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Research Article

SIMULTANEOUS DETERMINATION OF ASCORBIC ACID, DOPAMINE AND URIC ACID AT POLY (ANILINE BLUE) MODIFIED CARBON PASTE ELECTRODE: A CYCLIC VOLTAMMETRIC STUDY

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ABSTRACT

A cyclic voltammetric technique was used for the electropolymerisation of aniline blue on the surface of carbon paste electrode was applied for individual and simultaneous determination of ascorbic acid, dopamine and uric acid. The modified electrode exhibited stable and sensitive current responses toward dopamine. Due to its strong electrocatalytic activities towards dopamine, the modified carbon paste electrode can resolve the overlapped voltammetric waves of ascorbic acid, dopamine and uric acid into three well defined voltammetric peaks by using cyclic voltammetry. This property allows for the selective determination of dopamine in the presence of ascorbic acid and uric acid. The modified electrode has been successfully applied for the determination of dopamine, ascorbic acid and uric acid. The proposed method showed excellent stability and reproducibility.

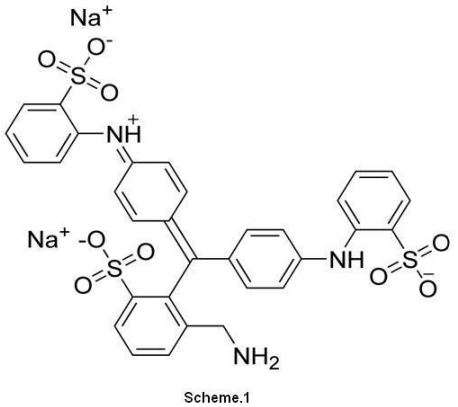
Keywords: Dopamine, Ascorbic acid, Uric acid, Electropolymerisation, Cyclic voltammetry

INTRODUCTION

Dopamine (DA), uric acid (UA) and ascorbic acid (AA) are three important neurotransmitters, widely distributed in the body of many mammals and exhibited message transfer in the brain and defense against disease [1-4]. Thus, it is important to develop sensitive, fast and selective methods for the detection of DA, UA and AA. In the past decades various chemically modified electrodes have been used to analyze these biological molecules [5–7]. Among these methods, polymer modified electrodes (PMEs) have many advantages in the detection of biomolecules because of their selectivity, sensitivity and homogeneity in electrochemical deposition, strong adherence to the electrode surface and chemical stability of the film [8, 9]. Various PMEs have been used to investigate electrocatalytic oxidation of DA, UA and AA [10–14].

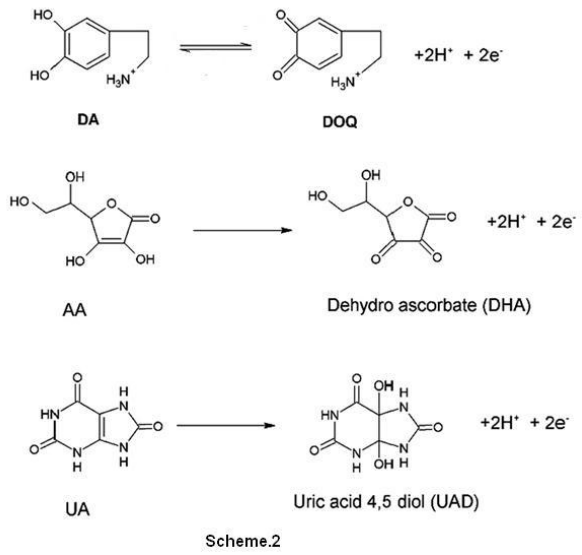
Several electrochemical approaches have been used to implement the above goals [15–22]. Among these methods, using polymer-coated electrode to determine DA in presence of AA and UA shows excellent selectivity and sensitivity. A poly (phenosafranine) film modified glassy carbon (GC) electrode exhibited potent and persistent electron mediating behavior followed by well separated oxidation peaks towards AA, DA and serotonin with activation over potential, which is 0.2 V lower than that of bare electrode for AA oxidation [23]. Roy describes an poly(N,N-dimethylaniline) modified electrode, which could separate the DA ,AA and UA oxidation peak potentials and could detect DA at its low concentration in the presence of higher concentration of AA and UA [24]. In addition there were reports of using hippuric acid [25], sulfosalicylic acid [26], styrene sulfonic acid [27], amino benzoic acid [28], aniline [29], cobalt hexacyanoferrate[30], 3,4-ethlenedioxy thiophene[31], eriochrome black-T [32], L-methionine [33]andtoludine blue [34] as monomer to modify electrode for the detection of DA in the presence of AA and UA.

