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Full Paper

Simultaneous Determination of Dopamine, Ascorbic Acid and Uric Acid at Poly (Crystal Violet) Modified Carbon Paste Electrode

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Abstract- A sensitive and selective electrochemical method for the determination of dopamine (DA) using a crystal violet polymer film modified on carbon paste electrode was developed. The crystal violet polymer film modified electrode poly (crystal violet) MCPE shows excellent electrocatalytic activity towards the oxidation of DA in phosphate buffer solution (pH 7.4). The separation of the oxidation peak potentials for dopamine–ascorbic acid (AA) and dopamine–uric acid (UA) were about 182 mV and 180 mV, respectively. The differences are large enough to determine AA, DA and UA individually and simultaneously. This modified electrode shows very good selectivity, sensitivity, stability and antifouling property.

Keywords- Poly (Crystal Violet) Modified Electrode, Simultaneous, Dopamine, Uric Acid, Ascorbic Acid

1. INTRODUCTION

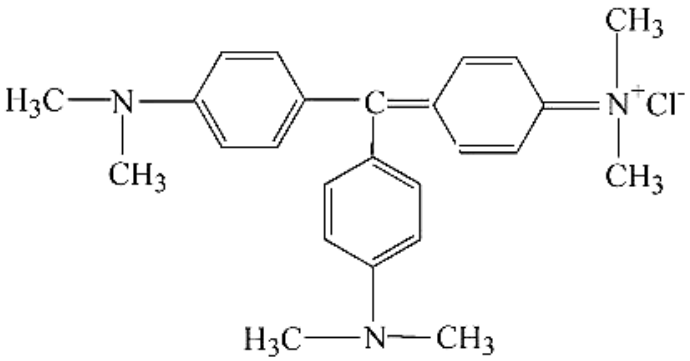
Dopamine (DA) is an important neurotransmitter molecule of catecholamines which is widely distributed in the mammalian central nervous system for message transfer. Normal levels of dopamine in the brain allow the usual freedom of movement, whereas excess DA in

the brain often creates pleasurable, rewarding feelings and sometime euphoria. One of the most well known and important effects of DA deficiency is Parkinson's disease [1,2]. Moreover, according to other recent clinical studies [3] it seems that the content of AA and DA can be used to assess the amount of oxidation stress in human metabolism, linked to cancer [4], diabetes mellitus [5], and hepatic diseases [6]. Low levels of DA are related to neurological disorders schizophrenia [7–9]. UA is the primary end product of purine metabolism. Abnormal levels of UA are symptoms of several diseases such as hyperuricaemia, Gout and Lesch–Nyan disease [10]. AA presents in both animal and plant kingdoms, is a vital vitamin in human diet and is very popular for its antioxidant properties. It has been used for the prevention and treatment of common cold, mental illness and infertility [11]. DA, UA and AA usually coexist in physiological samples, but there has an overlapping oxidation potential on the solid electrodes. Therefore it is essential to develop simple and rapid methods for their determination in routine analysis. Among many methods for determination of UA, DA and AA in biological samples, voltammetric method has shown to be a powerful tool. Moreover, the direct redox reactions of these species at the bare electrodes take place at very similar potentials and often suffer from a pronounced fouling effect, which results in rather poor selectivity and reproducibility. The ability to determine DA, UA and AA selectively has been a major goal of electroanalytical researches [12].

Exploration of many kinds of chemically modified electrodes to detect DA selectively has occurred in past years. Several approaches based on polymer-modified electrode [13-21], carbon ionic liquid electrodes [22-24], nano materials modified electrodes [25-28] and self-assembled monolayer's [29-33] have been tried to solving the problems. A number of researchers have employed polymeric film modified electrode to detect DA. So far different methodologies have been used for depositing polymeric films. Electropolymerisation is a good approach to immobilize polymers because adjusting the electrochemical parameters can control film thickeners, permeation and charge transport characteristics [34]. Recently dyes are used as modifier [35-38] has attracted more attention because of their novel electrode material which exhibits several excellent electrochemical properties and high electrochemical stability. Electropolymerisation methods provide certain advantages. For example, the long term operation stability of a poly (methylene blue) film modified electrode was much higher than that of the adsorbed mediator [38]. Meanwhile, an electropolymerisation-modified electrode has better electrocatalytic activity electropolymeryzed methylene blue was at least 10 times more active for NADH oxidation than the monomer [38]. Recently related works have been done by our research group [39-42].

Crystal violet is a well-known dye being used for various purposes can be electropolymeryzed to form a modified electrode. It is a sort of triphenylmethane dye, and its chemical structure is illustrated in scheme 1. Crystal violet has an open but ionized structure.

Therefore its polymerized product exhibits interesting properties, such as fast charge transfer and ion transport [43].



