

ELECTROCHEMICAL STUDIES OF TINIDAZOLE AT POLY (ERIOCHROME BLACK T) MODIFIED GLASSY CARBON ELECTRODE

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ABSTRACT

An electro polymerized Eriochrome Black T (EBT) modified glassy carbon electrode was fabricated and employed for the determination of Tinidazole (TNZ). The poly EBT modified GCE exhibited an enhanced electrochemical response towards Tinidazole by showing two prominent reduction potential peaks and one oxidation potential peak in alkaline media at 565mVs⁻¹, 927mVs⁻¹ and 665mVs⁻¹ respectively, and enhanced reduction and oxidation peaks current of 8.68μA, 25.02 μA and 0.9 μA respectively in 0.2M phosphate buffer solution (PBS) of pH 11.5. The effect of pH, concentration, and scan rate was studied at the bare glassy carbon electrode and poly EBT modified glassy carbon electrode were investigated. Low detection limit (LOD) and Low quantification limit (LOQ) of TNZ were detected. The modified electrode showed high sensitivity, Low detection limit, easy preparation and this modified electrode was successfully employed to determine Tinidazole in pharmaceutical drug samples.

Keywords: Eriochrome Black T, Tinidazole, Glassy Carbon Electrode, Cyclic Voltammetry.

INTRODUCTION

Tinidazole (1-(2-(ethylsulfonyl) ethyl) 2-methyl-5- Nitro-1H-imidazole) which is one of the nitro imidazole derivatives has been used as an antiprotozoal agent for many years in the treatment of infestations caused by *Trichomonas vaginalis*, *Entamoeba histolytica* and *giardia lamblia*^{1,2}. Now a days, the TNZ is used in the treatment of adult periodontitis. Moreover, TNZ, in combination with amino penicillin, can be used to treat children infected with *Helicobacter pylori*³.

Tinidazole is an anti-parasitic drug widely used throughout Europe and Third World as a treatment for a variety of amebic and parasitic infections like^{4,5} trichomoniasis, giardiasis and amoebiasis^{6,7}.

The most common side effects reported with TNZ are upset stomach, bitter taste, diarrhea and itchiness. Other side effects which are headache, physical fatigue, and dizziness. Intake alcohol while taking Tinidazole effects an unpleasant disulfiram like reaction, which includes nausea, vomiting, and increased blood⁸. Thus, the determination of TNZ is of great importance and various methods have

been developed. HPLC, Capillary electrophoresis, Voltammetry, spectrophotometric^{9,10} and chromatographic methods were commonly used to detect TNZ^{11,12}.

In this work, a simple, rapid and sensitive electrochemical procedure for the determination of Tinidazole at a glassy carbon electrode in the presence of Eriochrome Black T was proposed. The experimental results showed that the Eriochrome Black T had a distinct enhancement effect on the electrochemical response of TNZ at the glassy carbon electrode. Electrochemical parameters of the TNZ reduction and oxidation were investigated. The chemical structure shown in fig.1.

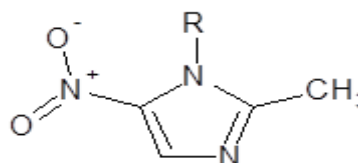


Fig. 1: Structure of Tinidazole

2 MATERIALS AND METHODS

2.1 Reagents

All reagents were of analytical grade, pure grade of TNZ was kindly supplied by SigmaAldrich. Sodium phosphate dibasic dehydrate, Potassium dihydrogen orthophosphate, Potassiumferrocyanide, Potassiumferricyanide, Potassiumchloride, Sodiumhydroxide was purchased from Himedia laboratories pvt.ltd. Mumbai. The pharmaceutical dosages containing TNZ were purchased from drug stores.

A stock solution of 0.25×10^{-3} M TNZ has been prepared in the double distilled water and further diluted with the same solvent to give appropriate concentration for the working range.

