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Research Paper

Electrochemical Studies of Ceftriaxone on Eriochrome Black-T Polymer Film Modified Glassy Carbon Electrode

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Abstract *- A novel electrochemical method was developed for the determination of ceftriaxone (CFRX) based on the polymerised film of Eriochrome Black-T (EBT) on the surface of glassy carbon electrode (GCE) in phosphate buffer solution (PBS) at pH 2.48 by cyclic voltammetric technique (CV). Cyclic voltammetric studies indicated that CFRX was oxidised irreversibly at high positive potential of 1169 mV at bare GCE, giving rise to a well defined oxidation peak at a potential of 1064 mV at poly(EBT) modified GCE. It was found that the oxidation peak current of CFRX at the modified GCE was greatly improved compared with that at the bare GCE. The effects of scan rate, pH and concentration were examined on poly(EBT) modified GCE. A linear relationship was obtained between the anodic peak current(I_{pa}) and the CFRX concentration in the range of $0.5X10^{-3}$ to $2.0X10^{-3}$ M with a correlation co-efficient of 0.9805. The proposed method was sensitive and simple. It was successfully employed to determine CFRX in pharmaceutical samples.*

Keywords: Ceftriaxone, Eriochrome Black-T, Cyclic Voltammetry, Electropolymerisation, Modified glassy carbon electrode.

Introduction:

Ceftriaxone (Figure 1) is a (6R,7R)-7-{2-(2-aminuteo-4-thiazolyl)-(z)-2[methoxy iminuteoacetamido]-3{[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-as-triazin-3-yl)thio]-methyl}-8-oxo-5 thia-1-azobicyclo[4,2,0]oct-2-ene-2-carboxylicacid, in the hydrated disodium salt and contains approximately 83mg of sodium per gram of ceftriaxone. It belongs to a group of antibiotics called cephalosporins and is a third generation parentral cephalosporin with a relatively long half-life which is stable to β-lactamases particularly those produced by Gram-negative organisms [1-6]. It has excellent anti Gram-negative activity. It contains a highly acidic heterocyclic system on the 3-thiomethyl group, this unusual ring system is believed to confer the unique pharmacokinetic properties to this agent. It kills bacteria by interfering in the synthesis of the cellwall. Ceftriaxone has been effective in treating infections due to other 'difficult' organisms such as multidrug- resistant *Enterobacteriaceae* [7-12].

The use of voltammetric techniques such as cyclic voltammetry and chronocoulommetry allow reaction peaks to be analyzed, which in turn permits the relatively straightforward determination of kinetic parameters [13]. Along this line, a cathodic stripping voltammetry method has been described for determining ceftriaxone [14–16]. However, few reports have dealt with the oxidation of cephalosporins at solid electrodes and the use of the anodic response for their determination [16–23]. Nevertheless, oxidation of the aminothiazole group, which substituted of the side-chain in position 7 of the cephem ring in some cephalosporins, was reported to enable development of a promising amperometric detection mode for liquid chromatography or possibly other flow analytical procedures [19, 20].

Drug analysis has an extensive impact on public health. Electrochemical techniques have been used for the determination of the drug's electrode mechanism. The redox properties of drugs can provide insight into their metabolic fate, their invivo redox processes and their pharmacological activity [24,25].The concept of chemically modified electrodes (CME's) is one of the exciting developments in the field of electroanalytical chemistry. Many different strategies have been employed for the modification of the electrode surface. The motivation behind the modifications of the electrode surface are, (i) improved electrocatalysis, (ii) freedom from surface fouling and (iii) prevention of undesirable reactions competing kinetically with the desired electrode process [26].

Electropolymerisation is a good approach to immobilise polymers to prepare polymer modified electrodes (PME's) as adjusting the electrochemical

parameters can coated film thickness permeation and charge transport characteristics. Polymer-modified electrodes have many advantages in the detection of analytes because of its selectivity and homogeneity in electrochemical deposition, strong adherence to electrode surface and chemical stability of the film [27-28]. Glassy carbon electrode has been very popular because of its excellent electrical and mechanical properties, wide potential range, extreme chemical inertness and relatively reproducible performance [29-34].

In this present work, a poly (Eriochrome Black T) film was fabricated on the surface of a GCE in 0.2M NaOH solution by Cyclic Voltammetry (CV). The polymer was found to be electrocatalytically active for the oxidation of ceftriaxone. With its good sensitivity, selectivity and stability, the polymer coated GCE has been used for the determination of ceftriaxone.

