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Full Paper

Poly(Succinic acid) Modified Carbon Paste Electrode for the Resolution of Dopamine in the presence of Ascorbic acid and Uric acid

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Abstract- A voltammetric electro chemical sensor was developed for the resolution study of dopamine and ascorbic acid by the electrochemically deposited succinic acid polymer on a carbon paste electrode at 50 mVs^{-1}/Ag–AgCl. This modified electrode exhibited an excellent electrochemical 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 scan rate was shows the process is diffusion controlled. The DPV study of 57 to 438 µM concentration range of DA shows 34 µM detection limit. The oxidation potentials of AA, DA and UA at the poly succinic acid modified carbon paste electrode were shifted slightly negatively and the peak currents were much larger than the bare carbon paste electrode. The limit of detection for AA, DA and UA were 0.7 mM, 1.0 µM and 79 µM respectively. This succinic acid modified carbon paste electrode showed many merits in constancy, sensitivity, capability, economy and also applicable for real sample analysis.

Keywords- Succinic acid, Ascorbic acid, Uric acid, Dopamine, Cyclic voltammetry, Differential pulse voltammetry

1. INTRODUCTION

In recent years the design, fabrication and application of novel electrochemical sensor has been of considerable interest [1]. Particularly the development of voltammetric sensors for the determination of neurotransmitters, such as dopamine (DA) and other catecholamines, has received a lot of interest. Among them DA has attracted much interest because the change in DA levels has proved to be very effective route toward understanding brain functions, such as learning and memory formation, physiological and pathological process of Parkinsons disease [2]. As a result of these discoveries, catecholamine drugs are now widely used in the treatment of bronchial asthma, hypertension, Parkinsons disease, myocardial infarction and cardiac surgery. Due to these the quantitative determination of DA in human physiological fluids is of considerable significance in both biochemical and clinical diagnoses, Consequently various electrochemical techniques have been proved to be advantageous in the selective and sensitive determination of DA concentrations. A major problem is however, the interference of AA, which is also present in biological fluids at very high concentrations, whereas DA levels are much smaller [3]. It is known that the direct electro-oxidation of DA and AA at bare electrodes is irreversible and requires high potentials. DA and AA are moreover, oxidized at nearly same potentials at bare electrodes [4,5] and oxidation often suffers from a pronounced fouling effect, which results in rather poor selectivity and reproducibility. Both sensitivity and selectivity are, therefore of equal importance in DA determination. The overlap of their voltammetric responses makes their simultaneous determination highly difficult [6]. One of the most common routes is to use a modified carbon paste electrode, which has the ability to eliminate the interfering substances from DA determination. The study of electrochemical determination with different modified electrode for sensitive and selective determination of DA has been reported [7-11]. Similarly some of organic redox mediators [12], nanoparticles [13] and self-assembled monolayer [14] have been applied as modification layer to construct the highly selective and sensitive DA biosensor. The traditional analytical methods for the detection of DA include chemiluminescence [15], fluorimetry [16], ultraviolet-visible spectrometry [17], and capillary electrophoresis (CE-luminescence) [18] also applicable.

The other methods like Ion exchange membranes are anionic and cationic nature has been developed to electrostatically accumulate oppositely charged analyte molecules. They are Nafion [19], polyester sulphonic acid [20], poly (4-vinylpyridine) [21], stearate [22], w-mercapto carboxylic acid [23], poly (monomericeugenol) [24], overoxidised poly (1-(2-carboxyethyl) pyrrole [25], 4-aminophenylacetic acid [26], ionic liquid [27], overoxidised polypyrrole [28,29].

Similarly in the electroanalytical chemistry some of the other modified electrodes like nanoparticles modified electrodes [30-36], carbon ceramic electrode [37], pyrolytic graphite electrode [38], boron-doped diamond electrode (BDD) [39], carbon ionic liquid electrode

[40], screen-printedcarbon electrode [41], electrochemically oxidized GCE [42], and carbon nanotube microelectrode [43] 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 electrodes [44,45]. Herein, we have reported the formation of poly succinic acid polymer composite via electrochemical method based on the number of cycles of redox reaction of succinic acid, which is more facile and less time consuming than other existing methods. We have studied the electrochemical determination of DA at poly succinic acid polymer modified electrode. The high surface area and good conductivity of the poly (Succinic acid) MCPE polymer composite allows the efficient oxidation of DA. Moreover, composite electrode showed greater electro catalytic 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.

