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Research Article

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Simultaneous determination of ciprofloxacin hydrochloride and enrofloxacin at poly (furosemide) modified carbon paste electrode by cyclic voltammetry

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ABSTRACT

A poly (Furosemide) modified carbon paste electrode (PFMCPE) was used for sensitive voltammetric determination of ciprofloxacin hydrochloride (CIP) and Enrofloxacin (ENRO). The surface morphology of PFMCPE was characterized by SEM. The electrochemical response of CIP and ENRO were investigated by cyclic voltammetric (CV) and differential voltammetric techniques (DPV). The effect of pH, concentration and sweep rate has been studied. The modified electrode was used for the simultaneous determination of CIP and ENRO in the phosphate buffer solution of pH 7.2. The low detection limit (LOD) and low quantification limit (LOQ) of ENRO and CIP were detected. This modified electrode was successfully used for the simultaneous determination of CIP and ENRO by resolving the overlapped voltammetric peaks by using cyclic voltammetry. The modified electrode showed high sensitivity, detection limit, high reproducibility, easy preparation and regeneration of the electrode surface.

Keywords: Enrofloxacin (ENRO), Ciprofloxacin Hydrochloride (CIP), Furosemide (FUR), Carbon paste electrode, Cyclic Voltammetry.

INTRODUCTION

The two important fluoroquinolones, namely Enrofloxacin (1-cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro- 4-oxo-3-quinolonecarboxylic acid) and ciprofloxacin (1-cyclopropyl-7-(1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid), were investigated and quantitatively analyzed by voltammetric methods. In several animal species, including pigs, Enrofloxacin (ENRO) is de-ethylated to its primary metabolite, ciprofloxacin (CIPRO) [1] and both ENRO and CIPRO are found in the bile and urine of animals receiving ENRO [2].

ENRO has been historically used as veterinary medicine for treatment of gastrointestinal and respiratory infections in several animal species, including pigs cursing diseases caused by gram-positive and negative bacteria. Enrofloxacin is used to prevent and treat pneumonia and E.coli bacterial diarrhea syndrome in cows and pigs. Due to these many problems, a lot of researchers have been carried out to remove antibiotics.

Several analytical techniques have been used for the determination of ENRO and CIPRO including liquid chromatography [3-6], capillary electrophoresis with diode array detection [7] and molecularly imprinted matrix solid-phase dispersion [8]. Electrochemical methods, such as polarography and voltammetry, have high sensitivity and are widely used in many areas of analytical chemistry. Recently, cathodic adsorptive stripping voltammetry on a static mercury drop electrode and principal-component regression have been applied for the determination of ENRO

in the presence of CIPRO [9] at trace levels. The linear sweep voltammetry (LSV) used for the first time with a multiwall carbon nanotube/glassy carbon electrode (MWCNT/GCE) for the determination of ENRO or CIPRO has been studied by Ali A. Ensafi et al.,[10].

In this work, cyclic voltammetry (CV) and differential pulse voltammetric (DPV) techniques were used for poly (FUR) modified carbon paste electrode which provides a sensitive method for the determination of ENRO and CIP simultaneously.

EXPERIMENTAL SECTION

Reagents

Ciprofloxacin Hydrochloride, Enrofloxacin and Furosemide were purchased from Merck, Himedia chemicals and all other chemicals were of analytical grade. The electropolymerisation of Furosemide was performed in 0.1 M phosphate buffer solution. The phosphate buffer solution was prepared from KH_2PO_4 and K_2HPO_4 and the pH was adjusted with 0.1N NaOH solution. The 10 mM stock solution of CIP was prepared by dissolving in water and ENRO dissolving in0.1N acetic acid respectively. Other chemicals used were of analytical grade except for spectroscopically pure graphite powder. All solutions were prepared with doubly distilled water.

Apparatus

Electrochemical measurements were carried out with a model-201 electrochemical analyzer (EA-201 chemlink systems) 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.

Preparation of bare carbon paste electrode

The bare carbon paste electrode was prepared by hand mixing of graphite powder 70% and silicon oil 30% in an agate mortar for about 30 min to get homogenous carbon paste. The paste was then packed into the cavity of a Teflon tube electrode (3 mm diameter). Before measurement, the modified electrode was smoothened on a piece of transparent paper to get a uniform, smooth and fresh surface.

Preparation of poly (Furosemide) modified carbon paste electrode (PFMCPE)

Electrochemical polymerization process of Furosemide (FUR) has been carried by using cyclic voltammetric in potential range -200 to 1400 mV at scan rate of 50 mVs^{-1} in phosphate buffer solution (PBS) of pH 4.5. The monomer concentration was usually 1 mM. After 10 cycles, the surface of the electrode was washed with doubly distilled water to remove the physically adsorbed material. This modified electrode was immersed in PBS and electrochemical determination of CIP and ENRO was carried out in a voltammetric cell in the potential range from -200 mV to1400 mV. The same procedure was applied for all the sample analysis and all electrochemical measurements were carried out at room temperature.

RESULTS AND DISCUSSION

Electropolymerisation of Furosemide on a carbon paste electrode

Electrochemical polymerization process has been carried out for Furosemide (FUR) by using carbon paste electrode modified with electropolymerised film of Furosemide. A solution of monomer Furosemide is oxidized to an activated form that polymerizes to form a polymer film directly on the electrode surface. This procedure results in few pinholes since polymerization would be accentuated at exposed (pinholes) sites at the electrode surface. Electro catalysis at a modified electrode is usually an electron transfer reaction between the electrode and solution substrate which, when mediated by an immobilized redox couple (i.e., the mediator) proceeds at a lower over potential and enhances the peak current. Electropolymerisation of Furosemide was fabricated in 0.1 M phosphate buffer solution containing 1 mM Furosemide on CPE. The film was grown on CPE by cyclic voltammetric scans between -200 to 1400 mV. The optimized scan number under the experimental conditions was determined as 10 for reaching the steady response. As shown in **Fig. 1,** in the first cycle, with the potential scanning from -200 to 1400 mV the two anodic peaks were observed at 885 & 1091 mV corresponding to the oxidation of Furosemide (FUR). The peak descended gradually with the increase in cyclic time; such decrease indicates the poly (FUR) membrane forming and depositing on the surface of the carbon paste electrode by electropolymerisation. FUR was oxidized to free radical at the surface of carbon paste electrode rapidly resulting in the possible structure of electropolymerised poly (FUR). After polymerization the poly (FUR) 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.

