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***Original Research Article***

# Electrocatalytic Oxidation of Cefixime at the Surface of Modified Carbon Paste Electrode with Synthesized Nano Zeolite

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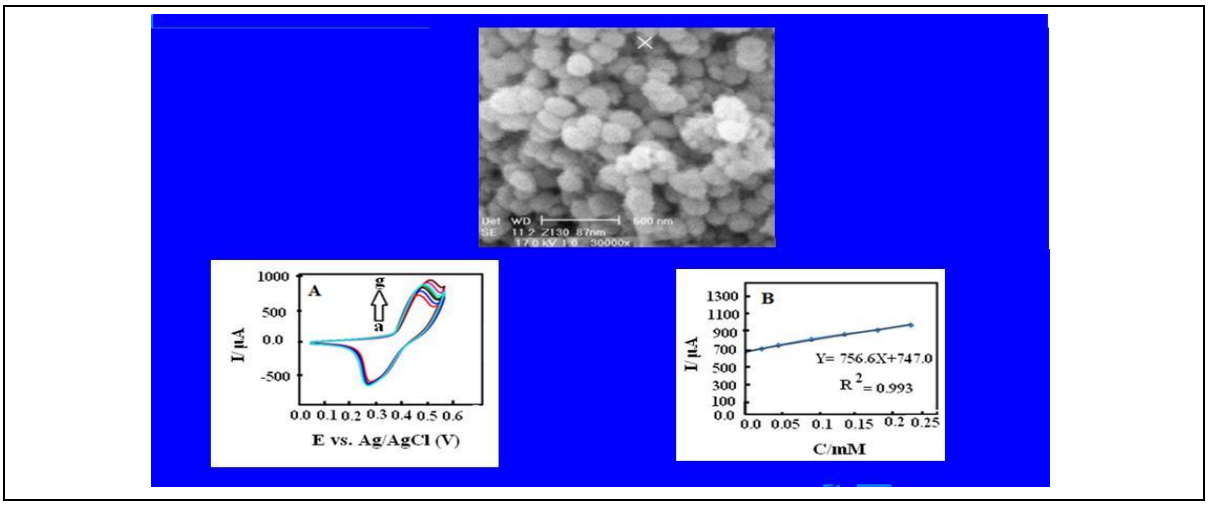
**ABSTRACT**

The aim of the present study was to determine the cefixime (CFX) through highly sensitive and simple electrocatalytic method. The electrocatalytic oxidation of CFX was performed on the surface of the modified carbon paste electrode (MCPE) with synthesized nano-sized ZSM-5 zeolite using the cyclic chronoamperometry and voltammetry methods. Also this work probed the application of the nano-zeolite in electrode structure and prepare zeolite MCPE. Due to the porous structure of the zeolite framework, the nickel (Ni) (II) ions were embedded into the zeolite framework through the immersing MCPE with synthesized zeolite in a 1.0 M Ni chloride solution. An excellent redox activity was practically seen for the $Ni^{2+}$/ $Ni^{3+}$ couple on the MCPE surface in alkaline solution. The Ni ions were acted as a mediator for the oxidation of CFX and catalyzed the electron transfer in this process. The CFX molecules were successfully oxidized on the surface of proposed electrode. The chronoamperometric method was used and catalytic reaction rate constant (K) was 3.5 $\times 10^6$ $cm^3/s^{-1}/mol^{-1}$ for the CFX oxidation. This electrocatalytic oxidation had a good linear response in the CFX concentration range of $25\times10^{-6}$– $25\times10^{-5}$ M with a regression correlation coefficient of 0.993, and the detection limit (3$\delta$) of the method was $26\times10^{-7}$ M. The diffusion coefficient of CFX molecules (D=$6.47\times10^{-5}$ $cm^2/s^{-1}$) was calculated based on the chronoamperometry studies.