2. EXPERIMENTAL PART

2.1. Apparatus and procedure

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 smoothened on a weighing paper [46].

2.2. 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 <20µm) 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.

Succinic 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.

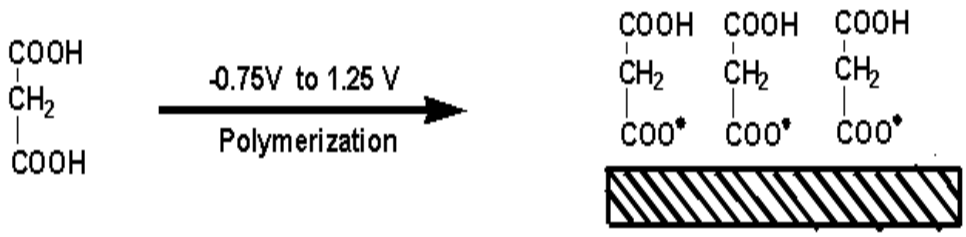
2.3. Preparation of bare carbon paste electrode and poly (Succinic acid) MCPE Paste electrode

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 Succinic acid on a carbon paste electrode in 0.1 M phosphate buffer solution of pH 7.0 containing 1.0 mM of Succinic 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.

3. RESULTS AND DISCUSSION

3.1. Electrochemical polymerization of succinic acid on the carbon paste electrode

The poly (Succinic acid) modified carbon paste electrode (MCPE) was prepared by placing 1.0 mM solution of Succinic acid monomer in 0.1 M PBS of pH 7.0 in an electrochemical cell. Over the potential range of -0.75 V to 1.25 V at a scan rate of 50 mV/s in 20 cycles as shown in Fig. 1. During the polymerization process, an anodic peak potential at 0.714 V was observed due to the formation of polymer layer of succinic 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, succinic acid was oxidized to form free radical which combines with the surface of CPE, resulting in the feasible structure of electro polymerized poly (Succinic acid) MCPE (Scheme 1), and then the modified carbon paste electrode was rinsed with distilled water.



Scheme 1. Electropolymerization of Succinic acid

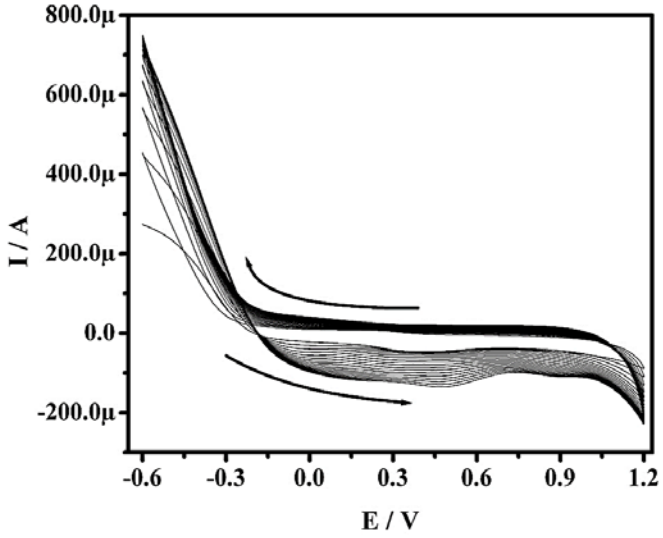


Fig. 1. Cyclic voltammograms for the electrochemical polymerization of 1 mM succinic acid on a carbon paste electrode at the scan rate of 50 mVs^{-1}

3.2. The response of DA at the bare CPE and poly (Succinic acid) MCPE

Fig. 2 shows the cyclic voltammograms of 0.1 mM DA at bare carbon paste electrode (dotted line) and the poly (Succinic acid) MCPE with scan rate of 50 mV/s. At the BCPE, the difference between the anodic peak potential (Epa) 0.355 V and the cathodic peak potential (Epc) 0.118 V is reversible voltammogram with ΔEp (Epa-Epc)=0.237 V. However, DA peak currents were significantly increased at the poly (Succinic acid) MCPE, with the anodic peak at Δ 0.250 V and the corresponding cathodic peak potential is 0.147 V and the resulting ΔEp =0.103 V. Compared with BCPE a remarkable enhancement in the peak currents with the reduction of over potential indicates the catalytic effect of the poly succinic 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 (Succinic acid) MCPE improves the electrode contact area for DA.