Aniline Blue also called aniline blue WS, China blue or soluble blue is a mixture of methyl blue and water blue as shown in scheme.1.



Scheme.1

Scheme 1: Structure of Aniline blue.



Scheme.2

Scheme 2: The scheme of oxidations of DA, AA and UA.

In this work poly (aniline blue) film was fabricated on the surface of a carbon paste electrode by cyclic voltammetric technique. The polymer was found to be electrocatalytically active for the electrooxidation of DA. The oxidation potential of DA could be well separated from those of AA and UA with its good sensitivity, selectivity and stability. The scheme of oxidations of DA, AA and UA is shown in scheme 2. Recently related works have been done by our research group [35-43].

MATERIALS AND METHODS

Apparatus and reagents

Cyclic voltammetric experiments were carried out with a model EA-201 Electro Analyser (Chemilink Systems, India) connected to a personal computer for control and data storage. All electrochemical experiments were performed in a standard three-electrode cell. The bare or poly(anilineblue) modified CPE was used as a working electrode, platinum electrode as counter electrode and saturated calomel electrode (SCE) as reference electrode.

Dopamine hydrochloride (DA), Aniline blue, Ascorbic acid (AA) and Uric acid were obtained from Himedia chemicals, Bangalore, India and were used as received. All other chemicals were of analytical grades. Phosphate buffer solutions (PBS) was prepared by mixing standard stock solutions of 0.2 M Na_2HPO_4 and 0.2 M $NaH_2PO_4.H_2O$. Freshly prepared solutions of DA, AA and UA were used in all experiments. Stock solution of aniline blue was prepared in DMF and other solutions were prepared with double distilled water.

Preparation of bare carbon paste electrode

The bare carbon electrode was prepared by hand mixing of graphite powder and silicon oil in the ratio of 70:30 (w/w) in an agate mortar until a homogenous paste was obtained. The prepared carbon paste was tightly packed into a PVC tube (3 mm internal diameter) and the electrical contact was provided by a copper wire connected to the paste at the end of the tube.

Preparation of pre treated and poly (aniline blue) modified CPE

1 mM aniline blue was placed in the electrochemical cell containing 0.05M H_2SO_4. The CPE was pretreated by scanning in the solution from –400 to 1200 mV at 100 mVs^{-1} for 10 times. After words , the same electrode enforced under sweeping from –400 to 1200 mV at 100 mVs^{-1} for 10 cycles in 0.01M NaoH containing 1 mM aniline blue (Fig.1). The poly (aniline blue) modified CPE. CPE after polymerization was rinsed with water and used for the determination of DA, AA and UA.

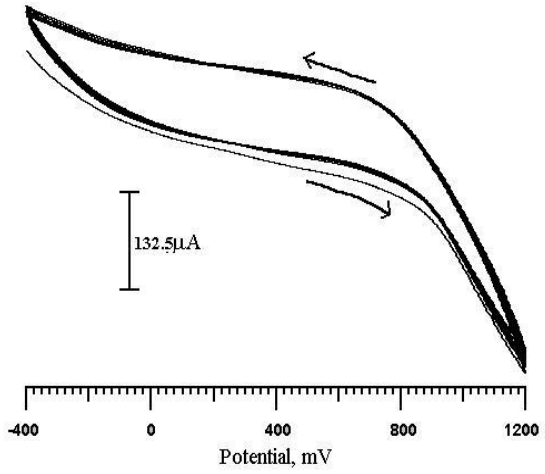


Fig. 1: Cyclic voltammogram of preparation of poly (aniline blue) film coated CPE. 1 mM aniline blue in 0.01M NaOH at 10 cycles with sweep rate of 100 mVs^{-1}

RESULTS AND DISCUSSIONS

Electrochemical investigation of potassium ferrocyanide at poly (aniline blue) modified CPE

