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## **Simultaneous Investigation of Dopamine and Ascorbic Acid at Poly (Tryptophan) Modified Carbon Paste Electrode: A Cyclic Voltammetric Study**

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**Abstract-** A stable modified carbon paste electrode based on the poly (tryptophan) film was prepared by electrochemical polymerization technique using cyclic voltammetry. The properties of the electrodeposited films, during preparation under different conditions and their stability were evaluated. It was found that the current response of DA and AA were greatly enhanced at this poly (tryptophan) modified carbon paste electrode. Moreover, the anodic over-potential of AA was significantly reduced for about 150 mV (*vs.* SCE) compared with that obtained at bare carbon paste electrode. The modified carbon paste electrode was applied to the study of electrocatalytic oxidation of DA and AA which resolved the overlapping of the anodic peaks of DA and AA in 0.1 mol L<sup>-1</sup> acetate buffer solution at pH 6.5. The separation of the oxidation peak potentials for ascorbic acid–dopamine was about 0.277 V. Interference studies showed that the modified electrode exhibits excellent selectivity towards DA and AA.

**Keywords-** Electropolymerisation, DA, AA, Poly (Tryptophan)/CPE, Cyclic Voltammetry

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## 1. INTRODUCTION

Dopamine is one of the most significant catecholamine, functioning as a neurotransmitter in the central nervous system and a medicament to drug addiction and Parkinson's disease [1, 2]. So it is important to establish a fast, sensitive and selective method for the detection of dopamine. Generally the electrochemical methods are very simple and advantageous for detection of dopamine. Ascorbic acid has been used in the prevention and treatment of common cold, mental illness, cancer and AIDS [3]. Usually ascorbic acid co-exists with dopamine in the biological fluids at a significantly high concentration range and it has the same oxidation potential as those of dopamine. However, the voltammetric response of dopamine is not resolved enough at ordinary electrodes, due to the interference of ascorbic acid. Therefore, the separation of the electrochemical response of AA and DA with reasonable sensitivities has been one of the major goal of both electroanalytical and bioelectrochemical research. In in-vivo and in vitro measurements, appropriately modified electrodes, where properly designed and biocompatible substrate transducers are introduced, can certainly create some novel bioanalytical tools [4]. Various approaches, such as the modification of electrode by polymer film [5] covalent modification [6] self-assembled monolayers [7] as well as the use of carbon paste electrode [8] and the electrochemical pretreatment of the electrode [9] have been developed to distinguish them selectively from each other. During recent years, great efforts have been devoted to the development of electrochemical sensors with electrosynthesized polymeric film electrodes because of its enhanced selectivity and sensitivity in addition to the homogeneity and the strong adherence to the electrode surface along with the excellent chemical stability of the film. It has been reported that electrodes modified with various films, such as Nafion [10], clay [11], conducting polymers [12–15] and others [16–18] at a physiological pH of 7.4 could absorb and even preconcentrate the cationic DA while effectively rejecting the negatively charged AA and other anionic interfering agents. The successful route to overcome the problems of selectivity is to modify the electrode surface because the modified electrode could decrease the over potential, improve the mass transfer velocity and effectively enrich the substance [19]. Various modified materials have been modified on various base electrodes to investigate and detect dopamine [20–24].

Tryptophan (Trp), systematic name (S)-2-amino-3-(1H-indol-3-yl)-propionic acid, is an amino acid essential in human nutrition and is an important metabolite. It is not only an essential building block in proteins but also a precursor for synthesis of a variety of biologically significant substances including vitamins, neurohormones, phytohormones, phytoalexin, and alkaloids. It is found that tryptophan to be a safe and reasonably effective sleep aid, probably because of its ability to increase brain levels of serotonin. It has been extensively studied of its electrochemical properties on the modified electrodes by using the cyclic voltammetric methods [25, 26].

Recently there are reports on electropolymerisation on carbon paste electrode [27-30]. The present work describes the fabrication of poly (tryptophan) modified carbon paste electrode for the determination of DA and AA using cyclic voltammetric technique. The poly (tryptophan) modified carbon paste electrode distinctly separated the oxidation peaks of AA and DA and applied this electrode for the real sample analysis. Hence this electrode can also be applied in the biomedical and analytical field.

## **2. EXPERIMENTAL**

### **2.1. Chemical Reagents**

Tryptophan, dopamine hydrochloride and ascorbic acid were obtained from Himedia chemical company. All other chemicals were of analytical grade. Freshly prepared solutions of DA and AA were used and the acetate buffer solution (ABS) was prepared by mixing standard stock solutions of 0.1 M CH<sub>3</sub>COOH and 0.1 M CH<sub>3</sub>COONa and adjusting their pH with 0.1 M CH<sub>3</sub>COOH or CH<sub>3</sub>COONa. All aqueous solutions were prepared using double distilled water.