3.3. Stability and reproducibility of the poly (Succinic acid) MCPE

The stability and reproducibility of the SAMCPE electrode was studied in a sensitive and selective manner for the determination of DA and is shown in Fig. 3. The anodic peak currents for dopamine were almost stable for 20 cycles. From these results the poly (Succinic acid) MCPE has produced stable anodic peak currents with negligible change in the redox peak currents of DA was observed.

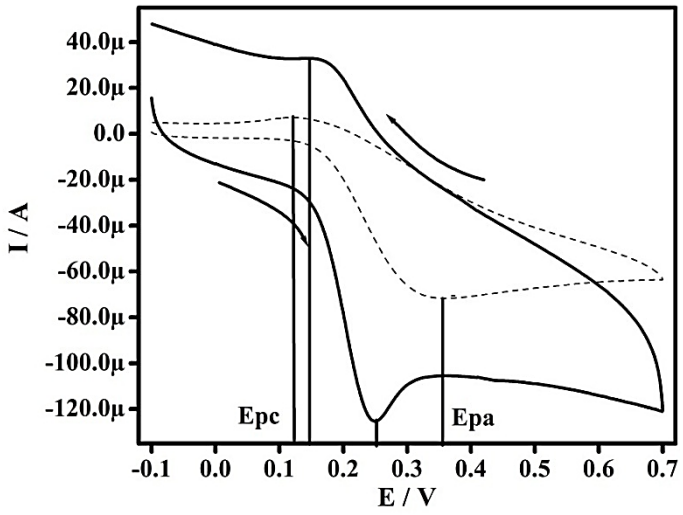


Fig. 2. Cyclic voltammograms of bare carbon paste electrode (dotted line), poly succinic acid carbon paste electrode using 0.1 mM DA in 0.1 M PBS (pH=7.0) at scan rate of 50 mV s^{-1}

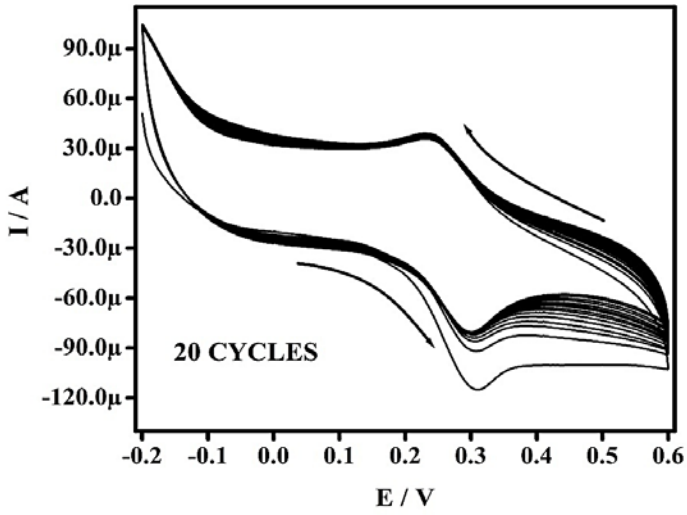


Fig. 3. Stability and repeatability study of poly (Succinic acid)MCPE at 0.1 mM DA

3.4. Effect of pH value on the determination of DA at the poly (Succinic acid) MCPE

The pH of the supporting electrolyte shows significant influence on the determination of DA by electrocatalysis of SAMCPE by affecting both peak currents and peak potentials. The effect of pH value on the determination of DA in PBS solution at poly (Succinic acid) MCPE was carefully investigated in a wide pH range of 5.5–8.0. Fig. 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 reaction of DA undergoes a two electron and two proton processes, which was consistence with that reported in the literature [47].