Phosphate buffer (0.2M) used as a supporting electrolyte has been prepared by dissolving a mixture of Sodium phosphate dibasic dihydrate and Potassium dihydrogen orthophosphate in 500 ML standard flask. pH was adjusted with an appropriate amount of 0.1M NaOH and ortho phosphoric acid.

2.2. Apparatus

Electrochemical detection were carried out with a model-201 electrochemical analyzer (EA-201 Chem link Systems) in a three-electrode system. The glassy carbon electrode having cavity of 3 mm diameter is used as working electrode. The Platinum electrode is used as counter electrode and saturated calomel electrode (SCE) is used as a Standard reference electrode for completing the circuit.

2.3. Pretreatment of Glassy Carbon Electrode (GCE)

Before the modification, the glassy carbon electrode surface was polished with a fine emery sheet and then rinsed with double distilled water. The electrochemical pretreatment of the GCE by cycling the potential between -1200 mV and +1000 mV at a scan rate of 100 mV/s for 10 times in 0.1 M H_2SO_4 solution.

2.4. Preparation of the Modified Glassy Carbon Electrode

The pretreated glassy carbon electrode was electro polymerized by immersing in 0.01mM NaOH containing 1mM solution of Eriochrome Black T, and the potential of range -400 to 1400mVs⁻¹ was applied for 20cycles. After polymerization the electrode was removed from the cell and washed with double distilled water to remove adsorbed impurities. The same procedure was applied for all the sample analysis and all electrochemical

measurements were carried out at room temperature¹⁰.

3 RESULT AND DISCUSSION

3.1. The Electrochemical Response of Potassium Ferrocyanide at Eriochrome Black T Modified Glassy Carbon Electrode (EBTMGCE)

Potassium ferro cyanide was used as the electrochemical redox probe to investigate the electrochemical properties of modified electrode. Electrochemical response of 1mM $\text{K}_4[\text{Fe}(\text{CN})_6]$ in 1M KCl has been studied at modified (EBTMGCE) and bare glassy carbon electrode in the range -200 to 600 mvs⁻¹ respectively by cyclic voltammetric technique. The cyclic voltammograms obtained are shown in fig1. The curve 'a' showed increase in the reduction peak current at modified (EBTMGCE) electrode compare to bare GCE curve 'b'. The cyclic voltammograms shows an oxidation peak potential at 225 mvs⁻¹ of peak current 12.1 μA and reduction peak potential at 196 mvs⁻¹ of peak current 6.5 μA respectively at bare glassy carbon electrode. Where as modified (EBTMGCE) electrode shows a oxidation and reduction peak potential at 306 and 153 mvs⁻¹ and peak current 19.5 μA and 11.2 μA respectively. The enhancement of peak current has been obtained at modified (EBTMGCE) electrode. The effective area of the modified electrode was found to be **0.086.cm²**.

3.2 Electrochemical Behavior of Tinidazole at Poly (EBT) Modified GCE

The electrochemical behavior of 0.1×10^{-3} mM TNZ was investigated in 0.2M phosphate buffer of pH 11.5 at (EBTMGCE) modified electrode using Cyclic Voltammetric technique. In the fig1.1 the curve 'a' shows the Cathodic peak potentials and Cathodic peak current of 565 mvs⁻¹, 925 mvs⁻¹ and 8.68 μA , 25.02 μA Anodic peak potential and Anodic peak current of 665 mvs⁻¹ and 0.9 μA at (EBTMGCE) modified electrode. The curve 'b' represents the Cathodic peak potential of 685 mvs⁻¹ at bare GCE. The curve 'c' represents blank solution of (EBTMGCE) modified electrode..

3.3. Effect of pH

The effect of pH on the reduction of 0.1mM of TNZ in 0.2 M pBS has been studied in the range of 3 to 12 at poly EBTMGCE which is shown in fig.2. The cathodic peak current increased sharply from pH 3 to 11.5, then it is decreased. Therefore the pH 11.5 was chosen as the optimum pH for our experiments¹³ in fig.2.