Potassium Ferrocyanide [$K_4Fe(CN)_6$] was used as the electrochemical redox probe to investigate the electrochemical properties of poly (aniline blue) modified CPE (Fig.2). The cyclic voltammograms (CVs) of poly (aniline blue) modified CPE showed that the redox peak current increased compared to bare CPE. At the bare CPE the cyclic voltammogram of [$K_4Fe(CN)_6$] (solid line) showed a pair of redox peaks with the anodic peak potential at 230 mV and the cathodic peak potential at 159 mV in 1M KCl. The poly (aniline blue) modified CPE shows a pair of redox peaks (dashed line). The anodic peak potential was located at 232 mV and the cathodic peak potential at 178 mV respectively. The results of the enhancement of peak current showed excellent catalytic activity of poly (aniline blue) modified CPE.

Electrochemical investigation of dopamine at the poly (aniline blue) modified CPE

Fig. 3 shows cyclic voltammogram of 1 mM DA in 0.2 M PBS (pH 7) at bare CPE and poly (aniline blue) film modified CPE was recorded. At bare CPE (solid line) a pair of redox peak showed electrocatalytic activity with anodic peak potential of 218 mV and cathodic peak potential of 117 mV. The separation in peak potential (ΔEp) is 101 mV and the ratio of redox peak current (ipa/ipc) was 1.84, which is the characteristic of a quasi-reversible electrode process. Under the identical condition poly (aniline blue) modified CPE (dashed line) shows redox peaks was observed with anodic and cathodic peak potential at 230 mV and 140 mV with the peak potential separation (ΔEp) 90 mV. The value of ipa/ ipc was about 1.7, and positive side shift in the peak potential was observed in modified electrode which is the characteristics of the reversible nature of the electrode, with remarkable increase in the current signals.

Effect of scan rate on the peak currents of dopamine

Fig. 4a shows the cyclic voltammograms of the poly (aniline blue) modified carbon paste electrode at various scan rates obtained in PBS (pH 7) containing 1 mM DA. The peak current for the anodic oxidation of DA is proportional to the scan rate in the range of 100 – 300 mV s^{-1} with a correlation coefficient of r^2= 0.99802, indicating that the catalytic reaction of DA at the surface of poly (aniline blue) MCPE is controlled by adsorption and the anodic potential shifted positively with the increase of scan rate indicating the quasi-reversible nature of the electrode reaction (Fig.4b).

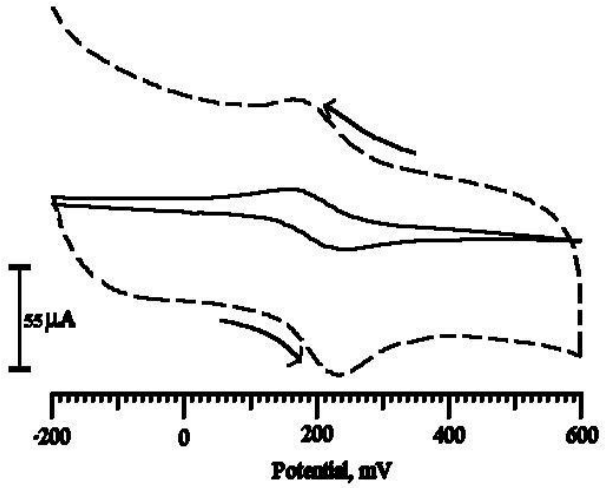


Figure.2

Fig. 2: Cyclic voltammograms for the electrochemical responses of K_4 [Fe (CN) $_6$] at bare (solid line) and poly (anilineblue) modified CPE (dotted line) in 1 M KCl containing 1 mM $K_4[Fe(CN)_6]$ at scan rate 50 mVs^{-1}

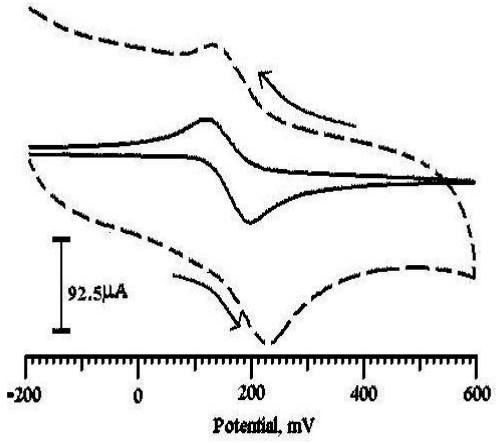


Figure.3

Fig. 3: Cyclic voltammogram of 1 mM DA in 0.2 M phosphate buffer solution of pH 7 at bare CPE (solid line) and poly(aniline blue)film coated CPE (dashed line). At scan rate 100 mVs^{-1}.