Material and Methods

Experimental Reagents

Ceftriaxone (CFRX) obtained from Micro labs, India and used as received. Eriochrome Black-T was purchased from G.S.Chemical Testing Lab and Allied Industries (Bombay, India). All reagents used were of analytical grade and used without further purification. The stock solution of the ceftriaxone (25mM) was prepared by dissolving it in double distilled water and kept in the dark under refrigeration to avoid any degradation of the drug. Other dilute standard solutions were prepared by appropriate dilution of stock solution in the phosphate buffer solution (PBS), pH 2.48. PBS was prepared from 0.2 M H_3PO_4 and adjusted pH with 0.2 M NaOH.

Apparatus

Electrochemical measurements were carried out with a model EA-201 electroanalyser (chemlink systems) a three electrode system was employed. The polyEBT modified glassy carbon electrode having an approximate area of 0.025 cm^2 is used as working electrode with a saturated calomel electrode as reference electrode (SCE) and the platinum electrode as auxillary electrode for all experiment.

Modification procedure

Before the modification, the glassy carbon electrode surface was polished with a fine emery sheet and then rinsed with distilled water. After each polishing step followed by electrochemical pretreatment of the GCE by cycling the potential between -1200 mV and +1000 mV at a scan rate of 100mV/s for 10 times in 0.10 M H_2SO_4 solution. The electrode was subsequently placed in a solution containing 0.20 M NaOH and 1 mM EBT and cyclic potential sweep was applied in the range of -400 to +1400 mV at a scan rate of 100 mV/s for 20 times. The polyEBT fabricated modified GCE after polymerisation washed with distilled water and data were recorded in pH 2.48 PBS.

Results and Discussion

Electropolymerisation of Eriochrome Black-T on a glassy carbon electrode

PolyEBT GCE was fabricated in 0.2 M sodium hydroxide solution containing 1mM of eriochrome black-T. The film was grown on GCE by cyclic voltammetric scans between -400 to +1400 mV. The optimized scan number under the experimental conditions was determined as 20 for reaching the steady response. As shown in Figure 2, in the first cycle, with the potential scanning from of -400 to +1400 mV the anodic peak was observed at +26 mV corresponding to the oxidation of eriochrome black-T monomer. The peak descended gradually with the increase in cyclic time, such decrease indicates the polyEBT membrane forming and depositing on the surface of the GCE by electropolymerisation. After polymerisation the polyEBT modified GCE was carefully rinsed with distilled water and was used for the determination of CFRX.

Electrochemical response of potassium ferrocyanide at polyEBT modified GCE

Potassium ferrocyanide was used as the electrochemical redox probe to investigate the electrochemical properties of poly EBT modified GCE. (Figure 3). The cyclic voltammogram of potassium ferro cyanide at polyEBT modified GCE (solid line in Figure 3) showed that the redox peak current increased than that of bare GCE (dashed line in Figure.3). At the bare GCE the cyclic voltammogram of $K_4Fe(CN)_6$ showed a pair of redox peaks, with the anodic peak potential at 258 mV and the cathodic peak potential at 188 mV in 1M KCl. However for the poly EBT modified GCE a pair of redox waves of $K_4Fe(CN)_6$ were observed with greatly increase of the peak current. The anodic peak potential was located at 248 mV and the cathodic peak potential at 187 mV respectively. The results of the enhancement of peak current showed excellent catalytic ability of polyEBT modified GCE. Electrocatalysis at a modified electrode is usually an electron transfer reaction between the electrode and solution substrate which when mediated by an immobilised redox couple (i.e., the mediator) proceeds at a lower potential than would otherwise occur at the bare electrode [35-37].

Electrochemical behaviour of ceftriaxone at polyEBT modified GCE

Cyclic voltammetry was utilized to investigate the electrochemical behaviour of ceftriaxone at the EBT polymer film GCE (Figure 4a), a bare GCE (Figure 4b) and cyclic voltammogram of bare GCE in blank solution containing 0.2M phosphate buffer solution at pH 2.48 (Figure 4c). It showed that only one oxidation peak at +1169 mV and a peak current of 6.9 µA at bare GCE, whereas an oxidation peak at 1064 mV and a peak current of 24.6 µA at the polyEBT GCE, in the potential range -100 to +1400 mV. No reduction peak was observed in the reverse scan, suggesting that the electrochemical reaction is a totally irreversible process. From the figure 4b, the oxidation peak at the bare GCE is broad due to slow electron transfer, while the response was considerably improved at the polyEBT film electrode and the peak potentials shifted to negative direction, the shape of the peak turns sharper and the peak current increased significantly in figure 4a.