3.4. Effect of Scan Rate

The effect of scan rates on the electrochemical response of 0.1mM TNZ at poly (EBT) modified glassy carbon electrode was studied at different scan rates. The peak current increases linearly with the scan rate in the range of 25,50,75,100,125, 150mvs⁻¹.The cyclic voltammograms were shown in fig.3. However linearity obtained for the plot of cathodic peak current versus scan rate with the correlation coefficient of 0.9930 shown in fig.3.1.The linear relationship with a correlation coefficient of 0.9988 obtained for the plot of cathodic peak current versus square root of scan rate in the range of 25 to150mvs⁻¹ which indicates the process is adsorption controlled is shown in fig.3.2. The relationship between the cathodic peak current and scan rate is explained by the plot of the logarithm of cathodic peakcurrent versus logarithm of scan rate as shown in fig 3.3.By considering the following equation.

$$\text{Log } I_{pc} = 0.340\text{logu} + 0.5813 \quad (R=0.9980)$$

3.5. Effect of Concentration

The effect of TNZ concentration in the range of 1×10^{-5} to 6×10^{-4} M has been studied by cyclic voltammetry in 0.2 M phosphate buffer (PBS) of pH 11.5 at the scan rate 50 mvs⁻¹. The reduction peak current increases with increase in concentration of TNZ which is as shown in fig3.5.indicates the linear relationship between the cathodic peak current (I_{pc}) with the TNZ concentration of 2×10^{-5} M, 4×10^{-5} M, 6×10^{-5} M, 8×10^{-5} M, 1×10^{-4} M, 2×10^{-4} M. The corresponding linear regression equation is shown in below.

$$I_{pc} (\mu A) = 5.7504 C (10^{-5}M) + 15.802 \\ (R = 0.9939) \dots \dots \dots$$

The low detection limit (LOD) and limit of quantification (LOQ) of TNZ were found to be

1.6 μ M and 5.6 μ M respectively .Related statistical data of calibration curves were obtained from five calibration curve (n=6).The LOD and LOQ were calculated from the peak current using the following equation

$$\text{LOD} = 3S/M \quad \text{and} \quad \text{LOQ} = 10S/M$$

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

APPLICATION STUDIES

Determination of Tinidazole In Pharmaceutical Dosages

The practical application of tinidazole in pharmaceutical formulations is used to analyze the compute of modified (EBTMGCE) electrode for practical purpose. The Tiniba IP tablets(Zydus healthcare rangpo)Were purchased from drug shop.A ten tablets of Tiniba IP (specified content of tinidazole is 300 mg) were accurately weighed, then transferred in to a 50 ml standard flask and dissolved in phosphate buffer solution(pH11.5).The prepared solution was examined using the modified (EBTMGCE) electrode.The determination amount of tinidazole in tiniba IP tablets obtained from cyclic voltammetry are given in table 1.The results were satisfactory,showing that the prepared method could be efficiently used for the determination of TNZ in pharmaceutical preparations.

CONCLUSION

In this work Eriochrome Black T is used as modifier for investigating the electrochemical behavior of Tinidazole. The poly (EBTMGCE) modified electrode enhanced both anodic and cathodic peak current of TNZ. Electrochemical process was found to be adsorption controlled. The modified electrode showed good sensitivity and reproducibility with its low cost and ease of preparation will have good application for further sensor development.

