Full Paper

Voltammetric Detection of Dopamine and Ascorbic Acid Using a Multi-Walled Carbon Nanotubes/Schiff Base Complex of Cobalt-Modified Glassy Carbon Electrode

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Abstract- The surface of the glassy carbon electrode (GCE) is modified with the composite of new Cobalt complex with a tetradentate Schiff base ligand derived from 3-ethoxysalicylaldehyde and 4,5-dimethyl orthophenylenediamine (CoOEtSal) and multi-walled carbon nanotube (MWCNT). The electrochemical oxidation of ascorbic acid (AA) and dopamine (DA) at the modified electrode was studied using the cyclic and differential pulse voltammetric techniques (CV and DPV). The effect of the scan rate and pH of the buffered solution on the electrode response is studied. An acceptable resolution of more than 285 mV for anodic oxidation waves of AA and DA is obtained using the modified glassy carbon electrode, and makes it very efficient for the simultaneous detection of these compounds. The Results show good peak resolution for AA and DA and the sub-micromolar detection limits for these compounds (0.07 and 0.04 µM for AA and DA respectively). The modified GCE was used successfully for the recovery of the analytes in human urine samples.

Keywords- Glassy carbon electrode; Multi-walled carbon nanotube; Cobalt Schiff base complex; Modified electrode; Dopamine

1. INTRODUCTION

In recent years, detection or quantification of physiological species in clinical or biological samples has been of notable interest, and many analytical methods have been applied for the detection of these biomolecules [1–7]. Dopamine (DA), a significant neurotransmitter, exists in the mammalian central nervous system and its primary content of DA is very low [8]. In the electrochemical detection of DA, the respective anodic peak is overlapped with the anodic peak of ascorbic acid (AA) that coexists with DA and results in a serious problem. Many kinds of research have been done in order to overcome this difficulty. Recently, the modification of electrodes has been accomplished in order to the determination of these biologically important compounds. Electrochemical oxidation of ascorbic acid has been done at the hierarchical meso-macroporous carbons networks aerogels-carbon nanorods modified GCE [9], MWCNTs@GONRs/AuNPs modified electrode [10], nanoporous gold modified electrode [11], platinum nanoparticles/graphene/GCE [12], platinum nanochains/MWCNT/graphene modified GCE [13], CeO_2-CNT/GCE [14], Cu_2O/CuO/ERGO modified Au electrode [15], molybdenum oxide/Prussian-graphite felt modified electrode [16], and porphyrin dye-MWCNT modified GCE [17]. Electrochemical detection of dopamine has been done at the surface of modified electrodes using gold nanoparticles/thiolated calix[n]arene/graphene nanosheets [18], upconversion nanoparticles/oligoaniline-crosslinked gold nanoparticles [19], poly(glyoxal-bis(2-hydroxyanil))-amino-functionalized graphene quantum dots-MnO_2 [20], nickel telluride-modified CPE [21], porphyrin-diazocine-porphyrin/fullerene/GCE [22], poly(L-methionine)/Au_1Pt_3/GCE [23], silicon carbide nanopowders modified GCE [24], manganese oxide nanofibers modified CPE [25], iron oxide nanoparticles modified CPE [26], pyrene-1-boronic acid-functionalized graphene-modified electrode [27] and a dopamine-imprinted chitosan film/porous ZnO NPs@carbon nanospheres/macroporous carbon electrode [28]. On the other hand, making an approach for convenient and selective detection of DA in the presence of AA is very important in analytical and clinical chemistry. Furthermore, the anodic potential of DA oxidation at the common solid electrodes is near to that of AA and causes to overlap the anodic peaks of these species [29,30]. The modification of various electrodes in order to separate the electrochemical responses for simultaneous detection of DA and AA has been done [11–15,24]. Among them, a widely used method is the application of nanomaterial composites [14,31–36]. Recently, poly(sodium 4-styrenesulfonate)-functionalized graphene [31], titanium nitride-reduced graphene oxide composite [32], multi-walled carbon nanotubes/ionic liquid crystal/graphene/18-crown-6 [33], CeO_2/reduced graphene oxide [34], Au-polydopamine nanospheres [35] and NiO/graphene oxide [36] were used to construction of various modified electrodes for determine DA in the presence of AA in the mixture samples.