### **2.2. Preparation of bare carbon paste electrode**

The bare carbon paste electrode (CPE) was prepared by hand mixing of graphite powder and silicon oil at a ratio 70:30 (w/w%) in an agate mortar until a homogenous paste was obtained. The paste was then tightly packed into a PVC tube (2 mm internal diameter) and the electrical contact was provided by a copper wire connected to the end of the tube.

### **2.3. Apparatus Required**

EA-201 Electro Analyzer (Chemilink systems) controlled by a personal computer was used for the cyclic voltammetric measurements. A conventional three electrode cell was used with a saturated calomel electrode (SCE) as a reference, a platinum wire counter electrode and bare or poly (tryptophan) modified electrode as working electrode. A digital pH meter MK VI (systronics) was used for the pH values.

## **3. RESULTS AND DISCUSSION**

### **3.1. Electropolymerisation of tryptophan at the CPE surface and its SEM analysis**

A poly (tryptophan) film modified carbon paste electrode was fabricated by electropolymerisation of tryptophan on bare carbon paste electrode. The experiment was performed in an acetate buffer solution (pH 6.5) containing 1 m mol L<sup>-1</sup> tryptophan with 20 segment cyclic voltammetric sweeps in the potential range from -500 mV to 1500 mV. As shown in Fig. 1a, the first anodic peak at 0.185 V, the second anodic peak at 0.781 V and

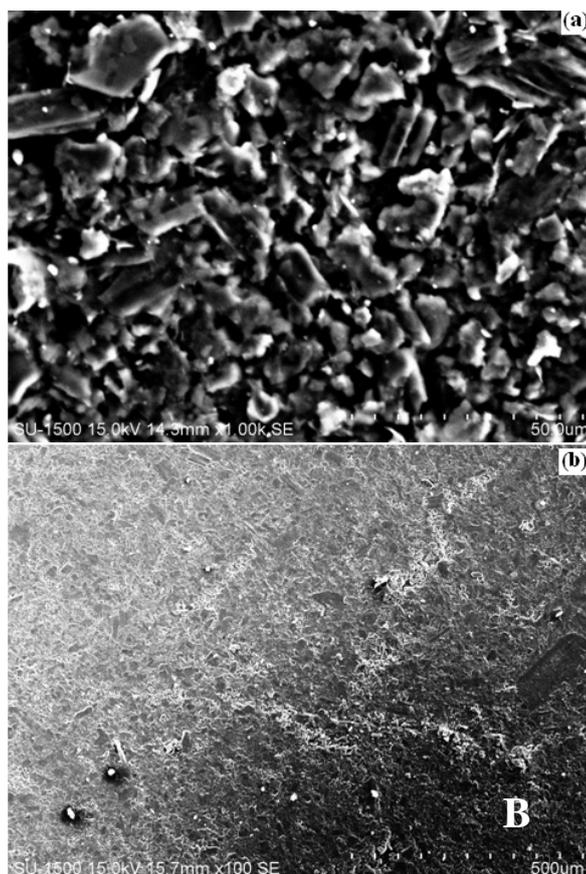
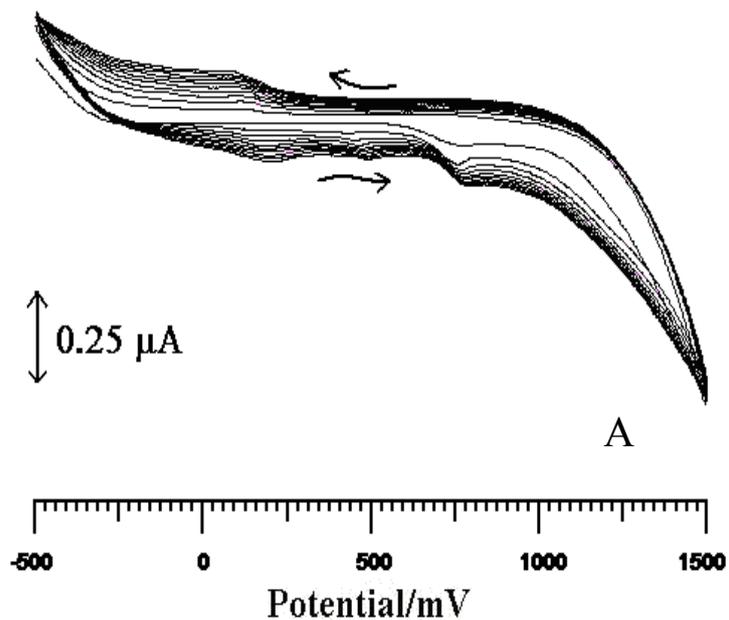
cathodic peak at 0.061 V were observed in the first scan. The oxidation and reduction peak current increases with increase in segments of voltammetric scan which indicates that the formation of an electroconductive polymer film on the electrode surface. After 20 segments of potential sweep the electropolymerisation procedure was terminated and the polymer film electrode was washed with distilled water to remove the physically adsorbed material. Then the film electrode was transferred to an electrochemical cell containing acetate buffer and cyclic voltammetric sweeps were carried out to obtain electrochemical steady-state. Thickness of the poly (tryptophan) film could be adjusted by the segments of voltammetric scan.