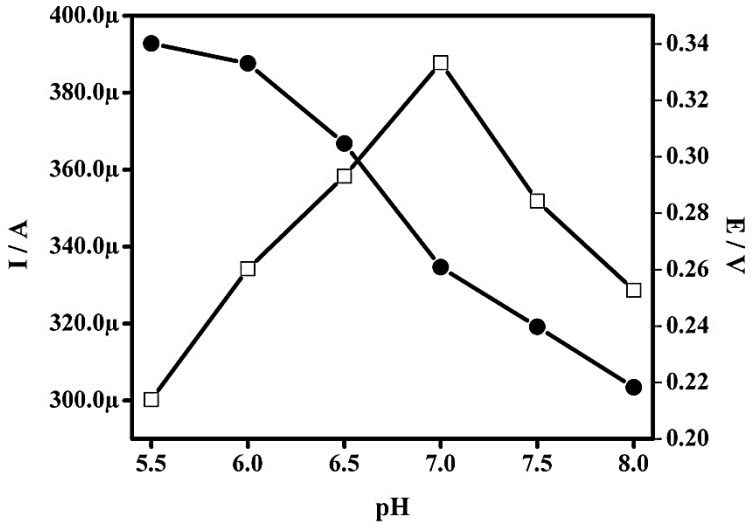


Fig. 4. Effect of pH on anodic peak current (I_{pa}) (-□-), anodic peak potential (E_{pa} (-●-) of 0.1 mM DA in 0.1 M phosphate buffer solution

3.5. Effect of scan rate

The effect of scan rate for 0.1 mM DA in 0.1 M PBS at pH 7.0 was studied by CV at poly (Succinic acid) MCPE. 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 (ν) was plotted and a good linearity between scan rates and peak current was obtained as shown in Fig. 5. The redox peak currents were proportional to $(\nu)^{1/2}$ The correlation coefficient (r^2) of I_{pa} and I_{pc} were 0.989 and 0.997 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 poly (Succinic acid) MCPE [48].

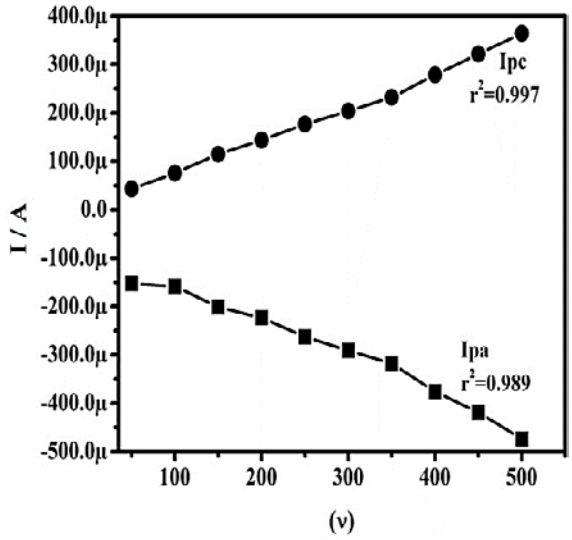


Fig. 5. Graph shows the linear relationship between the peak current and the scan rate

3.6. Concentration effect of DA

The differential pulse voltammetric technique was used for the analysis of DA concentration which was varied from 57 µM to 438 µM and is shown in Fig. 6 for the poly (Succinic acid) MCPE

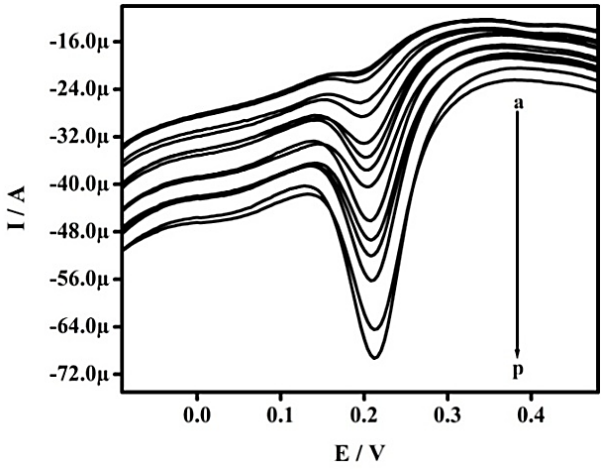


Fig. 6. Series of differential pulse voltammograms (57 to 438 µM) obtained for DA at poly (Succinic acid) MCPE in 0.1 M of pH 7.0 phosphate buffer solutions

The concentration of DA was increased from 57 to 483 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} (µA)=0.1263(C/µM)+12 µA (n=16, R^2=0.995) and is shown in Fig. 7. The detection limit and quantification limit were calculated by using the formulae [49-52].