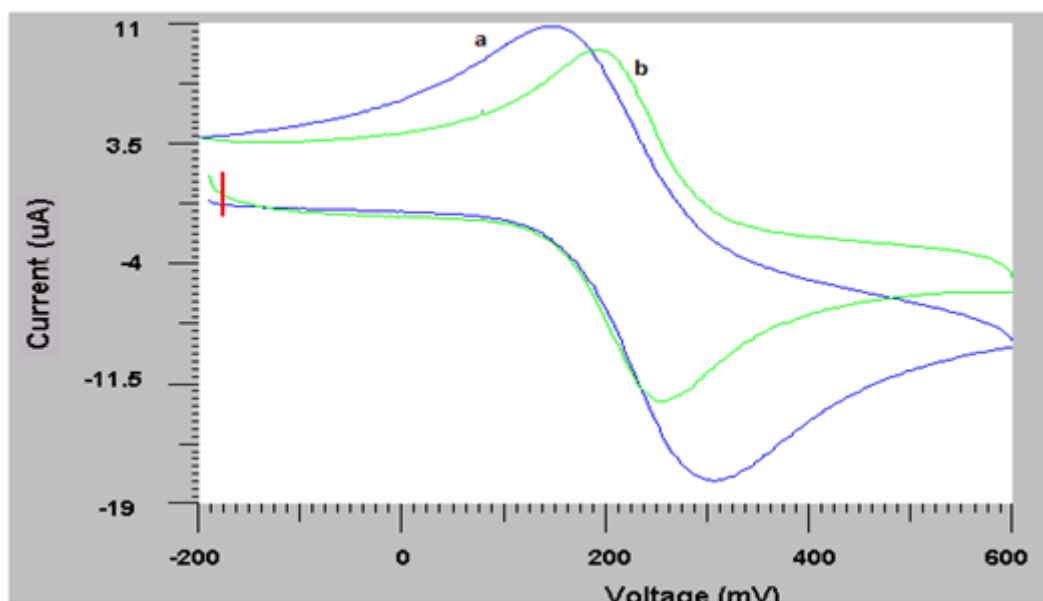


Fig. 1: Comparison of 1mM K₄ [Fe (CN) ₆] in 1 M KCl solution at Poly EBT MGCE curve (a) and bare GCE curve (b).

