Electrochemical Behavior of L-Tyrosine at Poly (Dicyclomine Hydrochloride) Film Modified Carbon Paste Electrode: A Cyclic Voltammetric Study

Deepak M.P[1], Mamatha G.P[2]

[1]Department of Chemistry, Kuvempu University, Jnana Sahyadri, Shankaraghatta, Shimoga, Karnataka, India.-577 451
[2]Department of Pharmaceutical Chemistry, Kuvempu University, Post Graduate Centre, Kadur, Chickmagalore, Dt. Karnataka, India.-577 548

Abstract: **An electrochemical method for the determination of L-Tyrosine (LTY) using a dicyclomine hydrochloride (DICY) polymer film modified carbon paste electrode. The surface morphology of poly (DICY) modified carbon paste electrode was characterized by SEM. The modified electrode showed excellent electro catalytic activity towards the oxidation of LTY in 0.1 M phosphate buffer solution of pH 6.5. The effect of pH, concentration and scan rate were studied at the bare carbon paste electrode and poly (DICY) modified carbon paste electrode were investigated. Increase of LTY concentration shows linear increase in oxidation peak current. The linear relationship was obtained between the anodic peak current (I_{pa}) and concentration LTY in range 2×10^{-5} M to 1×10^{-3} M with correlation coefficient of 0.9984. The low detection limit (LOD) and low quantification limit (LOQ) of LTY were detected. The cyclic voltammetric studies indicated that the oxidation of LTY at the modified electrode surface was irreversible; adsorption controlled and undergoes a one electron transfer process at the poly (DICY) film modified carbon paste electrode. The modified electrode showed high sensitivity, detection limit, high reproducibility, easy preparation and regeneration of the electrode surface.**

Keywords: **Carbon Paste Electrode, Cyclic Voltammetry (CV), Dicyclomine Hydrochloride (DICY), Irreversible, L-Tyrosine (LTY), Scanning electron microscope (SEM).**

1. INTRODUCTION

L-Tyrosine (LTY) or 4-hydroxyphenylalanine is one of the 22 amino acids that are used by cells to synthesize proteins. The word "tyrosine" is from the Greek *tyri*, meaning *cheese*, as it was first discovered in 1846 by German chemist Justus von Liebig in the protein casein from cheese [1, 2]. It is called tyrosyl when referred to as a functional group or side chain. Aside from being a proteogenic amino acid, tyrosine has a special role by virtue of the phenol functionality. It occurs in proteins that are part of signal transduction processes. It functions as a receiver of phosphate groups that are transferred by way of protein kinases (so-called receptor tyrosine kinases). Phosphorylation of the hydroxyl group changes the activity of the target protein. A tyrosine residue also plays an important role in photosynthesis. In chloroplasts (photosystem II), it acts as an electron donor in the reduction of oxidized chlorophyll. In this process, it undergoes deprotonation of its phenolic OH-group. This radical is subsequently reduced in the photosystem II by the four core manganese clusters.

There are number of electrochemical methods reported for the determination of tyrosine using various modified electrodes which include, L-serine polymer film [3], multiwall carbon nanotube [4], poly (9-aminoacridine) [5] and gold nanoparticles [6] modified with glassy carbon electrode, FTIR spectroscopic studies at poly crystalline platinum surface [7] and polyamide modified carbon paste electrode along with tryptophan [8] and iron(III) doped zeolite modified with carbon paste electrode along with dopamine [9] amperometry and cyclic voltammetry at carbon fiber microelectrodes applied to single cell analysis along with tryptophan [10]. Numerous methods have been reported for tyrosine determination mainly spectrophotometric, fluorimetric, flow injection, chemiluminescence, liquid chromatography-tandem mass spectrometry, gas chromatography-mass spectrometry and high-performance liquid chromatography [11-17].

Limited work has been done in the area of carbon paste electrode for the determination of L-Tyrosine. Therefore in the present work we used dicyclomine Hydrochloride polymer modified CPE to study the electrochemical behavior of L-Tyrosine and polymerization of dicyclomine hydrochloride has been established for the determination of L-Tyrosine for the first time. This electrode showed excellent electrocatalytic activity towards the oxidation of L-Tyrosine, the determination and sensitivity is significantly improved compared to bare CPE.

2. EXPERIMENTAL SECTION

2.1 Reagents:

Dicyclomine Hydrochloride (DICY) and L-Tyrosine (LTY) were purchased from Sigma Aldrich and all other chemicals were of analytical grade. The electropolymerisation of dicyclomine hydrochloride was performed in 0.1 M phosphate buffer solution. The phosphate buffer solution was prepared from KH_2PO_4 and K_2HPO_4, the pH was adjusted with H_3PO_4 and 0.1 N NaOH solutions. The stock solution of L-Tyrosine (10 mM) was prepared by dissolving in 0.1 N NaOH. Other chemicals used were of analytical grade except for spectroscopically pure graphite powder. All solutions were prepared with doubly distilled water. Freshly prepared LTY solution is used prior to measurements.