Scheme 1. Structure of crystal violet

2. EXPERIMENTAL PART

2.1. Reagents

DA, AA and UA were obtained from Himedia chemical company and were used as received. All other chemicals were of analytical grade. The phosphate buffer solution (PBS) was prepared by mixing standard stock solutions of 0.2 M Sodium dihydrogen phosphate (NaH_2PO_4) and di-Sodium hydrogen phosphate (Na_2HPO_4) and adjusting the pH with 0.2 M HCl or 0.2 M NaOH. Freshly prepared solutions of DA, AA and UA were used in all experiments. All the aqueous solutions were prepared using double distilled water.

2.2. Apparatus and Procedure

Cyclic voltammetry (CV) was performed on Model EA-201 Electroanalyser (ChemilinkSystem). All the experiments were carried out in a conventional three electrode electrochemical cell. The electrode system contained a carbon paste as working electrode (3.0 mm in diameter), a platinum wire counter electrode and saturated calomel electrode as reference electrode (SCE). The carbon paste electrode was prepared as fallows, 70% graphite powder and 30% silicone oil were mixed by hand in an agate mortar to produce a homogeneous paste. The paste was then packed into the cavity of a homemade carbon paste electrode and smoothed on a weighing paper .

2.3. Preparation of poly (crystal violet) film on carbon paste electrode

The paste packing procedure was same as that at the bare carbon paste electrode (BCPE). Electrochemical polymerizations of crystal violet at the carbon paste electrode was done by using cyclic voltammetric method in aqueous solution containing 2 mM of crystal violet in

0.2 M PBS (pH 7.4). Electropolymerisation was achieved by the formation of film that grew between of 0 to 2000 mV at a scan rate of 100 mVs^{-1} for 10 cycles using cyclic voltammetry as shown in Fig. 1. After that, the electrode was rinsed with water, and then cycled in the blank PBS over a potential range of -250 to 600 mV at a scan rate of 50 mVs^{-1}, until a reproducible voltammogram was attained.

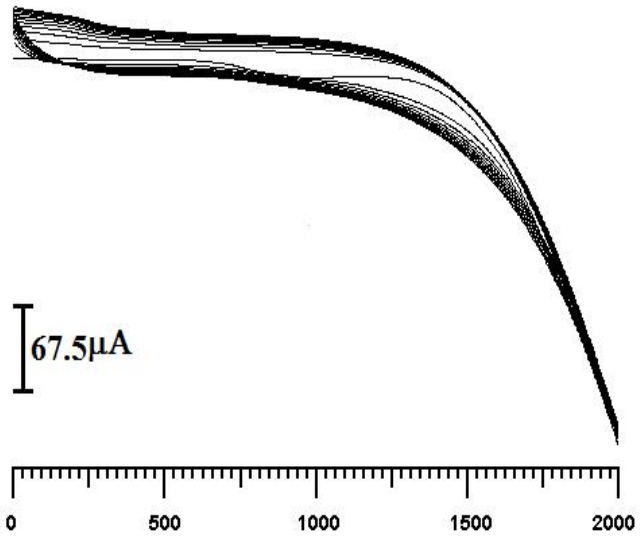


Fig. 1. Growth of poly (crystal violet) film on carbon paste electrode substrate by potential cycling in the range 0-2000 mV at a scan rate of 100 mVs^{-1}. Solution contains 1×10^{-3} M crytalviolet and 0.2 M phosphate buffer (pH 7.4).

3. RESULTS AND DISSCUTION

3.1. Surface morphology and stability of poly (Crystal violet) MCPE film

Morphology of both BCPE and MCPE were characterized by SEM. Fig. 2A shows the morphology of MCPE which is formed by electropolymerisation. Clearly, Crystal violet forms rather a smooth and uniform film on the surface of CPE and the image is entirely deferent from BCPE (Fig. 2B). The stability of the poly (crystal violet) MCPE checked by taking 20 multiple cycles in 0.2 M PBS containing 0.1×10^{-4} M DA (Fig. 3). From the figure it was observed that after the first cycle the anodic and cathodic peaks of DA remains constant, which shows that the electrode is highly stable.