The surface morphology of bare carbon paste electrode and poly (tryptophan)/CPE has been explained by using scanning electron microscopy and shown in the Fig. 1b. The surface of bare carbon paste electrode was irregularly arranged in flakes shaped structure of graphite. However, the poly (tryptophan) film coated carbon paste electrode has typical uniform arrangement of tryptophan over the flake shaped bare carbon paste electrode. This confirms that the carbon paste electrode was coated by poly (tryptophan) film. These results indicated that the poly (tryptophan) film coated carbon paste electrode produced a particularly large surface area structure, which could be expected to be an attractive platform for the construction of highly catalyzed electrochemical biosensors.

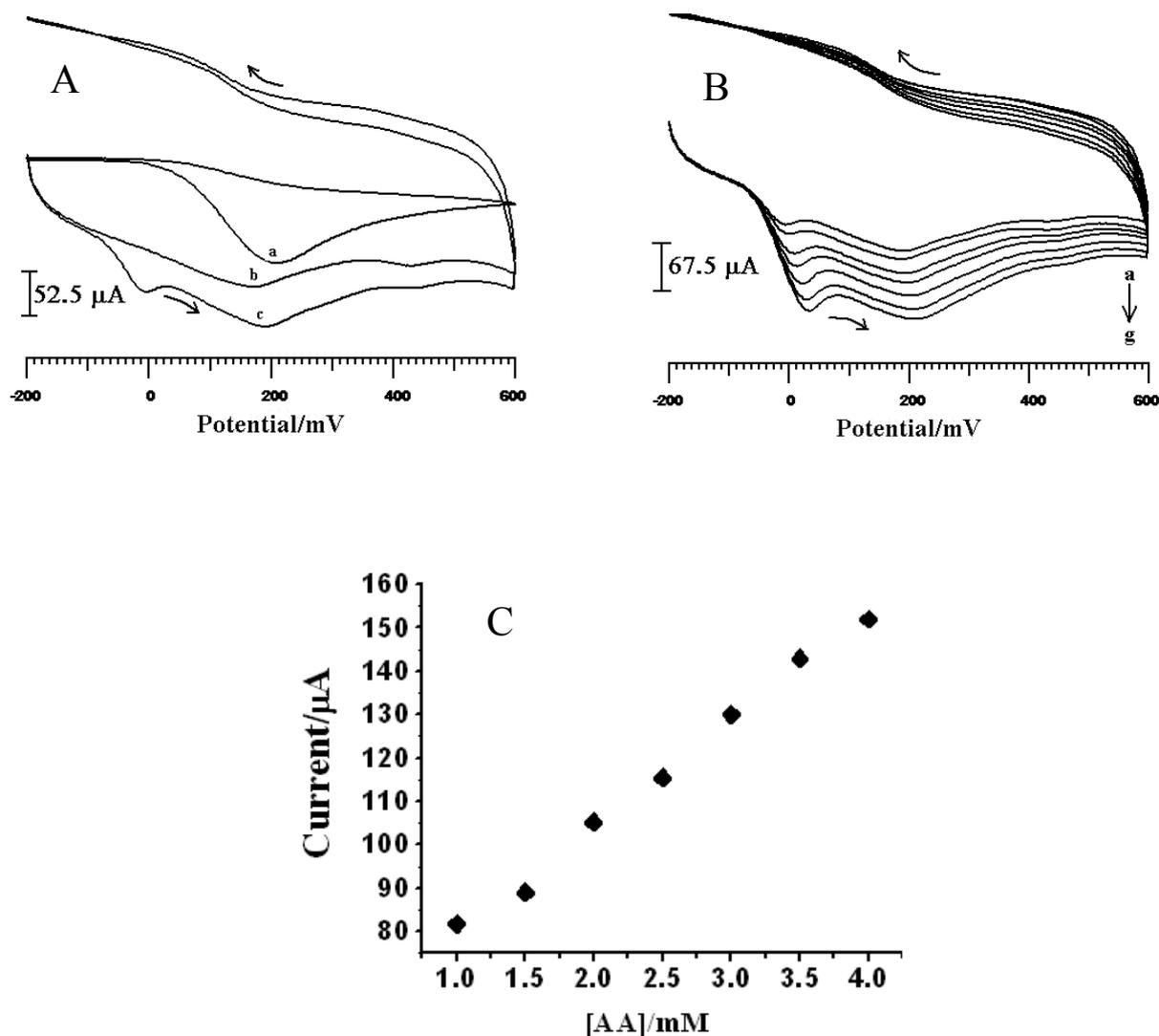
### 3.2. Electrochemical oxidation response of ascorbic acid at poly (tryptophan)/CPE

