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β-Cyclodextrine Modified Carbon Paste Electrode as a Selective Sensor for Determination of Piroxicam Using Flow Injection Cyclic Voltammerty

Parviz Norouzi*, Nazanin Ghaheri

Center of Excellence in Electrochemistry, Faculty of Chemistry, University of Tehran, Tehran, Iran

* Corresponding Author; Tel.: +98-21-61112788; Fax: +98-21-66495291

E-Mail: Norouzi@khayam.ut.ac.ir

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Abstract- A carbon paste electrode was modified with β-cyclodextrin to form a selective electrochemical sensor for determination of Piroxicam (PX). A novel sensitive technique which called fast Fourier transform cyclic voltammetry (FFT CV) was used for measurements of redox current of the component. To have a more defined and intense peak current for piroxicam determination, Tris-HCl buffer (pH=8.0; 0.05 mol L^{-1}) was used as supporting electrolyte. In this aqueous media, PX has been selectively accumulated on the surface of electrode by CD as a modifier. The drug presented one irreversible oxidation peaks at about 500 mV on modified carbon paste electrode vs. Ag/AgCl which produced a higher current. This property was used for analytical purposes by developing a stripping FFTCV method to determine piroxicam. Furthermore, signal-to-noise ratio has significantly increased by application of discrete fast Fourier Transform (FFT) method, background subtraction and two-dimensional integration of the electrode response over a selected potential range and time window. The effective parameters were optimized. As a result, CDL of 5.0×10^{-9} mol L^{-1} and LOQ of 8.0×10^{-9} mol L^{-1} were obtained. The electrode applied for determination of PX in urine and plasma and formulation samples. The recovery for spiked assay in urine and plasma samples was obtained 101-105% respectively and a good quantification of piroxicam was achieved in a commercial formulation. The effect of some interfering substances was investigated and electrode showed a good selectivity to piroxicam.

Keywords: β-cyclodextrne, Fast Fourier Transformation, Cyclic Voltammetry, Carbon Paste electrode, Piroxicam, Oxidation-Reduction, Sensor

1. INTRODUCTION

Molecular recognition at solid materials surface has attracted in these days. Researchers are trying to realize functional materials for chemical sensors. [1-3]. Cyclodextrins are non-reducing cyclic glucose oligosaccharides resulting from the cyclomaltodextrin glucano-transferase, catalyzed degradation of starch. The binding of cyclodextine is driven by the enthalpic and entropic gain on the reduction in the hydrophobe-aqueous surface and the release of water molecules from the cavity to the bulk phase. Such binding also allows cyclodextrins to be used to increase the water solubility of normally hydrophobic compounds or minimize undesirable properties such as odor or taste in certain food additives. Cyclodextrin complexes are now widely used in the pharmaceutical, food and cosmetic and toiletry fields [4]. The cavities have different diameters dependent on the number of glucose units. CD is also useful as biomimetic enzyme models [5-7], drug delivery systems [8] and electrode reaction modifiers [9]. The use of CDs for modified electrodes is a new challenge in the field of the modified electrodes. There are few published articles revealing carbon paste electrode modified with β-cyclodextrin [10, 11], furthermore, a review about immobilization of cyclodextrins, complexation abilities and analytical applications has been published [12]. In order to utilize possibilities of CD for modification of electrode as selective sensor, we investigate the electrochemical behavior of the piroxicam at modified β-CD carbon paste electrode to develop an electroanalytical method to determine this drug.

Piroxicam (PX) (4-hydroxy-2-methyl-N-2-pridyl-2H-1, 2-benzothiazine-3-carboxadiamide -1,1-dioxide) belongs to a class of non-steroidal pharmaceuticals with anti-inflammatory prope rties which are used to treat rheumatoid, arthritis and post operative inflammations. PX belongs to the oxicam group a class of enolic acids. Piroxicam is used to reduce the pain, inflammation, and stiffness caused by rheumatoid arthritis and osteoarthritis. Many diverse properties have been exhibited by oxicam group of drugs such as chemoprevention, chemosupression [13-15] and UV induced photosensitization of the skin [16]. The chemoprevention effect of PX is attributed to its property of reducing levels of prostaglandins which are associated with carcinogenesis in the colon [17]. The properties and therapeutic efficacy of PX have been extensively described [18, 19]. Many methods have been used for determination of piroxicam such as liquid chromatography-mass spectroscopy [20], thin layer chromatography [21], spectrofluorimetry [22], sensitive liquid chromatography determination [23], UV-high performance liquid chromatography [24], high performance thin layer chromatography [25], differential pulse voltammetric method [26]. At the other reported articles, it made clear that PX could be more effective in presence of β-CD [22,27] also too many clinical attempt had been done for use of PX in combination of CDs.