On the basis of quondam studies, Transition metal complexes specially Schiff base complexes showed the electron mediator properties in the electro-oxidation of various compounds [37,38]. Using the Schiff base complexes in the modification of electrodes result in a major drawback that is the poor selectivity of the electrode response. On the other hand, in this research, a new Schiff base complex of cobalt (Fig. 1) was used as an electron mediator in the modification of the GCE surface. The present work related to the voltammetric detection of DA in the presence of AA on the surface of the modified glassy carbon electrode by casting Schiff base complex of cobalt/MWCNTs suspension (MWCNTs/CoOEtSal). Preliminary, the reaction mechanism is studied using cyclic voltammetry (CV) in the various scan rates of potential and pHs. DPV technique and the MWCNTs/CoOEtSal-modified GCE were applied in the simultaneous determination of DA and AA in the mixture solutions. Sub-micromolar detection limits, acceptable accuracies, and very good resolution in these studies were obtained.
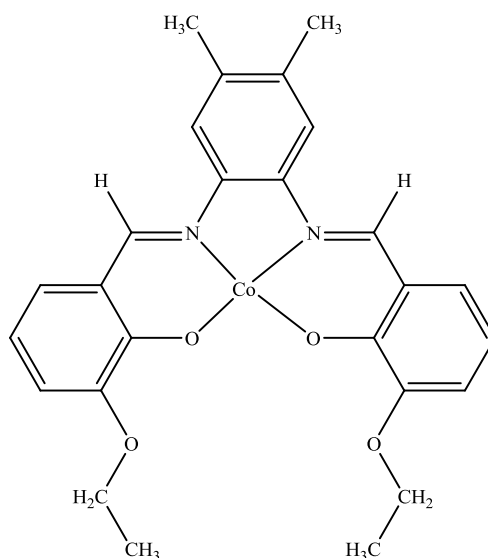

Fig. 1. Chemical structure of cobalt complex with tetradentate Schiff base ligand derived from 3-ethoxysalicylaldehyde and 4,5-dimethyl orthophenylenediamine

2. Materials and method

2.1. Apparatus and chemicals

A digital pH/mV/ion meter (CyberScan model 2500, Eutech Instruments) was used in order to make up the buffered solutions. A system of three common electrodes with one SCE, one counter electrode (platinum wire), and one glass carbon working electrode (GCE, unmodified or modified, diameter=2 mm) were used. Using a computerized potentiostat/galvanostat Autolab model 302 (Eco Chemie Utrecht, the Netherlands) controlled with General Purpose Electrochemical System (GPES) software, the CV and DPV studies were done.

Multi-walled carbon nanotubes (purity more than 95%) with O.D. between 10-20 nm, I.D. between 5-10 nm and tube length from 0.5 to 200 nm were purchased from Nanostructured & Amorphous Materials (USA). The synthesis of the Schiff base cobalt complex (Cobalt complex with a tetradentate Schiff base ligand derived from 3-ethoxysalicylaldehyde and 4,5-dimethyl orthophenylenediamine, CoOEtSal) has been done according to the reported paper [39]. Spectroscopic mineral oil (Nujol) and powder of Graphite (20 μm) were obtained from Merck. DA and AA were obtained from Aldrich. All aqueous solutions were made up using doubly distilled deionized water.

DA and AA stock solutions were freshly made up, purged with pure nitrogen gas for 2 min for voltammetric studies. In order to study the recovery for the determination of dopamine and ascorbic acid, the obtained sample of human urine, was filtered and diluted using the buffer solution (pH 4.0).

2.2. Preparation of modified electrode

Before the modification step, the GCE was polished with alumina slurry (0.1 μm) on a polishing cloth, rinsed thoroughly with water and, sonicated in water for 60 s and air-dried. 1.0 mg Schiff base complex of cobalt (CoOEtSal) and 1.0 mg MWCNTs were dispersed in 1.0 mL of doubly distilled water and, in order to obtain the homogenous suspension of MWCNTs/CoOEtSal, ultrasonicated for 15 min. For the preparation of the modified GCE, using a micropipette, 2 μL of different prepared suspensions (MWCNTs, CoOEtSal and MWCNTs/CoOEtSal in doubly distilled water) was applied directly onto a well-polished glassy carbon electrode and left to dry at room temperature.