2.2 Apparatus:

Electrochemical measurements were carried out with Electroanalyser model EA-201 chemlink system in a conventional three-electrode system. The working electrode was carbon paste electrode, having cavity of 3 mm diameter. The counter electrode was platinum electrode with a saturated calomel electrode (SCE) as a standard reference electrode for completing the circuit.

2.3 Modification Procedure:

2.3a. Preparation of bare carbon paste electrode:

The Bare Carbon paste electrode was prepared by hand mixing of 70% graphite powder and 30% silicon oil to produce a homogenous carbon paste which was then packed into the cavity of a homemade carbon paste electrode and smoothed on a weighing paper.

2.3b. Preparation of the Dicyclomine hydrochloride polymer film modified carbon paste electrode:

The polymer film modified electrode was prepared by electrochemical polymerization of DICY in 0.1 M phosphate buffer solution of pH 6.5 containing 1 mM DICY with cyclic voltammetric in the potential range 100 to 1400 mV at the scan rate of 100 mVs^{-1}. After 10 cycles, the surface of the electrode was washed with double distilled water to remove the physically adsorbed material, air dried and used for the electrochemical studies.

3. RESULT AND DISCUSSION

3.1 Electropolymerisation of Dicyclomine Hydrochloride (DICY) on Carbon Paste Electrode:

Electropolymerzation of dicyclomine hydrochloride (DICY) was performed on bare carbon paste electrode (BCPE). The cyclic voltammograms for the electropolymerisation of 1mM of DICY in 0.1 M phosphate buffer solution on CPE is shown in **Fig.1**, which displays the continuous cyclic voltammetric of 1mM DICY monomer by scanning in the potential range of 100 to 1400 mV for 10 cycles. During the electropolymerisation process, indiscernible peaks started to appear

after 5th cycle. An anodic peak at 861 mV potential was observed due to the formation of poly (DICY). The peak descended gradually with the increase in cyclic time; such decrease indicates the poly (DICY) membrane forming and depositing on the surface of the CPE by electropolymerisation. After polymerization the poly (DICY) modified carbon paste electrode was carefully rinsed with distilled water to remove the physically adsorbed material. Then the film electrode was transferred to an electrochemical cell and cyclic voltammetric sweeps were carried out to obtain electrochemical steady state.

The thickness of poly (DICY) film could be controlled by the cyclic number of voltammetric scans during the electrochemical modification. The effect of the thickness of poly (DICY) film on the electrochemical response was investigated by cyclic voltammetric technique. The current (I_{pa}) response of poly (DICY) films increase gradually as the number of cycles increases during film formation from 5 to 10 cycles. Afterwards I_{pa} starts to decrease by increasing the number of cycles which was examined up to 30 cycles **(Fig. 1a).**In order to obtain better oxidation peak and higher sensitivity of current for the electrochemical response of DICY, 10 scans were chosen to control the thickness of the poly (DICY) film.
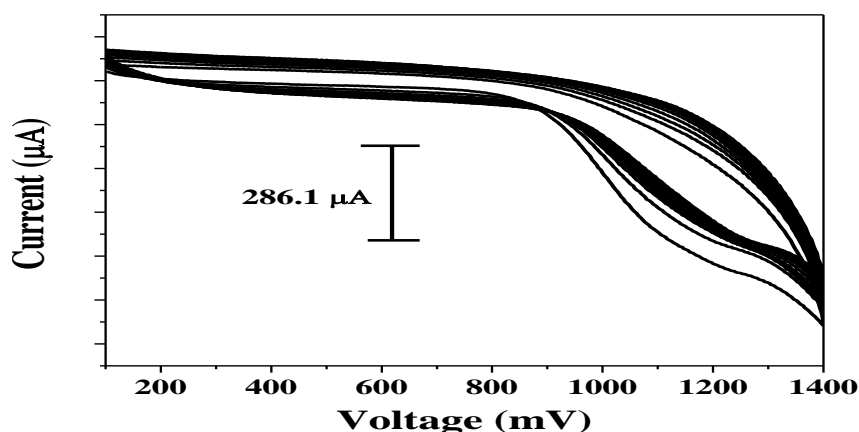

