

Electrochemical determination of piroxicam on the surface of pyrolytic graphite electrode modified with a film of carbon nanoparticle-chitosan

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Abstract The electrochemical behavior of the anti-inflammatory drug piroxicam is studied at the surface of a plain pyrolytic graphite electrode modified with chitosan-doped carbon nanoparticles. An electroactive surface was produced by drop-casting a suspension of the modifier and characterized by atomic force microscopy. A remarkable enhancement is found in studies on the cyclic voltammetric response towards piroxicam. This is described on the basis of the thin-layer mass transport regimes within the porous films, which leads to a considerable increase in the active surface area of the electrode. The electrode shows a linear response to piroxicam in the range of 0.05–50 μM , with a detection limit of 25 nM (at S/N of 3). The electrode was successfully applied to the determination of piroxicam in pharmaceutical and clinical preparations with satisfactory accuracy and precision.

Keywords Piroxicam · Carbon nanoparticles · Chitosan · Cyclic voltammetry · Modified electrode

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are a well known as antipyretic, analgesic and anti-inflammatory agents. They are prescript to reduce pain in different

arthritis and other post-operative conditions [1]. Some side effects have been reported for Piroxicam (PC) as a drug, such as gastrointestinal, headache, dizziness, skin rashes, palpitations, emeda and tinnitus [2, 3]. Several analytical methods, such as spectrophotometric [4–9], liquid chromatography [7, 10–14], high performance thin layer chromatography (HPTLC) [10, 15] and capillary electrophoresis [10, 16] have been used to determine the concentration of PC in pharmaceutical preparations and body fluids. Electroanalysis methods using various electrodes materials have been attracted more attentions in the determination of this drug [17–21].

Nanostructured materials particularly carbon nanoparticles, nanowires and nanotubes have attracted considerable interests and have become a vast area of research owing to their unique physical and chemical properties. These properties provide an important and feasible platform for electroanalysis particularly in the design of modified electrodes for electrochemical sensing [22–25]. Applications of carbon nanoparticles (CNPs) in electroanalytical studies display extraordinary advantages over conventional electrodes such as enhanced mass transport and catalysis, highly effective surface areas, high porosity, more absorption and reactive sites and control over the electrode macro-environment [26–32].

In the present work, a modified electrode consisting basal-plane pyrolytic graphite electrode coated with a thin film of chitosan/carbon nanoparticles (CS-CNP-PGE) was used for the investigation of the electrochemical behavior of PC. A considerable enhancement in the response of PC on the surface of the modified electrode is described based on an interlayer diffusional regime within the porous layer of film modifier. The fundamental theories for this new diffusion geometry have been previously described by Compton and co-workers [26, 27]. Based on these studies,

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the surface can be thought of as a porous layer in which, pockets of solution are trapped in between multiple layers of nanomaterials. In this model the oxidation of electroactive species within the porous layer is described using the model of a thin porous layer cell reflecting a large surface area resulted by the carbon nanomaterials. The effect of the experimental parameters such as pH, scan rate, accumulation time and potential and the volume of casted drop on the response of the modified electrode toward PC are investigated. The prepared electrode was successfully applied as a very sensitive sensor with an acceptable reproducibility and accuracy in the detection of nano-molar amounts of PC in pharmaceutical and clinical preparations using linear sweep voltammetry (LSV). The prepared modified electrode showed high reproducibility in both preparation and analytical determination and in comparison to previous reports for PC, showed very better analytical response characteristics e.g. detection limit and linear dynamic range.

Experimental

Reagents

A 0.05% chitosan (CS) solution (Aldrich, dissolved in a solution of 5% acetic acid). Functionalized carbon nanoparticles (ca. 8 nm diameter, with phenylsulfonate surface functional groups, Emperor 2000) were obtained from Cabot Corporation. Piroxicam (PC) was provided from Alhavi Pharmaceutical Company (Tehran, Iran) and applied without any further purification. All other chemicals which were of analytical reagent grade were purchased from Merck. Doubly distilled deionized water was used for all aqueous solution preparations. Fresh frozen plasma was prepared from Iranian Blood Research and Fractionation Holding Company (IBRFC).

Due to poor solubility and stability of PC in aqueous solutions, daily stock solutions of 0.5 mM PC were prepared in 10%v/v methanol/aqueous buffer solution and stored at 5°C in the dark [17]. In these experiments, 0.1 M acetate was used for pHs 4 and 5, and 0.1 M phosphate for pHs 3, 6 and 7. All Voltammetric investigations were performed in deoxygenated solutions by purging the pure argon (99.999% from Roham Gas Company). Pharmaceutical formulation of PC (100 mg capsules, Razi Pharmaceutical Co., Tehran, Iran) was purchased from the local pharmacy and dissolved in 10%v/v methanol/aqueous buffer solution and applied for the voltammetric determinations.