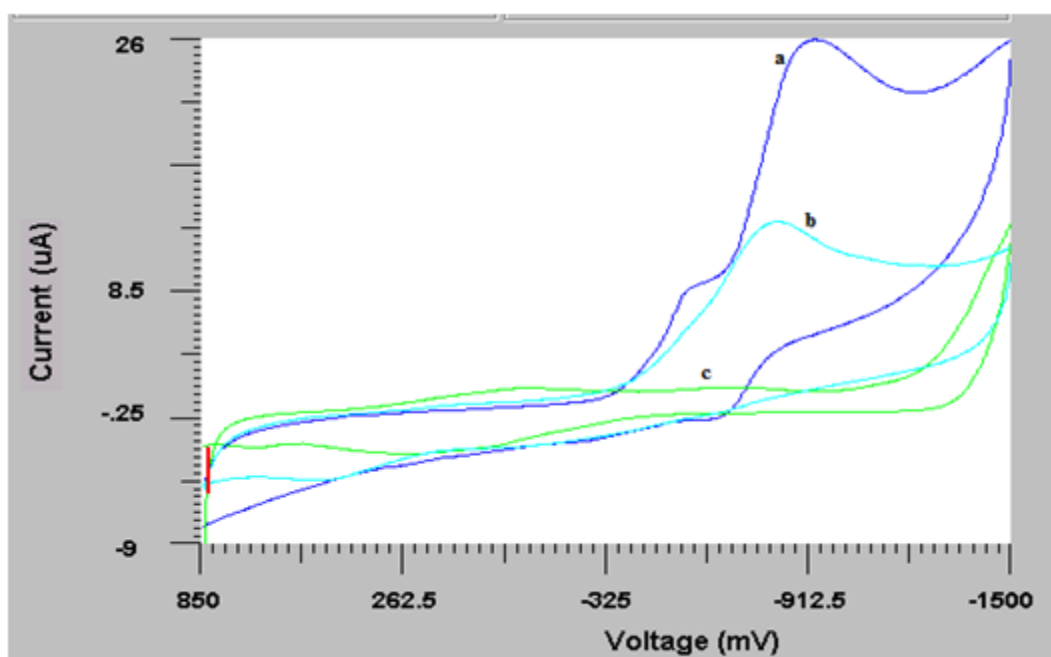


Fig. 1.1: cyclic voltammogram of 0.1mM TNZ at poly (EBT) MGCE curve (a), bare glassy carbon electrode curve (b), and blank solution in phosphate buffer of pH 11.5 at poly (EBT) MGCE (c); scan rate 50 mvs⁻¹

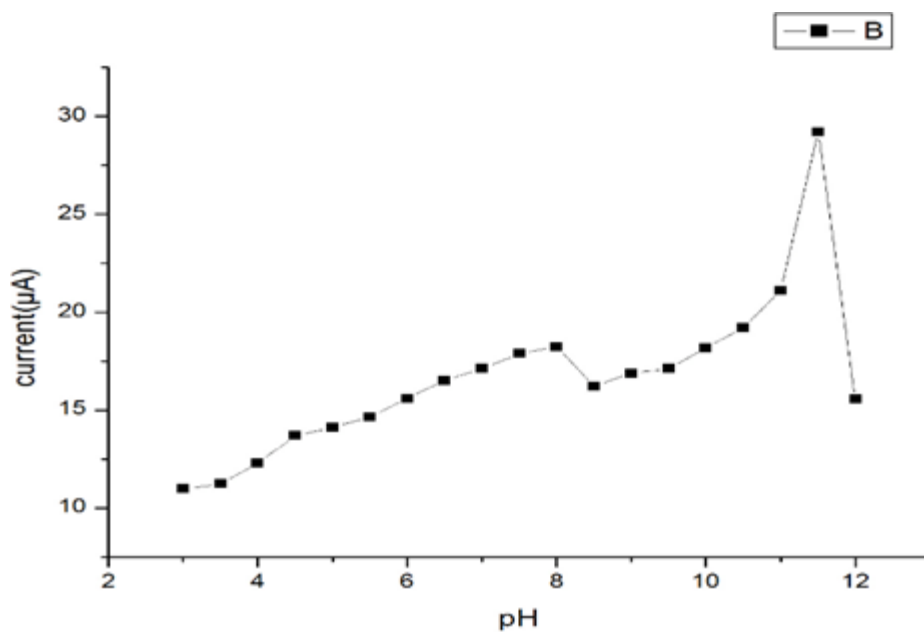


Fig. 2: plot of cathodic peak current v/s pH of (3 to 12)
1mM TNZ at poly(EBT) MGCE