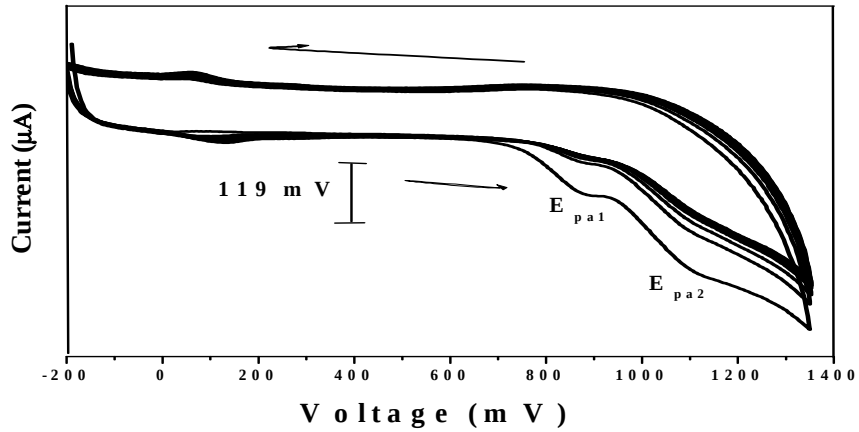


Fig. 1. Cyclic voltammograms for the electro polymerization of 1 mM of FUR 0.1M phosphate buffer solution on CPE, - 200 to 1400 mV, Scan rate 50 mVs^{-1}

Effect of the poly (FUR) film thickness on the electrochemical response of Ciprofloxacin hydrochloride (CIP) and Enrofloxacin (ENRO)

The thickness of poly (FUR) film could be controlled by the cyclic number of voltammetric scans during the electrochemical modification. The effect of the thickness of poly (FUR) film on the electrochemical response was investigated by cyclic voltammetric technique. The anodic peak current (I_{pa}) response of poly (FUR) 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 peaks and higher sensitivity of current for the electrochemical response of CIP and ENRO. 10 scans were chosen to control the thickness of the poly (FUR) film.

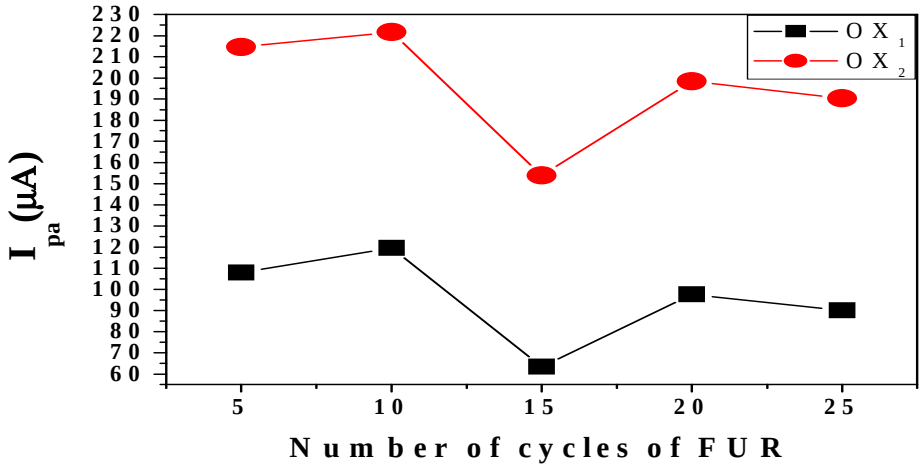


Fig. 1a. Anodic peak current v/s. Number of cycles of FUR

SEM Characterization of poly (FUR) modified carbon paste electrode (PFMCPE)

Fig. 2a and **Fig. 2b** explain the surface morphology of bare carbon paste electrode (BCPE) and 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 modified electrode had a typical uniform arrangement on the surface of PFMCPE [11].

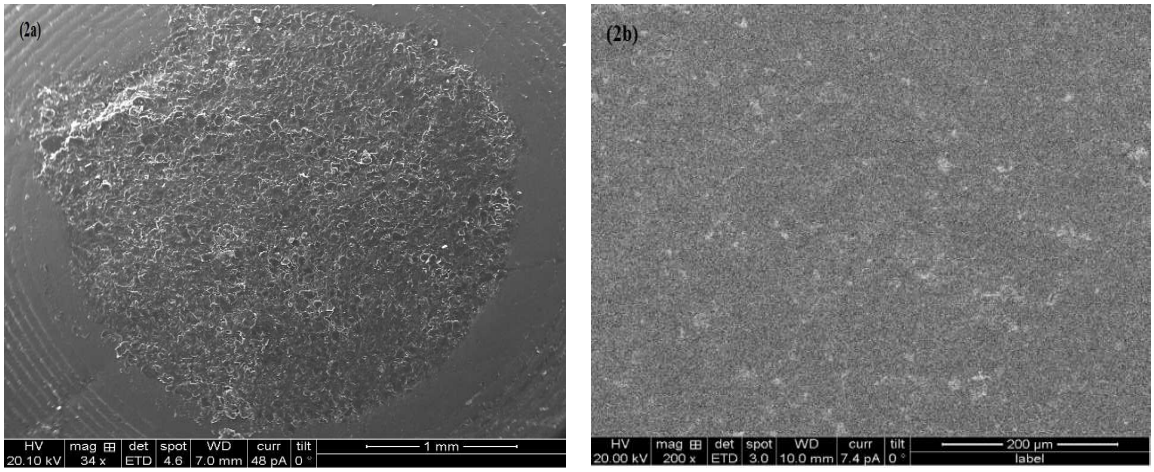


Fig.2. SEM images of bare carbon paste electrode (2a) and Poly (FUR) modified carbon paste electrode (2b)

Electrochemical investigation of potassium ferrocyanide at poly (FUR) modified carbon paste electrode (PFMCPE)

The electrochemical response of 1mM $K_4[Fe(CN)_6]$ in 1 M KCl at bare CPE and poly (FUR) modified carbon paste electrode (PFMCPE) are shown in the **Fig 3.** The cyclic voltammograms of $K_4[Fe(CN)_6]$ at PFMCPE (curve 'a') showed that the redox peak current increased than that of bare CPE (curve 'b'). At the bare CPE the cyclic voltammograms of $K_4[Fe(CN)_6]$ showed a pair of redox peaks, anodic peak potential E_{pa} at 245mV with peak current I_{pa} 6.45 µA and the cathodic peak potential E_{pc} at 124 mV with peak current I_{pc} 6.23. Whereas for poly (FUR) modified carbon paste electrode a pair of redox peaks of $K_4[Fe(CN)_6]$ were observed with greatly increase of the peak current. The anodic peak potential E_{pa} at 263 mV with peak current of 24.93 µA and the cathodic peak potential E_{pc} at 182 mV with peak current of 18.59 µA respectively. The results of the enhancement of peak current showed excellent catalytic ability of poly (FUR) modified carbon paste electrode. The bare Caron paste electrode is 0.024 cm^2, whereas the effective area of the modified electrode was found to be 0.032 cm^2.