The method which introduced in this paper is very sensitive, inexpensive and fast for detection of piroxicam. The fast fourier transform cyclic voltammetry (FFTCV) has recently been shown to be advantageous for environmental detection of several compounds [28-34]. The adaptation of this technology to piroxicam CV on a (cyclodextrine carbone paste electrode) CD/CPE could provide a substantial improvement for rapid and very sensitive analysis. Carbon-paste electrodes (CPEs), due to their ease of construction, renewability, and compatibility with various types of modifiers, and low background current compared to the solid graphite have been widely used as a suitable matrix [35]. In this paper a feature was designed to separate the voltammetric signal and background signal in time domain by using discrete fast Fourier transformation (FFT) method for determination of PX by using a CD-modified carbon paste electrode.

2. EXPRIMENTAL

2.1. Instrumentation

The Electrochemical instrument, ultra voltammetry, a homemade potentioastate were used for the present voltammetric measurements. All electrochemical experiments were done using a setup comprised of a PC PIV equipped with a data acquisition board (PCL-818H, Advantech Co.) was used to output an analog waveform to the working electrode and acquire current readings from the working electrode that connected to a custom made potentiostat. The card and accompanying dynamic link libraries allowed waveform generation and current sampling to be synchronized, which was essential in interpreting CV current response. The data could be interpreted in real time, or stored data could be loaded and reanalyzed to generate electropherograms. The algorithms used to interpret the current response from each waveform cycle were discussed before [36]. A typical three-electrode cell with Ag/AgCl as reference electrode, a platin wire as a counter electrode and the modified CPE as a working electrode was used. The pHs are adjusted using the Metrohm pH Meter Model 810 equipped with a combined glass electrode, which is calibrated regularly with buffer solutions (pH 4.00 and 8.00) at 25±1^{o}C.

2.2. Carbon paste electrode preparation

The modified carbon paste electrode was prepared from the spectral carbon powder. β-cyclodextrin and white waseline was purchased from Merck Co. (Germany). The CD-modified Carbone paste electrode CD/CPE) was prepared by hand-mixing of 1.25 g graphite powder and 0.45 g white waseline by addition of 200 μl of the CD solution (50 mg/ml) adequately in a gate mortar. The resulted mixture was then allowed to evaporate it water at about 60^{o}C. A portion of the resulting paste was then packed firmly into the electrode cavity (1.0 mm diameter) of a polytetrafluorethylene (PTFE) sleeve. The unmodified CPE was

prepared in a similar way without addition of CD. Electrical contact was established via a copper wire. The surfaces of all the modified and unmodified CPEs were carefully smoothed on weighing paper and rinsed with twice distilled water prior to each measurement.

2.2. Materials and reagents

All chemicals and reagents were of analytical grade quality. PX was a gift from Drug and Food quality control, Tehran, Iran. A stock solution of 1.0×10^{-5} mol L^{-1} of PX was prepared in doubly distilled water at 4°C. More dilute solutions were prepared daily with deionised water just before use. The phosphate buffer (pH 3-9), Tris-HCl, acetate and citrate buffer were prepared using analytical grade reagents (Merck, Germany). All the other solutions are in analytical grade and were made by double distilled water.

2.3. Stripping voltammetry

In this new method to improve the detector sensitivity, the FFT-CV technique was modified in the potential excitation waveform and current sampling and data processing that the potential program was shown in our previously papers [28]. The potential waveform consisted of three sections; a) electrode conditioning and b) accumulation part C) measurement the potential waveform contained three additional potential steps, E_{c1} to E_{c2} (for cleaning the electrode surface) and Es (for accumulation of PX). Based on our primary experiments PX can be adsorbed on the surface of electrode (repetitive cycles show decrease in current, data not shown). Most of the time the electrochemical method based on adsorptive behavior of component (stripping voltametry) are more sensitive. The time and the potential of adsorption of analyte were optimized. One of the important aspects of this method is application of a special digital filtration, which is applied during the measurement. In this method at the first, a CV of the electrode was recorded and then by applying FFT on the collected data, the existing high frequency noises were indicated. Finally, by using this information, the cutoff frequency of the analog filter was set at a certain value (where the noises were removed from the CV).