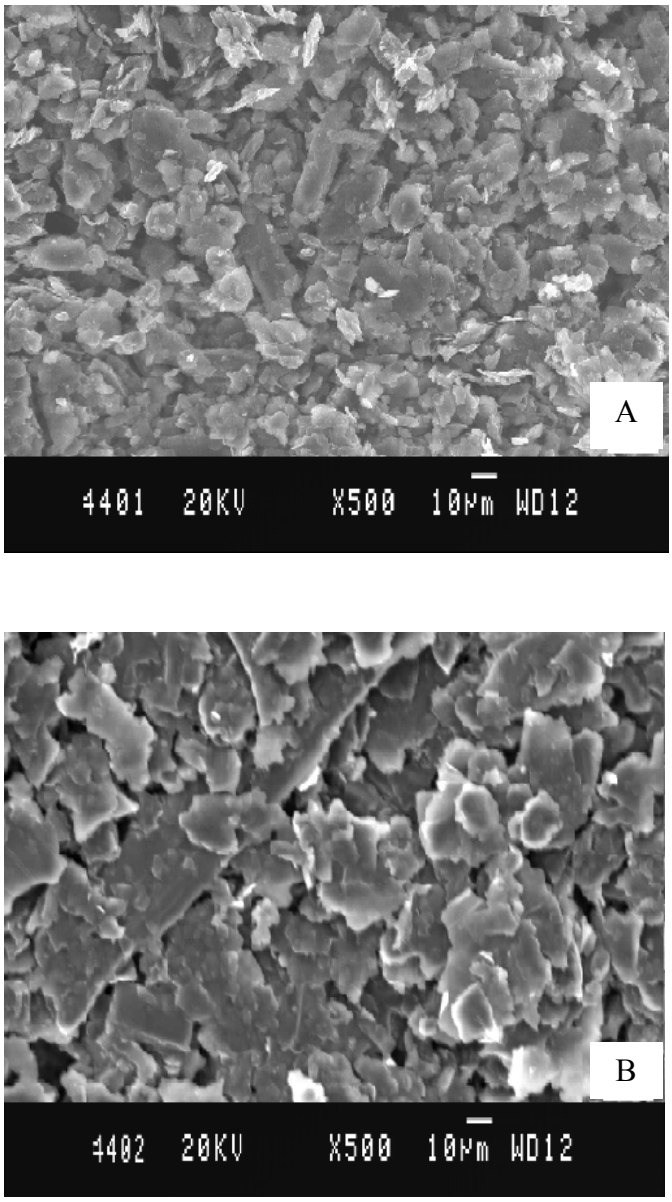


Fig. 2. SEM image of BCPE (A) and MCPE(B)

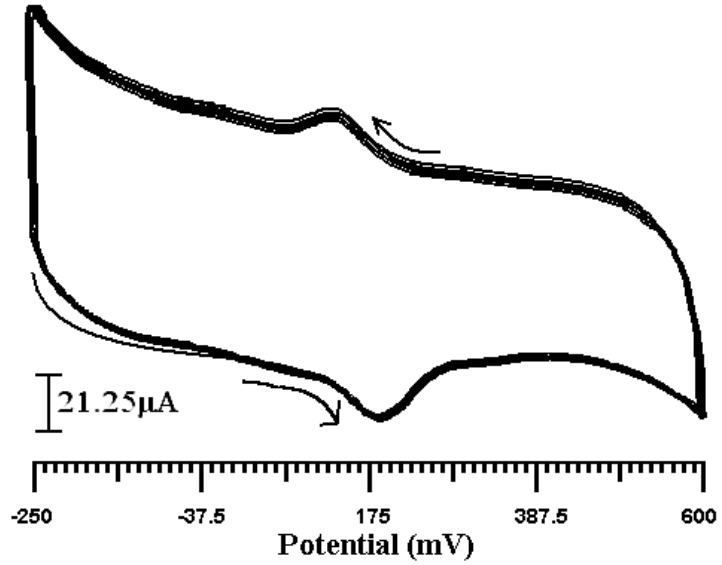


Fig. 3. Cyclic voltammograms for 20 multiple cycles of 0.1×10^{-4} DA in 0.2 M phosphate buffer solution pH 7.4 at scan rate 50 mV s^{-1}

3.2. Electrocatalytic oxidation of DA at poly (Crystal violet) MCPE

To test the electrocatalytic activity of the poly (crystal violet) MCPE toward DA oxidation, cyclic voltammetric responses were obtained in pH 7.4 PBS in the absence and presence of 0.1×10^{-4} DA at a BCPE and poly (crystal violet) MCPE (Fig. 4). At a BCPE (small dashed line) a pair of redox peak showed poor electrocatalytical activity with anodic peak potential of 160 mV and cathodic peak potential of 108 mV. Under the same condition poly (crystal violet) MCPE (solid line) gave birth to significantly enhanced peak current and more reversible electron transfer process to DA with slight shift in redox peak potentials. A well defined redox wave of DA was observed with anodic (E_{pa}) and cathodic peak potential (E_{pc}) at 193 mV and 133 mV respectively. The separation of peak potential (Δ_{Ep}) at the poly (crystal violet) MCPE was 60 mV which was accordance with Nernst reversible behavior. Intensive increase in peak was also observed owing to the improvement in reversibility of electron transfer process and the larger real surface of poly (crystal violet) film, at the same time in the absence of analyte the poly (crystal violet) MCPE doesn't shows any peaks (dashed line). This suggests an efficient oxidation reaction toward DA at the poly (crystal violet) MCPE.