Fig.1: Cyclic voltammograms for the electro polymerization of 1 mM of DICY in 0.1 M phosphate buffer solution on carbon paste electrode.
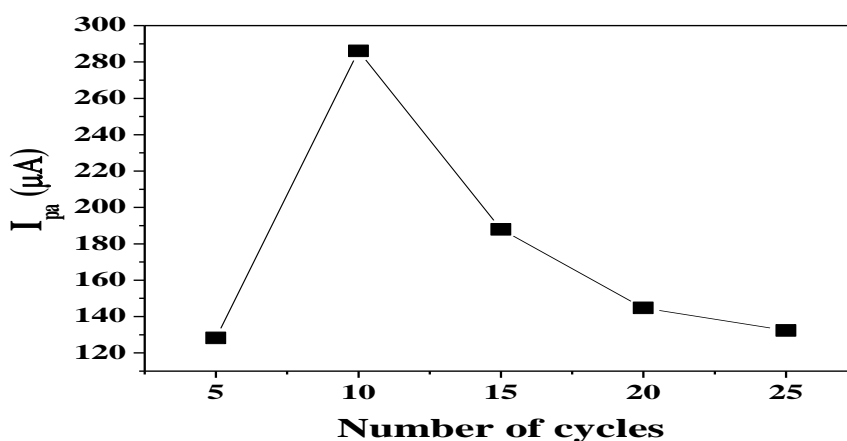

Fig.1a. Plot of anodic peak current vs. number of cycles of Dicyclomine hydrochloride.

3.2 SEM Characterization of poly (Dicyclomine Hydrochloride) modified carbon paste electrode:

Fig. 2 (a&b), explain the surface morphology of bare carbon paste electrode (BCPE) and poly (DICY) modified carbon paste electrode respectively using scanning electron microscope (SEM). The surface of bare CPE was formed by irregularly shaped micrometer-sized flakes of graphite. Whereas the modified electrode had atypical uniform arrangement of DICY molecules on the surface of CPE [18].
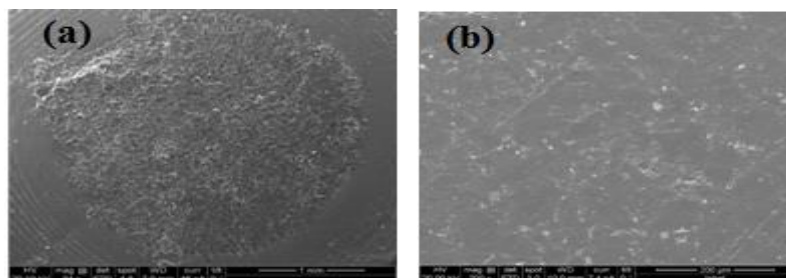
Fig.2: SEM images of bare carbon paste electrode (a) and poly (DICY) modified carbon paste electrode (b).

3.3 Electrochemical response of potassium ferrocyanide at poly (DICY) modified carbon paste electrode:

Fig. 3 shows the electrochemical response of 1 mM potassium ferrocyanide in 1M KCl at bare carbon paste electrode (BCPE) curve 'b' and at poly (DICY) modified carbon paste electrode curve 'a'. Anodic peak potential E_{pa} 290 mV with peak current I_{pa} of 13.38 µA and cathodic peak E_{pc} 50 mV with peak current I_{pc} of 8.23 µA respectively. After modification with poly (DICY) modified carbon paste electrode which shows enhancement of both electrochemical anodic peak potential (E_{pa}) 234 mV with peak current (I_{pa}) of 23.9 µA and cathodic peak potential (E_{pc}) 163 mV with peak current (I_{pc}) of 14.55 µA respectively were observed. The surface area of bare carbon paste electrode is 0.0258 cm^2. Whereas, effective surface area of the modified electrode was found to be 0.0365 cm^2.
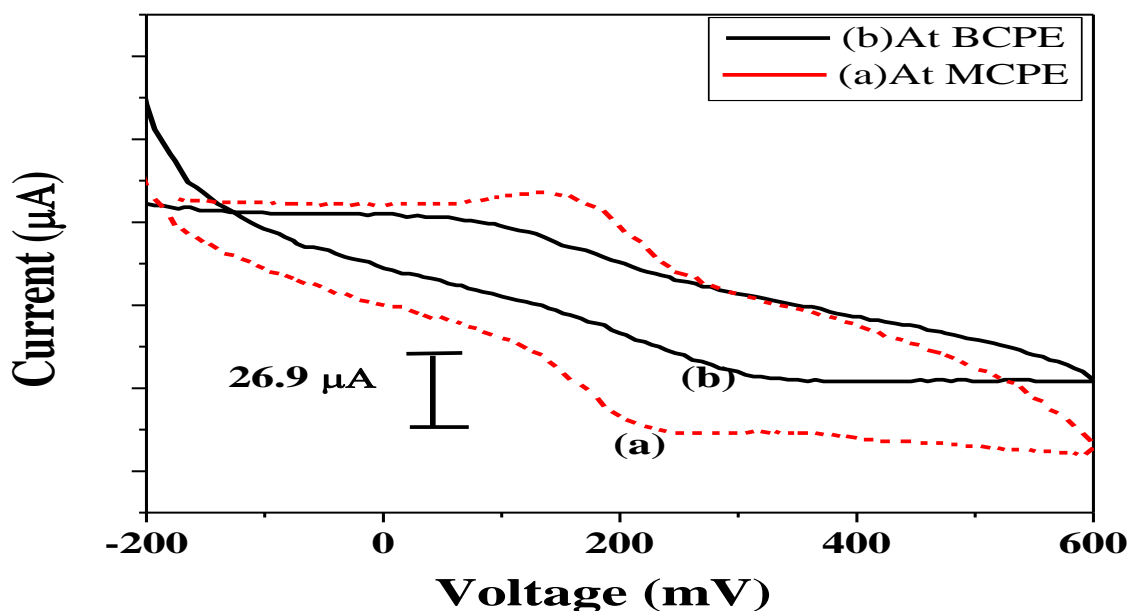