3. RESULTS AND DISCUSSION

3.1. Cyclic and differential pulse voltammetric studies

The potential application of MWCNTs/CoOEtSal composite at the surface of GCE as electrochemical sensing film for DA and AA was examined using CV and DPV. The cyclic voltammograms obtained in 1mM DA and AA in buffered solutions of pH 4.0 at the modified and unmodified GCE are shown in Fig. 2A. The cyclic voltammogram at the unmodified GCE shows a totally irreversible process for the oxidation of AA, and a quasi-reversible process for DA is obtained. The anodic peak potential of AA and DA is approximately 0.280 V and 0.390 V respectively. These results revealed that the presence of CoOEtSal at the surface of the GCE (CoOEtSal/GCE) has an efficient effect on decreasing the overpotential of the anodic oxidation of AA (about 100 mV) and improving its anodic peak current. On the other hand, by the addition of MWCNT into the modifier film at the surface of GCE (MWCNTs/CoOEtSal/GCE), an acceptable improvement in the electrode response to AA and DA has seen. In fact, a better modifier effect on the electrode response to AA be seen at the

MWCNTs/CoOEtSal/GCE. At the MWCNTs/CoOEtSal-modified GCE, a 140 mV diminish has resulted in the oxidation potential of AA. Furthermore, at the MWCNTs/CoOEtSal/GCE, the peak current of AA augmented from 3.5 to 31.2 µA and the DA peak current increased from 10.1 to 38.3 µA.

In the existence of MWCNT in conjunction with CoOEtSal can complete the catalytic properties of the modifier film at the surface of GCE in the electro-oxidation of AA, and an improvement in the peak current and diminish in the oxidation potential of AA is resulted. On the other hand, as can be seen in Fig. 2B the differential pulse voltammograms of the anodic oxidation of DA and AA at unmodified GCE overlap with each other and so the unmodified GCE isn't applicable for measurement of AA and DA in mixture solutions. But the responses of these biomolecules are much different at the modified GCEs. The results of differential pulse voltammetric studies of DA and AA in 0.1 M acetate buffer solution with pH 4.0 revealed that the presence of CoOEtSal at the surface of the modified GCE (CoOEtSal/GCE) caused a great decreasing in peak potential of AA and the peak current of this compound increases by about 400%.
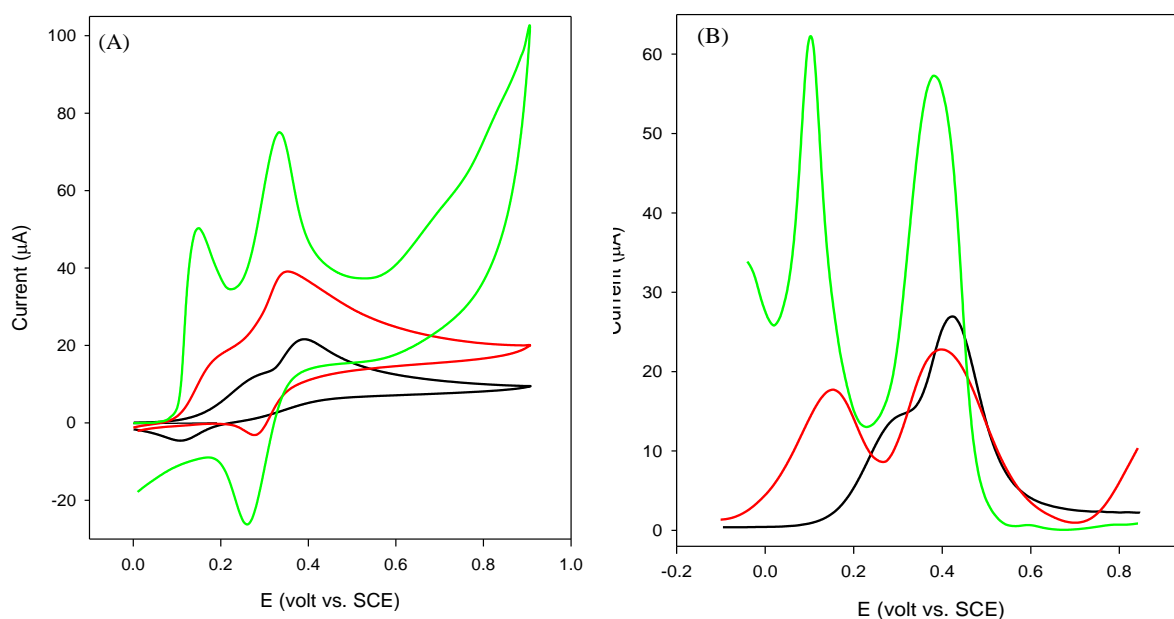

