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Preparation of Modified Electrode using Toluidine Blue O and Molybdenum Schiff Base Complex for Detection of Dopamine in the presence of Ascorbic Acid

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Abstract- The complex *cis*-dioxo-bis[3-methoxy-2,2-dimethylpropanediamine] molybdenum(VI) (*cis*-[Mo(O)$_2$L]) and toluidine blue O (TBO) are used for modification of carbon paste electrode (CPE). In order to study of the electrochemical behavior of ascorbic acid (AA) and dopamine (DA) at the surface of the modified CPEs, the differential pulse and cyclic voltammetric methods (DPV and CV) were used. These results reveal that by application of the modified CPE a peak resolution about 329 mV is obtained for AA and DA and the limits of detection (S/N=3) were 1×10^{-7} M and 4×10^{-7} M for DA and AA, respectively. Surface regeneration and the very easy preparation of the modified CPE together with the very good peak resolution and sub-micromolar detection limits designate the prepared CPE in this work appropriate for simultaneous voltammetric determination of DA and AA. The modified CPE was used successfully for recovery of the analytes in human plasma samples.

Keywords- Molybdenum schiff base complex, Toluidine blue O, Modified electrode, Dopamine

1. INTRODUCTION

Recently, the manufacture and design of new voltammetric sensors have been of notable interest [1,2]. Specially, detection of secretion neurotransmitters, e.g. dopamine (DA) through

the improvement of the electrochemical sensors received many interests. DA, a significant neurotransmitter, exists in mammalian central nervous system and its primary content of DA is very low [3-5]. A serious problem in detection of DA is the overlapping of the anodic peaks of DA and coexisting AA. The anodic oxidation potential of DA is close to that of AA at common solid electrodes and results in the lack of good resolution between their anodic peaks. Several methods have been applied to overcome this problem. In recent years, in order to the determination of these biologically important compounds, chemically modified electrodes (CMEs) have extensively been applied [6-26].

Previous works showed that Schiff base complexes are efficient electron mediators and can catalyze the oxidation process of different biological compounds, such as AA [27,28]. The major drawback in the using of the redox mediators in the modification of the electrode is lack of good resolution for simultaneous determination of different analytes in the mixed samples. On the other hand, the application of an electron mediator together with toluidine blue O in the preparation of modified electrodes, because of the electrostatic repulsion or attraction between the charged analytes and the modifier can separate the voltammetric peaks of different compounds and improve the selectivity for their simultaneous detections. Respect to the charge sign of various analytes and the modifier, the electrostatic interactions can be exclusive or inclusive that is important for the improvement of the voltammetric resolution between the peaks of these analytes. Electrocatalytic oxidation of ascorbic acid has been done at the graphene/ Fe3O4@SiO2–ionic liquid nanocomposite modified CPE [29], thionine/nafion modified CPE [18] and Triton X-100 modified glassy carbon electrode [30].

In the present work, the electrochemical oxidation of DA and AA at the surface of the modified carbon paste electrode containing the molybdenum schiff base complex/ toluidine blue O is studied. Modification of the carbon paste electrode using toluidine blue O results in increasing the anodic overpotential for DA oxidation. In order to obtain a higher sensitivity and selectivity in the voltammetric response of the modified electrode, the effect of toluidine blue O percent on the resolution between the anodic peaks of DA and AA is studied.

2. EXPERIMENTAL

2.1. Apparatus and chemicals

In order to make up the buffered solutions, a digital pH/mV/ion meter (CyberScan model 2500, Eutech Instruments) was used. A common three-electrode system was applied with a platinum wire as a counter electrode, a saturated Calomel reference electrode, and modified or unmodified carbon paste working electrode. Voltammetric measurements were performed using a computerized potentiostat/galvanostat Autolab model 302 (Eco Chemie Utrecht, the Netherlands) controlled with General Purpose Electrochemical System (GPES) software.