Fig. 2a shows the cyclic voltammograms of 1 mM AA in the acetate buffer solution of pH 6.5 at bare carbon paste electrode and a poly(tryptophan) film modified carbon paste electrode at 100 mVs<sup>-1</sup>. At the bare carbon paste electrode (a) an oxidation peak was observed at potential of about 155 mV and however at the poly (tryptophan)/CPE (c) strongly enhanced oxidation peaks were recorded at a potential of 5 mV which was an evidence for the good electrocatalytic oxidation of AA. The cyclic voltammogram (b) of the blank solution at poly (tryptophan)/CPE is shown in figure and the oxidation peak potential at 200 mV, was due to the effect of electropolymerisation of tryptophan over the bare carbon paste electrode .

The CV's showed successive enhancement of peak current on increasing ascorbic acid concentration is shown in the Fig. 2b. As the concentration increases the anodic peak potential ( $i_{pa}$ ) shifted slightly towards positive potential. The plot of anodic peak current vs. the concentration of ascorbic acid was found to be linear in the range of 1.0– 4.0 mM as shown in Fig. 2c, the correlation co-efficient was 0.99746.



**Fig. 1.** A) Cyclic voltammogram for electrochemical polymerisation of tryptophan on bare carbon paste electrode in  $0.1 \text{ mol L}^{-1}$  ABS pH-6.5 by 20 segments. Sweep rate  $100 \text{ mVs}^{-1}$  B) SEM image of bare carbon paste electrode (a) and poly(tryptophan)/CPE (b)