Fig. 2. (A) CV and (B) DPV responses of a mixture of 1.0 mM DA and 1.0 mM AA in the buffered solution of pH 4.0 using unmodified GCE (black), prepared CoOEtSal/GCE (red) and prepared MWCNTs/CoOEtSal/GCE (green). Sweep rate: 100 mVs^{-1}; pulse amplitude: 50 mV

Using MWCNTs/CoOEtSal/GCE, a 50 mV reduction is achieved in the oxidation potential of AA. At the MWCNTs/CoOEtSal/GCE the peak currents of DA and AA increased to 50.2 and 42.3 µA respectively. The resolution of the DA and AA anodic peaks in these

studies (285 mV) is reported to be more favorable than other sensors. The resolution of the DA and AA anodic peaks about 218 mV is obtained at the platinum nanoparticles/ graphene/GCE [12], about 200 mV at the titanium nitride-reduced graphene oxide modified electrode [32], about 150 mV at the Au-polydopamine nanospheres modified electrode [35] and about 166 mV at the NiO/graphene oxide modified electrode [36]. Modification of electrode with CeO_2/reduced graphene oxide resulted in a peak resolution of 190 mV for the simultaneous detection of DA and AA [34]. In comparison to the previous reports, by using the modified electrode in this work (MWCNTs/CoOEtSal/GCE) the anodic current decay is observed between AA and DA voltammetric waves, which have reached the capacitive background current, extremely reduces overlapping voltammetric DA and AA responses, and possesses more desirable accuracy in the simultaneous determination of these compounds in mixture samples.

3.2. The effect of pH and sweep rate

The other effect that must be optimized is solution pH. Therefore the redox behavior of the DA and AA at the surface of the finally modified GCE (MWCNTs/CoOEtSal/GCE) in the mixture solution of these compounds with various pH was studied and these results are shown in table 1. These results reveal that the best peak resolution between DA and AA has resulted in pH 4.0. Therefore the buffered solution with pH 4.0 was applied as supporting electrolyte in all voltammetric studies.

Table 1. Results of cyclic voltammetric studies of DA and AA in mixture solutions with different pH at the surface of MWCNTs/CoOEtSal/GCE

pH	AA		DA		ΔE_p
	$I_{p,a}$ (µA)	$E_{p,a}$ (mV)	$I_{p,a}$ (µA)	$E_{p,a}$ (mV)	
3	31.9	192	39.5	390	198
4	30.9	139	39.0	380	241
5	29.5	121	38.1	325	204
6	21.6	117	31.8	301	184
7	16.8	112	28.3	239	137

According to these results and quondam studies, the following mechanism may be suggested for the anodic oxidation of AA on the surface of the modified GCE containing CoOEtSal:

$$2Co(II)L \rightarrow 2Co(III)L + 2e^- \tag{1}$$

$$2Co(III)L + C_6H_8O_6 \rightarrow 2Co(II)L + C_6H_6O_6 + 2H^+ \tag{2}$$
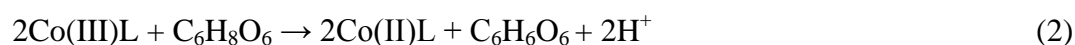
($C_6H_8O_6$: ascorbic acid, $C_6H_6O_6$: dehydroascorbic acid)

The results of cyclic voltammetric experiments at different potential scan rates showed

that peak currents of DA and AA proportional to the square root of the scan rate ($v^{1/2}$) increase in the range of 25-250 mVs^{-1}. These results confirm that the electrochemical reactions of DA and AA at the surface of the MWCNTs/CoOEtSal/GCE are diffusion-controlled.

