

Resolution of Dopamine in the Presence of Ascorbic Acid and Uric Acid by the Poly Oxalic Acid Modified Carbon Paste Electrode

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Abstract: A novel poly oxalic acid carbon paste electrode was fabricated by electro polymerization process and characterized in this study. This poly oxalic acid modified carbon paste electrode by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) exhibited an excellent electro catalytic activity towards the resolution of ascorbic acid (AA), dopamine (DA) and uric acid (UA) compared with bare carbon paste electrode. The effect of pH range from 5.5 to 8.0 was studied and the redox peak was pH dependent with a slope of 60 mV/pH and from the regression coefficient of the effect of scan rate shows that the process is adsorption controlled. The DPV study of 6.2 to 131 μM concentration range of DA shows 6 μM detection limit. The oxidation potentials of AA, DA and UA at the poly oxalic acid modified carbon paste electrode was shifted slightly negatively and the peak currents were much larger than the bare carbon paste electrode. DPV was used for the simultaneous determination of AA, DA and UA in their mixture, and the peak potential separations between AA and DA, DA and UA, AA and UA were 197 mV, 169 mV and 367 mV respectively. The limit of detection for AA, DA and UA were 0.93 mM, 7.4 μM and 13 μM respectively. This Oxalic acid modified carbon paste electrode showed many merits in stability, sensitivity, facility, economy and also applicable for real sample analysis.

Keywords: Oxalic acid, Ascorbic acid, Uric acid, Dopamine, Cyclic Voltammetry, Differential pulse voltammetry

Introduction

Neurotransmitters have received a considerable attention due to the key roles played by them in the central nervous systems of our body¹. Dopamine (DA) is an important neurotransmitter in the mammalian central nervous system. Low level of DA may result in neurological disorder such as Parkinson's disease and schizophrenia². Ascorbic acid (AA) is very popular for its antioxidant properties. Moreover, a number of studies have investigated the function of AA in gene expression and as a cosubstrate of many important dioxygenases³. Uric acid (UA) is the primary end product of urine metabolism. It has been

shown that extreme abnormalities of UA levels are the symptoms of several diseases, such as gout and hyperuricemia⁴. DA, UA and AA usually coexist in biological samples. Since the basal DA concentration is very low (0.01–1 μM) while the concentration of AA and UA is generally much higher than that of DA (100–1000 times)²⁻⁵, it is essential to develop sensitive and selective methods for their determination in routine analysis. However, a major problem encountered is that UA, DA and AA are oxidized at nearly the same potential with poor sensitivity at conventional electrodes with a pronounced fouling effect resulting in poor reproducibility. The overlap of their voltammetric responses makes their simultaneous determination highly difficult⁶. In order to overcome this goal, numerous materials such as organic redox mediators⁷, nanoparticles⁸ and self-assembled monolayer⁹ have been applied as modification layer to construct the highly selective and sensitive DA biosensor.

The traditional analytical methods have been employed for the detection of DA, such as Chromatography¹⁰, Spectrophotometry¹¹⁻¹², Chemiluminescence¹³, Fluorimetry¹⁴, Capillary Electrophoresis (CE-luminescence)¹⁵ and electrochemical methods¹⁶⁻¹⁸. Similarly in the electroanalytical chemistry some of the modified electrodes like nanoparticles modified electrodes¹⁹⁻²⁰, carbon ceramic electrode²¹, pyrolytic graphite electrode²², boron-doped diamond electrode (BDD)²³, carbon ionic liquid electrode²⁴, screen-printed carbon electrode²⁵, electrochemically oxidized GCE²⁶ and carbon nanotube microelectrode²⁷ are applicable for the simultaneous estimation of neurotransmitters.

Recently, polymer film and metal nanoparticles have been arose great attentions due to their wide applications in the fields of chemically modified electrodes²⁸⁻²⁹. Among various types of conductive polymers³⁰⁻³¹, polyimidazole (PIIm) has many attractive features, for example the film is very stable and hard to be taken off from the surface unless the electrode is heavily polished. It has high selectivity and sensitivity due to the chemically stable homogeneous film with controlled thickness.