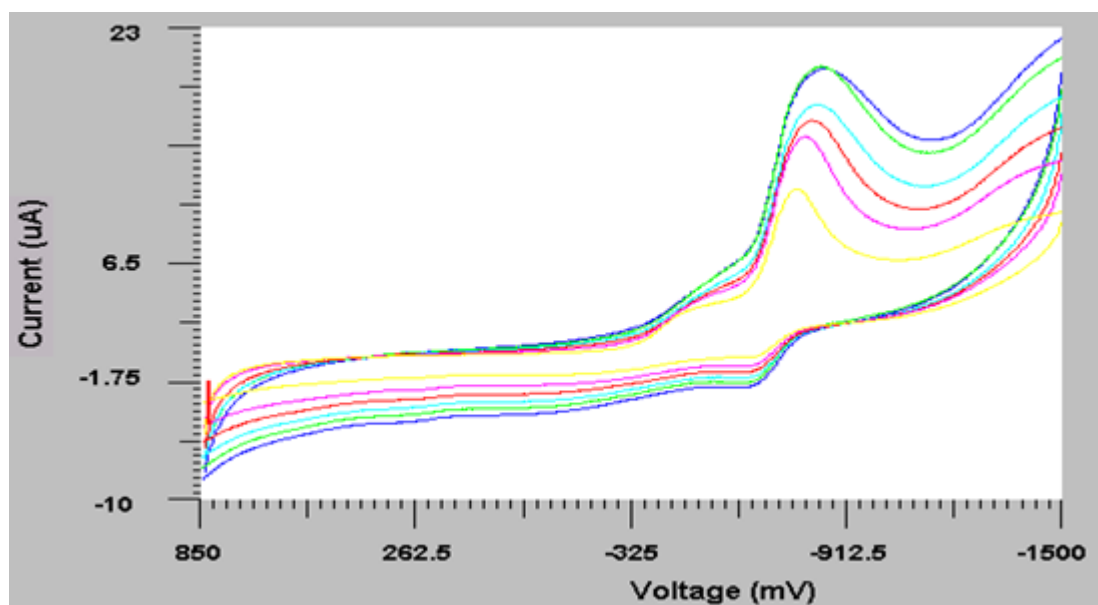


Fig. 3: plot of cathodic peak current versus scan rate of TNZ at poly EBT MGCE

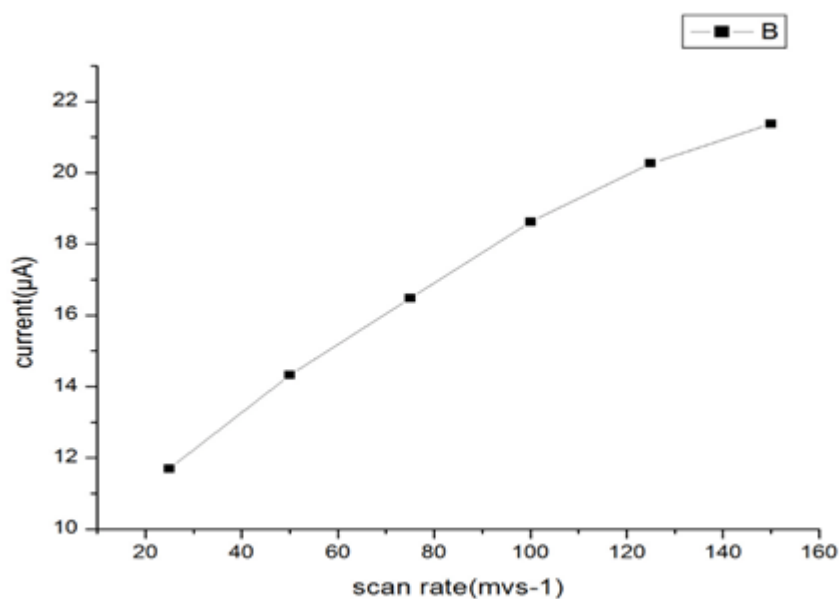


Fig. 3.1: Cyclic voltammograms of 0.1mM TNZ at poly (EBT) modified glassy carbon electrode with different scan rate 25, 50, 75, 100, 125, 150 mvs⁻¹