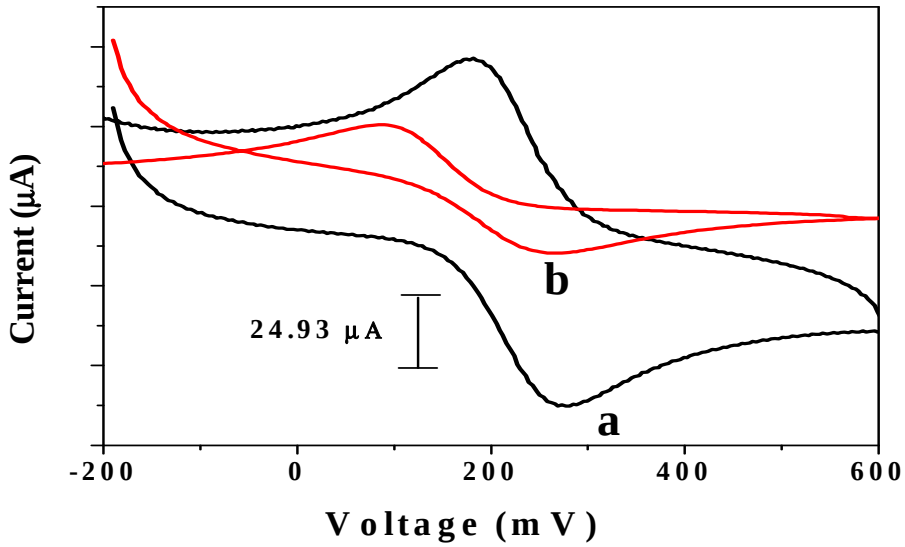


Fig. 3. Comparison of 1 mM $K_4[Fe(CN)_6]$ in 1 M KCl solution at poly (FUR) modified carbon paste electrode (a) and at bare carbon paste electrode (b)

Electrochemical response of ciprofloxacin hydrochloride (CIP) at poly (FUR) modified carbon paste electrode:

Ciprofloxacin hydrochloride (CIP) was investigated in 0.1 M phosphate buffer solution of pH 4.5 at PFMCPE using cyclic voltammetric technique. **Fig. 4** shows cyclic voltammograms of 0.1mM CIP at bare carbon paste electrode (curve 'b') and at PFMCPE. Curve's' represent cyclic voltammograms of blank solution at PFMCPE. Above studies

showed that one oxidation peak at 1090 mV potential with peak current 12.41 µA at bare CPE , whereas an oxidation peak at 1063 mV potential with peak current of 41.6 µA at poly (FUR) modified carbon paste electrode in the potential range from 500 to 1400 mV. The peak was observed in the irreverse scan, suggesting that the electrochemical reaction is a totally irreversible process.

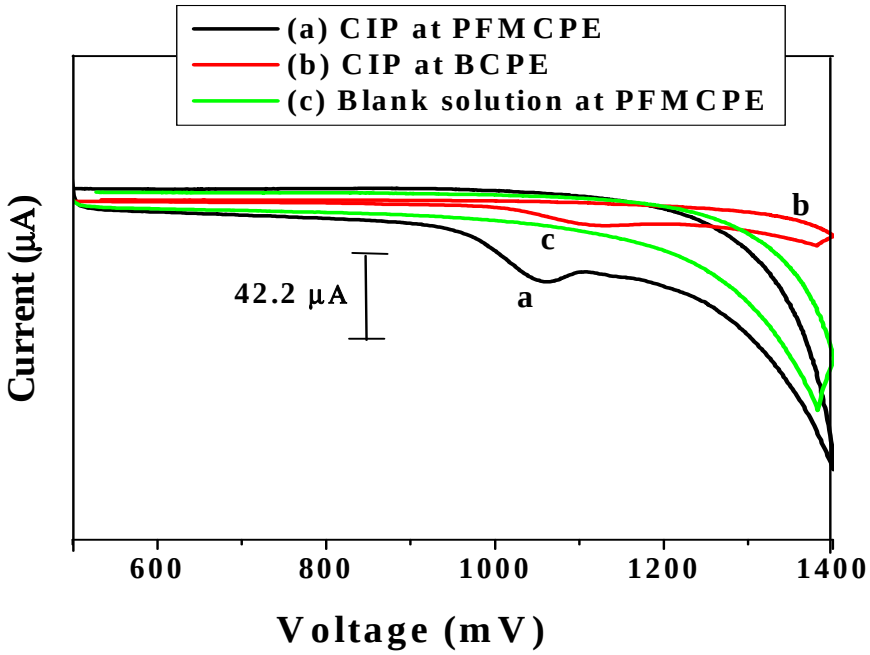


Fig. 4. Comparision of 0.1 mM CIP at poly (FUR) modified carbon paste electrode (a), bare carbon paste electrode (b) and blank solution in 0.1M phosphate buffer at poly (FUR) modified carbon paste electrode(c), scan rate of 10 mVs^{-1}

Electrochemical response of Enrofloxacin (ENRO) at poly (FUR) modified carbon paste electrode

Fig. 5 shows cyclic voltammograms of 0.1 mM ENRO at bare carbon paste electrode (curve 'b') and at PFMCPE (curve 'a'). The curve 'c' represents cyclic voltammograms of blank solution at PFMCPE. Two oxidation peaks at 891 and 1123 mV potential with a peak current 14.4 and 27.4 µA respectively at bare CPE, whereas two oxidation peaks at 891 and 1113 mV potential with peak current of 44.3 and 67.4 µA respectively at PFMCPE. The curve 'c' represents cyclic voltammograms of blank solution at PFMCPE in the potential range from -200 to 1400 mV. No reduction peak was observed in the reverse scan, suggesting that the electrochemical reaction is a totally irreversible process.