In potential ramp, the currents sampled and 4 first CVs of background stored and the current sampling of injection of drug and oxidation of it will compared by that first CVs. After preparing the solution, the measurements were carried out in the continuous fast Fourier transform stripping square wave voltammetric mode (FFTCV). A typical experiment consisted of three consecutive steps with the following experimental conditions: the pre-concentration at 0.4 V *vs.* Ag/AgCl for 0.2 s, sweep rate of 20 Vs^{-1} at the 0.05 M Tris-H|Cl buffer at pH=8.

2.4. Pharmaceutical preparation assay

Ten capsules (20 mg, Alhavi, Iran) were weighed in order to find the average mass of each capsule. Then the capsules were triturated and mixed. A 20.0 mg sample taken from the latter mixture was accurately weighed and dissolved in 25 ml of water/Ethanol in 50/1 ratio. The mixture was stirred and filtered into a 50.0 ml calibrated flask; the residue was washed several times with water. 1 ml of the filtered solution was diluted with 0.05 mol L^{-1} Tris-HCl in a 50 ml volumetric flask.

2.5. Determination of PX in human urine and plasma

1 ml of untreated urine containing 20 μg/ml PX was placed into a 10 ml volumetric flask and diluted with water to the mark. A 1 ml of this solution was diluted with pH=8 buffer solution to 10 ml into a volumetric flask. Then 20 μl aliquot was injected into the system.For the determination of PX in plasma, 100 μl aqueous PX solutions (50 μg/ml) were added to 100 μl of untreated plasma. The mixture was vortexed for 30 s. In order to precipitate the plasma proteins, the plasma samples were treated with 50 μl perchloric acid $HClO_4$ 15%. After that, the mixture was vortexed for a further 30 s and then centrifuged at 6000 rpm for 5 min. Then 50 μl aliquot of the obtained supernatant was injected into the system.The voltammograms were recorded according to the above recommended procedure. The voltammograms of samples without PX do not show any signal that can interfere with the direct determination and with the unchanged slope for calibration then, external calibration can be used.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of PX by using modified CD/CPE

A CPE containing 2 %w/w of β-CD, in comparison with CPE without CD, showed an effective activity in the electrochemical oxidation of PX. At Fig. 1 the schematic of β-CD was presented which shows the mechanism of interaction of PX with CD which confirmed by spectroscopic method [37].

Fig. 2 shows a relatively weak anodic wave for the electro-oxidation of PX on the surface of the unmodified electrode revealed that the electrode process is sluggish and it oxidized at potential about 500 mV. On the other hand, using CD-modified electrode, a well-defined and sharp anodic wave with a peak potential of 470 mV is obtained for PX. The anodic peak current at the CD/CPE is enhanced greatly by approximately 50% and is accompanied by a slightly negative shift about 30 mV in the oxidation potential. This indicates that the CD/CPE improves electrochemical reactivity toward the oxidation of PX compared with bare CPE. According to the literature, it is apparent that PX (ca. 6.0 Å wide and 13.7 Å long) is a little large to fit completely in cavity of CD but it can have an interaction with PX because PX is a molecule with electron donor groups able to form hydrogen bonds within the CD cavity [21].

The above differences in the voltammograms can be ascribed to the immobilization of the β-cyclodextrin on the carbon paste that leads to a modification of the electrode surface that cause a significant increase in the signal due to the drug accumulation on electrode modified with CD.

Specifically interaction of β-CD with PX has been demonstrated by spectroscopy [21]. After accumulation in open circuit, the effect of accumulation potential was investigated between -500-600 mV and the current was increased more. Thus, the optimized accumulation time and potential was studied which is explained in section 3.2.