3.3. Analytical characterization

The modified GCE in this work (MWCNTs/CoOEtSal/GCE) and the DPV method were applied as a beneficial technique with a low limit of detections in detections of DA and AA in an extensive range of their concentrations. Fig. 3 shows some of these obtained DPV responses. A linear range is obtained by drawing the anodic peak currents versus the concentration, that is 6.0×10^{-7} to 1.0×10^{-3} M for AA and 2.0×10^{-7} to 1.0×10^{-3} M for DA (Fig. 4). A slope of 0.05139 µA/µM ($R^2 = 0.9994$) is resulted for DA, and a slope of 0.04442 µA/µM ($R^2 = 0.9982$) for DA. On the basis of seven replicates, the relative standard deviations (R.S.D.) of these slopes for DA and AA are obtained 3.1 and 3.3% respectively and based on twelve measurements in a period of three months, were less than 3.4% for both DA and AA. So the prepared MWCNTs/CoOEtSal/GCE in this work revealed to be very stable.
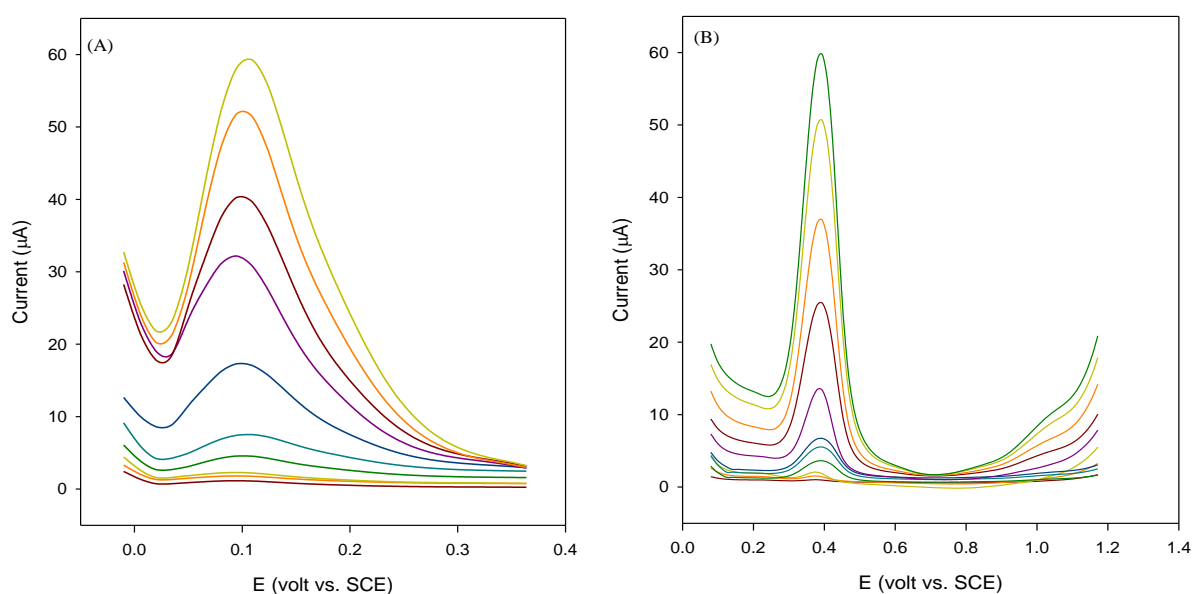

Fig. 3. DPVs at the surface of MWCNTs/CoOEtSal/GCE in buffered solution of pH 4.0 containing (A) 0.0, 0.005, 0.012, 0.065, 0.085, 0.25, 0.4, 0.6, 0.8 and 1.0 mM AA and (B) 0.0, 0.01, 0.025, 0.045, 0.085, 0.093, 0.20, 0.41, 0.62, 0.85 and 1.0 mM DA (down to up). Pulse amplitude: 50 mV