**GRAPHICAL ABSTRACT**

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## Introduction

Cefixime (CFX) as an oral third generation cephalosporin antibiotic is useful to treat the susceptible infections caused by bacteria including gonorrhea, otitis media, bronchitis, pneumonia as well as throat, ear, lung and urinary tract infections. The CFX is obtainable as a trihydrate form [1]. Cephalosporins known as semi-synthetic antibiotics are a member of β-lactam family. The β-lactam antibiotics are most frequently applied in human medicine since they have low toxicity and broad antibacterial activity spectrum [2-4]. Due to their pharmacokinetic and antibacterial properties, the cephalosporins are extensively exploited in the clinical therapy to treat the severe detection [5-8]. The review of literature reveals that the CFX in pure form and its formulation have been quantified with various techniques and methods such as derivative spectrophotometry [9,10], fluorometry [11,12], high-performance liquid chromatographic-electrospray ionization-mass spectrometry [13-15], high-performance liquid chromatography [16], high-performance thin-layer chromatography [17], liquid chromatography–tandem mass spectrometry and electroanalysis [18] techniques. On the other hand, the quantification of these sulfur-containing compounds, measured by spectrophotometry is not valuable because these compounds are not active against light [19], and their derivatization is sophisticated [20,21]. Therefore, an easy and simple technique should be applied to immediately detect the cephalosporins. Since then, the electrochemical have been extensively used to quantify this component analysis methods due to their advantages [22,23]. The aim of the current study was to develop a simple electroanalytical method to determine these drugs in pharmaceutical and pure dosage forms. Cephalosporins contain sulfur. The electrochemical studies of sulfur-containing compounds were conducted using platinum, gold and carbon electrodes; however, severe working conditions may lead to offensive current signals and electrode poisoning. To solve this issue, different modified electrodes have been used and introduced in literature. Due to their low cost, wide potential window, low electrical resistance and ease of modification, the carbon-based electrodes usually have been applied in electrochemical quantifications [24]. The chemical modification of carbon electrodes has been done using inorganic or organic materials. Recently, the electrochemists are paying more attention to the zeolite modified carbon paste electrodes (MCPEs) [25]. The zeolite-modified electrodes as a class of chemically modified electrode (CME) have newly attracted much interest [26-29]. The zeolite-modified electrodes form a subcategory of the so-called CMEs, which have been mostly studied and promoted by Murray *et al*. [30]. Microporous zeolite materials have interesting physical and chemical properties including catalytic activity, sorption and ion exchange capacity in various reactions. There are three important reasons for using the zeolite to prepare CMEs: zeolite has advantage of ion-exchange voltammetry with unique molecular sieving properties, it is linked to the development of novel electroanalytical devices, and it is related to their potential use in electrocatalysis [31, 32]. Zeolites with particle sizes <100 nm are considered as nano-sized zeolites. The nano-sized zeolites than conventional micron-sized ones are more effective adsorbent materials and catalysts since the former has higher surface areas. Decreasing the particle size declines the diffusion path lengths, leading to make active sites more readily accessible. From the academic and industrial perspective, the ZSM-5 among all types of zeolite is significant owing to its thermal stability, unique shape selectivity, acidity, environmental catalysis and oil refinery [33]. In the present study, a novel MCPE was made

through incorporation of Ni(II) into exchanged synthesized nano-ZSM-5 zeolite and mixing with graphite powder ($Ni^{2+}$ ZSM-5 /ZMCPE). Next, the modified electrode was used to determine CFX in 0.1 M NaOH solution using chronoamperometry and cyclic voltammetry methods. The nano zeolite MCPE was applied to delineate the electrocatalysis of CFX for the first time. Obtained results have suggested that the proposed electrode is applicable for determination of CFX in pharmaceutical and pure forms.

## Experimental

### *Reagents and materials*

Tetrapropylammonium hydroxide (TPAOH, 40% in water), tetraethyorthosilicate (TEOS), sodium hydroxide and aluminum isopropoxide (AIP) were applied to synthesize the nano-ZSM-5 zeolite. Besides, all materials including graphite powder (particle diameter: 0.10 mm), used to fabricate the working electrode substrate and nickel chloride were purchased from Merck Company. Paraffin oil prepared from the Fluka Company was applied as the pasting liquid for the carbon paste electrode. Twice-distilled water as a solvent was exploited in the ongoing study. The rest reagents were belonged to analytical grade. The CFX tablets were provided by the Farabi Pharmaceutical Company, Isfahan, Iran.