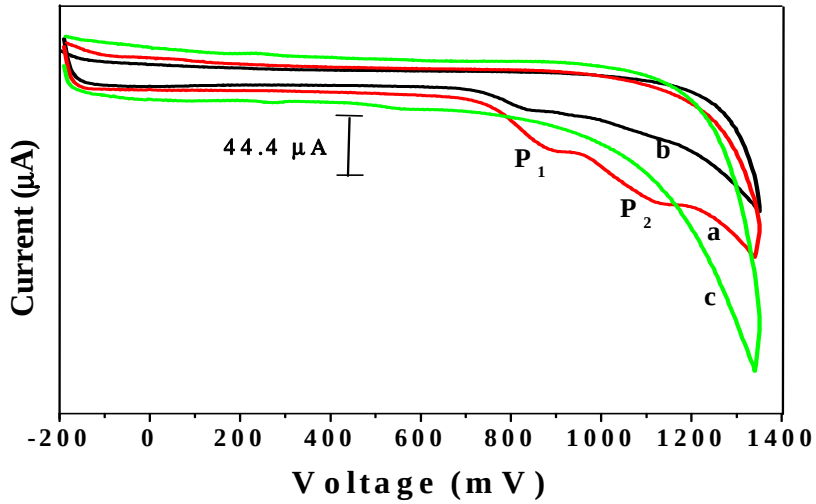


Fig. 5. Comparision of 0.1 mM ENRO at poly (FUR) modified carbon paste electrode (a), bare CPE (b) and blank solution in 0.1 M phosphate buffer at poly (FUR) modified carbon paste electrode(c), scan rate 50 mVs^{-1}

Effect of pH on CIP and ENRO

To optimize the electrochemical response of modified carbon paste electrode for the oxidation of CIP, the effect of pH on the electrode response was studied. As the pH increase from 2 to 9, the anodic peak current shifted towards the negative side the well oxidation peak arrived at pH 4.5 **(Fig. 6a)** and value of slope obtained was 42.23 mV/pH **(Fig. 6b)**. The plot of E_{pa} versus pH clearly indicates that the catalytic peak shift to a more negative potential with increasing the pH.

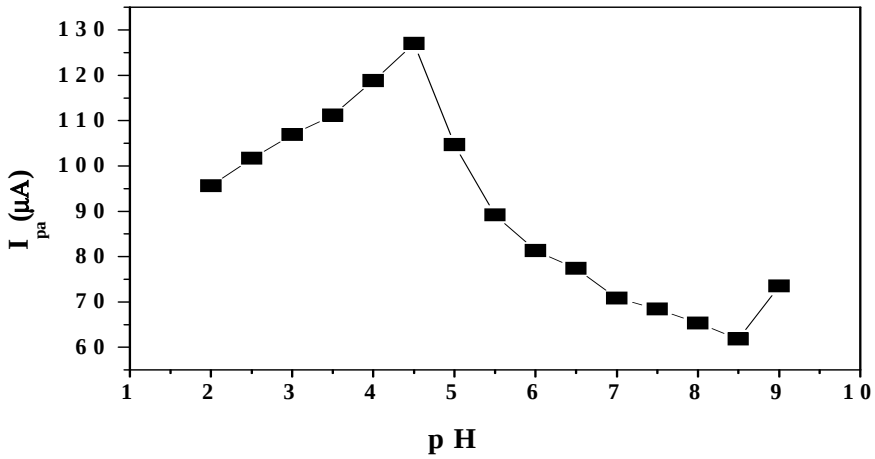


Fig. 6a. Plot of anodic peak current vs. pH (2 -9) of 0.1 mM CIP at the poly (FUR) modified carbon paste electrode

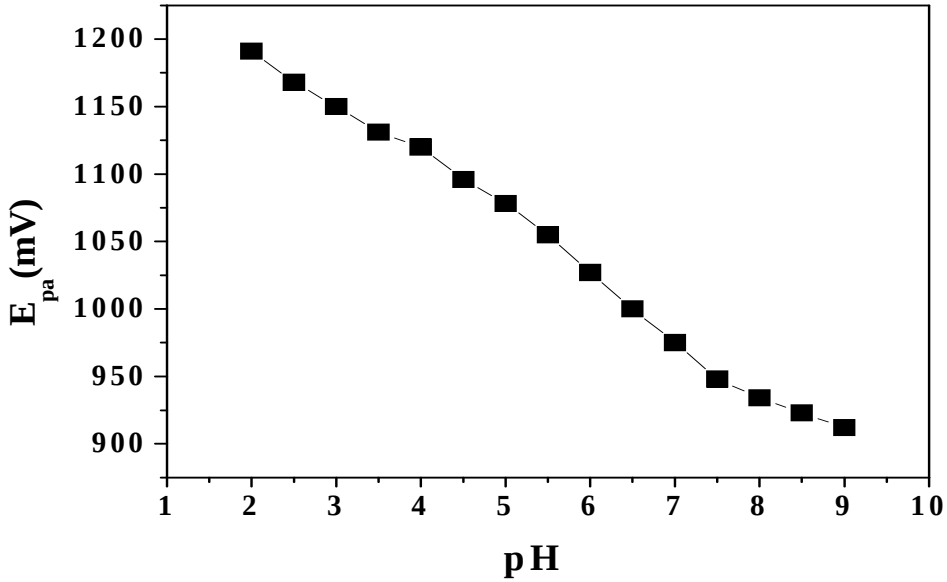


Fig. 6b. Plot of anodic peak potential vs. pH (2–9) of 0.1 mM CIP at poly (FUR) modified carbon paste electrode

To study effect of pH on ENRO, the electro oxidation of ENRO of 0.1 mM stock solution in 0.1 M PBS over pH range from 2 to 8.5 at a scan rate of 50 mVs^{-1} at PFMCPE using cyclic voltammetric technique has been studied. The anodic peak current decreases with increase of pH from 2 to 7 and becomes maximum and peak potential shifted negatively at pH 7. While pH beyond 7, 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. 7.**