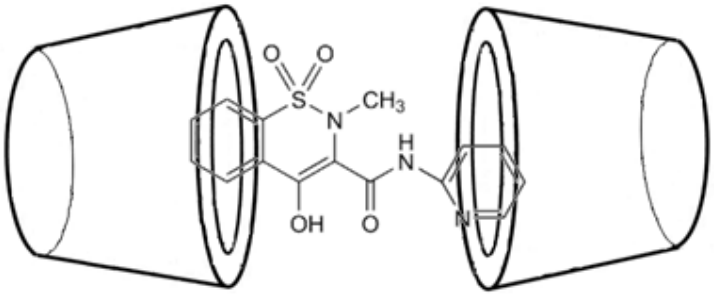

Fig. 1. The schematic of β-CD and PX interaction

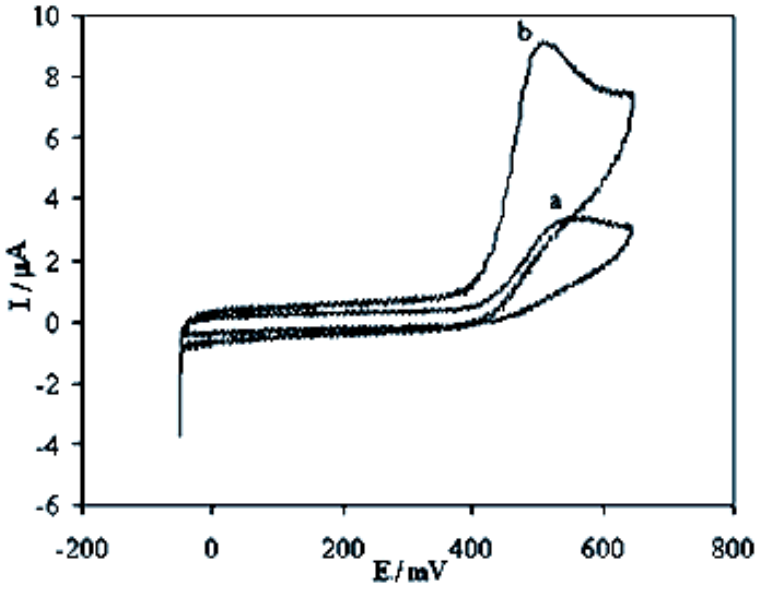

Fig. 2. The cyclic voltammogram of PX on the surface of a) bare and b) CD modified carbon paste electrode. [PX] = 1.0×10^{-6} M in 0.05 M Tris-HCl pH=8

3.2. Effect of buffer type and pH

PX is an enolic acid with a pK_a of 6.3 Therefore, when PX is dissolved in aqueous solvent medium, equilibrium is present between the dissociated and undissociated PX forms. However, since the purpose of this work was to determine piroxicam it is necessary to know the effect of pH on the response of PX oxidation. The influence of the solution of Tris-HCl buffer (pH 3.0–9.0), phosphate buffer (pH 3.0–9.0) and acetate buffer (pH 3.0–9.0) was also analyzed in the response of the peak current after pre-concentration of 20 s at 0.3 V. The results show the well defines shape of voltammogram and higher current at Tris-HCl buffer, so this buffer selected as a supporting electrolyte for further study. The dependence of peak current (I_p) and peak potential (E_p) of PX at concentration of 1.0×10^{-5} mol L^{-1} on pH was shown in Fig. 3 (a, b). As it can be seen, I_p is higher at pH value of 8. Furthermore, from Fig. 3(b) it was found that the peak potential is shifted to more negative values with increasing pH of 3.0-9.0, which could indicate the presence of chemical reaction (proton-transfer reaction) precedes the electrode process. Following equation displays correlation between peak potential and pH:

$$E(mV)=-24.6\ pH+671.47$$

The slope is close to that expected for a two electron/one proton reaction, at 25°C. That is 0.0592 (h/n) V/pH, where h and n are the number of protons and electrons involved in the electrode process [39]. This is in accordance with the literature data about mechanistic scheme of phenol moiety compounds to changed hydroxyl group to quinon group [40,41].