Effect of scan rate

The effect of scan rates on the electrochemical response of 1mM CFRX at poly (EBT) modified GCE was studied between the range 30 to 210 mV/s and the cyclic voltammograms were shown in figure 5a. From figure 5b, it was found that the oxidation peak current increases linearly

with the increase in scan rate with a correlation coefficient of 0.9982 and slope of 0.1787, which indicates an adsorption controlled process occuring at the polyEBT modified GCE. However linearity was also obtained for the plot of square root of scan rate vs. the oxidation peak current with a correlation coefficient of 0.9966 in figure 5c. Also the slope of log i_{pa} vs. log υ (figure 5D) was 1.23 which is larger than theoretical expected value 0.53 for a purely diffusion controlled process, this indicates that the process is adsorption controlled [38,39] .

Effect of pH

The influence of solution pH on the oxidation of 0.2mM CFRX at the polyEBT modified GCE using PBS of pH 2.48 to 6 were investigated by CV. It shows that, by increasing the pH of the PBS, a negative shift was observed in the oxidation peak potentials, showing that protons take part in these electrode reactions. Figure 6 shows the linear relationship between the anodic peak current and pH of the solution with a negative slope of 2.8404 mV and when pH value beyond 2.48, a great decrease of the oxidation peak current could be observed, then it decreased gradually with the further increasing the pH of solution. Therefore a pH of 2.48 was chosen for the subsequent analytical experiments.

Effect of ceftriaxone concentration

The variation of concentration of CFRX was studied at poly(eriochrome black-T) film modified GCE in 0.2 M phosphate buffer of pH 2.48 at a scan rate of 210 mV/s. Figure 7a shows the cyclic voltammograms of CFRX at poly(EBT) film modified GCE. The plot of i_{pa} versus concentration of CFRX showed the linear relationship between the anodic peak current i_{pa} and the CFRX concentration in the range of $0.5X10^{-3}$ M to $2.0X10^{-3}$ M with a correlation co-efficient of 0.9805 in Fig 7b.

Conclusion

In the present study, a novel method of constructing poly EBT modified GCE for the determination of ceftriaxone was developed. The electrochemical behavior of ceftriaxone on the modified electrode was investigated by cyclic voltammetry. The polyEBT film showed electrocatalytic action for the oxidation of ceftriaxone, characterizing by the enhancement of the peak current and the reduction of peak potential. Together with low cost and ease preparation, this film modified electrode seems to be of good utility for further sensor development.

Acknowledgement

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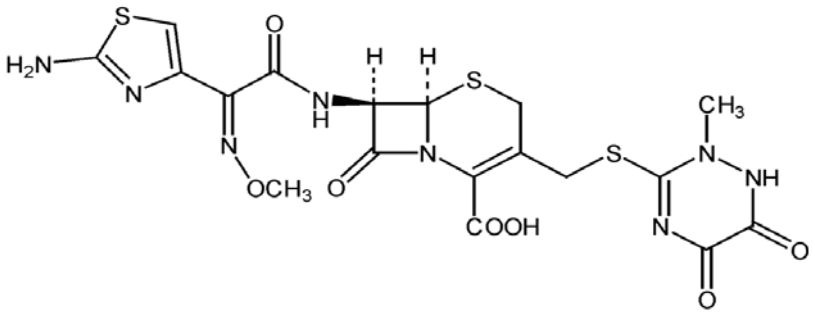

Figure 1: Chemical structure of ceftriaxone.