### *Synthesis and characterization of Nano-ZSM-5*

Nano-sized ZSM-5 was synthesized at the optimum condition according to the literature method [33]. The molar composition of the prepared clear $TPAOH/Al_2O_3/SiO_2/H_2O/EtOH/Na_2O$ solution was 5:0.25:25:480:100:0.1. Appropriate amounts of $H_2O$, NaOH, TPAOH and aluminum isopropoxide were mixed and agitated at 0 °C for 4 h to achieve a clear solution. Then, the TEOS was added to drop wise and agitated at room temperature for several hours to achieve complete hydrolysis of TEOS. The achieved sol was heated in an oil bath at 90 °C under atmospheric pressure (reflux) for 48 h. The product was then recovered by centrifugation at 12,000 rpm for 30 min, washed with deionized water, and dried at 120 °C for 10 h. The ZSM-5 crystals were calcined at 550 °C under air flow to remove the template.

The Fourier transform infrared (FT-IR) and X-ray diffraction (XRD) spectra were recorded using FT-IR spectrometer (Vector 22-Bruker) and X-ray diffractometer (GBC MMA instrument, CuK$\alpha$ radiation, 28 mA). The morphology and particle size of the nanocrystals were evaluated using the scanning imaging microscopy (SEM, Philips XL30 electron microscopy) technique.

### *Fabrication of working electrode*

Totally, 0.5 g of the nano-ZSM-5 zeolite was ground and immersed in 10 mL, 0.1 M Ni (II) chloride $6H_2O$ solution for 24 h. The ion-exchanged nano zeolite was washed using deionized water to remove the surface-adsorbed species. Then, the solid was dried in an oven at 373 K for 5 h (NiZSM-5). A mixture of 30% of nano-sized NiZSM-5 and 70% of graphite powder and paraffin oil, blended through hand mixing using a mortar and pestle was used to prepare the zeolite modified carbon paste [NiZSM-5/CPE]. Then, the obtained paste was placed in the bottom of a glass tube (internal radius: 1.3 mm). A copper wire lead fitted into the glass tube was used to make electrical connection. For 10 min, the zeolite modified electrode was put at an open circuit in a well-stirred aqueous solution of 1 M $NiCl_2$ to incorporate the zeolite MCPE [Ni/NiZSM-5/CPE] into more Ni (II) ions. In the same way, the CPE was prepared for comparison, but the zeolite was omitted in addition step. Moreover, the similar way was applied to make the ZSM-5/CPE but with no doped Ni (II) ions.

Through chronoamperometry and cyclic voltammetry methods, the CFX oxidation was evaluated at NiZSM-5/CPE and CPE in a 0.1 M NaOH solution. Finally, the gained results were compared with each other.

*Electrochemical instrumentation*

The electrochemical experiments were conducted using a galvanostat/potentiostat (Auto Lab PG302N). A platinum wire as an auxiliary electrode and an Ag|AgCl|KCl (3M) electrode as a reference electrode were used in the present study. The working electrode was carbon paste electrode modified with nano zeolite (NiZSM-5/CPE).

*Real sample preparation*

The real sample analysis was done with testing of CFX tablets which were containing 400 mg of cefeximin this way, 3 tablets of CFX containing 400 mg of cefexim were completely ground and homogenized. An accurate and appropriate amount of the homogenized powder was poured into a 100-mLcalibrated flask which contained 50 Ml of 0.1 mol/$L^{-1}$ sodium hydroxide solution. The flask's contents were sonicated for 10 min. The undissolved excipients were eliminated using filtration; and next *via* the same supporting electrolyte, the contents were diluted to appropriate volume.