Herein, we have reported the formation of poly oxalic acid polymer composite via electrochemical method based on the number of cycles of redox reaction of oxalic acid, which is more facile and less time consuming than other existing methods. We have studied the electrochemical determination of DA at poly oxalic acid polymer modified electrode. To the best of our knowledge, poly oxalic acid polymer composite has never been used for the electrochemical determination of DA. The high surface area and good conductivity of the poly oxalic acid polymer composite allows the efficient oxidation of DA. Moreover, composite electrode showed greater electrocatalytic oxidation for DA than other modified electrodes. Additionally, the practicality of this sensor towards the detection of DA in the commercial DA injection solutions has also been demonstrated.

Experimental

The electrochemical experiments were carried out with a CH-Instruments Model No. CHI610D Electrochemical work station with a connection to a personal computer was used for the electrochemical measurement and treating of data. All the experiments were carried out in a conventional three-electrode system. The electrode system contained a working carbon paste electrode with a homemade cavity of 3 mm diameter, a platinum wire as counter electrode and saturated calomel electrode as reference electrode. Bare carbon paste electrode was prepared by grinding 70% of graphite powder and 30% of silicon oil in an agate mortar by hand mixing for about 30 min to get the homogenous paste. The paste was packed into the cavity of CPE and smoothed on a weighing paper³².

Chemicals and solutions

Analytical grade Dopamine hydrochloride, Ascorbic acid, Uric acid, sodium dihydrogen phosphate, disodium hydrogen phosphate and silicon oil were procured from Himedia Chemicals. Fine graphite powder (particle size $<20\ \mu\text{m}$) was supplied by Sigma-Aldrich chemicals. All chemicals were of analytical grade and were used without further purification. Dopamine stock solution was prepared by dissolving known quantity of it in 0.1 M perchloric acid and Ascorbic Acid in double distilled water, the stock solution of Uric acid (25 mM) was prepared in 0.1 M sodium hydroxide solution. Phosphate buffer (pH 7.0) was prepared as per the literature with 0.1 M NaH_2PO_4 , Na_2HPO_4 and dissolved in double distilled water. Oxalic acid (25 mM) was prepared in double distilled water. The pH values were measured with Elico Li 120 pH meter. All measurements were carried out at the room temperature.

Preparation of bare carbon paste electrode and Oxalic acid polymer modified carbon Paste electrode

The bare carbon paste electrode was prepared by adding the graphite powder and silicon oil at the ratio of (70:30%) in an agate mortar to obtain a homogenous carbon paste. The polymer film modified CPE was prepared by the electrochemical polymerization of Oxalic acid on a carbon paste electrode in 0.1 M phosphate buffer solution of pH 7.0 containing 1.0 mM of Oxalic acid with cyclic voltammetric sweeps in the potential range of -0.8 V to 1.2 V at the scan rate of 50 mV/s. The surface of the electrode was washed with distilled water.

Results and Discussion

Electrochemical polymerization of oxalic acid on the carbon paste electrode

Oxalic acid polymer film modified CPE was fabricated with oxalic acid on the surface of CPE by cyclic voltammetric method in PBS (pH 7.0) containing 1mM of Oxalic acid. Electropolymerization was carried out between the potential -0.75V to 1.25 V at a scan rate of 50 mV/s in 20 cycles as shown in Figure 1. During the polymerization process, an anodic peak potential at 0.714 V was observed due to the formation of polymer layer of oxalic acid on the surface of the electrode. The redox peak current increases with an increase in the number of cyclic voltammetric scans, indicating that an electro-conductive polymer film was formed on the electrode surface. The reaction mechanism may be characterized as follows, Oxalic acid was oxidized to form free radical which combines with the surface of CPE, resulting in the feasible structure of electropolymerized poly OAMCPE (Scheme 1), and then the modified carbon paste electrode was rinsed with distilled water.