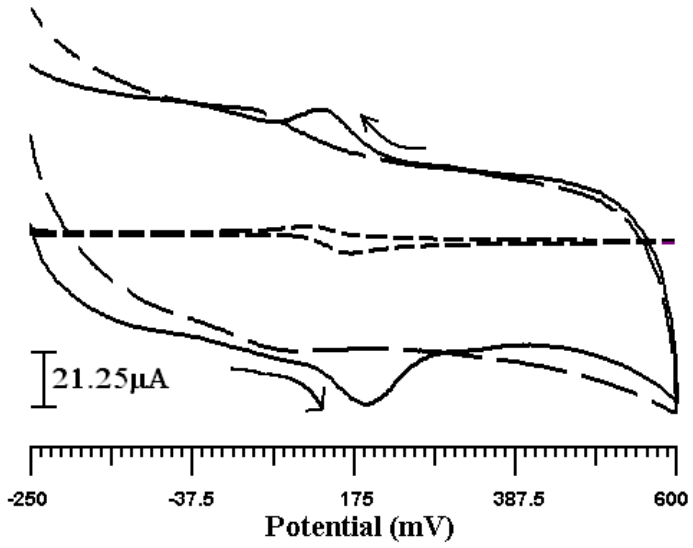
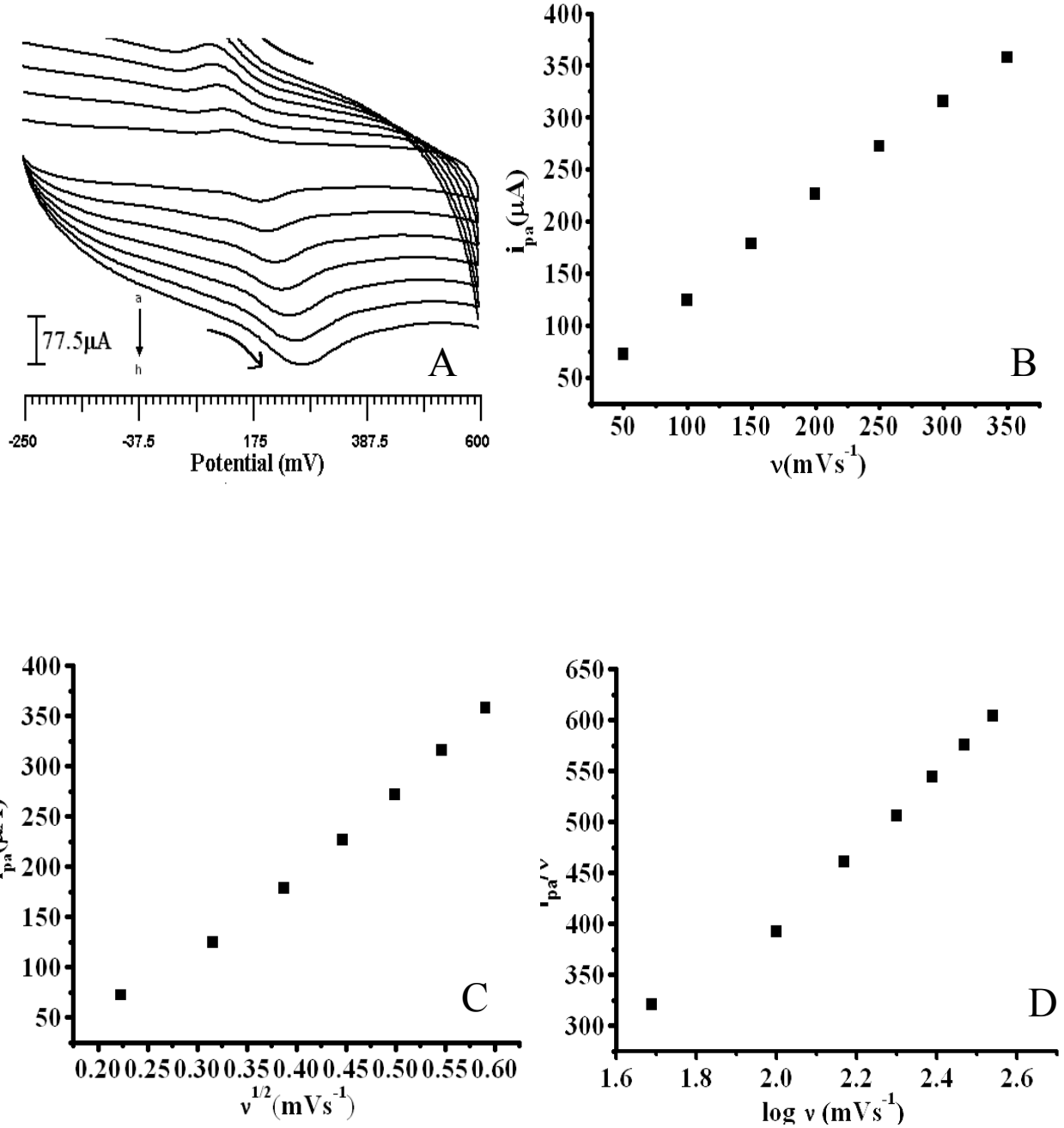