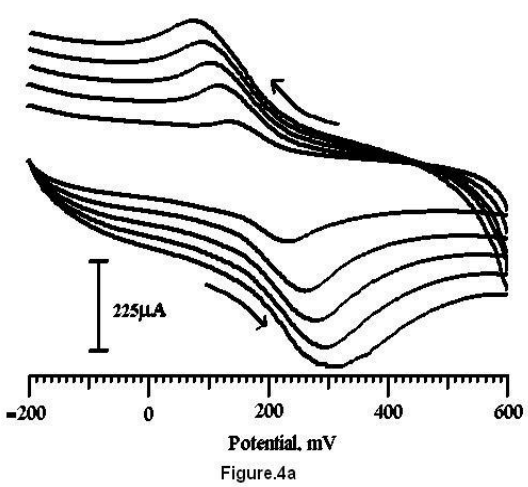


Figure.4a

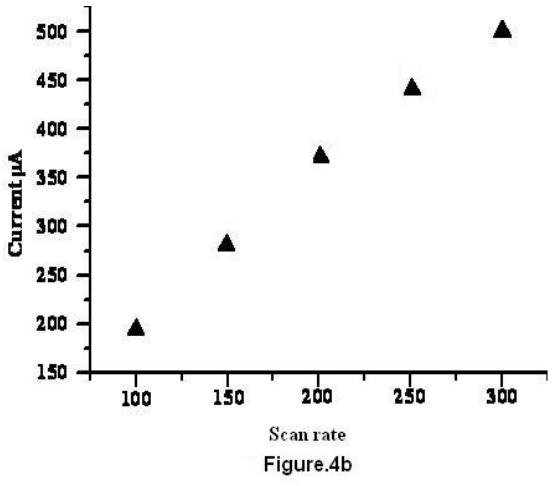


Figure.4b

Fig. 4: (a) Cyclic voltammograms of 1 mM DA on the poly(aniline blue) modified CPE at different scan rates (100, 150, 200, 250, 300 mV/s) in 0.2 M phosphate buffer solution pH 7.0 and (b) is the plot of the redox peak current versus the scan rate.

Effect of concentration of DA

The effect of the DA concentration on the voltammetric response of the poly (aniline blue) modified electrode was investigated. The cyclic voltammograms were obtained in a series of concentration of DA (Fig. 5a). Upon the addition of DA, there was a enhancement in the anodic and cathodic currents. The dependence of peak current on the concentration of DA shows two linearity's,from 1 to 1.5 mM, r^2=0.9985 and from 1.5 to 4 mM the correlation coefficient was r^2 = 0.9947(Fig.5b)