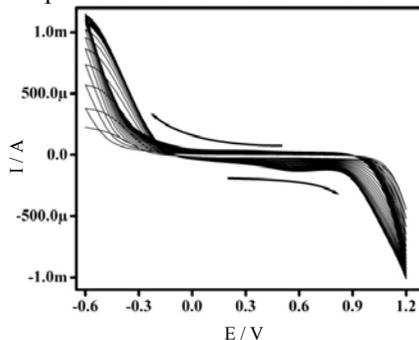
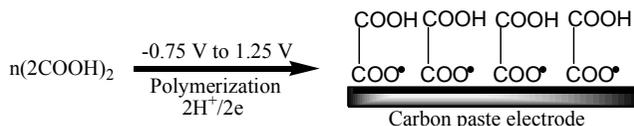


Figure 1. Cyclic voltammograms for the electrochemical polymerization of 1 mM Oxalic acid on a carbon paste electrode at the scan rate of $50\ \text{mVs}^{-1}$



Scheme 1. Electro polymerization of oxalic acid

The response of DA at the bare CPE and OAMCPE

Figure 2 shows the cyclic voltammograms of 0.1mM DA at bare carbon paste electrode (dotted line) and the OAMCPE with scan rate of 50 mV/s. At the BCPE, the difference between the anodic peak potential (Epa) 0.412 V and the cathodic peak potential (Epc) 0.200 V is reversible voltammogram with ΔE_p (Epa-Epc)= 0.212 V. However, DA peak currents were significantly increased at the OAMCPE, with the anodic peak at Δ 0.328 V and the corresponding cathodic peak potential is 0.246 V and the resulting ΔE_p = 0.037V. Compared with BCPE a remarkable enhancement in the peak currents with the reduction of over potential indicates the catalytic effect of the poly oxalic acid polymer layer. The mechanism may be the polymer layer combining with the hydrogen bond of the hydroxyl group of DA, which activates the hydroxyl and weakens the bond energy of O–H and improves the electron transfer rate. At the same time, high surface area of the poly oxalic acid improves the electrode contact area for DA.

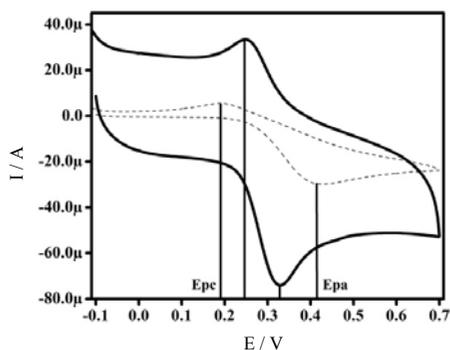


Figure 2. Cyclic voltammograms of bare carbon paste electrode (dotted line), poly oxalic acid carbon paste electrode using 0.1 mM DA in 0.1 M PBS (pH = 7.0) at scan rate of 50 mV s⁻¹

Stability and reproducibility of the OAMCPE

The stability and reproducibility of the OAMCPE electrode was studied in a sensitive and selective manner for the determination of DA and is shown in Figure 3. The anodic peak currents for dopamine were almost stable for 15 cycles. From these results the OAMCPE has produced stable anodic peak currents with negligible change in the redox peak currents of DA was observed.

Effect of pH value on the determination of DA at the OAMCPE

The pH of the supporting electrolyte shows significant influence on the determination of DA by electrocatalysis of OAMCPE by affecting both peak currents and peak potentials. The effect of pH value on the determination of DA in PBS solution at OAMCPE was carefully investigated in a wide pH range of 5.5–8.0. Figure 4 illustrates the dependences of the DA anodic peak current and anodic potential [E (V)] on the pH of buffer solution. It could be seen that the anodic peak current of DA increases with increasing pH value until it reaches

7.0, (shown with (-□-) square symbol) and then there was gradual decrease in the peak current of DA until it reaches 8.0. The anodic peak potential of DA shifts towards lower potential with the increase of the pH value of solution and depends linearly on the pH value in the range of 5.5–8.0 with a slope of 0.0603 V/pH. ($r^2 = 0.9721$) (shown with closed circles (-●-)). It demonstrates that the redox of DA undergoes a two electron and two proton processes, which was consistency with that reported in the literature³³.