For the synthesis of the complex *cis*-dioxo-bis[3-methoxy-2,2-dimethylpropanediamine] molybdenum(VI), to a solution of 1 mmol [$MoO_2(acac)_2$] (Merck, Darmstadt, Germany) in 50 mL methanol was added a solution of 1 mmol 3-methoxy-2,2-dimethylpropanediamine (Merck, Darmstadt, Germany) in 10 mL methanol and the reaction mixture was stirred for 120 min at reflux condition. Then the precipitated orange complexes were filtered and washed with methanol [31]. Spectroscopic mineral oil (Nujol), Graphite powder (20 μm), and toluidine blue O were purchased from Merck (Darmstadt, Germany). All the other chemicals were analytical reagent grade, purchased from Merck (Darmstadt, Germany). Using doubly distilled deionized water, all aqueous solutions were made up.

Stock solutions of DA and AA were freshly made up in a buffered solution and before the voltammetric experiments, purged with pure nitrogen gas (99.999%) for 120 s. The buffered solutions of DA and AA were deoxygenated by purging the pure nitrogen (99.999% from Roham Gas Company, Tehran, Iran), and then were used for voltammetric studies. Nitrogen gas was passed over the surface of the test solutions during the measurements, in order to avoid the influx of oxygen into the solution.

For the detection of the recovery in spiking of dopamine, the sample of fresh human serum, prepared from Razi Institute of Vaccine and Serum Co. (Tehran, Iran), was filtered and diluted using a 0.1 M acetate buffer solution of pH 4.0.

2.2. Preparation of modified electrode

To prepare the unmodified CPE, a suitable amount of mineral oil with powder of graphite (~25:75, w/w) was mixed by hand mixing in a mortar and pestle, then a portion of the resulted mixture was packed into the end of a polyamide tube (ca. 2.5 mm i.d.). A copper pin makes the electrical contact into the back of the composite, in the polyamide tube. The molybdenum Schiff base complex-modified carbon paste electrode was fabricated by mixing the Schiff base complex of molybdenum (3%, w/w) with powder of graphite and a suitable amount of mineral oil, and then the resulting composite was dissolved in dichloromethane in order to better homogeneity and reproducibility by polishing the electrode surface. For the fabrication of the molybdenum Schiff base complex-modified carbon paste electrode containing toluidine blue O, various percent of toluidine blue O together with 3 wt.% of Schiff base complex of molybdenum, powder of graphite and a suitable amount of mineral oil were mixed in an appropriate amount of dichloromethane. The solvent of the resulted mixture has been evaporated completely by stirring, and then air dried for one day and packed into the end of a polyamide tube.

3. RESULTS AND DISCUSSION

3.1. Voltammetric experiments in the mixed solutions of DA and AA

Making an approach for separation of anodic peaks and simultaneous detection of AA and DA is very significant in clinical and analytical chemistry. At the traditional solid electrodes, the anodic overpotential for oxidation of AA is the same as that of DA; furthermore, both of AA and DA are present simultaneously in mammalian brain which will cause to overlap the voltammetric responses of these species [33]. Many efforts have been made on the fabrication of the modified electrodes that are capable to separate their anodic peaks and make the feasibility of simultaneous determination of DA and AA [21-26].