Instruments

Voltammetric experiments were performed with a Metrohm Computrace Voltammetric Analyzer model 757 VA. A

conventional three-electrode system was used with a PGE working electrode (unmodified or modified), a saturated Ag/AgCl reference electrode and a Pt wire as the counter electrode. A digital pH/mV/Ion meter (CyberScan model 2500) was used for the preparation of buffer solutions, which were used as the supporting electrolyte in voltammetric experiments.

Procedures

To prepare a CS-CNP-PGE modified electrode, a PGE was polished with emery paper and sonicated in double-distilled water for 5 min, and then thoroughly washed with double-distilled water. In the next step, 16 mg CNP and 1.3 mg CS were dispersed into 10 mL of redistilled water by ultrasonication agitation for about 20 min, which leads to the formation of a homogenous and stable black suspension. The CS-CNP-PGE film was prepared by casting 6 μ L (in optimum condition of its voltammetric response) of the above suspension on the surface of PGE. The modified electrode was dried at room temperature and rinsed with distilled water before use. The prepared modified electrode was stored in room temperature and dry conditions, when is out of use in the determinations. AFM images predict a thickness of about 0.1 μ m for the deposited film of CNP-CS on the surface of PGE.

Results and discussion

Electrochemical behavior of PC

Cyclic voltammetric (CV) curves of PC (0.5 mM) at the polished bare PGE and the CNP-CS-PGE in 0.1 M phosphate buffer solution of pH 6.0 are shown in Fig. 1. Comparatively, the oxidation peak of PC at the CNP-CS-PGE appears at a lower potential (513 mV against 540 mV). Moreover, the anodic peak current at the modified electrode was increased considerably by a factor of 2.5. These results should be attributed to the unique physico-chemical properties of the composite of CNP, i.e. a combination of semi-infinite planar and thin layer diffusion (within porous modifier layer) effects that increases the active surface area [26, 27]. In the studied potential range, no cathodic peak was found, which indicates an irreversible charge transfer reaction in this system. This behavior is in agreement with those of the previous reports for PC oxidation [19, 20].

Based on the adsorption behavior of PC at CNP-CS-PGE, the accumulation time under open circuit showed a significant effect on the current of the oxidation of PC. By increasing the time to 100 s, the peak current increases and then changed slowly (Fig. 2). By considering the instability of PC in aqueous solutions in longer times, a maximum

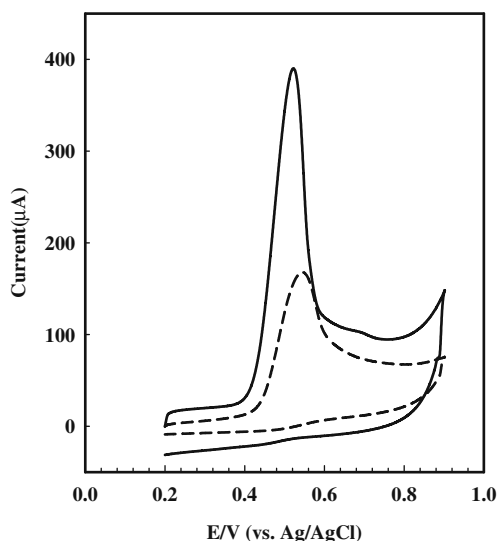


Fig. 1 CVs of 0.5 mM of PC on the surface of bare PGE (*solid line*), CNP-CS-PGE (*dashed line*). Supporting electrolyte was 0.1 M phosphate buffer solution of pH 6.0 and scan rate was 100 mVs^{-1}

accumulation time of 120 s at the open circuit condition was selected for all of the investigations.

Figure 3 shows CVs of 0.5 mM PC in buffered solutions of pH 6.0 at the modified electrode at different potential sweep rates from 25 to 300 mVs^{-1} . A linear relationship between $\log(I_{p,a})$ and $\log(v)$ was resulted with a slope of 0.59. Observation of a linear behavior between anodic peak current and the scan rate indicates an adsorptive controlled process for PC on the surface of the modified electrode.

Optimization of the amounts of the modifier on the surface of electrode has a predominant role on the