LOD=3S/M (1)

LOQ=10S/M (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 34 μM and the quantification limit is 25 mM.

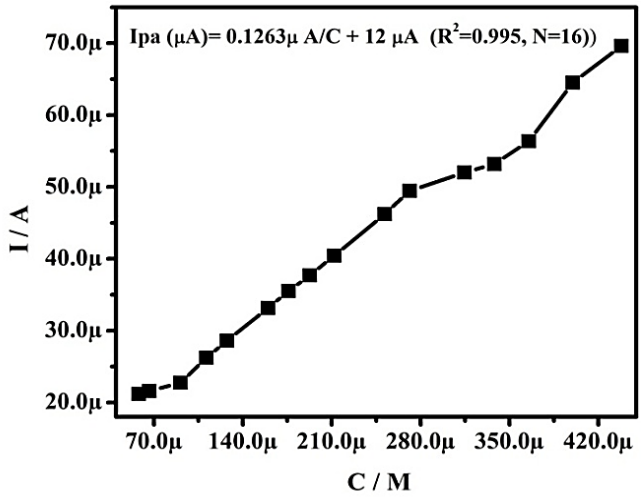


Fig. 7. Graph of I_{pa} *vs.* concentration of Dopamine

3.7. Resolution of DA with UA and AA

Fig. 8 is the cyclic voltammograms obtained for the electrochemical response of DA (10 μM), UA (100 μM) and AA (1 mM) at bare CPE (dotted line), the poly (Succinic acid) MCPE (thick line) 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 poly (Succinic acid) MCPE it showed a significant redox peak potential for DA&AA along with UA with various potential differences of DA, UA 76 mV DA, AA 282 mV and UA, AA 358 mV respectively.

The main objective of this study was to detect DA, UA, and AA simultaneously. The DPV results showed that the simultaneous determination of DA, UA, and AA with three well-distinguished anodic peaks with potentials at 352, 211 and 9 mV, corresponding to the oxidation of UA, DA, and AA, respectively, were observed at poly (Succinic acid)MCPE (Fig. 9A). 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 AA(1-4.8 mM), DA(10-48 μM) and UA(100-489 μM) showed a linear relationships with linear regressions for B (AA) $Y(\mu A) = 0.0065x + 6.392\times10^{-4}$, C (DA) $Y(\mu A) = 2.478x + 1.843\times10^{-5}$, D (UA) $Y(\mu A) = 0.293x + 7.914\times10^{-5}$, the correlation coefficient for these linear graphs was 0.808, 0.9873 and 0.947 respectively with poly (Succinic acid) MCPE which is shown in Fig.8. B, C, D respectively.

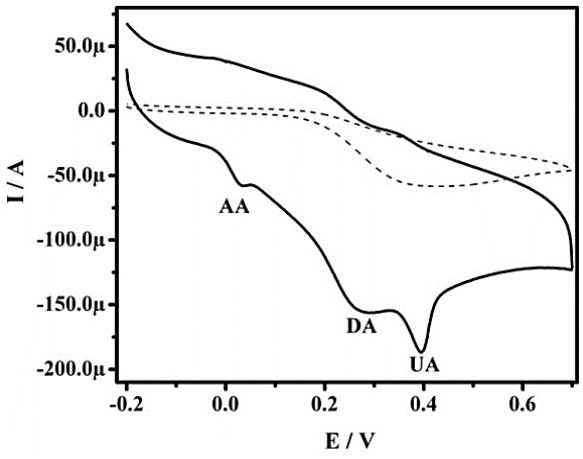


Fig. 8. Cyclic voltammograms obtained for the electrochemical response of 10 µM of DA 200 µM of UA, 1 mM of AA at bare CPE (dotted line) and at poly (Succinic acid) MCPE (thick line) in 0.1 M phosphate buffer solution of (pH7.0), scan rate 50 mVs^{-1}