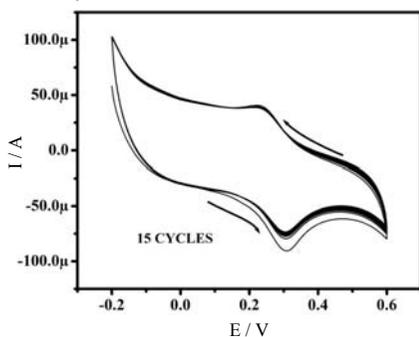


Figure 3. Stability and repeatability study of OAMCPE at 0.1 mM DA

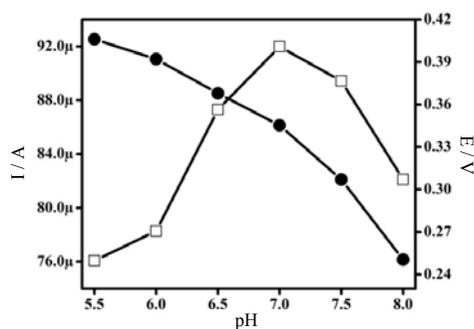


Figure 4. Effect of pH on anodic peak current (I_{pa}) (-□-), anodic peak potential (E_{pa}) (-●-) of 0.1 mM DA in 0.1 M phosphate buffer solution

Effect of scan rate

The effect of scan rate for 0.1 mM DA in 0.1M PBS at pH 7.0 was studied by CV at OAMCPE. The modified electrode shows an increase in the redox peak currents with an increase in the scan rate (50 to 500 mVs^{-1}). The graph of redox peak current (I_{pa}) vs. scan rate (v) was plotted and a good linearity between scan rates and peak current was obtained as shown in Figure 5. The redox peak currents were proportional to $(v)^{1/2}$. The correlation coefficient (r^2) of I_{pa} and I_{pc} were 0.9968 and 0.99735 respectively, which indicates that the electrode reaction was an adsorption controlled process. In the above cyclic voltammograms, a pair of cathodic and anodic peaks of DA were appeared symmetrically and the potential separation of the two peaks was close to $60/n$ mV ($n=2$) at a low scan rate. It could be inferred that there was a quasi-reversible reaction on the Oxalic acid modified carbon paste³³.

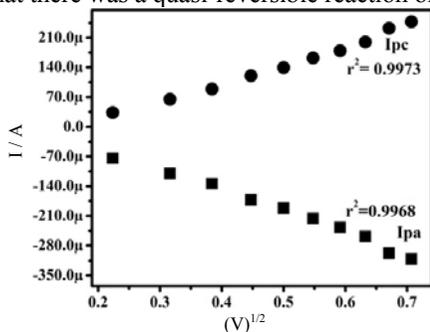


Figure 5. Graph shows the linear relationship between the peak current and the scan rate

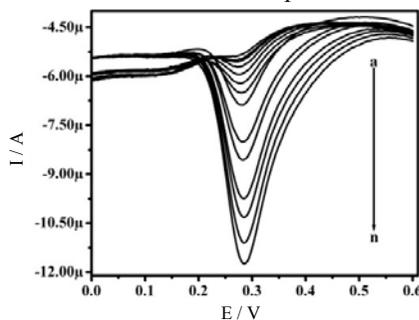


Figure 6. Series of differential pulse voltammograms (6.2 to 131 μM (a to n)) obtained for DA at OAMCPE in 0.1 M of pH 7.0 phosphate buffer solution

Concentration effect of DA

The differential pulse voltammetric technique was used for the analysis of DA concentration which was varied from 6.2 μM to 131 μM and is shown in Figure 6 for the OAMCPE.

The concentration of DA was increased from 6.2 to 131 μM and the corresponding graph of I_{pa} versus concentration of DA showed an increase in the anodic peak current with the linear regression equation as $I_{pa} (\mu\text{A}) = 0.0517(C/\mu\text{M}) + 5 \mu\text{M}$ ($n=14, R^2=0.998$) and is shown in Figure 7. The detection limit and quantification limit were calculated by using the formulae³⁴⁻³⁶.

$$\text{LOD} = 3S/M \quad (1)$$

$$\text{LOQ} = 10S/M \quad (2)$$

Where S is the standard deviation and M is the slope obtained from the calibration plots. The detection limit of dopamine was found to be 6 μM and the quantification limit is 22 μM .

Resolution of DA with UA and AA

Figure 8 is the cyclic voltammograms obtained for the electrochemical response of DA (10 μM), UA (100 μM) and AA (1 mM) at bare CPE (curve a), the OAMCPE (curve b) in 0.1 M phosphate buffer solution of pH 7.0. At bare CPE, a well redox peak potential of DA & AA was not observed and with OAMCPE it showed a significant redox peak potential for DA & AA along with UA with various potential differences of DA, UA and AA 489, 344, and 97 mV respectively.