In the present work, for simultaneous voltammetric detection of these compounds, the molybdenum Schiff base complex-modified CPE containing toluidine blue O was used. The electrochemical behavior of 0.1 mM of both DA and AA in a buffered solution of pH 4.0 at the surface of the unmodified CPE and the molybdenum Schiff base complex-modified carbon paste electrode containing various wt.% of toluidine blue O is studied by cyclic voltammetric method. The results of this study are shown in Fig. 1A. Fig. 1B shows the differential pulse voltammograms (DPVs) of five prepared electrodes in this solution. These figures revealed that at the surface of the unmodified CPE, only a quasi-reversible wave can be observed for DA and a distinguished wave cannot be obtained for AA, therefore this electrode (unmodified-CPE) isn't suitable for the simultaneous voltammetric detection of DA and AA. By introducing the molybdenum Schiff base complex in the matrix of carbon paste electrode (*cis*-[$Mo(O)_2L$/CPE), a little resolution between anodic peaks of DA and AA is obtained but the detection of each compound in the presence of the other isn't possible because of the overlapping of their anodic peaks. Application of *cis*-[$Mo(O)_2L$]-modified CPE containing toluidine blue O, results in more resolution of anodic peaks for DA and AA, because of positive shift of DA anodic peak. As can be seen in Fig. 1, at the *cis*-[$Mo(O)_2L$]-modified CPE including 10 wt.% of toluidine blue O, the complete resolution between anodic peaks of DA and AA is obtained. In fact, in solutions of $pH \leq 5$, DA contains protonated amine group and mostly exists in cationic form. The positive shift in anodic peak potentials of DA under the experimental condition (buffered solution of pH 4.0) is a result of the electrostatic repulsion effects between the positive charge of toluidine blue O at the surface of modified CPE and cationic form of dopamine in the solution. A better resolution does not obtained by using higher percent of toluidine blue O, whereas the sensitivity of electrode response to DA is decreased because of the resulted anodic overpotential and kinetic limitation for DA. Moreover, this investigation revealed that using of higher percent of toluidine blue O in modification of the electrode caused to limit the voltammetric detection limit for DA and AA (increase the capacitive background current). These efficacies can be obviously seen by comparing of the CVs or DPVs for the different modified CPEs in Fig. 1.

As a result, the molybdenum Schiff base complex-modified CPE containing 10 wt.% toluidine blue O was selected for simultaneous detection of DA and AA.

The resulting resolution between the anodic peaks of DA and AA in this investigation (329 mV) is significantly more desirable than other reported voltammetric sensors. The peak resolution for DA and AA about 180 mV using differential pulse voltammetry is obtained at the carbon dots/ferrocene derivative functional Au NPs and graphene- modified electrode [34] and about 150 mV at the polypyrrole/Cu_xO–ZnO modification electrode [35]. Application of nanoparticles of γ-WO_3 in the modification of the electrode resulted in a peak resolution of 133 mV for simultaneous detection of AA and DA [36]. In comparison to the previous works, the decay of anodic current between the anodic peaks of DA and AA is taken place close to the capacitive background by using the molybdenum Schiff base complex-modified CPE containing toluidine blue O. The resulted decay of current significantly causes to decrease the overlapping of the anodic waves of DA and AA, and simultaneous detection of these compounds in mixture samples possesses a more desirable accuracy. Moreover the reproducibility of the detections is improved, due to more stability of DA and AA in slightly acidic condition (pH 4.0).