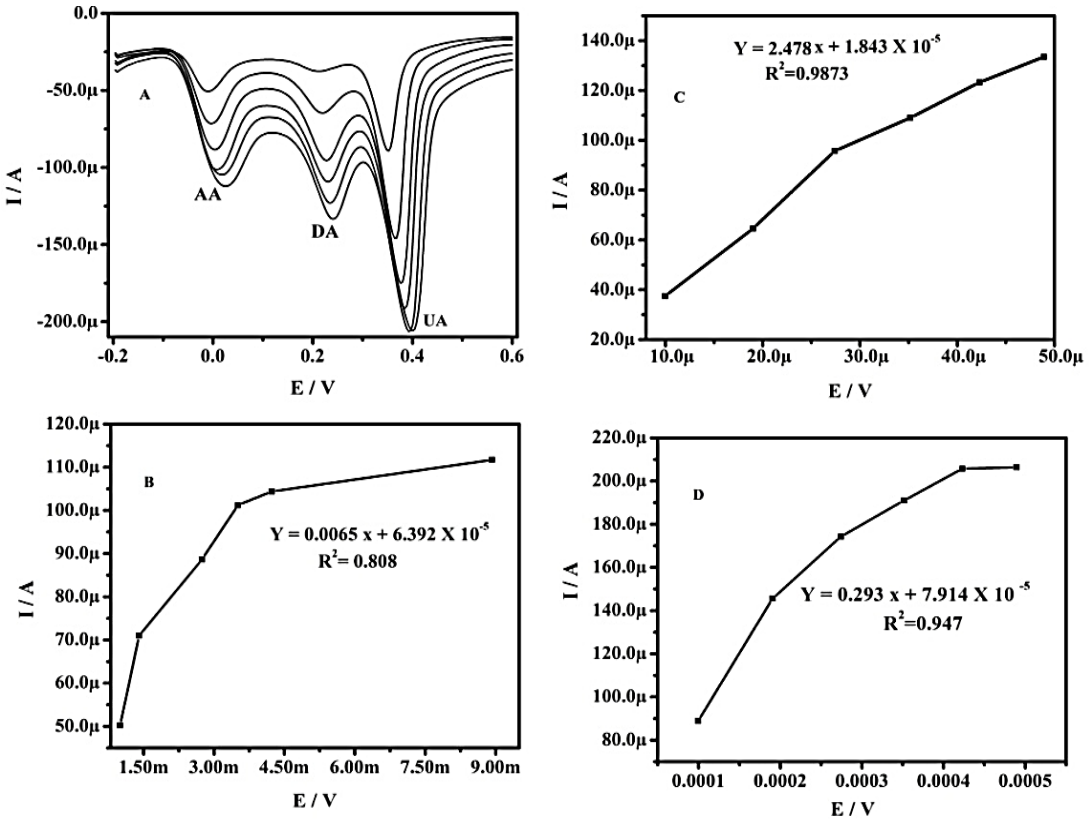


Fig. 9. (A) Differential pulse voltammograms of SAMCPE in 0.1M phosphate buffer solution (pH 7.0) containing different concentrations of DA, UA, and AA (from inner to outer) mixed solutions of 10+100+1, 20+200+2, 30+300+3, 40+400+4, 50+500+5 and 60+600+6, respectively, among three set values first value 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 AA (B), DA (C), and UA (D) versus concentration, respectively

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

Fig. 10 A, B, C shows the differential pulse voltammograms of poly (Succinic acid) MCPE for a fixed concentration of UA (100 µM), DA (10 µM) and AA with concentrations varying from 1 to 4.80 mM.