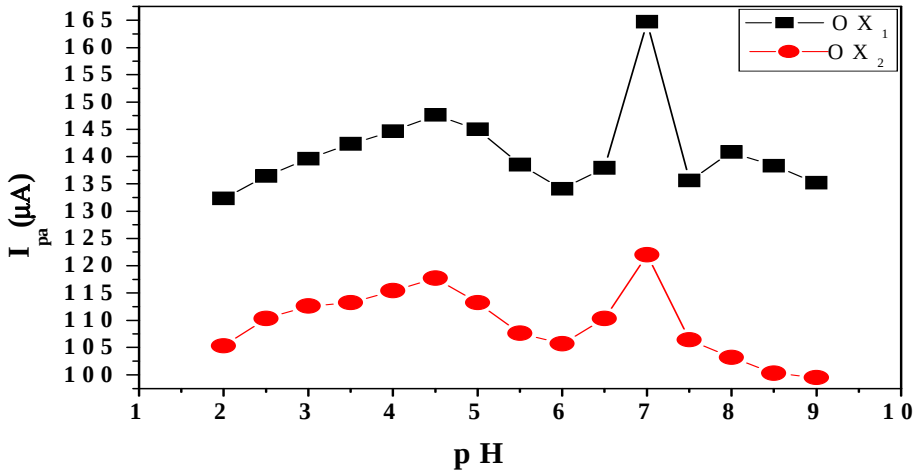


Fig. 7. Plot of anodic peak current vs. pH (2 - 9) of 0.1 mM ENRO at poly (FUR) modified carbon paste electrode

Effect of scan rate on CIP and ENRO

The effect of scan rates on the electrochemical response of CIP at poly (FUR) modified carbon paste electrode was studied and the cyclic voltammograms are shown in **Fig. 8a**. It was found that with the increase of the scan rate, oxidation peak current increased gradually and the oxidation peak potential shifted towards more positive potential. A linear relationship of anodic peak current versus scan rate in the range from 10 to 150mVs^{-1}with correlation coefficient of 0.9926 is shown in **Fig. 8b.** However, the linear relationship with a correlation coefficient of 0.9837 obtained between the anodic peak current and square root of scan rate in **Fig. 8c** with the linear regression equation is given by

$I_{pa}(\mu A) = 0.0128v^{1/2} + 17.380$ R = 0.9823……(1)

This revealed that a diffusion controlled process occurring at poly (FUR) modified carbon paste electrode.

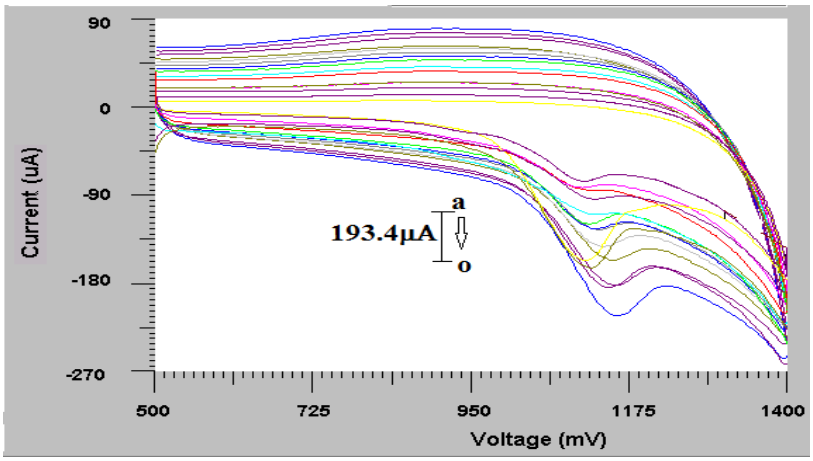


Fig. 8a. Cyclic voltammograms of 0.1 mM CIP at poly (FUR) modified carbon paste electrode with different scan rates (a) 10, (b) 20, (c) 30, (d) 40, (e) 50, (f) 60, (g) 70, (h) 80, (i) 90, (j) 100 (k) 110 (l) 120 (m) 130 (n) 140 (o) 150 mVs^{-1}

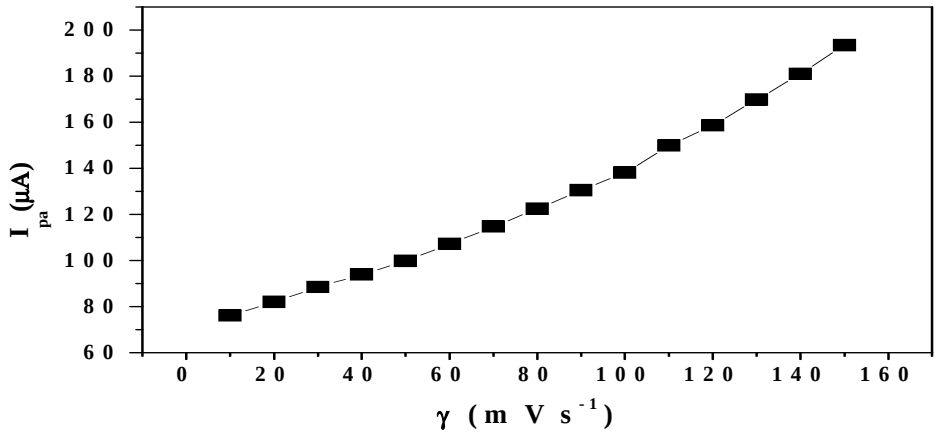


Fig. 8b. Plot of anodic peak current vs. scan rates of CIP at poly (FUR) modified carbon paste electrode

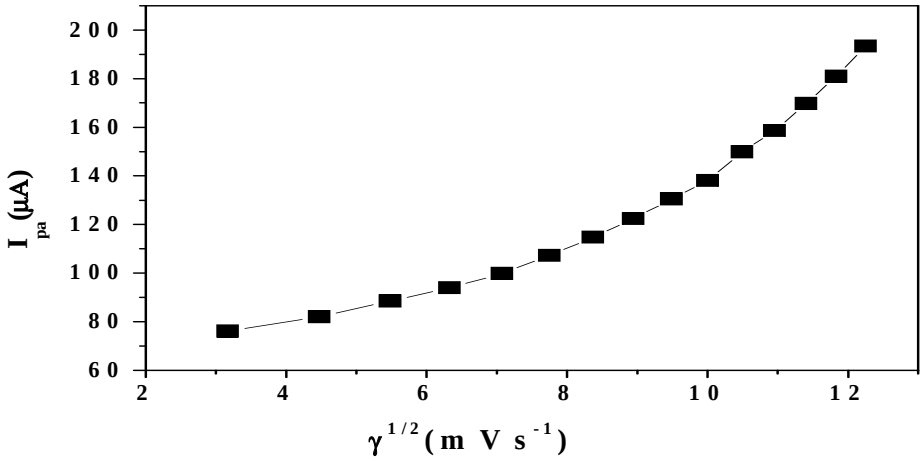


Fig. 8c. Plot of anodic peak current vs. square root of scan rates of CIP at poly (FUR) modified carbon paste electrode