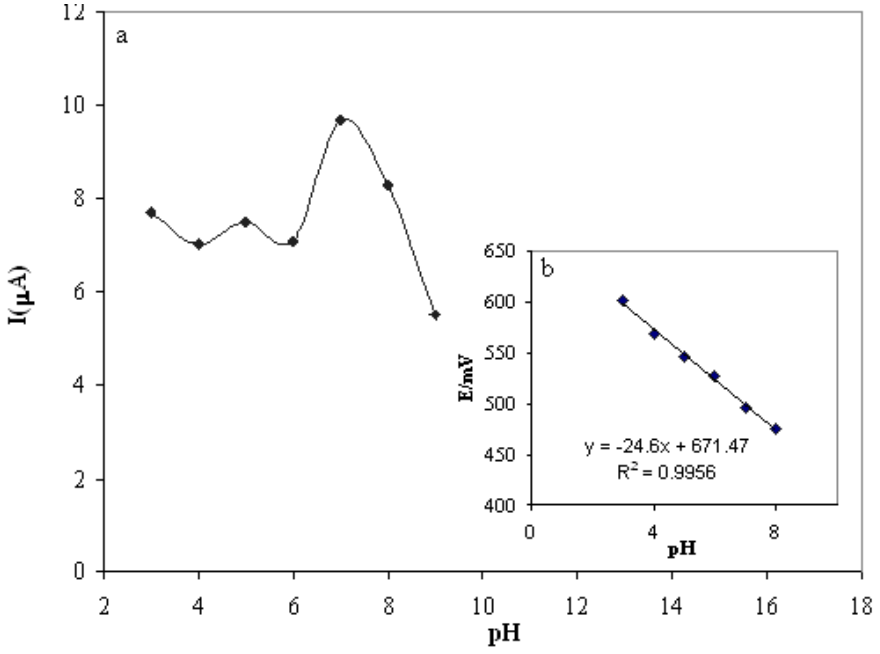

Fig. 3. Effect of pH on the oxidation of PX, A) The dependence of current to pH, [PX]=1.0×10^{-6} min 0.05 M Tris-HCl pH=8 and B) The dependence of anodic potential of PX to pH; the results are based on five replicate measurements

3.3. Optimization of sweep rate and flow arte

In order to investigate the influence of the scan rates and the eluent flow rate on the sensitivity of the detector response, solutions having a concentration of 1.0×10^{-6} mol L^{-1} of PX were injected. At different scan rates (from 10 to 30 Vs^{-1}) and the eluent flow, the responses of the detector to the injected sample were recorded. The flow rate of eluent was checked to get the best S/N between 0.5-3.0 mL min^{-1}. The results are presented in Fig. 4.As it is clear from the Fig. 4.the detector exhibits the maximum sensitivity at 15 $Vs^{-1}$of scan rate and 0.5 ml/min of the flow rate. The effects of the sweep rate on the detection performance can be taken into consideration from three different aspects: first, speed in data acquisition, second, kinetic factors of oxidation of the PX, and finally the flow rate of the eluent which controls the time window of the solution zone in the detector. The main reason for investigation of scan rates is to find the best sweep rate which did not make any limitation for redox behavior of PX and also get enough data during passing the PX zone on front of electrode.

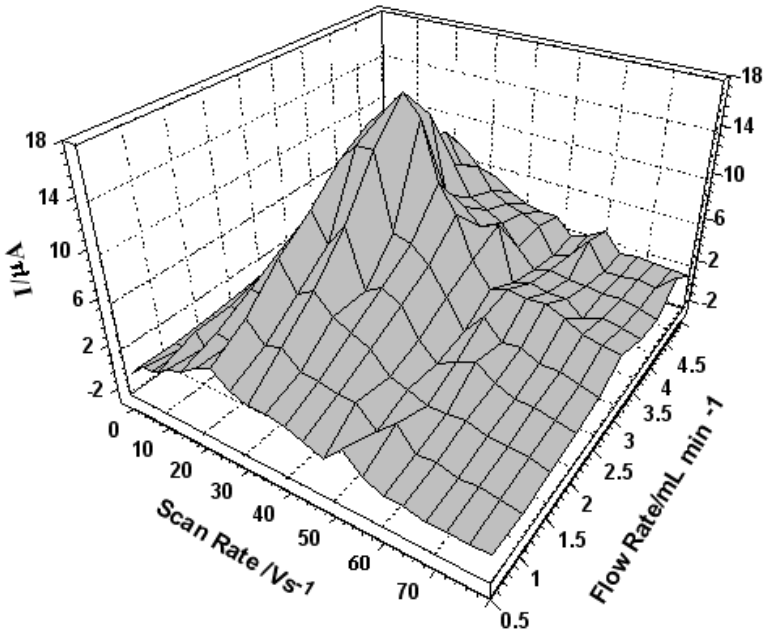

Fig. 4. The effect of the sweep rate and flow rate on the response of electrode to injections of 1.0×10^{-6} M PX in 0.05 M Tris-HCl and the effect of flow rate

3.4. Effect of accumulation potential

The dependence of the peak current on the accumulation potential was evaluated over the range of -0.5 to 0.5 V for 1.0×10^{-6} mol L^{-1} of PX at 0.05 mol L^{-1} Tris-HCl buffer pH=8.0 in

the presence of the drug, for an accumulation period of 0.2 s. The results obtained shown that the I_p values are maximum for an accumulation potential 0.4 V and after that the current decrease (see Fig. 5). So, the potential 0.4 V was selected as a best accumulation potential. It is in accordance with pK_a of drug, because PX has negative charge in this pH and can be accumulated on positive potential.