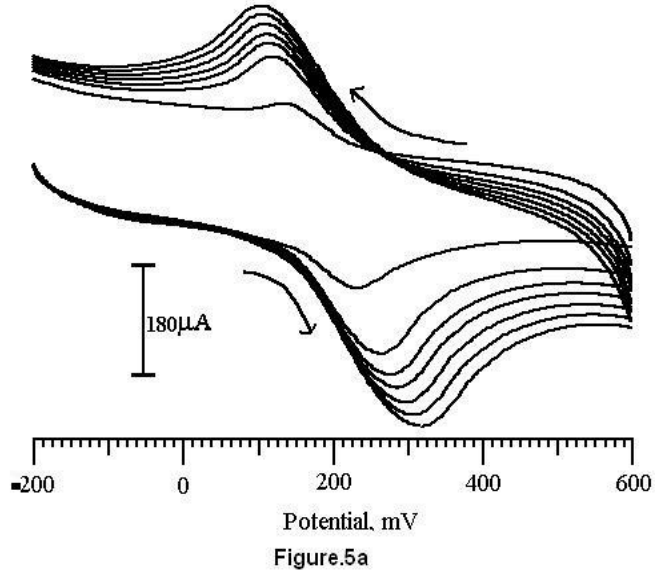


Figure.5a

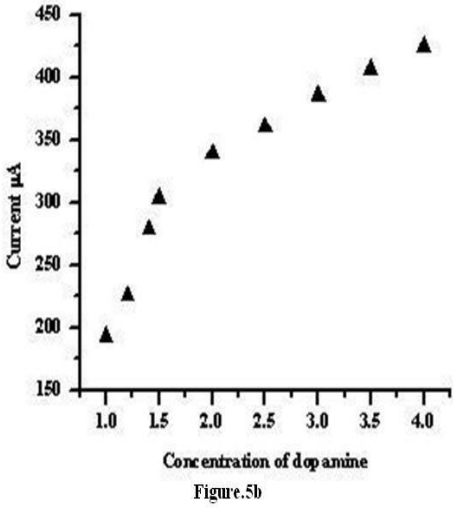


Figure.5b

Fig. 5: (a) Cyclic voltammogram of DA at different concentration (1 mM to 4 mM). (b) Graph of current vs concentration of DA.

Effects of pH of DA solution

The effect of solution pH on the response of DA was investigated over the range of 6 to 10. Fig. 6a shows, anodic peak potential shifted towards negative direction with increasing pH. The anodic peak potential for DA is pH dependent. The voltammograms of DA were recorded in 0.2 M PBS at different pH by cyclic voltammetric technique. The voltammogram demonstrates the pH dependence of DA at poly (aniline blue) at sweep rate of 100 mVs^{-1}. The anodic peak potential of DA shifted from 301 mV to 139 mV with respect to the pH from 6 to 10. The potential diagram was constructed by plotting the graph of calculated E_{pa}vs pH of the solution (Fig. 6b).The slope observed at -38 mV/pH.

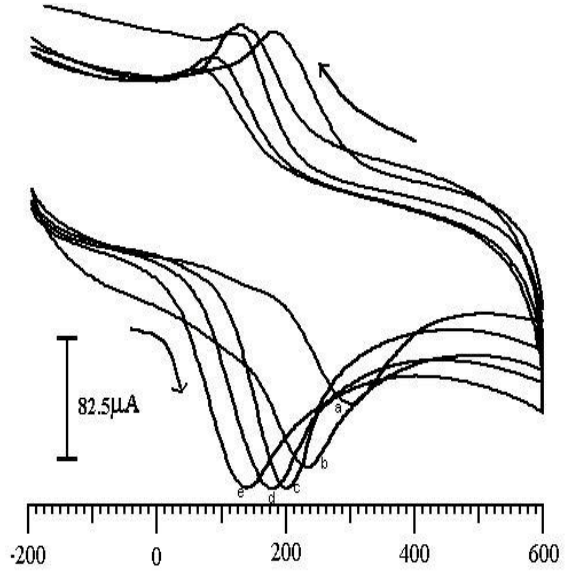


Figure.6a

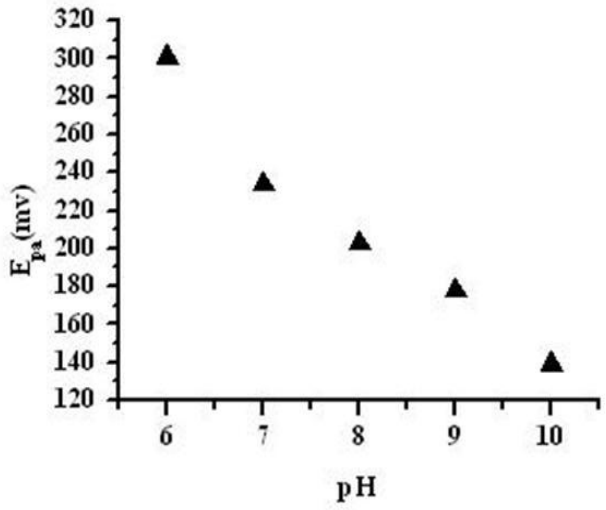


Figure.6b

Fig. 6:(a) Cyclic voltammograms obtained at the poly(aniline blue) modified CPE in 0.2 M phosphate buffer solutions in pH values, (a) 6 (b) 7 (c) 8 (d) 9 (e)10 containing 1 mM DA at scan rate of 100 $mVs^{-1}$and (b) the plot of standard redox peak potential (E_{pa}) of DA on cyclic voltammograms versus pH values