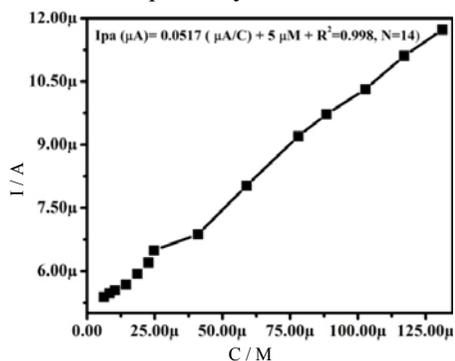


Figure 7. Graph of I_{pa} vs. concentration of Dopamine

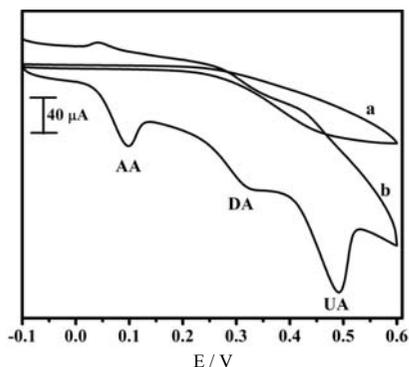


Figure 8. Cyclic voltammograms obtained for the electrochemical response of 10 μM of DA 200 μM of UA, 1 mM of AA at bare CPE (curve a) and at OAMCPE (Curve b) in 0.1 M phosphate buffer solution of (pH7.0), scan rate 50 mVs^{-1}

The main objective of this study was to detect DA, UA, and AA simultaneously. The applicability of the OAMCPE for the simultaneous determination of DA, UA, and AA was demonstrated by simultaneously changing the concentrations of DA, UA, and AA. The differential pulse voltammetric results showed that the simultaneous determination of DA, UA and AA with three well-distinguished anodic peaks with potentials at 426, 258 and 9 mV, corresponding to the oxidation of UA, DA and AA, respectively, were observed at OAMCPE (Figure 9 A). In contrast, the bare electrode showed no significant separation in the voltammetric signals of these substances. The corresponding graphs of the anodic peak current versus various concentrations of DA (10-48 μM), UA (100-489 μM), AA (1-4.8 mM) showed a linear relationships with linear regressions for B (DA) $Y(\mu\text{A}) = 0.751x + 1.654 \times 10^{-4}$, C

(UA) $Y(\mu\text{A}) = 0.2755x + 4.296 \times 10^{-5}$, D (FA) $Y(\mu\text{A}) = 0.0136x + 2.157 \times 10^{-5}$, the correlation coefficient for these linear graphs was 0.991, 0.994 and 0.991 respectively with OAMCPE which is shown in Figure 9. B, C, D respectively.