According to Randles Sevick's equation, increases in the scan rate increase the peak current. Poly (FUR) modified carbon paste electrode showed increase in the peak current with increase in the scan rate from 10 to 100 mVs^{-1} in the presence of 0.1 mM ENRO in 0.1 M phosphate buffer solution of pH 7. The plot of anodic peak current against scan rate is shown in **Fig. 9a**.The corresponding linear regression equation is

$I_{pa}(\mu A) = 0.912\nu + 22.68$ R= 0.9957……. (2)

As shown in **Fig. 9b** the linear relationship with a correlation coefficient of 0.9837 obtained between the anodic peak current and square root of scan rate in the range of 10 -100 mVs^{-1}. The corresponding linear regression equation is

$I_{pa}(\mu A) = 0.12051\nu^{1/2} + 12.787$ R = 0.9837……(3)

This revealed that a diffusion controlled process occurring at poly (FUR) modified carbon paste electrode.

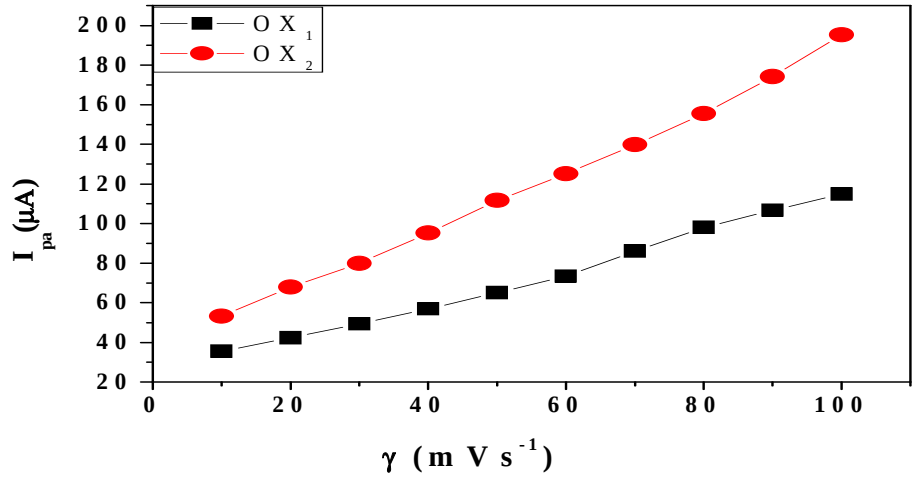


Fig. 9a. Plot of anodic peak current vs. scan rates of ENRO at poly (FUR) modified carbon paste electrode

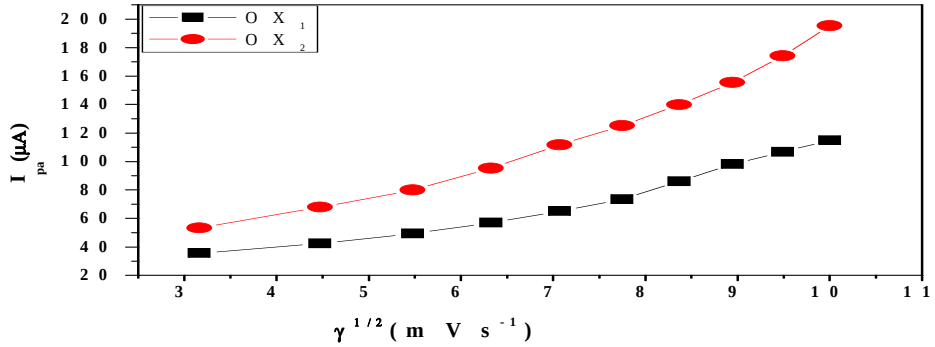
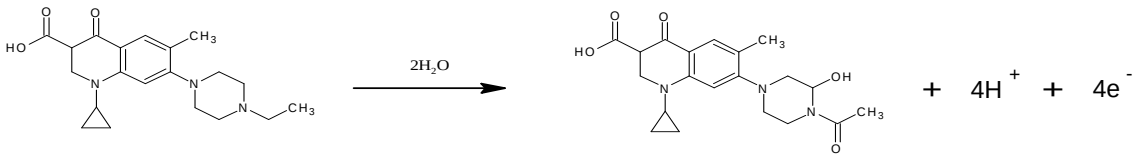
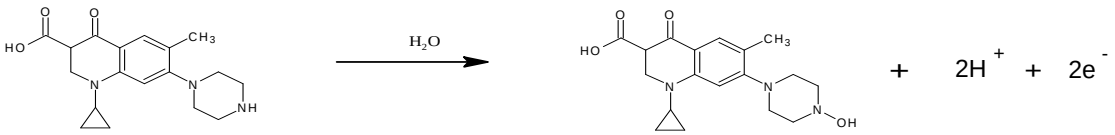


Fig. 9b. Plot of anodic peak current vs. square root of scan rates of ENRO at poly (FUR) modified carbon paste electrode

According to Laviron's theory [12], the slope is equal to $RT/\alpha n_{\alpha}F$. Then the value of αn_{α} was found to be 0.4542. For a totally irreversible electrode reaction process of CIP and ENRO the n_{α} was calculated as 1.646 and 3.705 respectively. This indicated that two electrons were involved in the oxidation process of CIP and ENRO at poly (FUR) modified carbon paste electrode. The electrochemical reaction process for CIP and ENRO at poly (FUR) modified carbon paste electrode can therefore be summarized as in **scheme I**.