Electrochemical response of ascorbic acid at the poly (anilineblue) modified CPE

Since ascorbic acid is the major interfering substance in the electrochemical measurement of DA, its voltammetric behaviour at poly (aniline blue) modified CPE was studied. Fig.7a shows cyclic voltammogram of 1 mM AA in 0.2 M PBS (pH 7) at bare CPE (solid line) and poly (aniline blue) film modified (dashed line) CPE at 100 mVs^{-1}. Bare carbon paste electrode shows only one broad peak at 197 mV. However at poly (aniline blue) film modified CPE, peak was recorded at -18 mV, which was an evidence for the electrocatalytic oxidation of AA. Fig. 7b shows the cyclic voltammograms of AA at the poly (aniline blue) modified CPE at different scan rates. The oxidation peak potential was observed to shift positively with the increase of the scan rate.

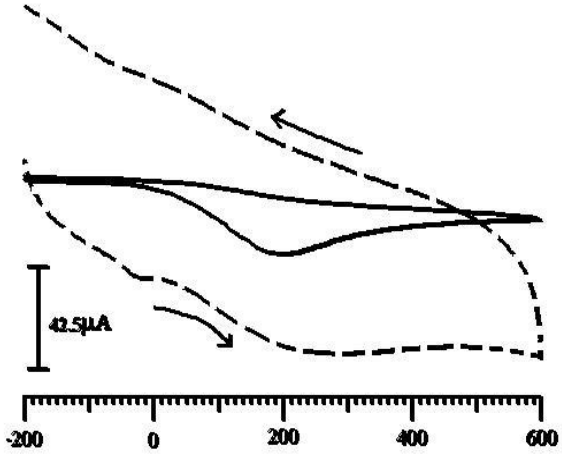


Figure.7a

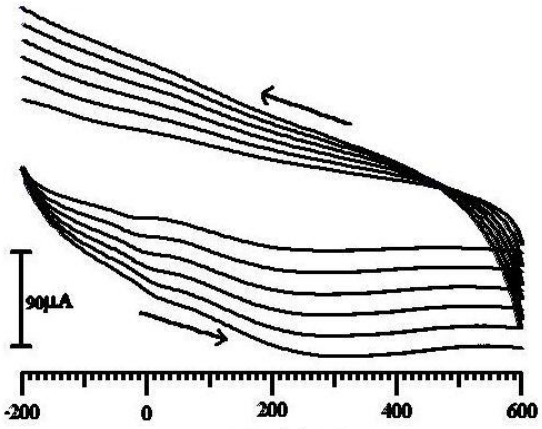


Figure.7b

Fig. 7: (a) Cyclic voltammograms obtained for the oxidation of 1 mM AA at poly(aniline blue) modified CPE (dashed line) and bare CPE (solid line) at scan rate 100 mVs^{-1}. (b) Cyclic voltammograms for the oxidation of 1 mM AA at different scan rate 100- 300 mVs^{-1}in 0.2 M phosphate buffer solution (pH 7.0)

Oxidation of UA at poly (aniline blue) modified CPE

In pH 7 PBS, the poly (aniline blue) modified CPE also possessed oxidation for UA (Fig. 8). Bare CPE and poly (aniline blue) coated electrode shows only one oxidation peak was observed at pH7 in PBS, which confirmed that electrochemical reaction of UA was an irreversible process. At the bare CPE the oxidation peak of UA was observed at around 308 mV. Meanwhile at the poly (aniline blue) modified CPE the anodic peak potential was observed at 351 mV. It was observed that the poly (aniline blue) film at electrode coat intensively catalysed the electrochemical oxidation of UA at pH7 in PBS. The effect of pH on electrochemical reaction of UA at poly (aniline blue) film coated electrode was also examined. With pH increasing from 6 to 10, the Epa shifted toward more negative potential (data not shown).