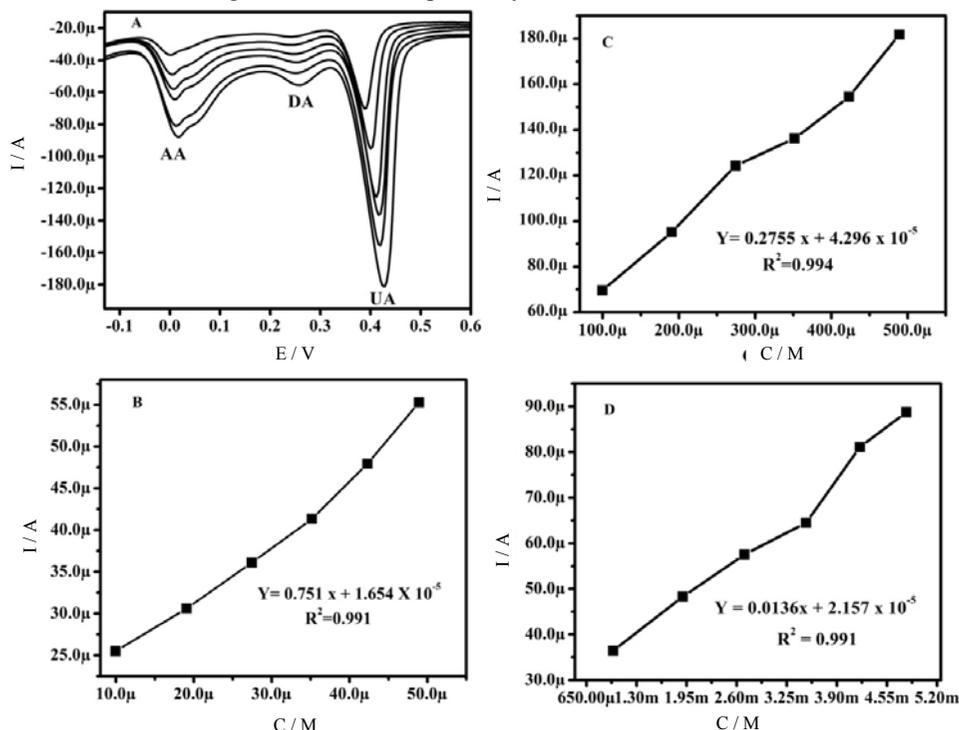


Figure 9. (A) Differential pulse voltammograms of OAMCPE in 0.1 M phosphate buffer solution (pH 7.0) containing different concentrations of DA, UA and AA (from inner to outer) mixed solutions of 9.9+99+0.95, 19+190+1, 27+274+2, 35+351+3.5, 42+423+4.2 and 48+489+4.89, respectively, among three set values first value is the concentration of DA in μM , the second value is the concentration of UA in μM and the last value is the concentration of AA in mM. Remain plots of the peak currents as a function of DA (B), UA(C) and AA (D) *versus* concentration, respectively.

Effect of various concentration changes of DA in the presence AA, UA / UA in the presence AA, DA / AA in the presence DA, UA.

Figure 10 A, B, C shows the differential pulse voltammograms of OAMCPE for a fixed concentration of UA (100 μM), DA (10 μM) and AA with concentrations varying from 1 to 4.80 mM. Similarly UA (100 μM), AA (1 mM) and DA with concentrations varying from 10 to 48 μM and DA (10 μM), AA (1 mM) and UA concentrations varying respectively in 0.1 M phosphate buffer (pH 7.0). Obviously anodic peak currents increase with increasing concentrations of AA, DA and UA while the anodic peak currents of UA, DA and UA, AA and DA, AA remain constant. A good linearity between I_{pa} and the concentrations variation of AA, DA, UA were obtained respectively and is shown in the Figure. 10 D,E and F respectively. Furthermore, it was observed that in the presence of high concentrations of remain analytes the detection of lower concentration of AA, DA and UA is still possible.

The OAMCPE showed a good selectivity for the electrochemical detection of DA, AA and UA in the presence of remaining analytes. The corresponding graphs of anodic peak current *versus* concentration of AA, DA, UA showed a linear regression $Y(\mu\text{A}) = 0.007x + 3.791 \times 10^{-5}$, $R^2 = 0.9879$, $Y(\mu\text{A}) = 0.659x + 1.598 \times 10^{-5}$, $R^2 = 0.9944$ and $Y(\mu\text{A}) = 1.6325x + 3.189 \times 10^{-6}$, $R^2 = 0.9870$ respectively and the detection limits of AA 0.93 mM, DA 7.4 μM and UA 13 μM were found.