## Results and discussion

*Nano zeolite characterization*

The FT-IR spectrum of the synthesized zeolite is represented in Figure 1a. The bands around 820, 1000 and1200 $cm^{-1}$ were characteristics of $TO_4$ (T = Si, Al) tetrahedron units. The band near 739 $cm^{-1}$ was assigned to the symmetric stretching of external linkages and the one near 600 $cm^{-1}$ was attributed to a structure-sensitive vibration caused by the double five-member rings of the external linkages. The presence of absorption bands around 460 $cm^{-1}$ was characteristic of the ZSM-5 crystalline structure. The XRD pattern of the synthesized nano-ZSM-5 is illustrated in Figure 1b with comparisons of main peaks at $2\theta$=7.9, 8.9, 23.2 and 24.5 with the reference sample. The crystallization of almost pure ZSM-5 phase was determined [33,34]. The SEM image of synthesized ZSM-5 zeolite is represented in Figure 2. The formation of regular spherical crystallites with the mean particle size of 63 nm was found. The obtained results demonstrated that the nano MFI structure was synthesized with fine crystal size and shape.

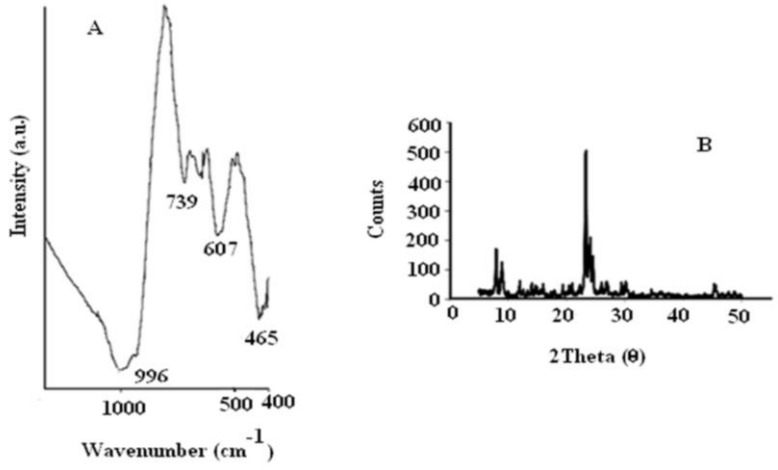
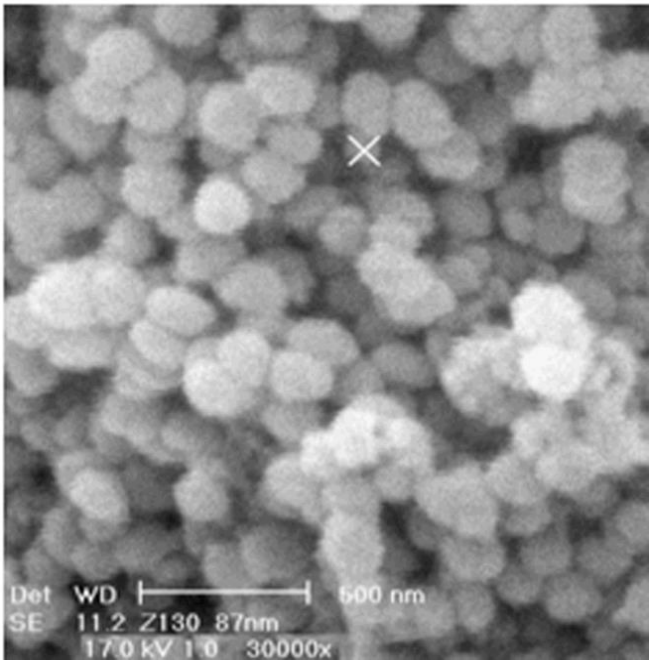



**Figure 1.** (A) FT-IR spectra and (B) X-ray pattern of synthesized nanozeolite

**Figure 2.** SEM image of synthesized nano zeolite




*Cyclic voltammetry studies*

*Electrochemical behavior of prepared working electrode (Ni/NZMCPE)*

Based on the external electron transfer of zeolite proposed by Walcarius, for the means of electron transfer of electroactive species onthe surface of modified electrode, the $Ni^{2+}$ ions in the zeolite pores of electrode moved to the surface of electrode via ion exchange between $Ni^{2+}$ ions in the zeolite framework and electrolyte cations ($Na^{+}$) [35].