Fig. 3. Comparison of 1 mM K$_4$[Fe(CN)$_6$] in 1 M KCl solution at poly (DICY) modified carbon paste electrode(a)and bare carbon paste electrode (b).

3.4 Electrochemical behavior of L-Tyrosine at poly (Dicyclomine Hydrochloride) modified carbon paste electrode:

Fig. 4 shows cyclic voltammograms of 0.1 mM L-Tyrosine in 0.1 M phosphate buffer solution of pH 6.5 at bare carbon paste electrode (curve 'b') and at poly (DICY) modified carbon paste electrode (curve 'a').The curve 'c'represents cyclic voltammogram of the blank solution at poly (DICY) modified carbon paste electrode. Above studies showed that only one oxidation peak at 1009 mV potential with peak current of 12.49 µA at bare CPE, whereas an oxidation peak at 991mV potential with peak current of 28.70 µA at the poly (DICY) modified carbon paste electrode respectively in the potential range 100 to 1300 mV. No reduction peak was observed in the reverse scan, suggesting that the electrochemical reaction is a totally irreversible process and the oxidation peak current at the bare CPE is broad due to slow electron transfer, while the response was considerably improved at the poly (DICY) modified carbon paste electrode and the peak potentials shifted to negative direction, the shape of the peak turns sharper and the peak current increased significantly.
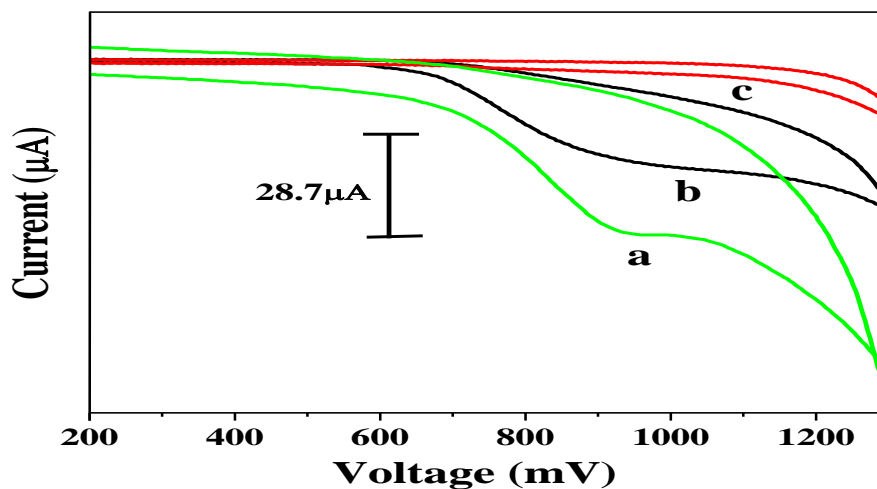
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Fig.4: Comparision of 1 mM LTY at poly (DICY) modified carbon paste electrode (a), bare carbon paste electrode (b) and blank solution in phosphate buffer at poly (DICY) modified carbon paste electrode (c); pH 6.5, scan rate 50 mVs^{-1}.

3.5 Effect of pH:

The pH influence was investigated by cyclic voltammetric measurement at different pH values between 3.5 and 9.0 as shown in **Fig. 5a**. The maximum response of current was observed at pH 6.5. In order to obtain the maximum bioactivity and optimal sensitivity, phosphate buffer solution of pH 6.5 and scan rate 50 mVs^{-1} were selected for our experiments. The oxidation peak current increases with increase of pH from 4.5 to 6.5 and becomes maximum and peak potential shifted negatively. While pH beyond 6.5, a great decrease of the oxidation peak current could be observed, then it decreased gradually with the further increase in pH of the solution as shown in **Fig. 5a** and the oxidation peak potential decrease with increase of pH as shown in **Fig. 5b**. A linear relationship was obtained between the anodic peak potential and pH of the solution in the range 3.5 – 9. The corresponding linear regression equation is

E_{pa} (mV) = 1086 – 28.89pH   (R = 0.99149)……………………….. (1)