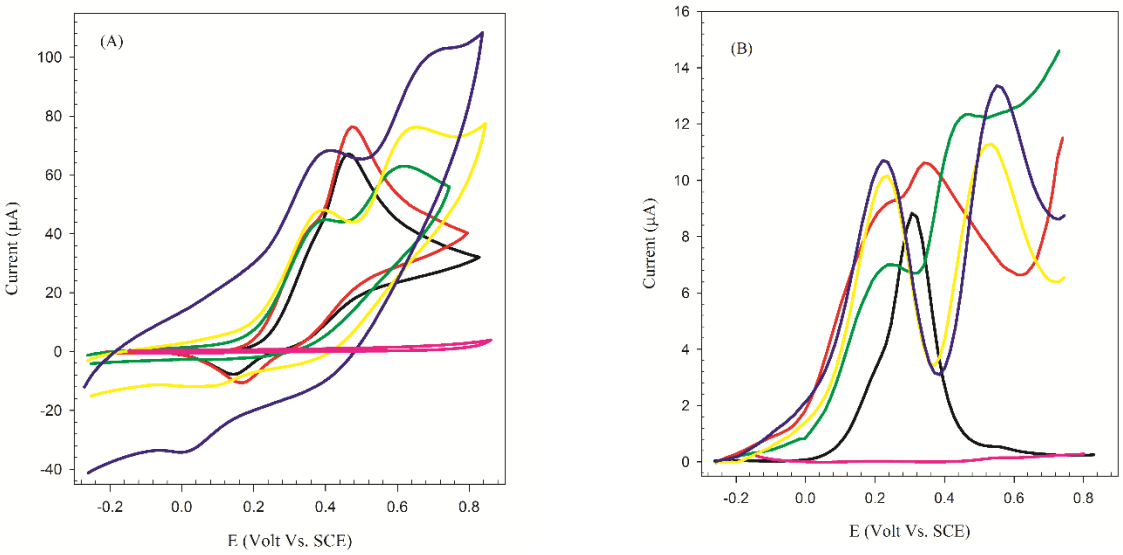

Fig. 1. (A) CV and (B) DPV responses of blank buffered solution of pH 5.0 using *cis*-[$Mo(O)_2L$]/modified CPE containing 10%TBO (pink) and of a mixture of 0.1 mM DA and 0.1 mM AA in the same buffer at the surface of unmodified CPE (black), CoL/modified CPE containing 0% (red), 5%(green), 10% (yellow) and 15% TBO (dark blue). Sweep rate: 100 mVs^{-1}; pulse amplitude: 50 mV

3.2. The effect of pH and sweep rate

Voltammetric studies of the buffered solutions with different pHs containing AA and DA were carried out to determine the optimized pH for acquiring the good sensitivity and an excellent resolution between their anodic peaks. In these experiments, 0.1 M phosphate was applied in preparation of buffered solutions of pH 3.0, 6.0 and 7.0, and 0.1 M acetate for pHs 4.0 and 5.0. Table 1 shows the peak potentials and peak currents of cyclic voltamograms obtained at the surface modified CPE in the mixture solutions of DA and AA with different pHs. These results reveal that the best peak separation is resulted in pH 4.0. Therefore in all voltammetric studies, the buffered solution with pH 4.0 was applied as supporting electrolyte.

Table 1. Variation of peak potential and peak current of cyclic voltammograms for mixture solutions of DA and AA with pH using *cis*-[$Mo(O)_2L$/modified CPE containing 10 wt.% of toluidine blue O

pH	AA		DA		ΔE_p
	$I_{p,a}$ (μA)	$E_{p,a}$ (mV)	$I_{p,a}$ (μA)	$E_{p,a}$ (mV)	
3	21.2	398	23.1	671	273
4	19.9	362	22.5	660	298
5	19.5	351	22.0	630	279
6	17.6	349	21.8	619	270
7	16.8	345	21.3	608	263

In order to investigate of the effect of the potential scan rate, cyclic voltammetric experiments were carried out in the buffered solution with pH 4.0. The results revealed that the anodic peak currents ($I_{p,a}$) of DA and AA increase linearly with increasing the square root of the scan rate ($\upsilon^{1/2}$) in the range of 20–150 mVs^{-1}. These results corroborate the diffusion-controlled anodic oxidation of DA and AA at the prepared CPE surface.

3.3. Analytical characterization

The differential pulse voltammetric method using the molybdenum Schiff base complex-modified CPE containing 10 wt.% of toluidine blue O was applied as a useful method with low limits of detection for detections of DA and AA in a wide range of their concentrations. Supporting electrolyte for these experiments was buffered solutions of pH 4.0. Fig. 2 shows some obtained DPV waves in these experiments. By drawing the anodic current signal versus the concentration (the calibration curves), a linear range is obtained that is 8.0×10^{-7}–0.6×10^{-3} M for AA and 5.0×10^{-7}–1.0×10^{-3} for DA (Fig. 3). A slope of 0.093696 μA/μM (R^2=0.9990) is resulted for AA, and a slope of 0.065066 μA/μM (R^2=0.9988) for DA.

The relative standard deviations (R.S.D.) for these slopes on the basis of five replicates were 3.0 and 3.1% for DA and AA, respectively and were less than 3.4% for both DA and AA, based on seven measurements in a period of two months. So the prepared modified CPE in this work revealed to be very stable.