$$Ni^{2+}_{(Z)} + 2Na^{+}_{(S)} \rightarrow Ni^{2+}_{(i)} + 2Na^{+}_{(Z)}$$

The subscripts of z, s and i explained the zeolite framework, solution and interface between the zeolite and solution, respectively. The oxidation of CFX was studied at the unmodified and modified electrodes by cyclic voltammetric experiments in 0.1 mol/$L^{-1}$ NaOH. The electrochemical response of CPE and Ni/NiZSM-5/CPE in the 0.1 M NaOH solution is illustrated in Figure 3. The electrochemical response of the CPE in the absence of CFX is displayed in Figure 3a). The addition of 0.25 mmol/$L^{-1}$ CFX to the alkaline solution had no effect on the electrochemical response of the CPE (Figure 3b) for analytical purpose. Well-defined stable redox peaks were observed for Ni/NZMCPE (Figure 3c). This phenomenon was ascribed to the oxidation of Ni (II) to Ni (III) with a peak potential of 0.59 V on the surface of modified electrode and to the decrease of Ni (III) to Ni (II) with a peak potential of 0.3 V vs. Ag/AgCl. After adding CFX (0.3 mmol/$L^{-1}$), the cathodic and anodic peak current decreased and increased, respectively (Figure 3d).The microprous structure of nano zeolite allowed the Ni ions to act as a good mediator for oxidizing the CFX molecules. This behavior as a typical observation which is expected from the mediated oxidation (EC mechanism) is illustrated in the following:

$$Ni(OH)_2 + OH^{-} \longrightarrow NiOOH + H_2O + e^{-}E$$

$$NiOOH + CFX \longrightarrow Ni(OH)_2 + \text{product}$$

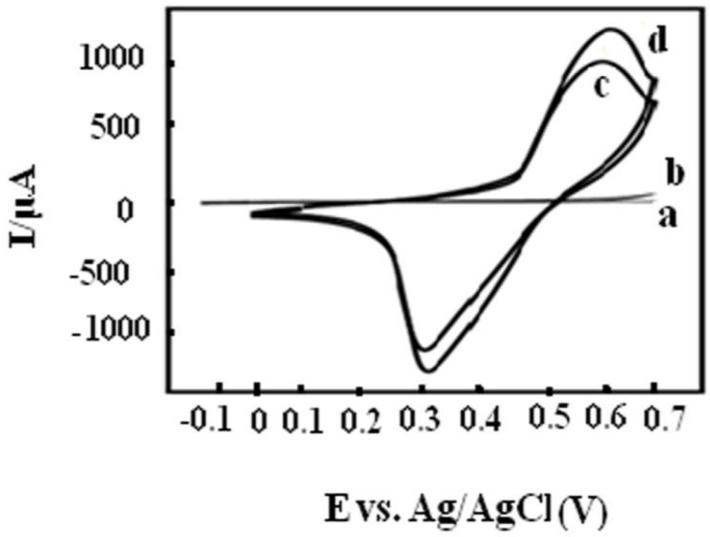



**Figure 3.** Cyclic voltammogram of carbon paste electrode (CPE) in the (a) absence and (b) presence of 0.25mM CFX and zeolite modified electrode (Ni/NiZSM-5/CPE) in the (c) absence and (d) presence of 0.25mM CFX in 0.1 M NaOH at scan rate of 20 $mVs^{-1}$

*Electrocatalytic determination of CFX*

To further clarify the electrochemical oxidation mechanism of CFX on the modified electrode, the effect of the CFX concentration on the cyclic voltammetric responses of this proposed electrode was studied (Figure 4a). As illustrated in Figure 4A, the Ni/NiZSM-5/CPE represents the linear increase of a well-defined catalytic oxidation current when the CFX concentration enhances. The calibration plot for the analysis of CFX (Figure 4A) displays a linear dependence of the anodic peak current on CFX concentrations in the range of 0.025–0.25 $mmol/L^{-1}$ and a regression correlation coefficient of 0.993. A limit of detection (LOD) of 0.0026 $mmol/L^{-1}$ was obtained from three times the standard deviation of the blank per the slope of the calibration plot.