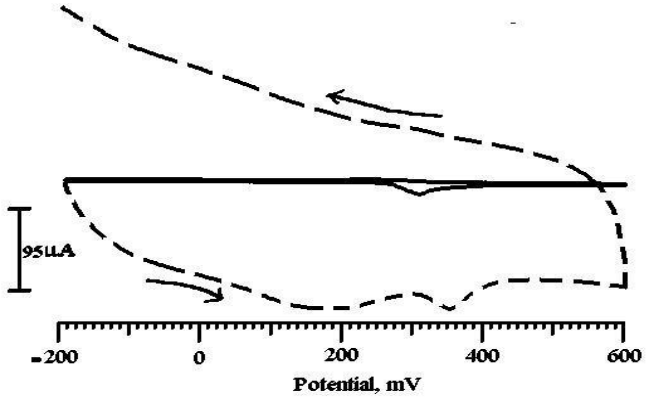


Figure.8

Fig. 8: Cyclic voltammogram of 0.1 mM UA in 0.2 M phosphate buffer solution of pH 7 at bare CPE (solid line) and poly (aniline blue) film coated CPE (dashed line).

Simultaneous determination of DA, AA and UA at poly (aniline blue) modified carbon paste electrode

DA, AA and UA coexist in the extra cellular fluid of the central nervous system and serum. Since they have similar oxidation potentials at most solid electrodes, separate determination of these species is a great problem due to overlapped signals. In order to evaluate the sensitivity and selectivity of the present system for the quantification of DA, AA and UA, the electrochemical behaviour of their mixture at the poly (aniline blue) modified carbon paste electrode was studied. Based on the electro catalytic action of poly (aniline blue) film to DA, AA and UA, it was supposed that the poly (aniline blue) modified electrode could conspicuously improve the voltammetric resolution of DA, AA and UA. the cyclic voltammogram of mixture solution containing 0.5 mM DA, 0.5 mM AA and 0.5 mM UA in PBS of pH 7 were recorded with the scan rate of 100 mV/s at a bare CPE and poly(aniline blue) modified CPE as shown in the Fig. 9.

The voltammogram of the bare carbon paste electrode overlapped peak appeared at 189 mV, the three well separated oxidation peak

of AA, DA and UA respectively was found at 14 mV, 242 mV and 384 mV. The difference of the oxidation peak potential for DA-AA, UA-AA and UA- DA were 228 and 370 and 142 mV respectively. Meanwhile it could be noticed that the peak current of DA, AA and UA were enhanced strongly at poly (aniline blue) modified CPE and peak potential of DA and UA were approximately identical to that at the pretreated electrode. It was further identified that poly (aniline blue) modified CPE possessed higher active surface area and excellent electrocatalytic activity for the oxidation of DA, AA and UA.

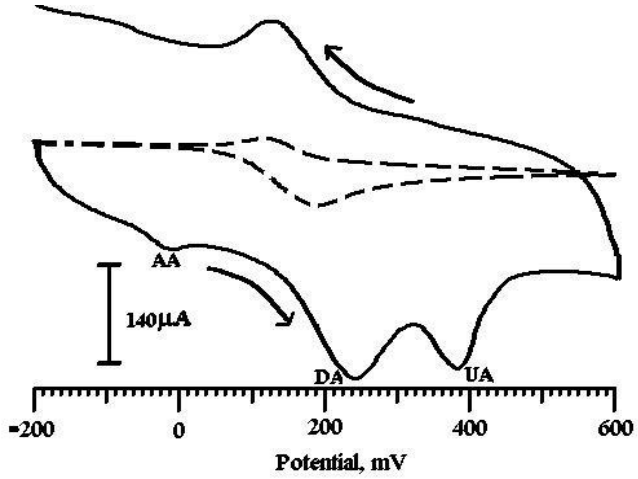


Fig. 9: Simultaneous determination of 0.5 mM DA, 1 mM AA and 0.2 mM UA at bare CPE (Dashed line) and at poly (aniline blue) film coated CPE (solid line).

CONCLUSION

The electropolymerisation of aniline blue on the carbon paste electrode produces a stable polymeric film. The redox potential of DA shifts in the negative direction with increasing solution pH. The poly (aniline blue) modified carbon paste electrode exhibited remarkable electrocatalytic activity towards the oxidation of DA and clearly resolved the mixed voltammetric signal of DA, AA and UA into three well defined voltammetric peaks. With its good selectivity and sensitivity, it is expected that the poly (aniline blue) modified carbon paste electrode could hold great application in the fields of electroanalytical chemistry and biosensors.

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