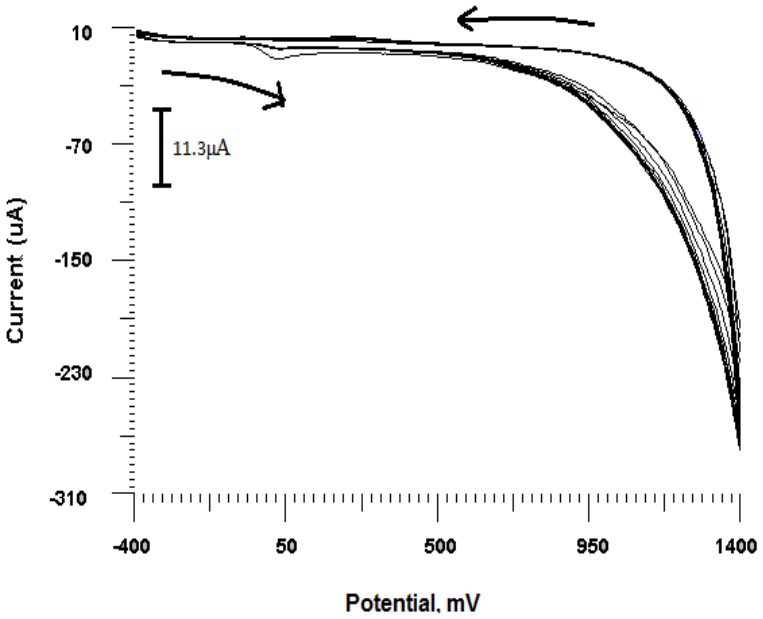

Figure 2: Cyclic voltammograms for the electropolymerisation of 1mM Eriochromeblack-T in 0.2M NaOH solution on a GCE. Initial potential -400 mV, Terminal potential 1400 mV. Scan rate: 100mVs^{-1}.

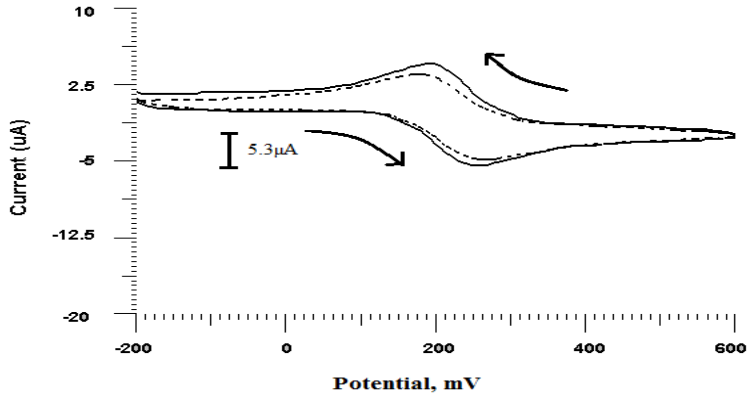

Figure 3: Cyclic voltammogram of 1mM potassium ferrocyanide in 1M KCl at bare GCE (dashed line) and modified GCE (solid line). Scan rate: 50mVs^{-1}.

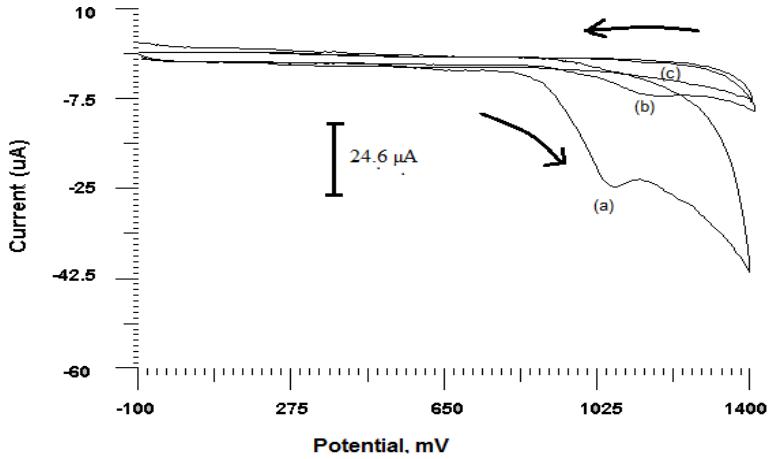

Figure 4: Typical cyclic voltammograms of 1.0 X 10^{-3} molL^{-1} ceftriaxone at the pallet modified GCE (a), a bare GCE (b), and without ceftriaxone (c) in phosphate buffer (pH 2.48), scan rate: 100mVs^{-1}.