Fig. 4. Cyclic voltammogram of 0.1×10^{-4} M DA in 0.2 M phosphate buffer solution of pH 7.4 at BCPE (dashed line) and poly (crystal violet) MCPE (solid line), big dashed line is for blank solution

3.3. Effect of scan rate

To understand the electrode behaviour, scan rate was varied between 50 to 350 mVs^{-1} and the change in current response of 0.1×10^{-4} M DA was observed (Fig. 5A). The anodic peak current was found to increase linearly with scan rate Fig. 5B. Such behaviour indicates involvement of adsorption complications in the electrochemical behaviour of DA [44]. In addition, we studied the relationship between I_{pa} and $\nu^{1/2}$ (Fig. 5C). The I_{pa} was proportional to the $\nu^{1/2}$, which suggested a diffusion-controlled process in solution, with a correlation coefficient of 0.9993. Also the slope of log I_{pa} *vs*. log ν (Fig. 5D) was 0.82 which is larger than theoretical expected value 0.53 for purely diffusion controlled process [45]. Further from the plot of $I_{pa}/\nu^{1/2}$ *vs*. log ν indicated an increase in the peak current with an increase in sweep rate (Fig. 5E) confirming that the reaction at the surface of electrode has adsorption complications. These results reveal that the anodic process is dominated by adsorption and diffusion of DA simultaneously.


77.5µA
a
h
A
-250
-37.5
175
387.5
600
Potential (mV)
400
350
300
250
200
150
100
50
i_{pa}(µA)
B
50 100 150 200 250 300 350
ν(mVs^{-1})
C
0.20 0.25 0.30 0.35 0.40 0.45 0.50 0.55 0.60
ν$^{1/2}$(mVs^{-1})
650
600
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i_{pa}/ν
D
1.6 1.8 2.0 2.2 2.4 2.6
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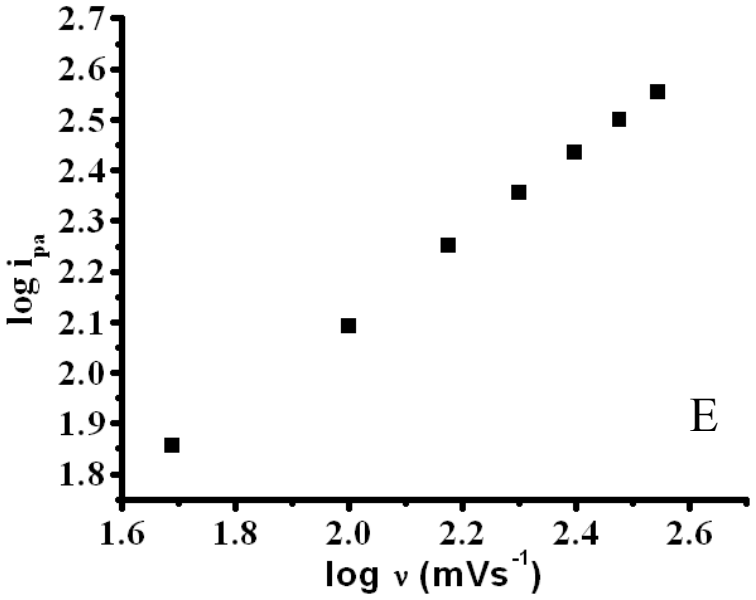

Fig. 5. (A) Cyclic voltammograms of poly (crystal violet) MCPE in the presence of 0.1×10^{-4} DA with the varying scan rate. Cyclic voltammograms were measured in 0.2 M phosphate buffer (pH 7.4). Scan rate (mVs^{-1}): 50 (a); 100 (b); 150 (c); 200 (d); 250 (e); 300(f); 350 (g) (B) Plot of I_{pa} *vs*. Scan rate (C**)** Linear relationship between I_{pa} *vs*. square root of scan rate (D**)** Plot of I_{pa} /$v^{1/2}$ *vs.* log v (E) Variation of the logarithm of I_{pa} with the logarithm of the scan rate

3.4. Effect of DA concentration

The concentration effect of DA was carried out by varying the concentration at poly (crystal violet) MCPE. As shown in Fig. 6A. By increasing the concentration of DA from 0.1×10^{-4} M 0.5×10^{-4} M the I_{pa} and I_{pc} goes on increasing with shifting E_{pa} towards positive and E_{pc} with negligible shifting. 0.1×10^{-4} M to 0.5×10^{-4} M DA concentrations showed the E_{pa} was shifted from 212 mV to 258 mV. The graph of I_{pa} *vs*. concentration of DA was plotted, showed increase in electrochemical peak current, (Fig. 6B). The graph obtained linearly increase in peak current with increase in the DA concentration and I_{pa} is proportional to concentration of DA.