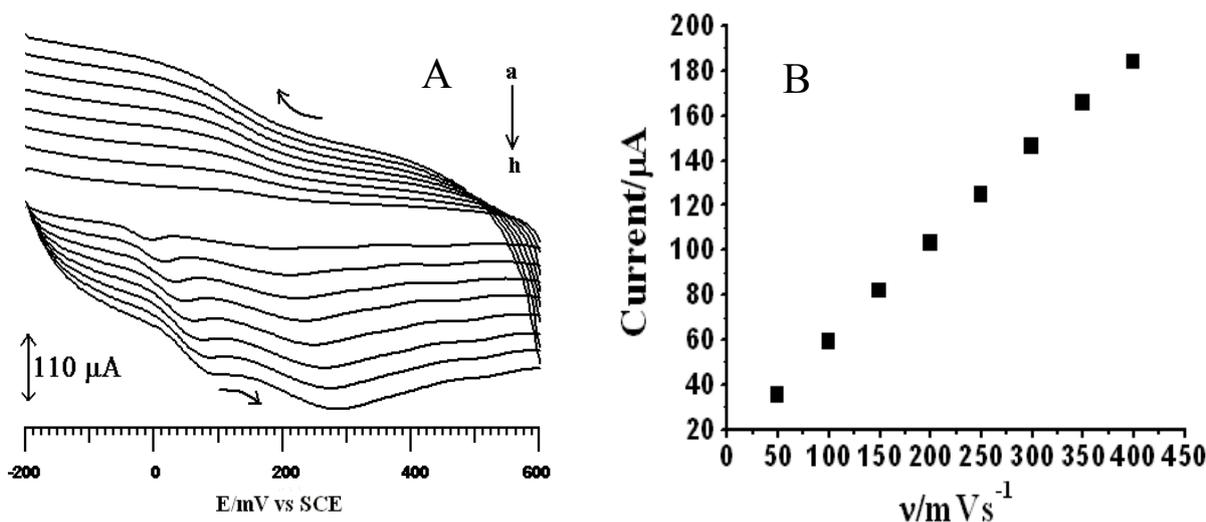


**Fig. 2.** A) Cyclic voltammograms at a bare CPE (a) and poly(tryptophan)/CPE (b,c) in presence of  $1 \times 10^{-3}$  mol L<sup>-1</sup> AA (a,c) and in the absence of AA (b) in 0.2 mol L<sup>-1</sup> ABS (pH 5.0): Scan rate 100 mVs<sup>-1</sup> B) Cyclic voltammograms of AA at different concentration(a-g; 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 m mol L<sup>-1</sup>) in 0.1 M ABS (pH 6.5): Scan rate 100 mVs<sup>-1</sup> C) Effect of concentration on the oxidation peak current of  $1 \times 10^{-3}$  mol L<sup>-1</sup> AA at poly(tryptophan)/CPE

### 3.3. Effect of scan rate variation on ascorbic acid

Fig. 3a shows the cyclic voltammogram of oxidation of ascorbic acid at the poly(tryptophan)/CPE at different scan rates. The oxidation peak potential was shifted positively with the increase in scan rate and in addition, exhibited a linear relation to the square root of

scan rate ( $v$ ) with correlation coefficient 0.9993. The results indicated that the oxidation of ascorbic acid at the poly (tryptophan)/CPE was an adsorption-controlled process (Fig. 3b).



**Fig. 3.** A) Cyclic voltammograms of  $1 \times 10^{-3}$  M AA at different scan rate (a-h; 50, 100, 150, 200, 250, 300, 350, 400  $\text{mVs}^{-1}$ ) in 0.1 M ABS (pH 6.5) B) Effect of scan rate on the oxidation peak current of  $1 \times 10^{-3}$  mol  $\text{L}^{-1}$  AA at the poly(tryptophan) electrode

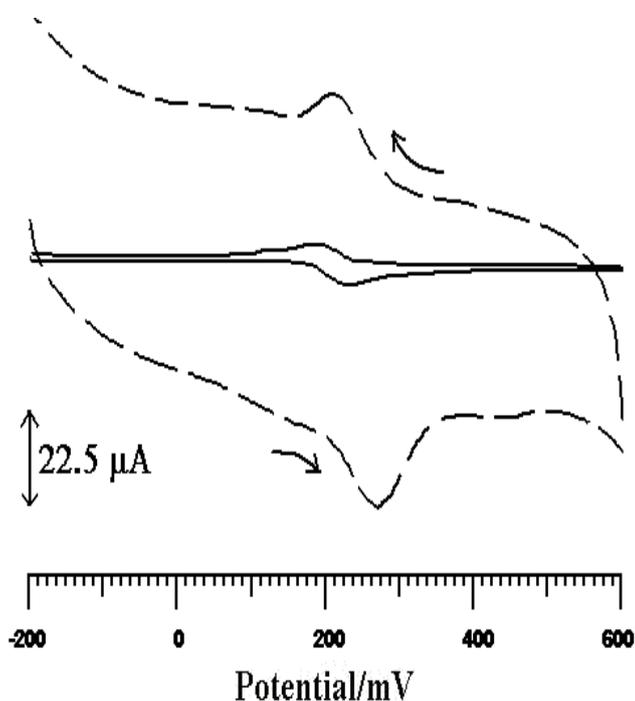
### 3.4. Electrochemical behavior of dopamine at poly (tryptophan)/CPE

The electrochemical behaviour of DA on the poly (tryptophan)/CPE was investigated and the cyclic voltammogram is shown in Fig. 4. At the bare CPE, the cyclic voltammogram of DA showed a pair of redox peaks at pH 6.5 (solid line). The anodic peak current ( $i_{pa}$ ) was 3.8  $\mu\text{A}$  and cathodic peak current ( $i_{pc}$ ) was 2.7  $\mu\text{A}$ . The ratio of redox peak currents ( $i_{pa}/i_{pc}$ ) was 1.4074 which was the characteristics of a quasi reversible electrode process. However, for the poly (tryptophan)/CPE a pair of well defined redox waves of DA (dashed line) were observed with a greatly increase of the redox peak currents. The anodic peak current was 39  $\mu\text{A}$  and the corresponding cathodic peak current was 26.5  $\mu\text{A}$ , the value of  $i_{pa}/i_{pc}$  was about 1.4716. The results of the enhancement of peak currents showed that an excellent catalytic properties of the poly (tryptophan) modified CPE for the electrochemical oxidation of DA.