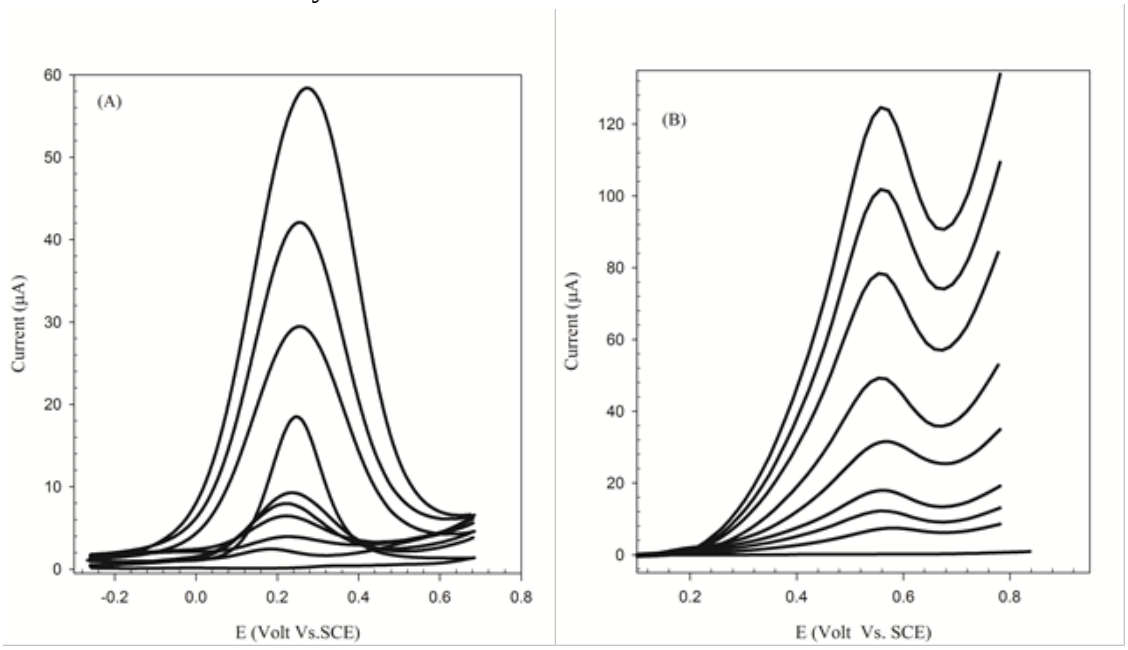

Fig. 2. Differential pulse voltammograms of buffered solution of pH 5.0 containing (A) 0.0, 0.01, 0.02, 0.04, 0.06, 0.1, 0.15, 0.3, 0.4 and 0.6 mM AA and (B) 0.0, 0.04, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mM DA (down to up). Pulse amplitude: 50 mV

The differential pulse voltammograms obtained in solutions containing 5×10^{-5} M AA and five various amounts of DA from 4×10^{-5} to 2×10^{-4} M are shown in Fig. 4A. The waves obtained in solutions including 5×10^{-5} M DA and various amounts of AA in the range of 1×10^{-5} to 1×10^{-4} M is represented in Fig. 4B. Using the modified CPE in this work, a linear range for AA in buffered solutions of pH 4.0 is acquired in the range from 1×10^{-6} to 1×10^{-4} M and for DA from 2×10^{-6} to 8×10^{-4} M. The respective limits of detection (S/N=3) were 4×10^{-7} M and 5×10^{-7} M for DA and AA, respectively. The resulted limits of detection and linear ranges were very similar to the detections in solutions containing only one of DA or AA. In the presence of 5×10^{-5} M AA, the calibration curve slope for DA was 0.092779 µA/µM (R^2=0.9983), which was about 98% of the resulted slope value for the separate DA solutions. This slope for AA, in the presence of 5×10^{-5} M DA was 0.063764 µA/µM (R^2 = 0.9976).