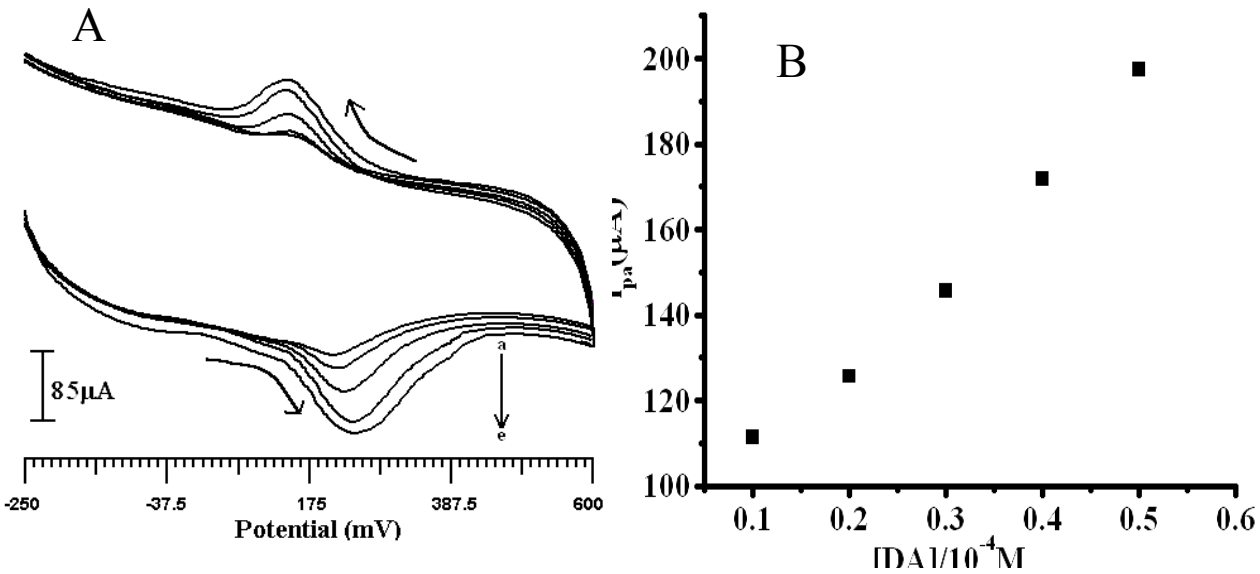

Fig. 6. (A) Cyclic voltammograms obtained for different concentration of dopa (0.1×10^{-4} to 0.5×10^{-4}) in 0.2 M phosphate buffer solution at Ph7.4 scan rate 50 mVs^{-1} (B) Plot of anodic peak current *vs.* concentration of DA

3.5. Effect of solution pH

The influence of solution pH and current response of DA at the poly (crystal violet) MCPE in 0.2 M PBS was investigated. (Fig. 7A) the peak current of DA increased with increasing solution pH until it attained about 7.4, and then the current decreased when the pH increased further. However the better sensitivity and shape of the voltammogram of the peak at pH 7.4 suggested it as optimal pH value. We further, studied the relationship between the anodic peak potential of dopamine and pH (Fig. 7B) and were found that the anodic peak potential decreased with increase in pH indicating that equal number of protons takes part in the reactions. The pH 7.4 was the physiological condition and the response current of DA was the highest at this pH, it was chosen as the experiment pH value in the electrochemical detection of DA.