*Effects of scan rate in presence of CFX*

Cyclic voltammograms of the Ni/NiZSM-5/CPE at the presence of 0.25 $mmolL^{-1}$ CFX at various scan rates were recorded at ν = 10–100 $mV.s^{-1}$ (Figure 5A). As the scan rate of potential was enhanced, the peak potential for the catalytic oxidation of CFX increasingly changed into positive potentials, indicating that there was a kinetic limitation in the reaction between the redox sites of the CFX and Ni/NiZSM-5/CPE. Also, there was a linear relation between the anodic peak current at the modified electrode and scan rate in the range of 10–100 $mV.s^{-1}$ ($y = 19.88x + 873.2$, $R^2=0.993$). This linear behavior might demonstrate that the peak current resulted mainly from the surface adsorbed species, representing the formation of the Ni (II) CFX complex on the surface of the modified electrode (Figure 5B). To achieve information on the rate determining step, b as a tafel slope was drawn based on the following equation, which is valid for a totally irreversible diffusion controlled process [36].

$$E_p = b/2 \log \nu + \text{constant} \quad (1)$$

On the basis of the mentioned equation, the slope of $E_p$ versus log ν plot is b/2, where b indicates the inverse of tafel slope. Figure 5c illustrates that the slope of the $E_p$ versus log ν plot is 0.053V for CFX and b=0.106 V. This slope indicated that a one-electron-transfer process was the rate-limiting step, supposing a transfer coefficient of $\alpha$=0.55 for CFX.

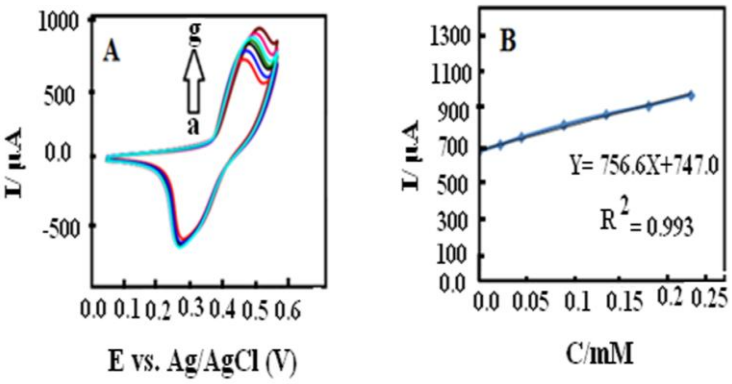



**Figure 4.** (A) Current–potential curves for oxidation of CFX at the Ni/NiZSM-5/CPE in 0.1 mol/L$^{-1}$NaOH solution at scan rate of 20 mV.s$^{-1}$ with different concentrations of CFX: (a) 0.0, (b) 0.025, (c) 0.05, (d) 0.1, (e) 0.15, (f) 0.2 and (g) 0.25 mmolL$^{-1}$ (B) Plot of $I_p$ versus CFX concentration

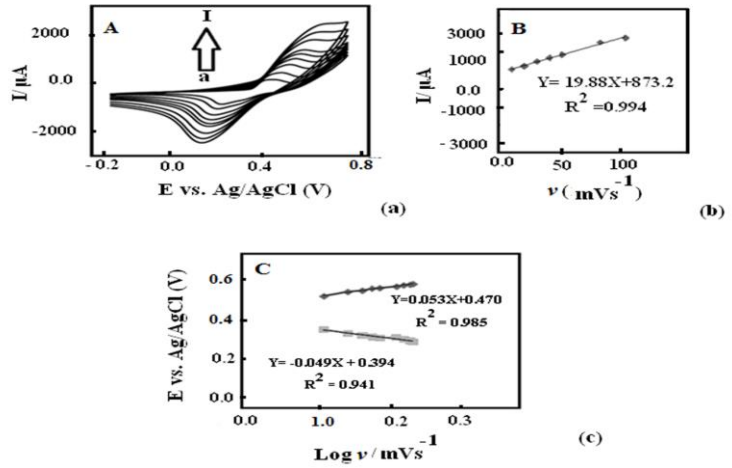



**Figure 5.** (A) Cyclic voltammogramsof 0.25 mol L$^{-1}$ CFX at Ni/NiZSM-5/CPE with different scan rates (a–l) 10, 20, 30, 40, 50, 70, 90, 100,110,120,130 and 140 mV.s$^{-1}$, respectively. (B) Variations of $I_{pa}$ versus ν. (C) Dependence of the peak potential, $E_p$ on log ν for the oxidation of CFX at the Ni/NiZSM-5/CPE