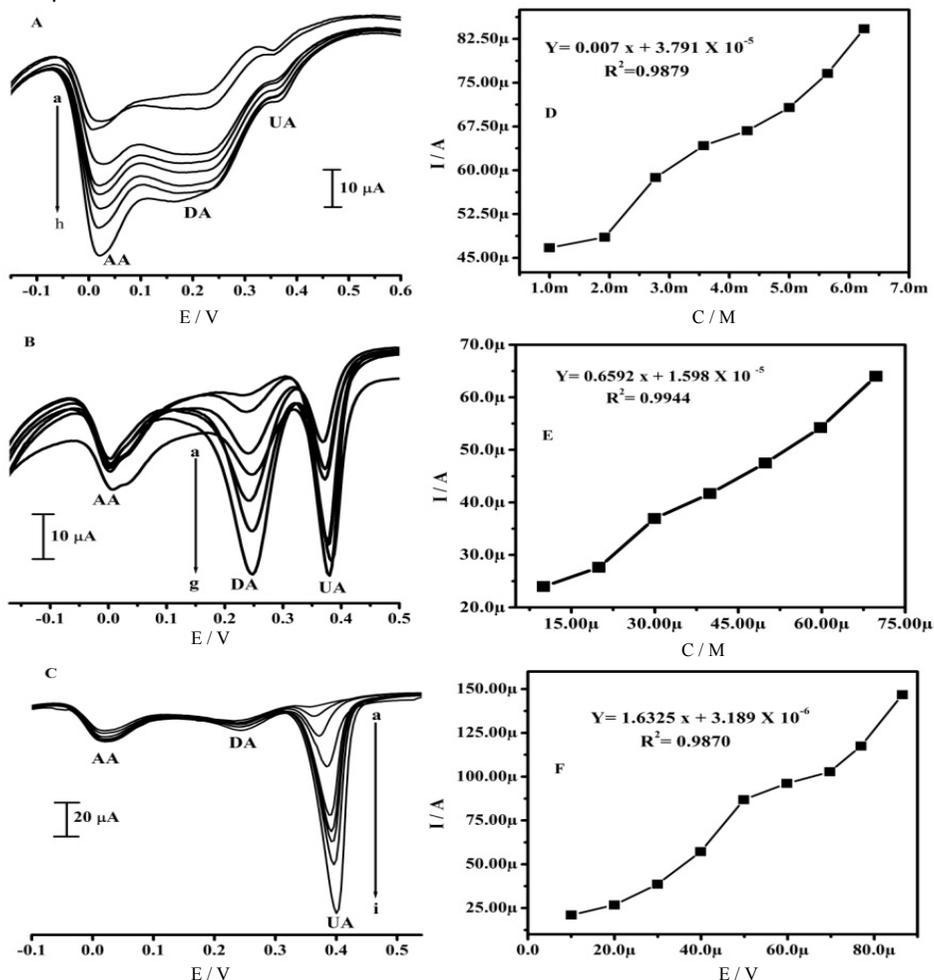


Figure 10. A) Differential pulse voltammograms at the OAMCPE for UA, DA and with different concentrations of AA, B) UA, AA and with different concentrations of DA, C) DA, AA and with different concentrations of UA, in 0.1 M phosphate buffer solution of pH 7.0. The plots, D) shows I_{pa} *versus* AA concentration, E) shows I_{pa} *versus* DA concentration, F) Shows I_{pa} *versus* UA concentration variations.

Application to real system

Practical application of modified electrode was demonstrated by quantitative determination of DA in human blood serum samples (obtained from the Health Centre, Sri Venkateswara

University, Tirupati, Andhra Pradesh, India). The procedure was as followed: 2 mL of human serum sample without any pretreatment was diluted to 100 mL with pH 7.0 phosphate buffer. Different volumes of this solution were mixed with a known volume concentration of DA solution and also of known concentration, to obtain different concentrations of spiked DA. Similarly, a drug injection capsule containing 200 mg of dopamine hydrochloride in 5 mL sterilized water (Neon laboratories Private Ltd, India) was suitably diluted to provide different known standard concentrations of DA which were analyzed by DPV using the OAMCPE. Each experiment was carried out at least 5 times and the results were presented in Table 1. The obtained recovery and relative standard deviation (RSD) were good, indicating the efficiency of the OAMCPE.

Table 1. Determination of DA in drug injection and human blood serum sample (n=5)

Samples	Spiked DA sample, mM	Found, mM	Recovery, %	RSD, %
Blood serum	0.1	0.095	95	3.5
	0.2	0.194	97	3.2
	0.3	0.297	99	1.4
Drug injection	0.1	0.097	97	3.0
	0.2	0.203	101	2.5
	0.3	0.294	98	1.1

Conclusion

A simple and sensitive electrochemical sensor was fabricated based on electrochemically polymerized carbon paste electrode surface with Oxalic acid for the determination of DA in the presence of AA, UA. The results demonstrated that the electrooxidation of DA at the surface of the oxalic acid modified electrode was occurred at a potential of about 0.0843 V which is less positive than that of the bare carbon paste electrode. The peak separations with detected potential differences of 168, 256 and 424 mV between DA-UA, DA-AA and UA-AA respectively were large enough to determine DA, UA and AA individually and simultaneously. Finally, the applicability of this electrode was tested for the determination of DA in pharmaceutical and also in clinical samples.

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