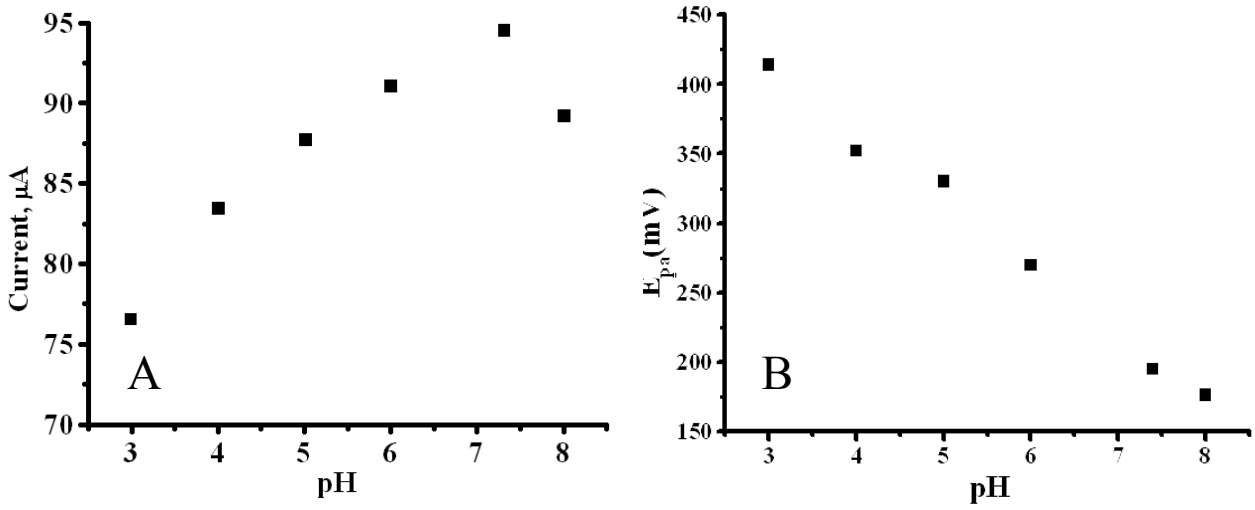

Fig. 7. (A) Plot of anodic peak current (I_{pa}) *vs.* (B) Plot of anodic peak potential *vs.* pH

3.6. Oxiation of ascorbic acid and uric acid at the poly (Crystal violet) MCPE

Fig. 8 indicates the electro-catalytic behavior of AA at the poly (crystal violet) MCPE (solid line) and the BCPE (dotted line) in the aqueous PBS of pH 7.4. The cyclic voltammogram of AA in the PBS produced a single irreversible oxidation peak at both the electrode surfaces. The oxidation of AA occurred at around 107 mV at the BCPE whereas the same occurred at the poly (crystal violet) MCPE at about -54 mV of high negative potential with an increase in the peak current compared to that of the BCPE. From the above results thus, it was evident that the poly (crystal violet) MCPE stood for its effective catalysis for the oxidation of ascorbic acid.

Fig. 9 illustrates the cyclic voltammogram of UA with pH 7.4 PBS at BCPE (dotted line) and poly (crystal violet) MCPE (solid line). It can be seen that voltammetric peak of UA in the 7.4 (pH) PBS appeared at about 318 mV at the BCPE, the peak was rather broad suggesting slow electron transfer kinetics, presumably due to the fouling of the electrode surface by the oxidation product, the poly (crystal violet) MCPE makes only a minor difference in peak potential of UA oxidation (328 mV) in a solution with pH 7.4. On the other by using poly (crystal violet) MCPE, a remarkable increase in anodic peak current from -23.6 to -69.9 is resulted for UA.

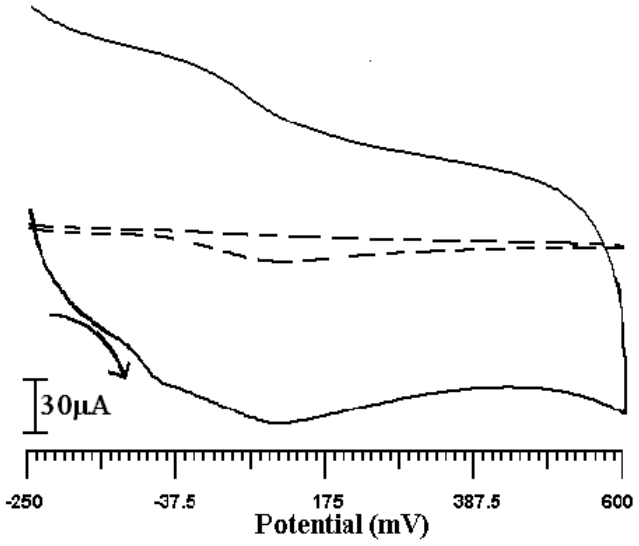

Fig. 8. Cyclic voltammograms of poly (CV) MCPE (solid line) and BCPE (dotted line) in the presence of 2×10^{-3} AA in 0.2 M phosphate buffer (pH 7.4) Scan rate 50 mVs^{-1}