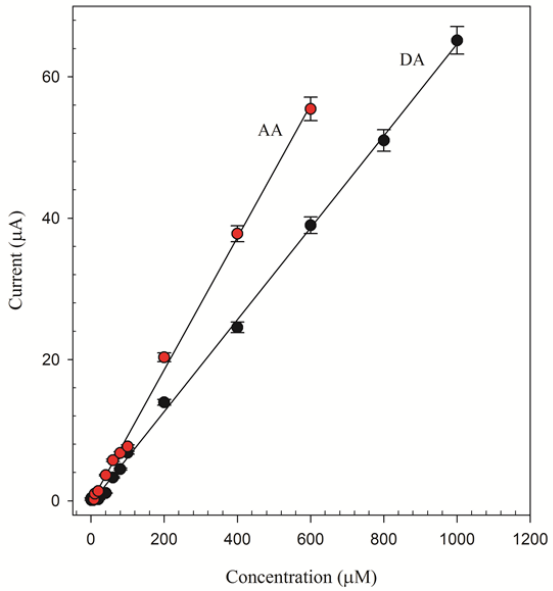

Fig. 3. Linear calibration curves of current signals versus DA and AA concentration in the range 10.0 to 1000.0 µM

To evaluate the validity of the proposed method, the molybdenum Schiff base complex-modified CPE containing 10 wt.% of toluidine blue O was applied for simultaneous determination of AA and DA in human urine samples.

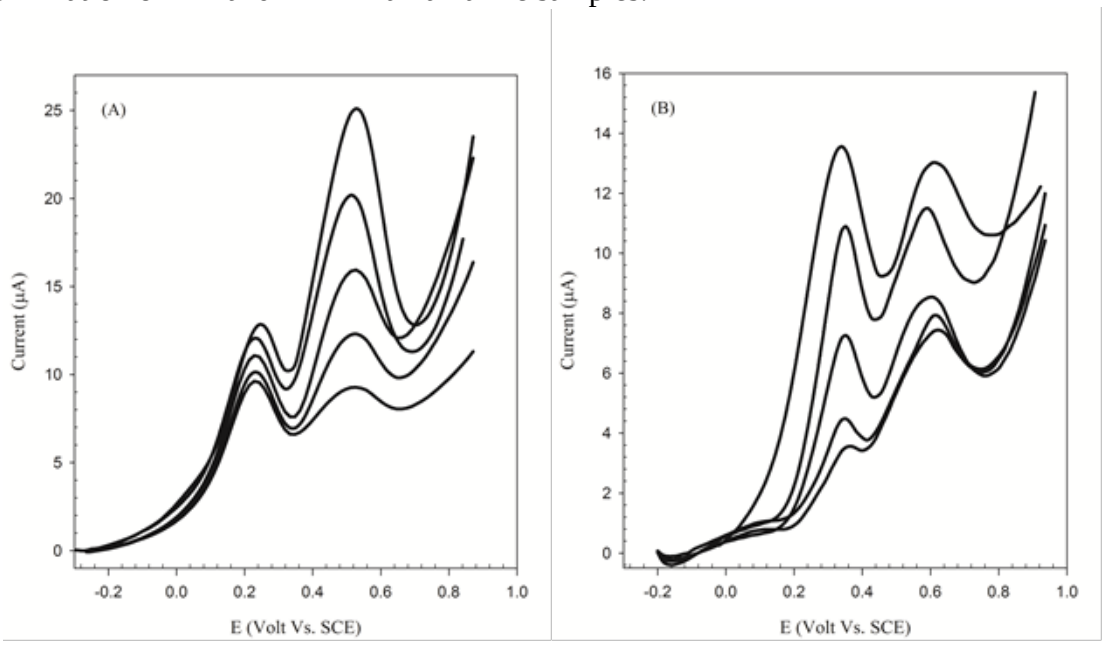

Fig 4. Differential pulse voltammograms for buffered solution of pH 4.0 containing (A) 0.05 mM AA (constant) and various concentrations of DA: 0.04, 0.06, 0.1, 0.12, 0.2 mM. (B) 0.05 mM DA (constant) and various concentrations of AA: 0.01, 0.02, 0.04, 0.06 and 0.1 mM. Pulse amplitude: 50 mV