### 3.5. Effect of scan rate variation on dopamine

The effect of the scan rate on the peak current and peak potential at the modified carbon paste electrode in 0.1 mol  $\text{L}^{-1}$  acetate buffer were investigated in the range of 50-400  $\text{mVs}^{-1}$  by cyclic voltammetry in the presence of 0.1 m mol  $\text{L}^{-1}$  dopamine at pH 6.5 is shown in the Fig. 5a. The Fig. 5b has shown the anodic peak current increased linearly with the scan rate,

which indicates an adsorption-controlled oxidation process occurred at the poly (tryptophan)/CPE.



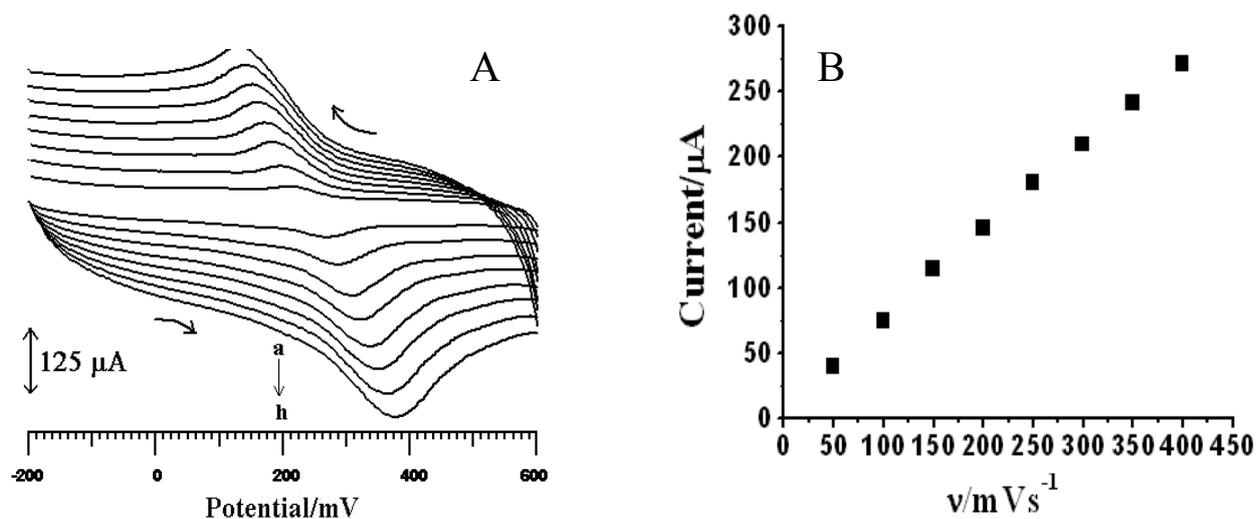
**Fig. 4.** Cyclic voltammogram of CPE (solid line) and poly(tryptophan)/CPE (dashed line) in 0.1 M ABS pH-6.5 containing  $1.0 \times 10^{-3}$  M DA

### 3.6. Effect of concentration of dopamine

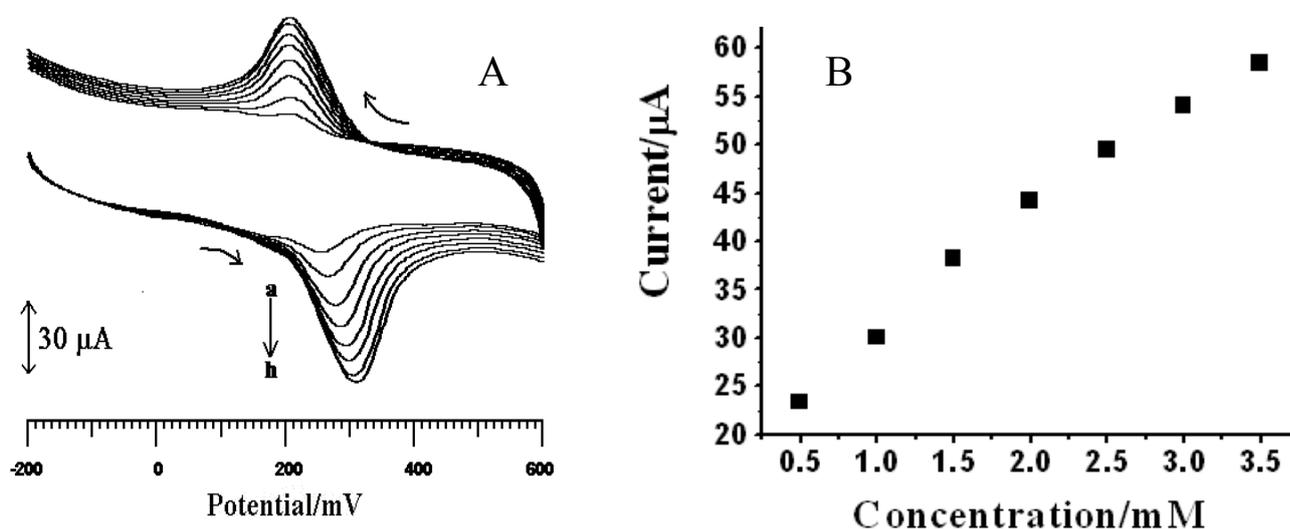
Fig. 6a reveals that the cyclic voltammograms of successive enhancement of peak current on increasing dopamine concentration. The plot of peak current vs. the respective concentration of dopamine was found to be linear from the range of 0.5 to 3.0 mM as shown in Fig. 6b with a correlation coefficient of 0.9939.