3.5. Effect of accumulation time

As expected, the extent pre-concentration is a function of the accumulation time (t_{acc}). The dependence of peak current on accumulation time was studied at concentration level of PX 1.0×10^{-5} mol L^{-1} at the Tris-HCl buffer pH=8 (see inset of Fig. 5). The peak current increased with increasing accumulation time till 0.2 s and after that it remains constant. For a period longer than 0.2 s, the saturation of the carbon paste electrode by drug to make a fouling of the electrode was observed. Hence, an accumulation time of 0.2 s were chosen to evaluate the best work conditions to the proposed method.

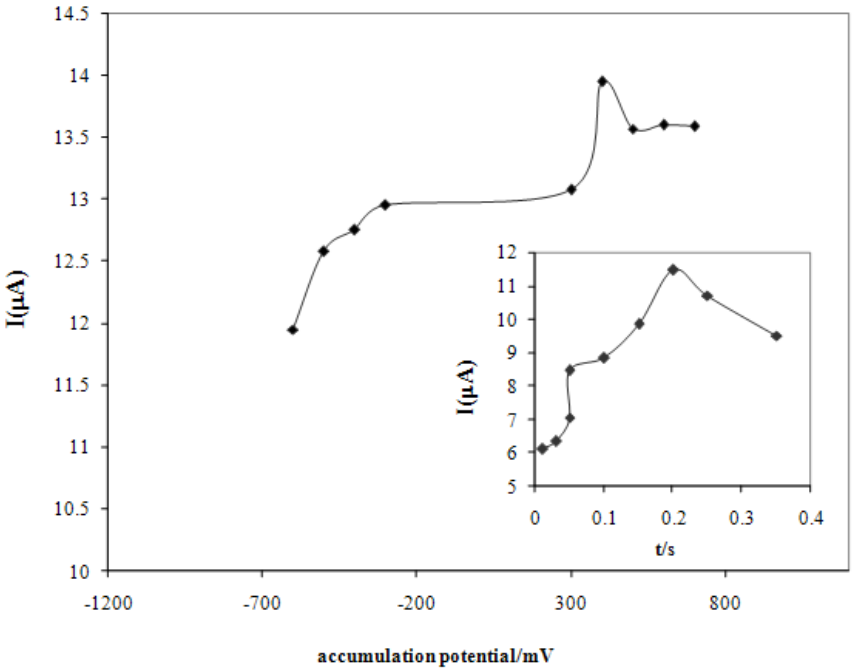

Fig. 5. The effect of the accumulation time on the electrode response to injections of 1.0×10^{-6} M, PX in 0.05 M Tris-HCl. Inset: the effect of the accumulation potential on the response. The results are based on five replicate measurements

3.6. Analytical application

Fig. 6 presents the 3D graph of injection of 5.0×10^{-5} mol L^{-1} PX that shows a sequence of CVs recorded during the flow analysis for determination of the drug. The volume of the

injection was of 50 μL into the eluent solution containing 0.05 mol L^{-1} Tris-HCl. The time axis of the graph represents the time of the flow injection experiment. In the absence of PX, the shape of the CV curves is typical for Tris-HCl buffer on the CD/CPE electrode. In order to provide an FFTCV quantitative procedure, the dependence between PX concentration and peak current (I_p) was conducted at both CD/CPE optimized conditions. For quantitation the calibration plot method, with solutions of PX in Tris-HCl (pH=8.0), was used. The experimental conditions were set at optimum values in order to obtain the best detection limits. As mentioned above the electrode response could be expressed in various ways as peak heights or peak areas. For this reason, the magnitude of injection peaks depends on the choice of the data processing methods. The calibration plot (Fig. 7) is described by the following regression curve Y(Ip/μA)=20.906X(C/μM)+0.3469 (R^2=0.9928) for concentrations range between 0.01-2.4 μ mol L^{-1}. Where I_p is the peak current and C is the PX concentration. The repeatability of the measurement was calculated from ten independent runs obtaining a variation coefficient of 2.20% for CD/CPE. The detection limit and quantification limit was 5.0×10^{-9} mol L^{-1} and 8.0×10^{-9} mol L^{-1} were calculated based on the 3σ and 10σ [38]. The repeatability of the measurements is determined as the R.S.D. value (n=5): 3.2% for 1×10^{-6} mol L^{-1}.