The DPVs resulted in solutions containing 0.05 mM DA and six different AA concentrations in the range of 8.5×10^{-5} to 1×10^{-3} M are shown in Fig. 5A. The DPVs

obtained in solutions containing 4.5×10^{-4} M AA and different concentrations of DA from 7.3×10^{-5} to 1×10^{-3} M are shown in Fig. 5B. Using the prepared MWCNTs/CoOEtSal/GCE in this work, in buffered solutions of pH 4.0 a linear range for DA is acquired in the range from 4×10^{-7} to 8.0×10^{-4} M and for AA from 1×10^{-6} to 1.0×10^{-3} M. The respective detection limits (S/N = 3) were 6×10^{-8} M and 1.0×10^{-7} M for DA and AA, respectively. The resulted linear ranges and detection limits were very similar to the experiments in solutions containing only one of DA or AA. In the solution containing 0.5 mM DA, the slope of the calibration curve for AA was 0.04375 µA/µM ($R^2 = 0.9990$), which was about 98.5% of the obtained slope value of the AA solutions. This slope for DA, in the solution containing 4.5×10^{-4} M AA was 0.05046 µA/µM ($R^2 = 0.9976$).
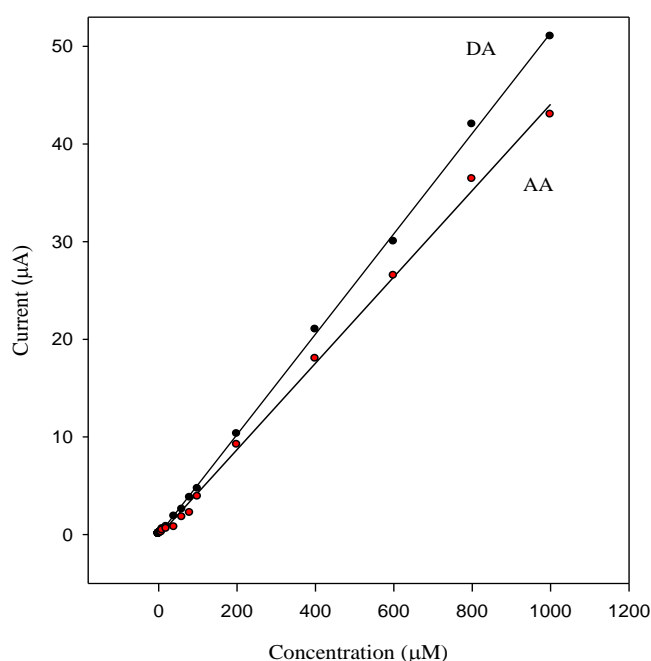

Fig. 4. Anodic peak current at MWCNTs/CoOEtSal/GCE *vs.* concentration of DA and AA in the range 0.1 to 1000.0 µM