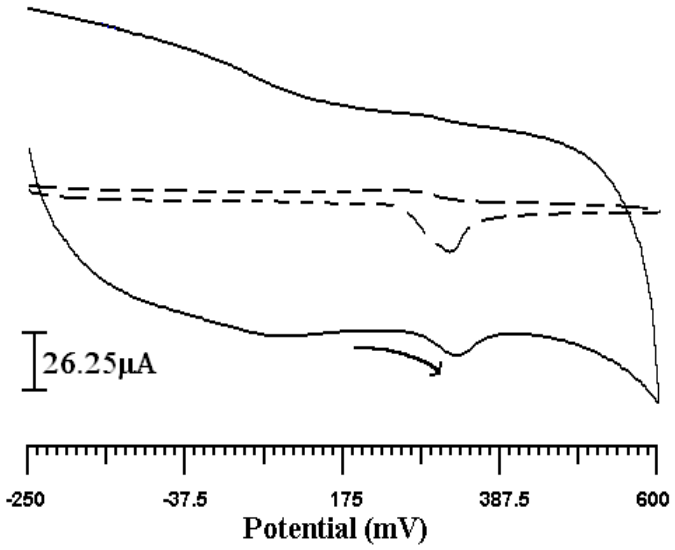

Fig. 9. Cyclic voltammogram of 0.1×10^{-3} UA at poly (CV) MCPE in phosphate buffer Solution of pH 7.4 at 50 mVs^{-1} scan rate

3.7. Separation of electrochemical responses to AA, DA and UA at poly (Crystal violet) MCPE

In order to establish a sensitive and selective method for the quantification of AA, DA and UA, the ability of the modified electrode to promote the voltammetric resolution of AA, DA and UA was investigated. The CV of the mixture solution of 2×10^{-3} M AA, 0.1×10^{-4} M DA and 0.1×10^{-3} M UA in pH 7.4 PBS (Fig. 10) shows one broad and overlapped anodic peak at (353 mV) at BCPE (dotted line). So the peak potentials for DA, AA and UA are indistinguishable at a bare BCPE. Therefore, it is impossible to deduce any information from the broad and overlapped voltammetric peak. But at poly (crystal violet) MCPE, the overlapped voltammetric peak is resolved into three well-defined CV peaks (solid line) at about around -27, 238 and 395 mV corresponding to the oxidation of AA, DA and UA, respectively. The separation of the oxidation peak potentials for AA–DA, DA–UA and AA–UA are about 265, 157 and 422 mV, respectively.

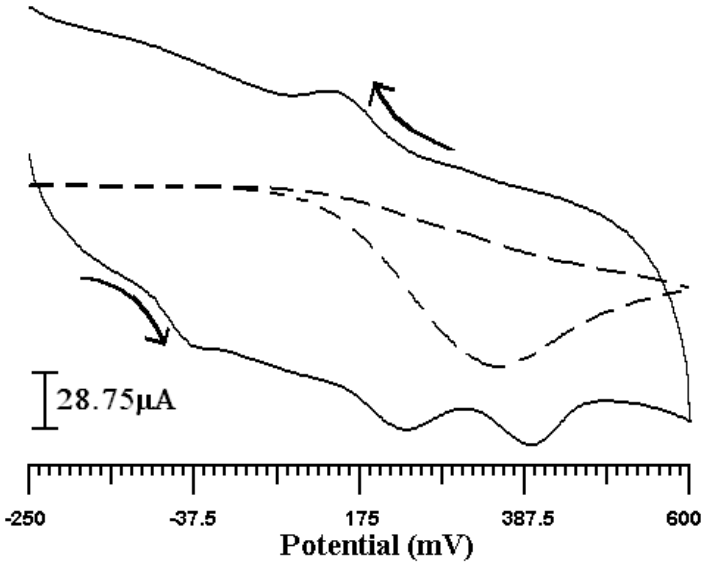

Fig. 10. Cyclic voltammogram for the solution containing mixture of 0.1×10^{-4} DA, 2×10^{-3} AA and 0.1×10^{-3} UA in 0.2 M phosphate buffer system of pH 7.4 at bare CPE (dashed line) and poly (CV)MCPE (solid line), Scan rate 50 mVs^{-1}

4. CONCLUSION

The electropolymerisation of crystal violet on the carbon paste electrode produces a stable polymeric film. This electrochemical sensor exhibited both strong electro-catalytic activity toward DA, UA and AA oxidation and significant surface accumulation of DA and UA but repulsion of AA in neutral solutions. The scan rate effect proved that the electrode process was controlled by both adsorption and diffusion process in PBS .buffer. A linear relationship between the peak current and the concentration of DA was obtained in a range of (0.1×10^{-4} to 0.5×10^{-4}). The anodic peak potential (E_{pa}) was proportional with the solution pH in the range of 3.0–7.0. The modified electrode was very suitable and effective for simultaneous determination of AA, UA and DA with good sensitivity, stability and selectivity. The simple fabrication procedure, reproducibility, high stability, and antifouling Properties suggest that this electrode is an attractive candidate as a sensor for practical applications.

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