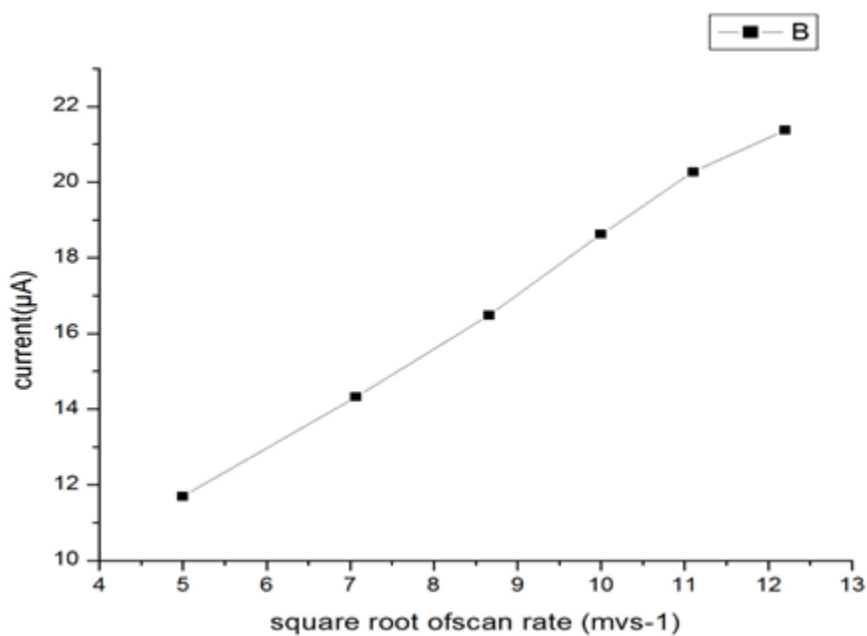


Fig. 3.2: plot of logarithm peak current vesus logarithm scan rate of TNZ at poly EBT MGCE

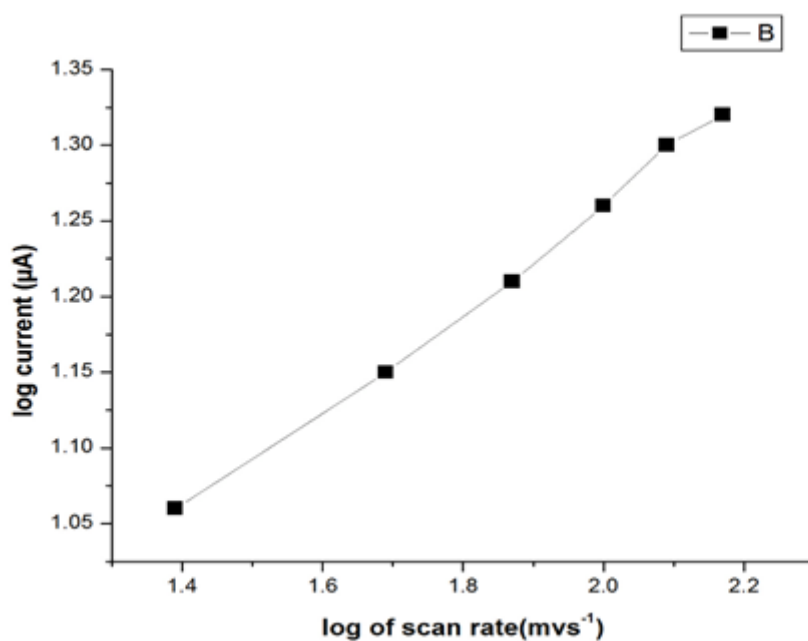


Fig. 3.3: plot of logarithm peak current versus logarithm scan rate of poly EBT MGCE