With a negative slope of 28.89 indicating that the number of electrons and protons are equal in the electrochemical oxidation of LTY at poly (DICY) modified carbon paste electrode.
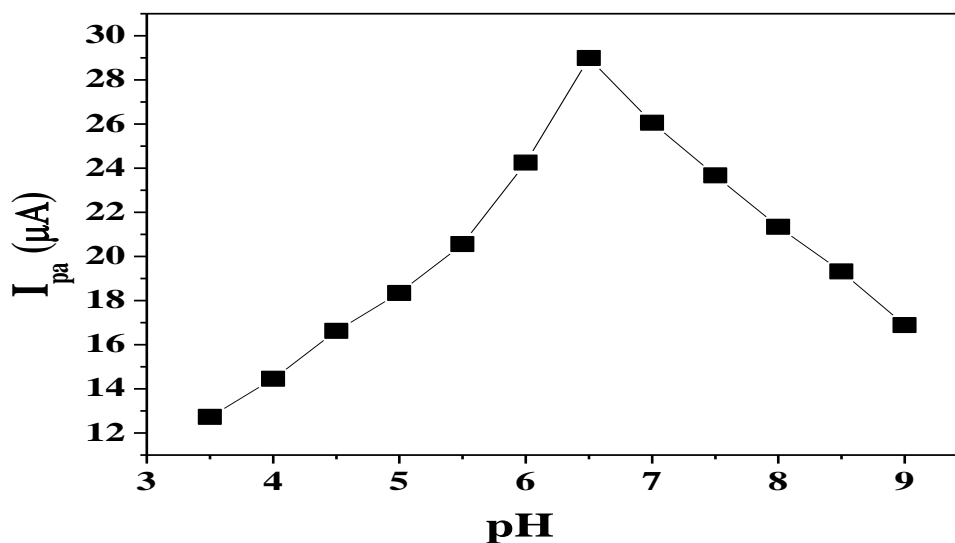

Fig.5a: Plot of anodic peak current vs. pH (3.5 – 9.0) of 0.1 mM LTY at Poly (DICY) modified carbon paste electrode.
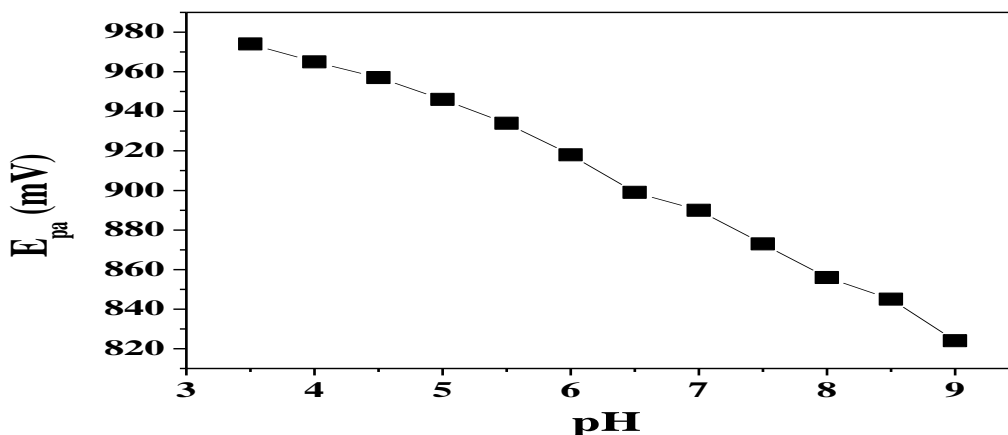
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Fig.5b: Plot of anodic peak potential vs. pH (3.5 – 9.0) of 0.1 mM LTY at poly (DICY) modified carbon paste electrode.

3.6 Effect of scan rate:

The effect of scan rates on the electrochemical response of 0.1 mM LTY at poly (DICY) modified carbon paste electrode was studied at different scan rates. Redox peak current increase linearly with the scan rate in the range 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mVs^{-1}.The cyclic voltammograms were shown in **Fig. 6a**. However linearity obtained for the plot of the anodic peak current vs. scan rate with a correlation coefficient of 0.9943 shown in **Fig. 6b.**The linear relationship with a correlation coefficient of 0.9988 obtained between the peak current and square root of scan rate in the range of 10-100 mVs^{-1}is shown in the **Fig. 6c** which indicates the process occurring is adsorption controlled. The relationship between the anodic peak potential and scan rate is explained by plotting the anodic peak potentials vs. natural logarithm of scan rate **(Fig. 6d)** by considering the liner regression equation given by

E_{pa}(mV) = 0.01443lnυ +0.43687   R= 0.99754……………………………. (2).

The relationship between the anodic peak current and scan rate is explained by plot of the logarithm of anodic peak current vs. logarithm of scan rate **(Fig. 6e)** by considering the following equation:

logI_{pa}= 0.8266logυ -0.15702   R= 0.9873…………………………… (3)

The slope of 0.82 is close to the theoretically expected value of 1.0 for an adsorption controlled process [19] and as a result the peak potential shifts towards positive side.