### 3.7. Effect of pH

The effects of pH on electrochemical response of the poly (tryptophan)/CPE towards the determination of dopamine solutions were investigated. It can be seen that the anodic peak currents of DA increased slightly with an increased in the solution pH until it reaches 6.5 and again it decreases as the pH increases which was shown in Fig. 7. Therefore the optimum solution pH-6.5 was selected for all electrochemical investigation.



**Fig. 5.** A) Cyclic voltammogram of  $1 \times 10^{-4}$  mol L<sup>-1</sup> DA at different scan rate (50, 100, 150, 200, 250, 300, 400 mVs<sup>-1</sup>) in 0.1 M ABS pH 6.5 B) Effect of scan rate on the oxidation peak current of  $1 \times 10^{-4}$  M DA at the poly(tryptophan)/CPE



**Fig. 6.** A) Cyclic voltammogram for different concentration of DA (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 m mol L<sup>-1</sup>) at 100 mVs<sup>-1</sup> in 0.1 M ABS pH 6.5 B) Effect of concentration of DA on its oxidation peak current at poly(tryptophan)/CPE

### 3.8. Simultaneous determination of dopamine and ascorbic acid at poly (tryptophan) / CPE

Based on electrocatalytic action of the poly (tryptophan) film to AA and DA, it was supposed that the poly (tryptophan)/CPE could conspicuously improve the voltammetric resolution of DA and AA. To ascertain the presumption the cyclic voltammogram of a mixture solution [0.1 M ABS (pH 6.5) containing 0.1 m mol L<sup>-1</sup> DA and 1 m mol L<sup>-1</sup> AA] were reported with the scan rate of 50 mVs<sup>-1</sup> at bare CPE and a poly (tryptophan)/CPE. As shown in Fig. 8 the cyclic voltammogram at the bare carbon paste electrode (solid line) appear the seriously overlapped peaks while two well separated oxidation peaks of DA and AA can be found at poly (tryptophan)/CPE (dashed line). The difference in the oxidation peak potentials for DA and AA were 277 mV which was enough large separations to allow the simultaneous determination of DA and AA in a mixture. Meanwhile it could be noticed that the peak currents of DA and AA were enhanced strongly at the poly (tryptophan)/CPE. It was further identified that the modified electrode possessed the higher active surface area and leads to an excellent electrocatalytic properties for both the oxidation of DA and AA.

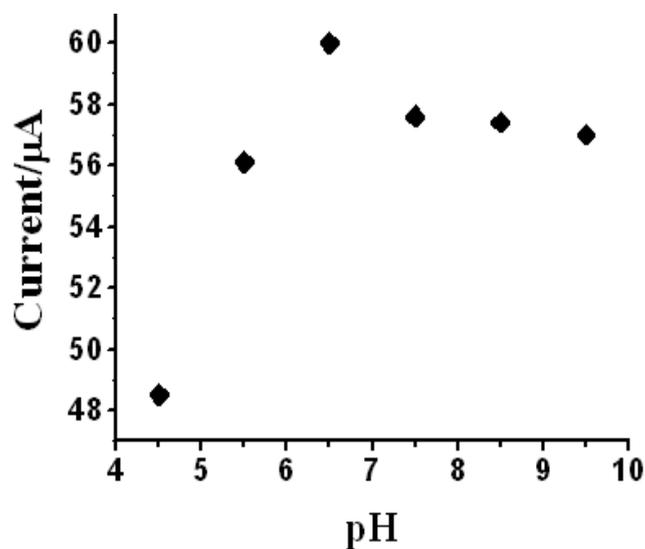
### 3.9. The stability and reproducibility of poly (tryptophan)/CPE