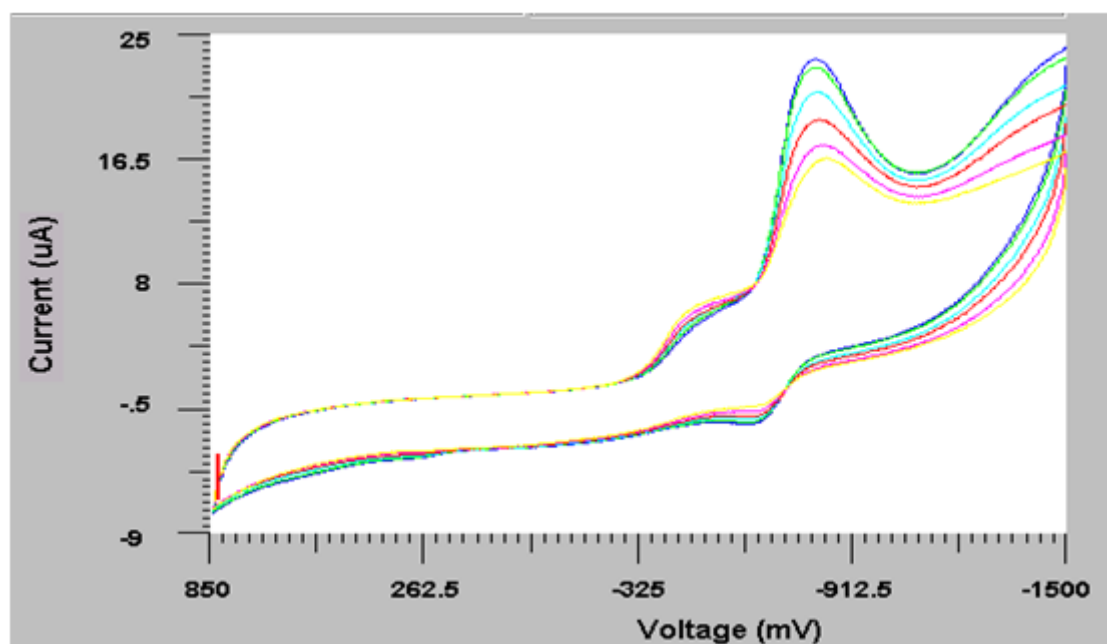


Fig. 3.4: Cyclic voltammogram of variation concentration of TNZ 2×10^{-5} M, 4×10^{-5} M, 6×10^{-5} M, 8×10^{-5} M, 1×10^{-4} M, 2×10^{-4} M, cathodic peak current at poly(EBT) modified glassy carbon electrode; pH 11.5 scan rate 50 mVs^{-1}

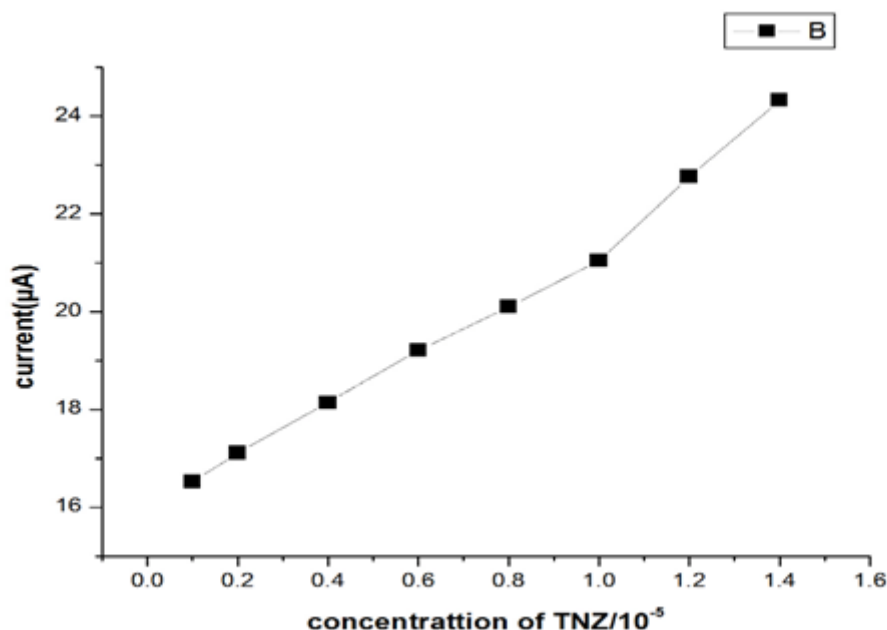


Fig. 3.5: plot of cathodic peak current v/s concentration of TNZ at poly (EBT) modified glassy carbon electrode

Table 1: Determination Results of Tinidazole In the Commercial Tiniba Tablets

Sample No.	Specified (mg/tab)	Detected (mg/tab)	Recovery(%)	RSD value
1	300	291.5	97.17	1
2	300	299.0	99.66	
3	300	301.67	100.55	

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