*Chronoamperometric studies*

Chronoamperometry was investigated onthe surface of the Ni/NiZSM-5/CPE electrode for evaluating the rate constant of the electrocatalytic oxidation of CFX using Cottrell equation. Figure 6 displays the chronoamperograms of CFX at Ni/NiZSM-5/CPE. Figure 6 represents the current-time profiles which were obtained through setting the working electrode potential at 0.6 V (in first step) and 0.1 V (in second step) versus Ag/AgCl in the 0.1 M NaOH solution for different concentrations of CFX. For the chemical reaction, the rate constant between the redox sites and CFX at the surface of Ni/NiZSM-5/CPE was evaluated using chronoamperometry in accordance with the method described in the literature [37].

$$I_C/ I_L=\gamma^{1/2} [\pi^{1/2} \text{erf}( \gamma^{1/2}) + \exp(-\gamma/(\gamma^{1/2}] \quad (2)$$

Where $I_C$ is the catalytic current of the Ni/NiZSM-5/CPE in the presence of CFX, $I_L$ is the limiting current in the absence of CFX and $\gamma = KC_0t$ ($C_0$ is the bulk concentration of CFX) is the argument of the error function. In the cases, where $\gamma$ is more than 1.5, the error function is almost equal to 1, and the equation (2) can be transformed and reduced to:

$$I_C/I_L= \gamma^{1/2}\pi^{1/2} = \pi^{1/2} (K C_0 t)^{1/2} \quad (3)$$

Where $C_0$, K, and t are the CFX concentration (mol/cm$^{-3}$), catalytic rate constant (cm$^3$/s$^{-1}$/mol$^{-1}$) and time elapsed (s) respectively. From the slope of the $I_C/I_L$ versus $t^{1/2}$ plot, the value of K can be simply calculated for a given concentration of CFX. Figure 6b exhibits one such plot constructed from the chronoamperogram of the Ni/NiZSM-5/CPE in the absence and presence of 2.5 mM CFX. The mean value was 3.5× 10$^6$ cm$^3$/s$^{-1}$/mol$^{-1}$ for K.

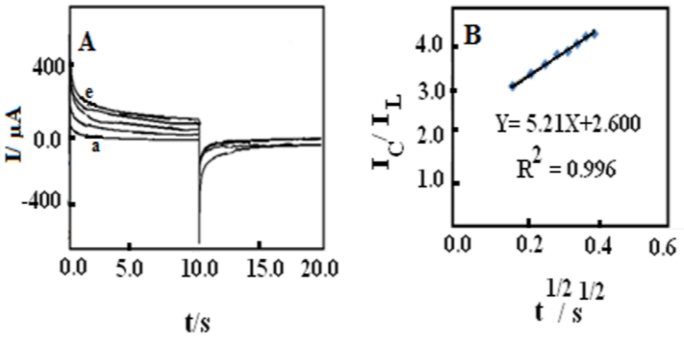



**Figure 6**. Thechronoamperograms obtained at the Ni/NiZSM-5/CPE in the absence (a) and presence of (b) 0.5, (c) 1.0, (d) 1.5, and (e) 2.5 mmol$L^{-1}$ of CFX in 0.1 M NaOH solution. First and second potential steps were 0.6 and 0.1 V vs. Ag/AgCl, respectively. (B) Dependence of $I_C/I_L$ on $t^{1/2}$, derived from the data of chronoamperograms of (a) and (e) in (A)

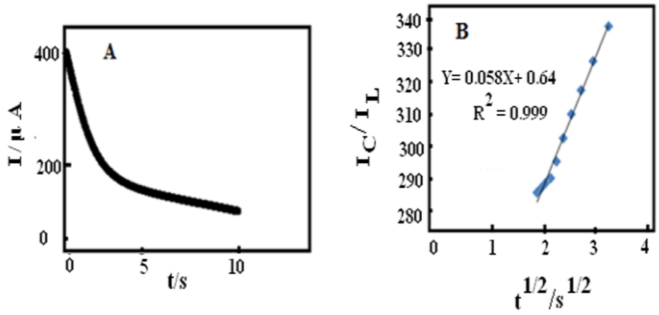



**Figure 7.** (A) Chronoamperogramof 2.5mM of CFX at the surface of modified electrode in 0.1 M NaOH solution and (B) oxidation peak current versus $t^{-1/2}$ from (A)