ENRO



CIP

Scheme I: Probable oxidation mechanism of ENRO and CIP

Effect of Ciprofloxacin Hydrochloride and Enrofloxacin concentration at poly (FUR) modified carbon paste electrode (PFMCPE)

The effect of concentration of CIP and ENRO were studied at poly (FUR) modified carbon paste electrode in 0.1 M phosphate buffer solution of pH 6andpH 7.2with scan rate of 10 mVs^{-1} and 50 mVs^{-1} respectively by CV. The anodic peak current of CIP and ENRO increase with increase in concentration as shown in **Fig. 10a** and **Fig. 10b**. The plot of anodic peak current vs. concentration showed that concentration is proportional to electrochemical peak current is shown in **Fig. 11a** and **Fig. 11b**, a linear concentration range was found to occur from 8×10^{-5} to 1.0×10^{-3} M and can be described by the linear regression equations for CIP and ENRO respectively were expressed as:

I_{pa} (µA) = 94.794 C (10^{-5} M) + 32.379 R= 0.9915…….. (4)

I_{pa} (µA) = 39.108 C (10^{-5} M) + 35.108 R= 0.9936…… ……. (5)

The limit of detection (LOD) and limit of quantification (LOQ) were 1.508, 4.526 µM for CIP and 1.335, 4.451µM for ENRO. The LOD and LOQ were calculated on the peak current using the following equation: LOD= 3S/M and LOQ=10S/M, Where S is standard deviation and M is the slope of calibration plot.

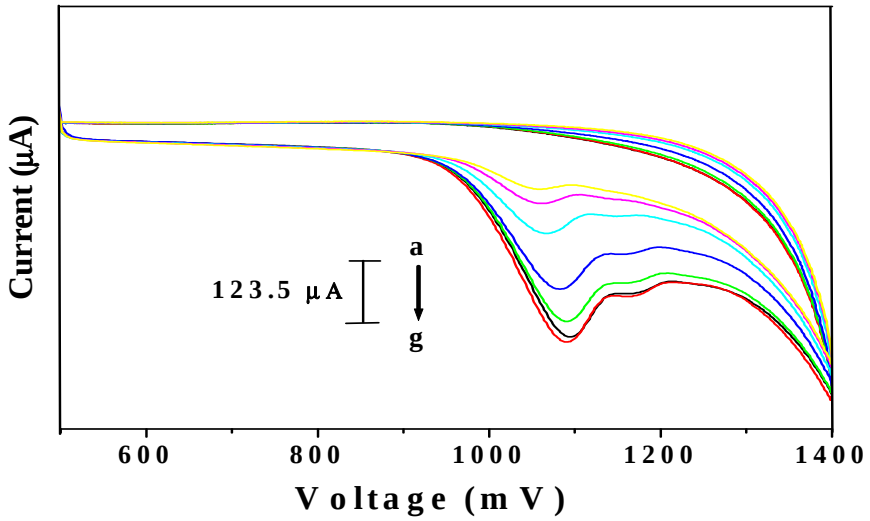


Fig. 10a. Effect of variation of concentration of CIP (a) 8×10^{-4} M, (b) 1×10^{-4} M, (c) 2×10^{-4} M, (d) 4×10^{-4} M, (e) 6×10^{-4} M, (f) 8×10^{-4} M, (g) 1×10^{-3} M on anodic peak current at poly (FUR) modified carbon paste electrode; scan rate 10 mVs^{-1}

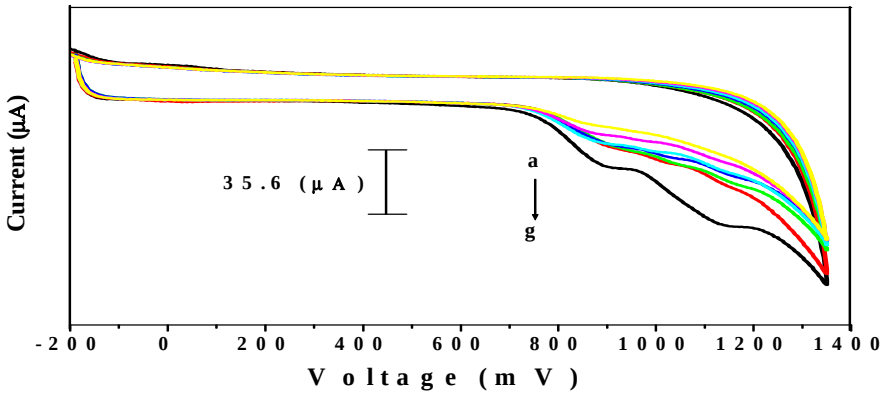


Fig. 10b. Effect of variation of concentration of ENRO (a) 8×10^{-4} M, (b) 1×10^{-4} M, (c) 2×10^{-4} M, (d) 4×10^{-4} M, (e) 6×10^{-4} M, (f) 8×10^{-4} M, (g) 1×10^{-3} M on anodic peak current at poly (FUR) modified carbon paste electrode; scan rate 50 mVs^{-1}

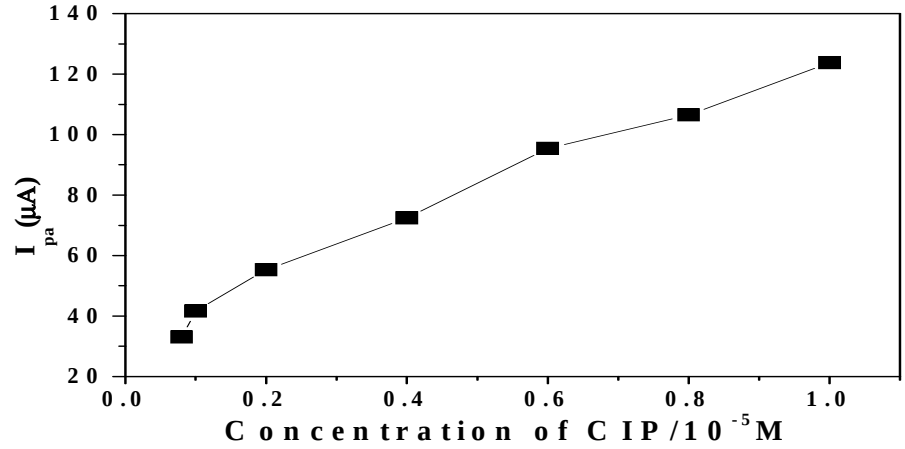


Fig. 11a. Plot of anodic peak current vs.CIP concentration at poly (FUR) modified carbon paste electrode