According to Laviron's theory [20] the slope is equal to RT/αn_αF. As for a totally irreversible electrode reaction on the basis of the above discussion, the n_α was found to be 0.806, which indicated that one electron was involved in the oxidation process of LTY at the poly (DICY) modified carbon paste electrode. Since the equal number of electrons and protons took part in the oxidation of LTY, therefore one electron and one proton transfer were involved in the electrode reaction process. The electrochemical reaction process for LTY at poly (DICY) modified carbon paste electrode can therefore be summarized as in **Scheme I.**

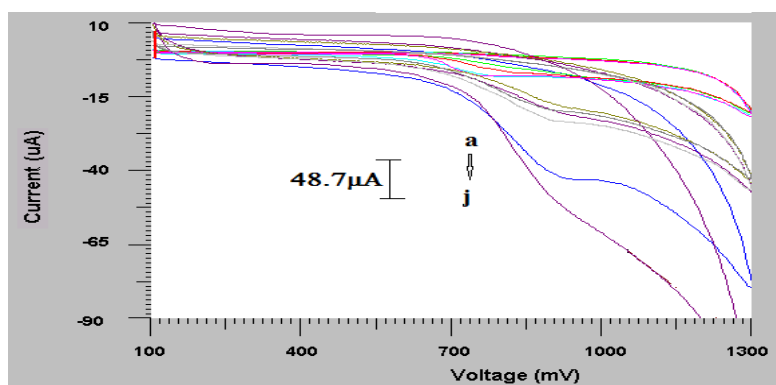**Fig.6a: Cyclic voltammograms of 0.1 mM LTY at poly (DICY) modified carbon paste electrodewithdifferentscan rates (a) 10, (b) 20, (c) 30, (d) 40, (e) 50, (f) 60, (g) 70, (h)80, (i) 90, (j) 100mVs^{-1}.**
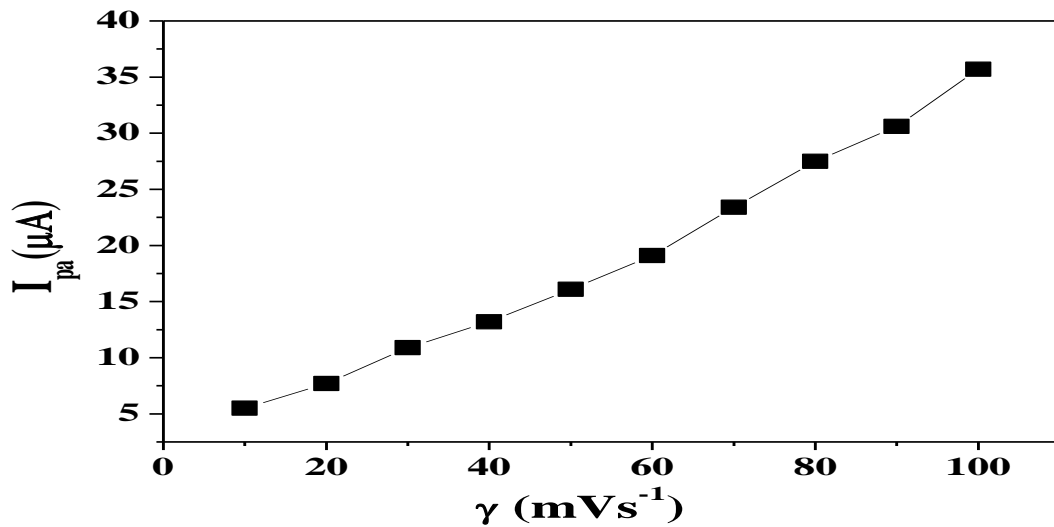
Page | 12

Fig.6b: Plot of anodic peak current vs. scan rates of LTY at poly (DICY) modified carbon paste electrode.
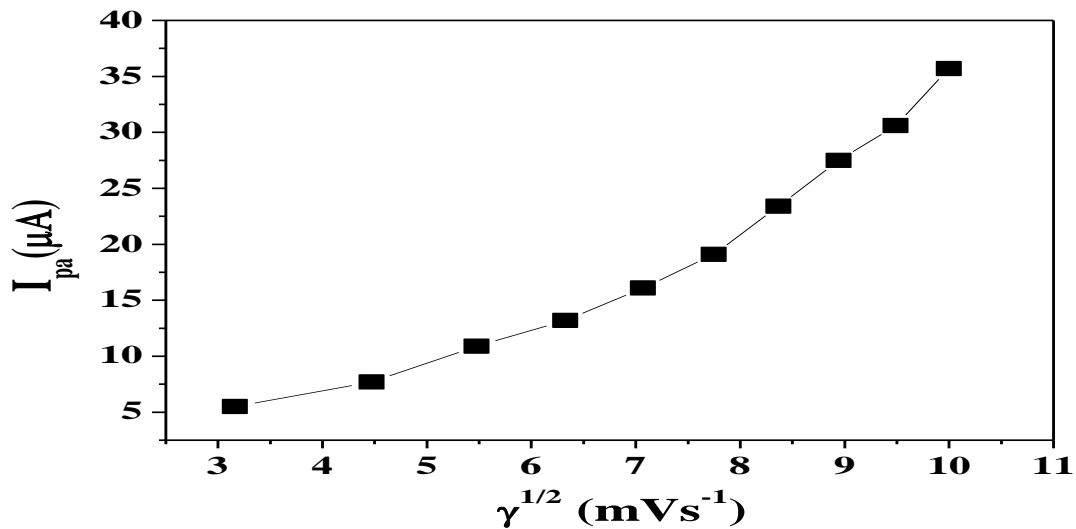