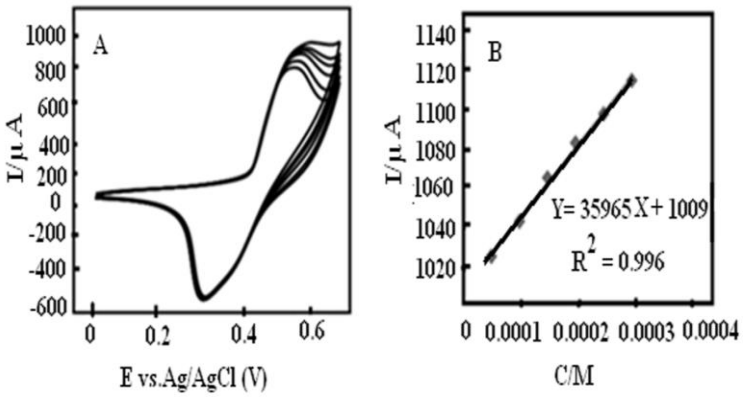



**Figure 8.** (A) The cyclic voltammograms of Ni/NiZSM-5/CPE in 0.1 M NaOH solution after addition of standard concentration of CFX , (a) 5 , (b) 10, (c) 15, (d) 20, (e) 25 and 30 mM, (B) the anodic peak current versus CFX concentration, derivated from A

The diffusion coefficient of CFX molecules was delineated through studying the chronoamperometry for oxidation process of CFX at the surface of modified electrode (Figure 7).

According to the Cottrell equation (Equation 4):

$$I = nFAC^{*}D^{1/2}\pi^{1/2}t^{-1/2} \quad (4)$$

The proposed electrode with the surface area of A= 0.053 $cm^2$ and electron transfer n=1, the diffusion coefficient was calculated to be $6.47\times10^{-5}/cm^2/s^{-1}$.

*Real sample analysis*

As the electrochemical sensor has high selectivity, the CFX can be determined in real samples. The CFX was delineated via standard addition method in tablet for evaluating the applicability of the proposed sensor (Figure 8). It was observed that the drug concentrations which were assessed by this method had a good compatibility with the

reported values. The reported values in vials and tablets as well as the values of drugs experimentally assessed are illustrated in Table 1. The current work can be compared with other works in Table 2.

**Table 1.** Determination of cefixim in pharmaceutical preparation (n = 6)

| Compounds | Amount labeled (mg) | Amount found (mg) | RSD (%) | Recovery (%) |
|---|---|---|---|---|
| Cefixime (tablet) | 400 | 409 | 2.38 | 95 |

**Table 2.**Comparison of the current study with other studies

| Method | LOD | LDR | Reference |
|---|---|---|---|
| RF-HPLC | 0.05 µg/mL | 100 ng/mL-30 µg/mL | 38 |
| Spectrophotometry | 1.08 µg/mL | 10-130 µg/mL | 39 |
| Cyclic voltammetry | 1.3 µg/mL | 12.6–25×126.7 µg/mL | This work |

## Conclusion

The novel, specific, precise, simple and accurate electrochemical method using the modified electrode was developed and validated to determine the pure form and pharmaceutical formulation of CFX. The zeolite MCPE was doped with Ni ions using theion exchange method, and Ni ions acted as a suitable catalyst for electrooxidation of CFX. By using the cyclic voltammetry and chronoamperometry methods, the kinetical parameters of CFX such as catalytic reaction rate constant and charge-transfer coefficient for oxidation of CFX were attained. Based on the experimental results, the catalytic oxidation current of CFX at the Ni/NiZSM-5/CPE can be applied to determine the CFX in the aqueous solution so that the good linear dynamic range of $25\times10^{-6}$– $25\times10^{-5}$ M and detection limit of $26\times10^{-7}$M can be obtained. The proposed electrode can be prepared and used to determine the CFX in the pharmaceutical products.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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