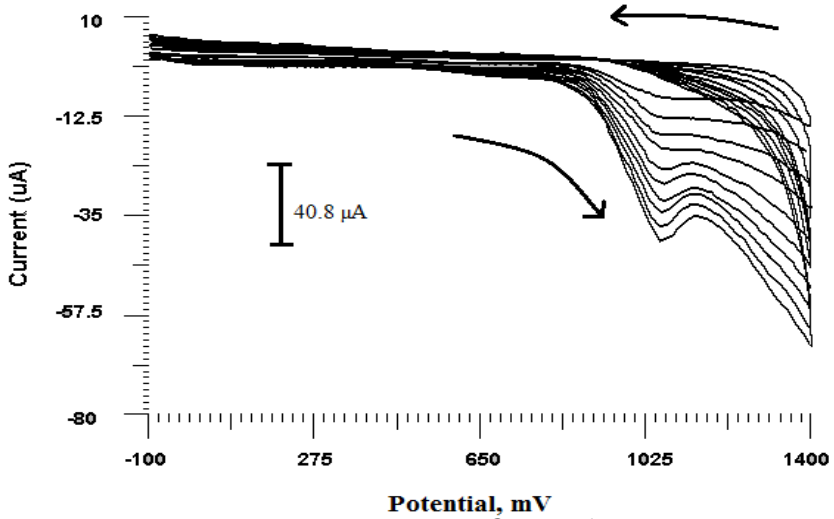

Figure 5a: Cyclic voltammograms of 1.0X10^{-3} molL^{-1} at the polyEBT GCE in PBS (PH 2 .48) with different scanrates were 10, 30, 50, 70, 90,110, 130, 150, 170, 190, & 210 mV/s respectively

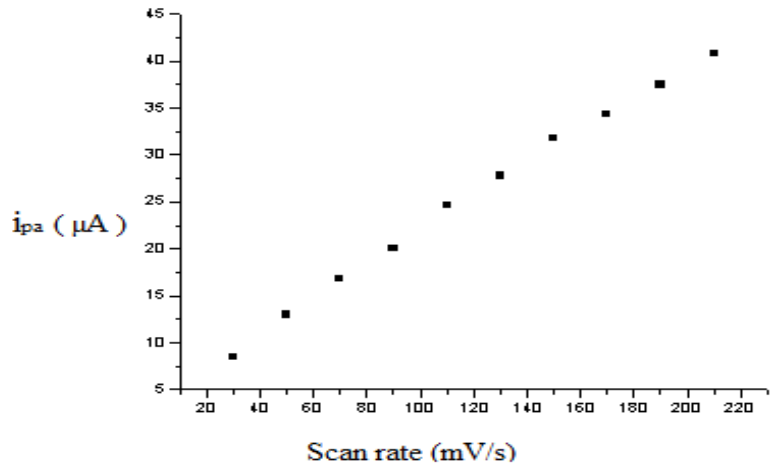

Figure 5b: The plot of Oxidation peak current versus Scan rates

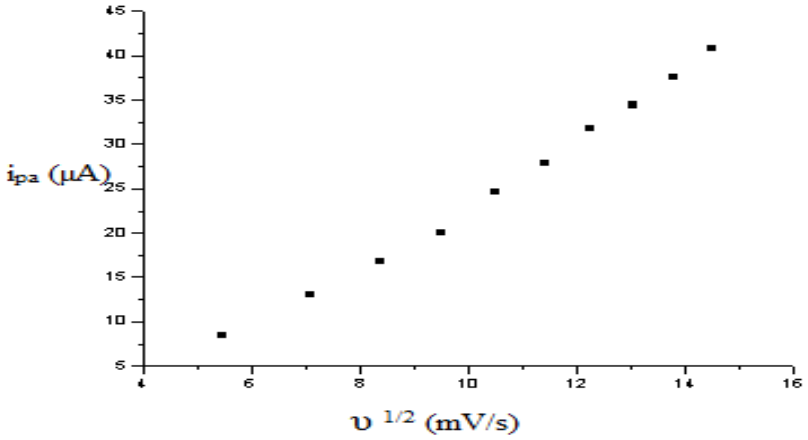

Figure 5c: Linear relationship between the peakcurrents and the square root of scanrates.

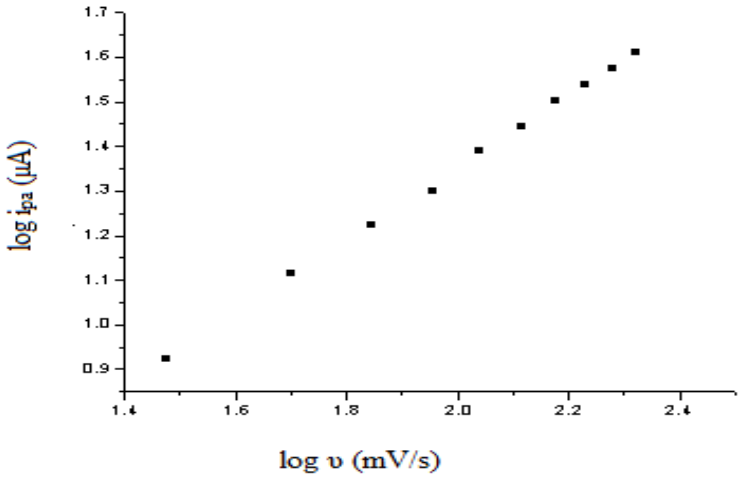

Figure 5d: Variation of the logarithm of peak current with the logarithm of the sweep rate.