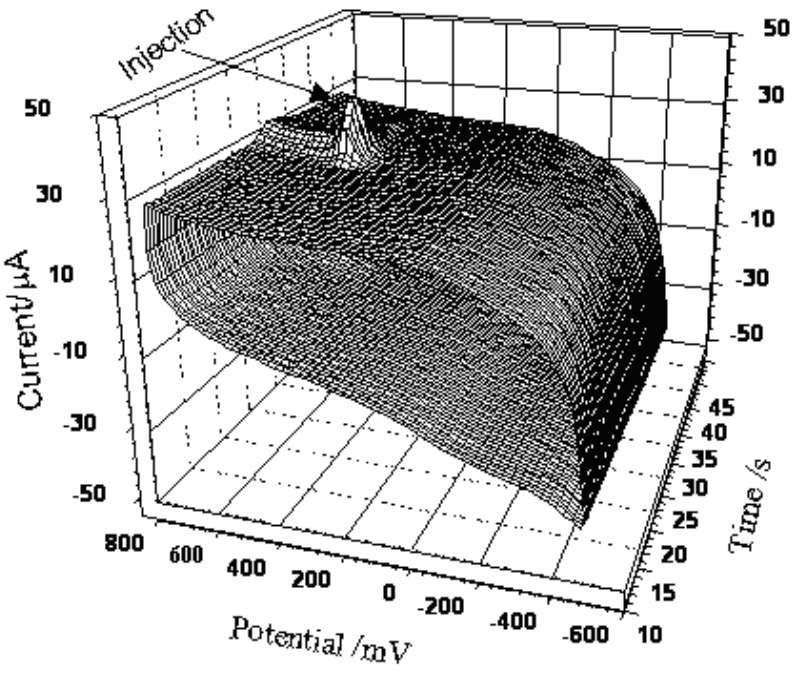

Fig. 6. 3D Cyclic voltammogram of PX during a flow-injection experiment. The eluent was 0.05 M Tris-HCl pH=8, the flow rate, and the sweep rate was 0.5 mL min^{-1} and 15 Vs^{-1}, respectively. The accumulation time was 0.2 s at 0.4 V. The injected solution (50 μL) contained 5.0×10^{-5} M PX