The MWCNTs/CoOEtSal-modified GCE and DPV method was applied for the simultaneous detection of DA and AA in human urine samples in order to estimate the reliability of this method. The prepared sample of fresh human urine was filtered and diluted using the buffer solution (pH 4.0). To prevent real-time matrix interference, only dilute urine samples are added to the electrochemical cell and placed in the AA and DA linear ranges. Buffered solutions of pH 4.0 were used to dilute the urine samples before the experiment. To determine the accuracy of the results, real samples were used using the method of standard addition. The results are shown in Table 2. The obtained recovery of the spiked samples is in the ranged from 98.1% to 102.6% and demonstrated that the MWCNTs/CoOEtSal-modified GCE was sensitive and reliable enough for the detection of DA and AA in these samples.
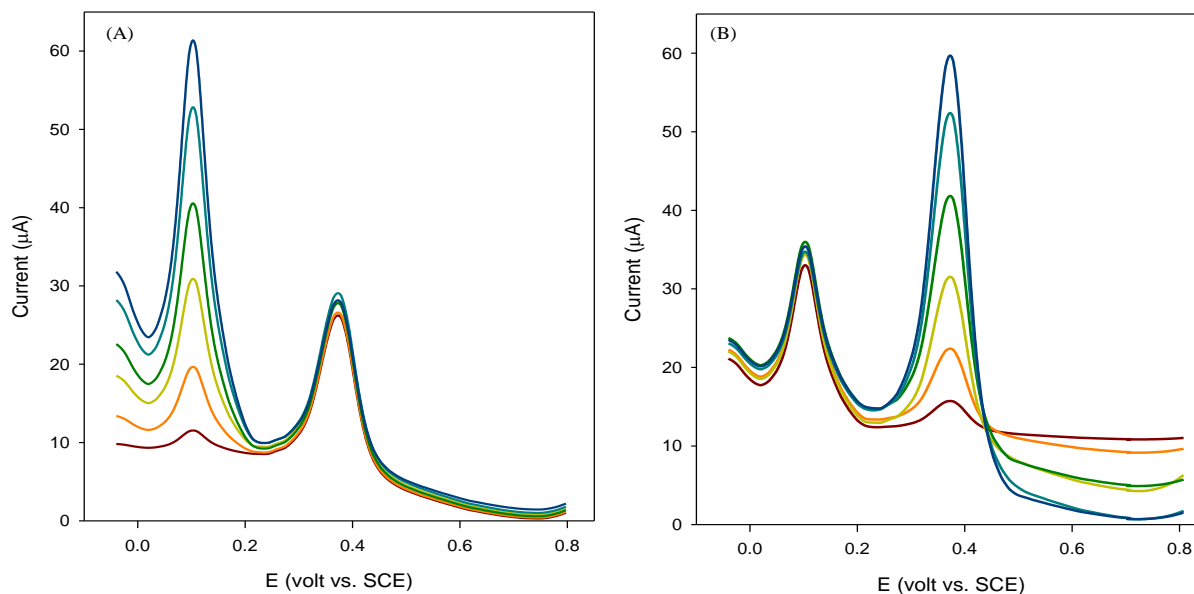
Fig 5. DPVs at the surface of MWCNTs/CoOEtSal/GCE in buffered solution of pH 4.0 containing (A) 0.5 mM DA (constant) and various concentrations of AA: 0.085, 0.22, 0.37, 0.60, 0.83 and 1.0 mM. (B) 0.45 mM AA (constant) and various concentrations of DA: 0.073, 0.19, 0.40, 0.60, 0.83 and 0.1 mM. Pulse amplitude: 50 m

Table 2. Analysis of DA and AA in samples of human urine (n = 7) at MWCNTs/CoOEtSal-modified GCE

Sample	Species	Added (µM)	Found (µM)	Recovery (%)
Urine 1	AA	0.00	Not detected	-
		150.0	151.8 (±1.9)	101.2
	DA	0.00	Not detected	-
		25.0	25.1 (±0.3)	100.5
Urine 2	AA	0.00	Not detected	-
		175.0	178.3 (±2.1)	101.9
	DA	0.00	Not detected	-
		40.0	51.3 (±0.8)	102.1
Urine 3	AA	0.00	Not detected	-
		200.0	198.2 (±1.2)	99.1
	DA	0.00	Not detected	-
		45.0	44.1 (±0.7)	98.1
Urine 4	AA	0.00	Not detected	-
		160.0	164.2 (±1.4)	102.6
	DA	0.00	Not detected	-
		30.0	29.5 (±0.4)	98.4

4. CONCLUSION

The MWCNTs/CoOEtSal-modified GCE in this study is able to improve the selectivity and the peak resolution between DA and AA. In the present work, the application of DPV method and MWCNTs/CoOEtSal-modified GCE results in an acceptable resolution of more

than 285 mV for anodic oxidation waves of AA and DA and make it very efficient for simultaneous detection of these compounds. The appropriate sensitivity and selectivity, and sub-micromolar detection limit indicate the prepared MWCNTs/CoOEtSal-modified GCE is useful in the manufacture of an attainable approach for the concurrent measurement of AA and DA in clinical and pharmaceutical samples.

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