 500.0 + 1100.0 + 950.0, 300.0 + 650.0 + 1200.0 + 1275.0, 400.0 + 800.0 + 1475.0 + 1700.0 and 470.0 + 900.0 + 1600.0 + 2000.0 respectively. Insets (A), (B), (C) and (D) plots of I_p *vs.* DA, AC, FA and NAC concentrations respectively.

3.4. Electrocatalytic determination of DA

The electrocatalytic peak current of DA oxidation at the surface of the modified electrode can be used for the determination of DA in solution. Therefore, square wave voltammetry (SWV) experiments were performed using modified electrode in phosphate buffer solution containing various concentrations of DA. The results show that the electrocatalytic peak current of DA oxidation at the surface of modified electrode was linearly dependent on the DA concentrations. The mediated oxidation peak currents of DA at the surface of a modified electrode were proportional to the concentration of the DA within the ranges 1.2 μM to 900.0 μM with a current sensitivity of 0.046 μA/μM in the SWV. The detection limit (3σ) was 0.57 μM.

3.5. Simultaneous determination of DA, AC, FA and NAC

To our knowledge, there is no report on the simultaneous determination of DA, AC, NAC and FA using modified electrodes and specially modified carbon nanotube paste electrodes. Therefore, the main objective of this study was to detect DA, AC, NAC and FA simultaneously using 5ADBCNPE. This was performed by simultaneously changing the concentrations of DA, AC, NAC and FA, and recording the SWVs. The voltammetric results showed well-defined anodic peaks at potentials of 285, 490, 785 and 1030 mV, corresponding to the oxidation of DA, AC, FA and NAC, respectively, indicating that simultaneous determination of these compounds is feasible at the 5ADBCNPE as shown in Fig. 5.

The sensitivity of the modified electrode towards the oxidation of DA was found to be 0.045 μA μM^{-1}. This is very close to the value obtained in the absence of AC, FA and NAC (0.046 μA μM^{-1}), indicating that the oxidation processes of these compounds at the 5ADBCNPE are independent and therefore, simultaneous determination of their mixtures is possible without significant interferences.

3.6. Real sample analysis

One milliliter of a DA ampoule was diluted to 10 mL with PBS (0.1 M, pH 7.0); then, a 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 SWV was recorded between 0.0 and 0.5 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 five times for each sample, and the average amount of DA in the injection was found to be 203 mg/5 mL, a value in well agreement with the value on the ampoule

Table 1
Determination of DA, AC, FA and NAC in mixtures. All the concentrations are in μM (n = 3).

No	DA Injection	AC Added	FA Added	NAC Added	DA			AC			FA			NAC		
					Found	Rec. %	RSD %	Found	Rec. %	RSD %	Found	Rec. %	RSD %	Found	Rec. %	RSD %
1	50.0	0	0		49.5	99.0	1.1	–	–	–	–	–	–	–	–	–
2	50.0	270.0	250.0	1000.0	49.7	99.4	2.0	269.0	99.6	3.4	253.9	101.6	1.9	1038.0	103.8	1.4
3	100.0	0	0		103.5	103.5	1.2	–	–	–	–	–	–	–	–	–
4	100.0	400.0	500.0	1500.0	99.5	99.5	2.3	403.0	100.7	2.8	504.5	100.9	2.6	1550.0	103.3	2.0
5	200.0	0	0		204.5	102.2	2.3	–	–	–	–	–	–	–	–	–
6	200.0	450.0	700.0	1900.0	197.3	98.6	3.2	445.9	99.1	1.1	690.2	98.6	1.5	1919.9	101.0	1.2

label (200 mg/5 mL). Also, to a series of 10 mL volumetric flasks, different capacity of the diluted DA injection solution together with standard AC, FA and NAC solutions were added and diluted to the mark with phosphate buffer. The SWVs were recorded and the anodic peak currents for each of DA, AC, FA and NAC were measured at their own oxidation potentials. According to the results listed in Table 1, very good recoveries for the determinations of DA, AC, FA and NAC were obtained with high reproducibility, which indicates that the sensor can be applied for the analysis of these compounds with no significant influence from each other.

4. Conclusion

In this paper, we have constructed a novel modified carbon-paste electrode for the detection of DA. The results of this study indicated that the electrode exhibited linear response over a wide concentration range (1.2 μM to 900.0 μM with a detection limit of 0.57 μM). Importantly, the proposed electrode was successfully applied for simultaneous determination of DA, AC, FA and NAC. Also, the constructed electrode was used for determination of DA, AC, FA and NAC in real samples.

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