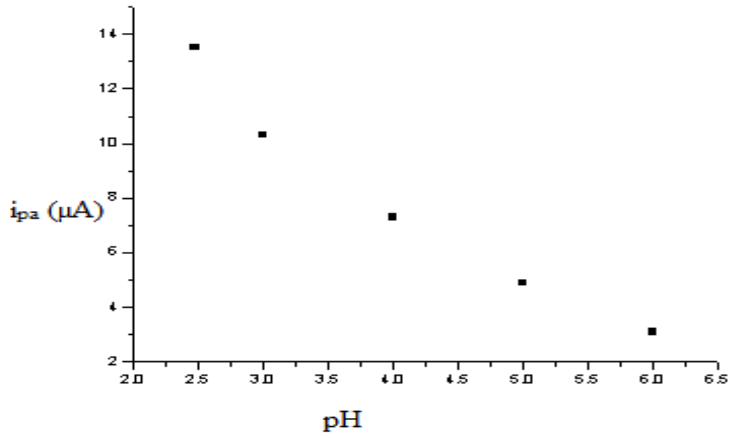

Figure 6: Dependance of the oxidation peak current on the solution pH

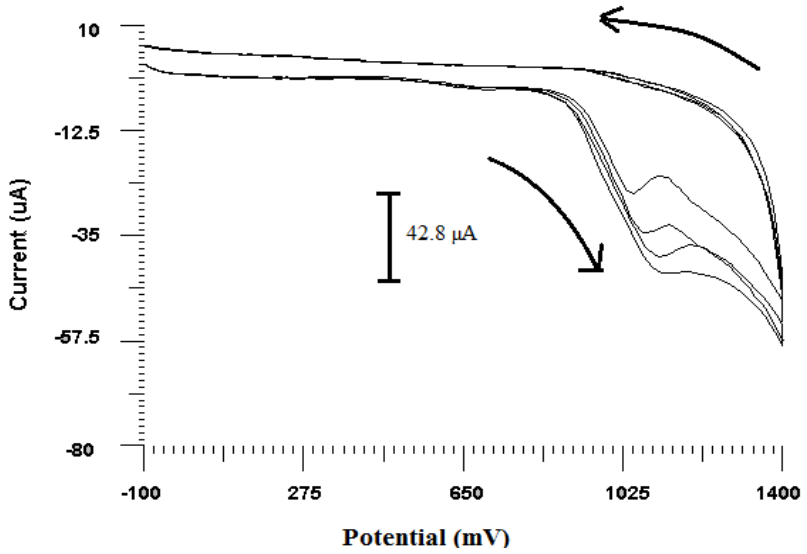

Figure 7 a: Cyclic voltammogram of variation of concentration of ceftriaxone from 0.5mM to 2.0mM in presence of phosphate buffer solution at pH 2.48.

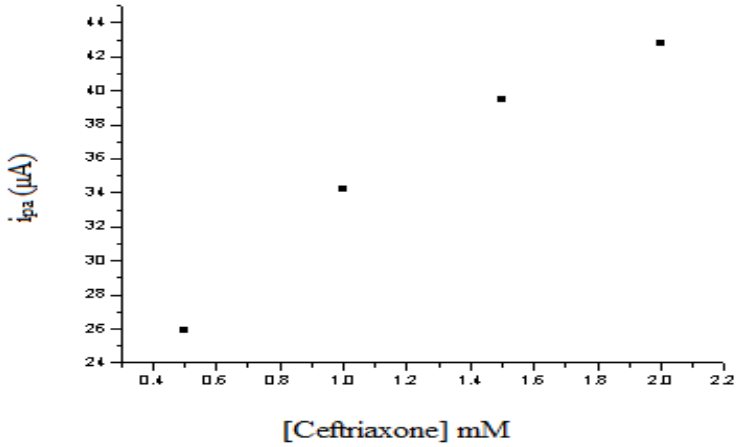

Figure 7 b. Effect of variation of concentration of ceftriaxone on the anodic peak current in phosphate buffer solution of pH 2.48