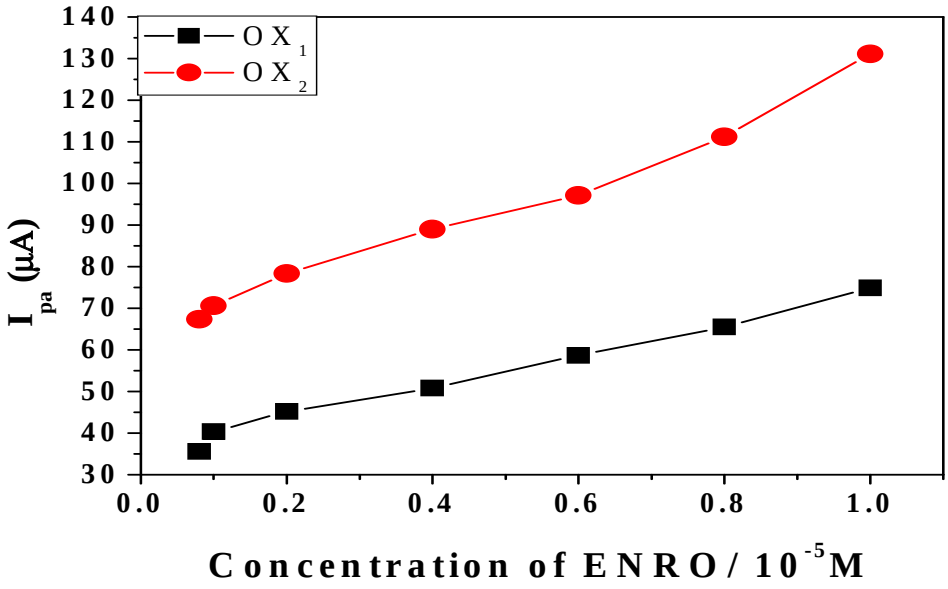


Fig. 11b. Plot of anodic peak current vs.ENRO concentration at poly (FUR) modified carbon paste electrode

Simultaneous detection of ENRO and CIP at ploy (FUR) modified carbon paste electrode (PFMCPE)

In order to examine the sensitivity and selectivity of poly (FUR) modified carbon paste electrode the electrochemical behavior of a mixture of 0.1mM ENRO and 0.5 mM CIP in 0.1M PBS of pH.7.2was investigated using cyclic voltammetry. **Fig. 12** shows the cyclic voltammograms obtained for ENRO and CIP coexisting at bare CPE and at PFMCPE. At bare CPE curve 'b'shows unable to separate the voltammetric signals of ENRO and CIP. Only broad voltammetric signals for ENRO and CIP were observed at approximately 1008 mV. Therefore it is impossible to use bare electrode for the voltammetric determination of CIP in the presence of ENRO. For the PFMCPE voltammetric signals are resolved into three peaks potentials at 840 and 970 mV for ENRO and at 1144 mV for CIP are observed (Curve 'a'). This is because ENRO exist as anionic form in pH 7.2, phosphate buffer solution and hence the electrostatic repulsion between the ENRO anions and the negatively charged groups on the electrode surface retarded the electron transfer and shifted the oxidation potential of ENRO towards more negative value so that the oxidation peak of CIP could be separated from that of ENRO. The ENRO is readily oxidized well before the oxidation potential reached, so the catalytic oxidation of ENRO is possible at the PFMCPE. The separation between the oxidation peaks of CIP and ENRO was 189 mV. Therefore the simultaneous determination of CIP and ENRO at poly (FUR) modified carbon paste electrode is possible.

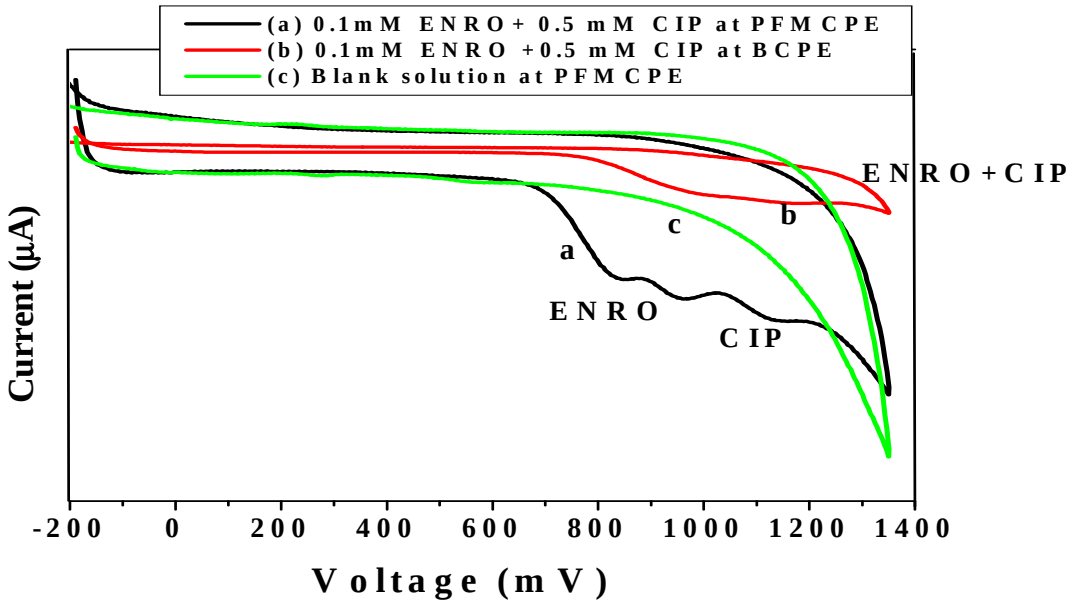


Fig. 12. Cyclic voltammograms at poly (FUR) modified carbon paste electrode (a), bare carbon paste electrode (b) in presence of 0.1 mM ENRO and 0.5 mM CIP, blank solution in 0.1 M PBS at poly (FUR) modified carbon paste electrode (c); pH 7.2, scan rate 50 mVs^{-1}

CONCLUSION

This modified electrode exhibited high electrocatalytic activities towards the oxidation of CIP and ENRO by significantly increasing their oxidation over potentials and enhancing the peak currents. The electrochemical response is diffusion controlled and irreversible in nature for CIP and ENRO respectively. The probable reaction mechanism involved in the oxidation of CIP and ENRO were also proposed. Peak separation between CIP and ENRO could be obtained using cyclic voltammetry, indicating that the poly (FUR) modified carbon paste electrode facilitated their simultaneous determination. This electrochemical sensor showed excellent selectivity and high sensitivity.

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