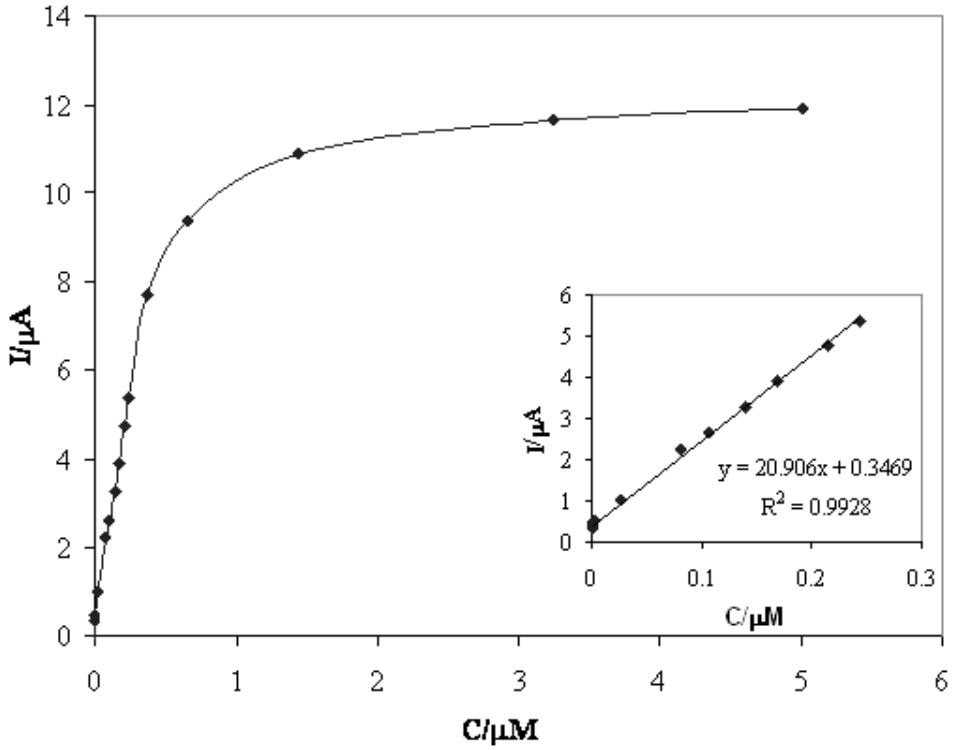

Fig. 7. Calibration curves obtained for PX on CD/CPE electrode in 0.05 M Tris-HCl pH=8 at the sweep rate of 15 Vs^{-1}, accumulation time 0.2 s and accumulation potential 0.4 V obtained in flowing solution by flow rate of 0.5 mL min^{-1}

3.7. Interferences and selectivity

Some ions and species commonly existing in biological samples were chosen for the study on selectivity of the piroxicam sensor. The effect of interferences on the determination of piroxicam was investigated with the concentration of PX fixed at 2.0×10^{-7} mol L^{-1}. The results presented in Table 1 reveal that following species caused no interference when existed in specified molar excesses of 100 times for citrate, carbonate, Na^{+},Ca^{2+},Mg^{2+},K^{+}, and 20 times of Glucose. Comparison of the detection limit of the proposed method with the other reported methods it is immediately obvious that the sensitivity of the method is superior to all previously reported methods. The data reveals that the detection limit of the method is about 120 times lower than the most sensitive reported method [21,22] (see Table 3).

3.8. Accuracy and precision

The accuracy of the proposed method was determined by using standard pure material of PX and applying the optimized analytical approaches with three spiking replicates at three concentration levels covering the linearity range (20.0, 50.0, and 100.0nmol L^{-1}) The

obtained mean recoveries of data collected by replicating the procedure within three consecutive days have been ranged from 98.60 to 102.35%.

Table 1. Interferences of different species to the CV determination of PX with proposed modified electrode

Interferences	**Concentration (mol L^{-1})**	**Recovery (%)**
Citrate	2.0×10^{-5}	101
Carbonate	2.0×10^{-5}	99
Na^{+}	2.0×10^{-5}	98.2
Ca^{2+}	2.0×10^{-5}	102
Mg^{2+}	2.0×10^{-5}	101.6
Glucose	40.0×10^{-7}	98.3

3.9. Assay of capsules

The method developed in the present study was applied for the determination of PX in capsules from the Iranian market. The results showed a percent recovery of 99.91 % and a R.S.D. of 1.20 %.

3. 10. Assay in human urine and plasma

As a preliminary evaluation of the validity of the proposed electrochemical sensor, the recovery of piroxicam in plasma and urine samples was testified. The results showed that the CD modified CPE could be used for the determination of piroxicam, with satisfactory recovery results of 101–105 %.(Table 2). The results of analysis of spiked human plasma (n=10) is shown in Table 2 too. The results are satisfactory, accurate and precise. No interference was noticed from the urine content after just dilution with the supporting electrolyte. The major advantage of the method as applied to plasma and urine is that no prior extraction step is required.

Table 2. Application of the proposed method to the determination of PX in spiked humane plasma and urine

	Added (μgmL^{-1})	Found (μgmL^{-1})	Recovery (%)
Plasma	50	50 ± 0.5	101
Urine	20	21 ± 0.3	105

Data obtained from ten replicates at each concentration. Interpolated concentration data expressed as mean ± S.D

Table 3. Comparison of the detection limit of the proposed method with the other reported

Method	Detection limit	Ref. No.
Spectrofluorimetric	2000 nM	21
Thin layer chromatography	600 nM	22
FFTCV	5 nM	This work

4. CONCLUSION

An effective accumulation of the drug molecules in β-CD as the electrode modifier has been found. This modification by cyclodextrine helps to improve the sensivity of drug determination by increasing the current. The electrochemical methods for pharmaceutical and biological samples analysis have been proven to be fast, precise, and simple to perform and produce low cost results, in which the interference from excipients of the drugs and interference of the biological fluids do not interfere with the determination, and, consequently, more extraction procedures are not needed. The above methods can be suggested as a good alternative for the routine quality control of this antibiotic drug in pharmaceutical formulation. Also, application of FFT-CV to high-performance liquid chromatography is being considered.

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