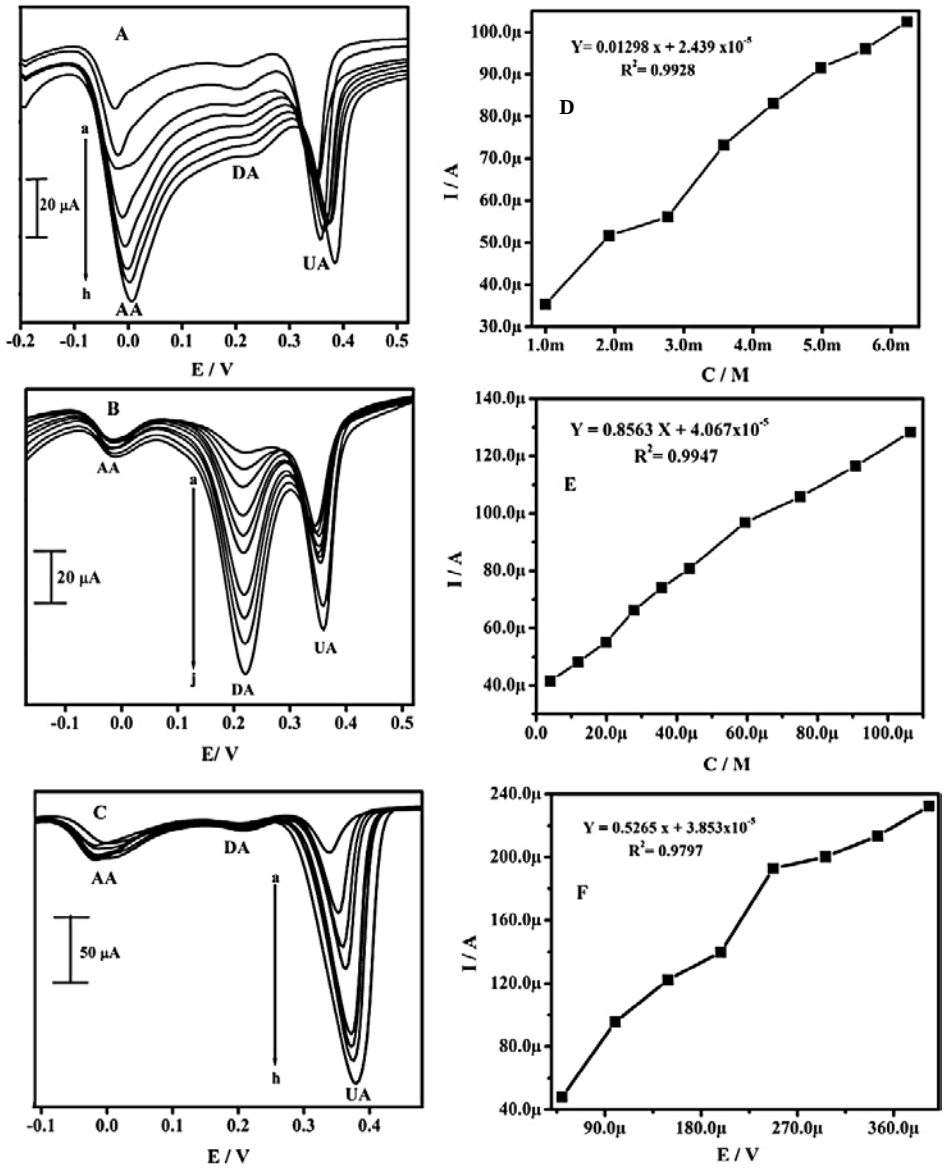


Fig. 10. A) Differential pulse voltammograms at the poly (Succinic acid) MCPE for UA, DA and with different concentrations of AA; B) UA, AA and with different concentrations of DA, C) DA, AA and with different concentrations of UA, in 0.1 M phosphate buffer solution of pH 7.0. The remain plots; D) shows I_{pa} versus AA concentration; E) shows I_{pa} versus DA concentration; F) Shows I_{pa} versus UA concentration variations

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 Fig. 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 SAMCPE 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 $Y(\mu A)=0.0129x+2.439\times10^{-5}$ $R^2=0.9928$, $Y(\mu A)=0.8563x+4.067\times10^{-5}$ $R^2=0.9947$ and $Y(\mu A)=0.5265x+3.853\times10^{-6}$ $R^2=0.979$ respectively and the detection limits of AA 0.7 mM, DA 1.0 µM and UA 79 µM were found.

3.9. 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.

Table 1. Determination of DA in drug injection and human blood serum sample (n=3)

Samples	Spiked DA sample (mM)	Found (mM)	Recovery (%)	RSD (%)
Drug injection	0.1	0.094	96	3.5
	0.2	0.175	98	3.2
	0.3	0.29	99	1.4
Blood serum	0.1	0.096	97	3.0
	0.2	0.179	98	2.5
	0.3	0.275	97	1.1

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 poly (Succinic acid) MCPE. 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 SAMCPE.

4. CONCLUSION

The simple and sensitive electrochemical sensor was developed based on electrochemically polymerized carbon paste electrode surface with Succinic acid for the determination of DA in the presence of AA, UA. The results demonstrated that the electro oxidation of DA at the surface of the poly (Succinic acid) MCPE was occurred at a potential of about 0.103 V which is less positive than that of the BCPE. The peak separations with detected potential differences of 76, 282, and 358 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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