Fig.6c: Plot of anodic peak current (I_{pa}) vs. square root of scan rates of LTY at poly (DICY) modified carbon paste electrode.
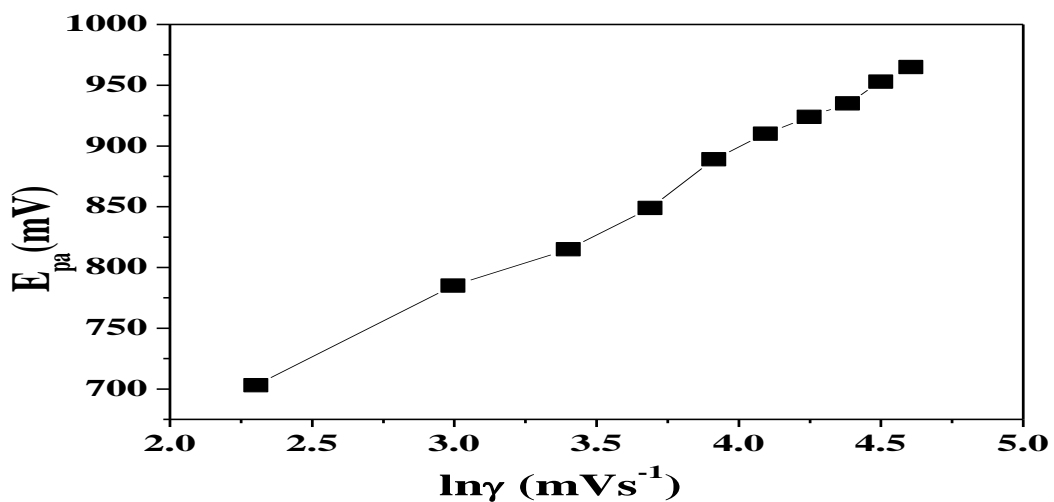

Fig.6d: Plot of anodic peak potential vs. natural logarithm of scan rates of LTY at poly (DICY) modified carbon paste electrode.

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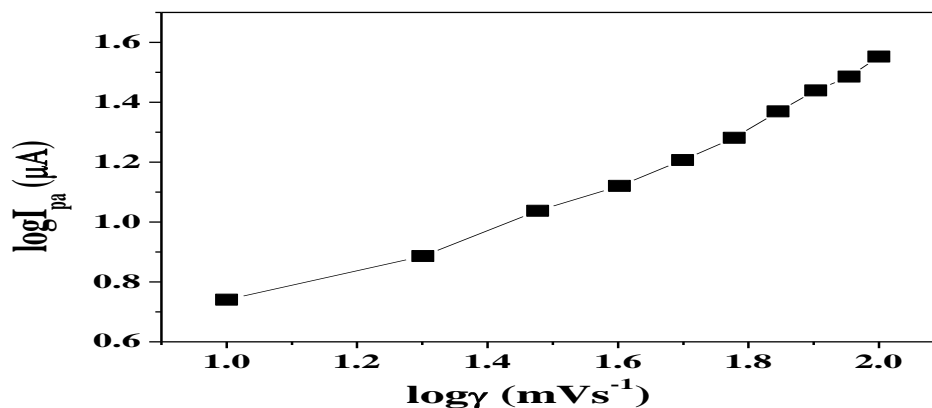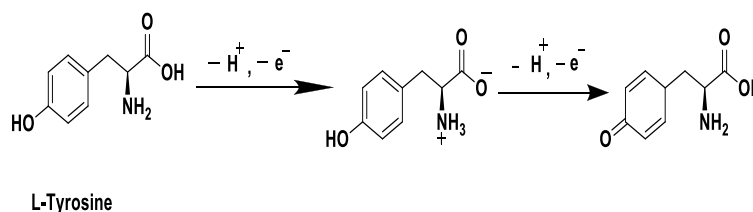
Fig.6e: Plot of logarithm of anodic peak current vs. logarithm of scan rates of LTY at poly (DICY) modified carbon paste electrode.

Scheme.I: Probable Reaction Mechanism of L-Tyrosine.