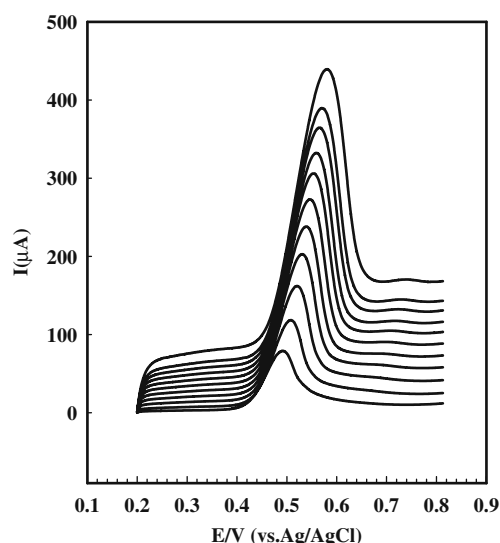


Fig. 3 CVs of 0.5 mM of PC at the CNP-CS-PGE at different scan rates in 0.1 M phosphate buffer solution of pH 6.0

voltammetric response for oxidation of PC. Various volumes of the modifier suspension were casted on the surface of the electrode. The results showed that the oxidation peak current for 0.5 mM PC increased quickly when the volume of CNP-CS suspension casted on the surface of PGE has been increased to $6 \mu\text{L}$. Further increase caused a gradual decrease in the anodic peak current of PC. This can be related to increased resistance of the modified electrode to electron transfer and sluggish mass transfer process for PC, which arises from large film thickness of the deposited suspension on the PGE. As a result, $6 \mu\text{L}$ of

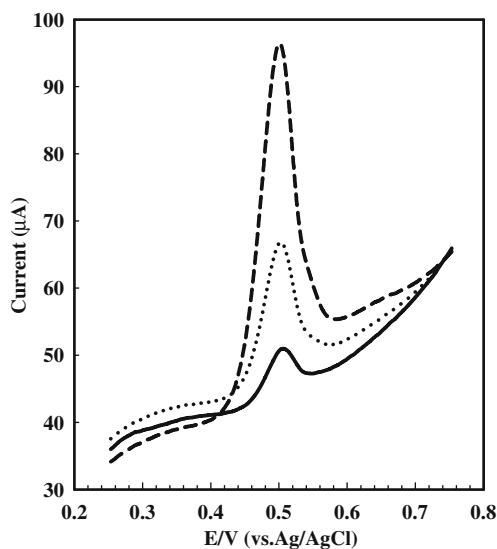


Fig. 2 LSVs of $5.0 \mu\text{M}$ on the CNP-CS-PGE for various accumulation times; 0 s (*solid line*), 60 s (*dotted line*), 120 s (*dashed line*). Supporting electrolyte was 0.1 M phosphate buffer solution of pH 6.0 and scan rate was 100 mVs^{-1}

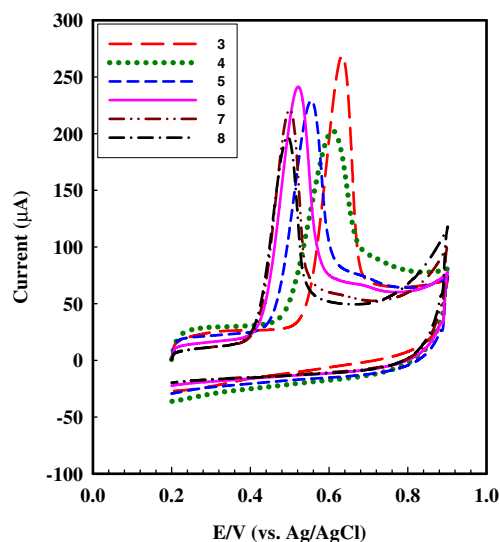


Fig. 4 CVs of 0.5 mM of PC at the CNP-CS-PGE in various pHs of buffer solution from 3–8. Scan rate was 100 mVs^{-1}

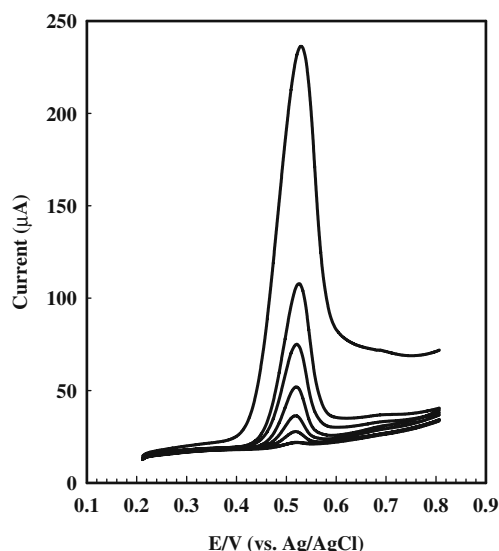


Fig. 5 LSVs for various concentrations of PC in the range of (down to up) 0.05–50 μM in 0.1 M phosphate buffer solution of pH 6.0. Scan rate was 100 mVs^{-1}

the modifier suspension was selected as the optimum volume for preparation of the modified electrode.