Table 2. Determination of AA and DA in human urine samples (n=6) using the molybdenum Schiff base complex-modified CPE containing 10 wt.% of toluidine blue O

Sample	Species	Detected (μM)	Added (μM)	Found (μM)	Recovery (%)
Urine 1	AA	-	200.0	203.2	101.6
	DA	-	30.0	29.7	99.0
Urine 2	AA	-	210.0	212.8	101.3
	DA	-	50.0	51.3	102.6
Urine 3	AA	-	205.0	203.9	99.5
	DA	-	40.0	39.1	97.8
Urine 4	AA	-	180.0	184.9	102.7
	DA	-	45.0	46.4	103.1

For six replicates in the spiked range of DA and AA concentration, R.S.D. was less than 3.0%

Table 3 shows a comparison of analytical properties for the detection of DA and AA at the prepared electrode in this work and various electrodes.

Table 3. Various modified electrodes: DA and AA analytical properties

Electrode	Method	ΔE_p (mV)	Linear range (μM)		DL (μM)		Ref.
			DA	AA	DA	AA	
Carbon Paste-modified GCE	DPV	187	3.84 - 27.0	19.2 - 135	1.5	8.3	[6]
CPE modified with $[Cu(bp)(H_2O)_2]_n$	DPV	200	0.05 - 30.0	0.05 - 30.0	0.04	0.02	[18]
GCE modified with CuNP/p-TA_{OX}	DPV	205	0.6 - 21.6	240 - 750	0.03	5.0	[20]
Polyethylene oxide-modified GCE	DPV	216	5.0 - 40	300 - 1700	0.25	50	[25]
CCE modified MWCNTs-MIP	DPV	300	1.72-11.58	16.83-226	0.21	2.24	[26]
GCE modified with ZnO-Cu_xO-PPy	DPV	150	0.1-130	0.2-1.0	0.04	25.0	[35]
GCE modified with γ-WO_3	DPV	133	0.1-600	_	0.024	_	[36]
cis-$[Mo(O)_2L]$ /TBO/ CPE	DPV	329	0.5-1000	0.8-600	0.1	0.4	This work

Note: CP, carbon paste; GCE, glassy carbon electrode; $[Cu(bp)(H_2O)_2]_n$, polymer of 4,4'-dicyanamidobiphenyl Cu(II) complex; CuNP, Copper nanoparticles; p-TA_{OX}, overoxidized poly(3-amino-5-mercapto-1,2,4-triazole; MWCNTs, multiwalled carbon nanotubes ; MIP, molecularly imprinted polymer; PPy, polypyrrole; γ-WO_3, monoclinic structure of Tungsten trioxide nanoparticles.

In order to avoid the interferences of the real samples matrix and fit into the linear ranges of AA and DA only diluted urine samples were added into the electrochemical cell. The urine samples were diluted with solutions of pH 4.0 before detection. To ascertain the correctness of the results, real samples were analyzed by the standard addition method and the diluted samples mentioned above were spiked with certain amounts of AA and DA and then detected. The recovery of the spiked samples ranged from 97.8% to 103.1% (Table 2), indicating that the molybdenum Schiff base complex-modified CPE containing 10 wt.% of toluidine blue O was reliable and sensitive enough for the determination of AA and DA in real samples.

4. CONCLUSION

The molybdenum Schiff base complex-modified CPE containing toluidine blue O prepared in the present work can enhance the selectivity and resolution of voltammetric responses of DA and AA. This modified CPE has been revealed to be capable to separate the anodic peaks of DA and AA. Resulted resolution is much better than the previous reported works. Application of the modified CPE in differential pulse voltammetric method in this work, results in a good resolution more than 320 mV for anodic peaks of AA and DA making it very appropriate and efficient for simultaneous detection of these compounds. Surface regeneration and very easy preparation of the modified electrode together with the acceptable selectivity and sensitivity, sub-micromolar detection limit and good reproducibility of the voltammetric response represent the prepared modified system is very effective in the fabrication of accessible tools for the simultaneous detection of DA and AA in pharmaceutical and clinical preparations.

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