3.7 Effect of L-Tyrosine (LTY) concentration and detection limit:

The effect of LTY concentration was studied by cyclic voltammetry in 0.1M phosphate buffer solution (PBS) of pH 6.5 at the scan rate 50 mVs^{-1}. The oxidation peak current increases with increase in concentration of LTY. **Fig. 7a** and **Fig. 7b** shows the linear relationship between the anodic peak current (I_{pa}) with LTY concentration in the range from 2×10^{-5} M to 1×10^{-3} M. The corresponding linear regression equation is

I_{pa} (μA) =26.5481 C (10^{-5} M) + 2.26913　　(R = 0.9984)…………….. (4)

The limit of detection (LOD) and limit of quantification (LOQ) of LTY were found to be 0.638 μM and 2.128 μM respectively. Related stastical data of calibration curves were obtained from five different calibration curves (n=5).

The LOD and LOQ were calculated from the peak current using the following equation:

LOD = 3S/M and LOD = 10S/M

Where S is standard deviation and M is the slope (sensitivity) of calibration plot.

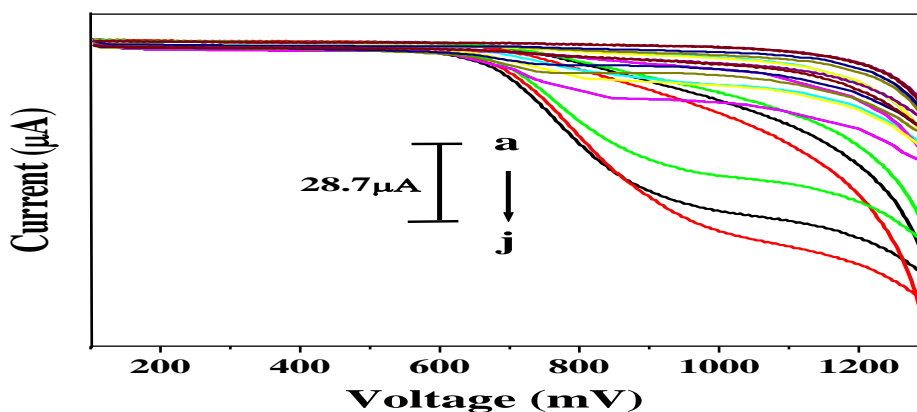**Fig.7a. Effect of variation of concentration of LTY (a) 2×10^{-5} M, (b) 4×10^{-5} M, (c) 6×10^{-5} M, (d) 8×10^{-5} M, (e) 1×10^{-4}M, (f) 2×10^{-4} M,(g) 4×10^{-4}M, (h) 6×10^{-4}M,(i) 8×10^{-4} M, (j) 1×10^{-3}Monanodic peak current at poly (DICY) modified carbon paste electrode; pH 6.5, scan rate 50 mVs^{-1}.**

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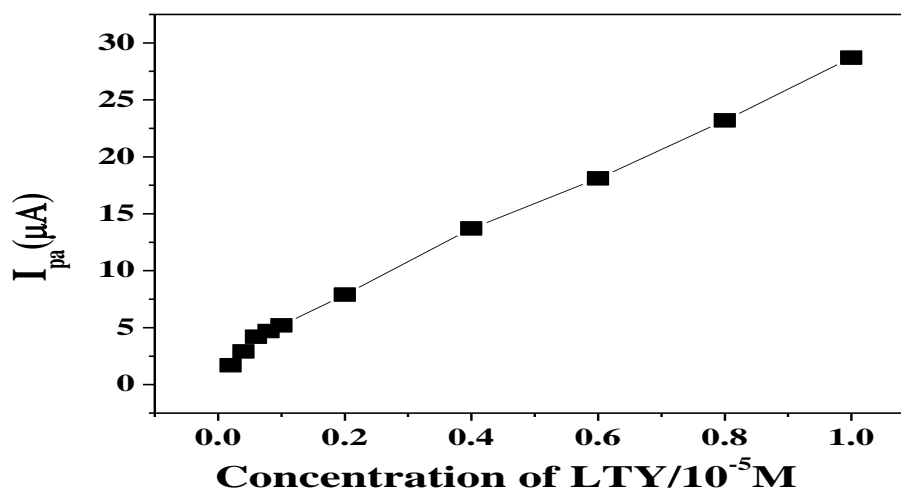
Fig.7b: Plot of anodic peak current vs. LTY concentration at poly (DICY) modified carbon paste electrode.

4. CONCLUSIONS

In the present study, the poly (Dicyclomine Hydrochloride) modified carbon paste electrode based on the electropolymerisation has been prepared for the electrochemical investigation of LTY. Results showed that the oxidation peak current of LTY was improved at poly (DICY) modified carbon paste electrode. The electrochemical response is diffusion controlled and irreversible in nature for LTY. A linear concentration range was found to occur from 2×10^{-5} to 1×10^{-3}M by CV. The probable reaction mechanism involved in the oxidation of (LTY) is also proposed.

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