The reproducibility of the modified electrode surface was examined using cyclic voltammetric data from five separately prepared poly (tryptophan)/CPE, obtained in optimum solution of pH 6.5. The calculated RSD for various parameters (4-5%) indicated that surface reproducibility was satisfactory. The fabricated poly (tryptophan)/CPE was stored at room temperature, was tested over a two-week period. After recording the cyclic voltammograms of the stored poly (tryptophan)/CPE in presence of dopamine solution, the peak potential for DA oxidation was unchanged and the current signals showed less than 5% decrease relative to the initial response. The antifouling properties of the modified electrode toward DA oxidation and its oxidation products were also investigated by recording the cyclic voltammograms of the modified electrode before and after use in the presence of DA. Cyclic voltammograms were recorded in the presence of DA after having cycled the potential 10 times at a scan rate of 50 mVs<sup>-1</sup>. The peak potentials were unchanged and the currents decreased by less than 3%.

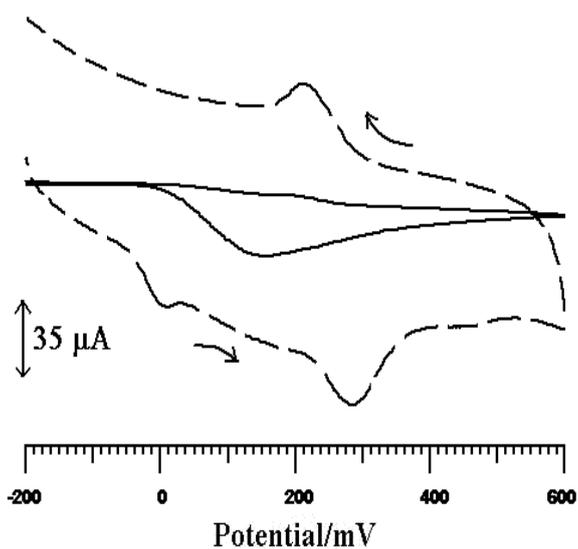
### 3.10. Determination of DA in dopamine injection

The dopamine hydrochloride injection solution (40.00 mg mL<sup>-1</sup>) was diluted with 0.1 mol L<sup>-1</sup> acetate buffer solution. An aliquot of 10 ml of this test solution was placed in the electrochemical cell and the potential was controlled between -0.2 and 0.6 V for cyclic scan rate 50 mVs<sup>-1</sup>. I<sub>pa</sub> was measured at the oxidation potential of DA. The results are shown in Table 1. The average determination results of DA in the injection were 40.2 mg mL<sup>-1</sup>, which were satisfactory corresponding to the value that was given by injection specification. This

procedure was repeated four times and the relative standard deviation obtained for six measurements were 2.45%-3.23%. Diluted DA injection was analyzed with respect to the anodic peak current of the standard DA solution ( $5 \times 10^{-6}$  mol L<sup>-1</sup>) and the recovery was between 98.2% and 103.1% for six measurements.



**Fig. 7.** Effect of pH on the oxidation peak current of  $1 \times 10^{-4}$  mol L<sup>-1</sup> DA at poly (tryptophan)/CPE



**Fig. 8.** Cyclic voltammograms for simultaneous determination of  $1 \times 10^{-4}$  mol L<sup>-1</sup> DA and  $1 \times 10^{-3}$  mol L<sup>-1</sup> AA at bare carbon paste electrode (Solid line) and poly(tryptophan)/CPE (dashed line) in 0.1 M acetate buffer. Sweep rate: 50 mVs<sup>-1</sup>

**Table 1.** Determination of dopamine injection sample

Sl/No.	DA Added ( $\mu\text{M}$ )	DA Found ( $\mu\text{M}$ )	Recovery (%)	RSD (%)
1	05	4.95	99.30	2.45
2	05	5.15	103.1	3.23
3	05	4.94	98.8	2.94
4	05	5.10	102	2.98
5	05	5.13	102.6	3.09
6	05	4.91	98.2	3.12

#### 4. CONCLUSION

The poly (tryptophan)/CPE exhibited highly electrocatalytic activity to the oxidation of DA and AA in  $0.1 \text{ mol L}^{-1}$  acetate buffer solution at pH 6.5. The modified electrode displays higher selectivity in voltammetric measurements of DA and AA in their mixture solution. The separations of the oxidation peak potentials for AA and DA was 277 mV by cyclic voltammetric method. With the good sensitivity and the wide linear range, the proposed method provides a possibility for simultaneous detection of AA & DA in their biological samples with the good reproducibility and recovery of the real samples.

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