The pH of the supporting electrolyte has a significant influence on the oxidation of PC at the modified electrode. The effect of pH was studied in the range of 3–8 and the peak potential was found to shift toward more negative potential by increasing the pH (Fig. 4). In these investigations E_p varied linearly with pH according to Eq. 1. Based on these results pH with maximum peak current was selected for the determination of PC.

$$E_p(\text{V}) = 0.719 - 0.033\text{pH} \quad (R^2 = 0.991) \quad (1)$$

The resulted slope for variation of E_p versus pH is in conformity with an electrode process consisting two

electrons and a proton and can be attributed to the oxidation of amide group in the structure of PC [20].

It is well known that PC has two ionizable functional groups with two known dissociation constants; the acidic ($-\text{OH}$) hydroxyl ($K_a=5.1$) and the basic ($-\text{N}$) pyridine groups ($K_b=1.91$) [28]. According to the selected pH for studies, it can be stated that PC has a partial negative charge and, more over to the $\pi - \pi$ interactions with the modifier film, has an electrostatic attraction with the positive sites of CS at the surface of the modified electrode.

Analytical application and the response repeatability of CNP-CS-PGE

Under the optimized conditions, the oxidation peak currents showed a linear relationship with PC concentrations over the range of 0.05–50 μM (with a slope of $3.856 \mu\text{A}/\mu\text{M}$) in 0.1 M phosphate buffer solution of pH 6.0 (Fig. 5). In these measurements, based upon linear extrapolation of the calibration curve [33], a theoretical detection limit ($S/N=3$) of 25 nM is resulted for the voltammetric determination of PC. The surface of the modified electrode was easily refreshed by two successive potential cycling between 0.0 and 1.5 V in the buffer solution, after each step of the determination. The RSD for the slope of the calibration curve, calculated from 3 replicate measurements using a single electrode, was 5.2%. The modified electrode could be used for more than 600 scans during several days without any considerable changes in its response. The results indicate that the prepared modified electrode has high repeatability and stability in its voltammetric responses toward PC.

Various techniques have been reported for the determination of PC which include flow injection-fluorescence detection [15], spectrophotometric [5, 8], UV spectrophotometry-HPLC [7], spectrofluorimetric [9], HPLC [12], capillary zone electrophoresis equipped with HPLC spectrophotometer detector [16], LC coupled with tandem mass spectrometry

Table 1 Analytical results for PC determinations using various methods

Method	Detection Limit (ppm)	Linear Range (ppm)	Reference
Spectrophotometry	0.013	0.12–2.25	5
UV spectrophotometry/ HPLC	–	2.4–20.0	7
Spectrophotometry	–	0.33–4.08	8
Spectrofluorimetry	0.01	0.03–0.2	9
HPLC	0.055	–	12
LC/Tandem MS	0.0005	0.0005–0.2	14
Europium sensitized fluorescence	0.023	0.1–1	15
CZE/HPLC Spectrophotometry	10	40–500	16
Electrochemistry using MWCNT modified CPE	0.1	0.15–5	19
Voltammetry using CNP-CS-PGE	0.0083	0.166–16.567	Present work

[14] and electrochemical detection using multi-walled carbon nanotube paste electrode [19]. In comparison to the above mentioned methods, the electro-analytical procedure in the present work shows some remarkable advantages e.g., simplicity in practice, high sensitivity, very low detection limit, reasonable selectivity, low cost and rapid response toward PC. Table 1 shows the results of the detection limit and linear range obtained for PC determinations with various methods in comparison to the present work.

The modified electrode was used for the determination of PC in its capsule forms using the standard addition method. The standard-addition calibration plot, in the range of 0.1–10.0 μM showed a slope of 3.925 $\mu\text{A}/\mu\text{M}$. In addition, the recovery for the determination of PC in drug samples (based on the comparison of the slopes of the calibration curves) was obtained to be 101.7%. The RSD for the slope of the replicated standard additions was 4.5% ($n=5$). It is observed that the matrix of the pharmaceutical sample has no interfering effects on the electrochemical responses of the PC.

The prepared modified electrode was used for the direct analysis of PC in human blood plasma samples without any further pretreatment. In these investigations, human blood serum samples were spiked with standard concentrations of PC in the range of 0.1–10.0 μM . The average recovery percentage in the studied concentration range was 93%. It seems that the components in the serum does not show any noticeable effects on the detection of PC. Therefore, the modified electrode in this work can be considered as a very good candidate for the sensitive voltammetric determination of PC in pharmaceutical and clinical preparations with acceptable accuracy and precision.

Conclusions

The results of the present work demonstrate that the pyrolytic graphite electrode modified with chitosan-doped carbon nanoparticles is able to accumulate the PC for performing sensitive voltammetric detections. The results showed that the presence of the modifier film on the surface of the electrode dramatically affect the sensitivity of the electrochemical responses toward PC. This enhancement can be explained based on the thin layer diffusion within the porous modifier film, moreover to the semi-infinite planar diffusion model. The constructed sensor was used as a sensitive device for the determination of PC in the concentration range of 0.05–50 μM with a detection limit of 25 nM.

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