

Electroanalytical Determination of Antibacterial Ciprofloxacin in Pure Form and in Drug Formulations

Abdel-Nasser Kawde · Md. Abdul Aziz ·
Nurudeen Odewunmi · Nouri Hassan ·
Abdulnaser AlSharaa

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Abstract A simple, rapid and sensitive electroanalytical method was developed to measure ciprofloxacin (CPF) concentration at an electrode surface made of a new composite material, electrochemically activated glassy carbon paste. Square-wave adsorptive stripping voltammetry comparisons of CPF measured at GCPE, glassy carbon and carbon paste electrode surfaces yielded the highest CPF signal at GCPE surfaces. When pH dependence was assessed, 0.1 M phosphate buffer solution, pH 5.0, showed the best anodic peak for CPF oxidation. When the conditions of the voltammetric method were optimized, a GCPE electrode surface 1.6 mm in diameter yielded a dynamic calibration curve ($R^2 = 0.97$) at CPF concentrations of 10–750 ppb. The limits of detection (3σ) and quantification were 12.2 ppb (33 nM) and 10 ppb (27 nM) CPF, respectively. The new composite electrode yielded reproducible results, with a relative standard deviation of 6.96 % (intraday) and 6.47 % (interday) for five repeated measurements of 0.5 ppm CPF. Finally, when this method was used to determine the concentration of CPF (active ingredient) in Floxacillin tablets dissolved in pure buffer and in buffer containing 10 % fresh urine, the recoveries of CPF were 100 and 105 %, respectively. This method thus allows the direct determination of CPF in urine samples, making it suitable for routine analysis in clinical laboratories.

Keywords Ciprofloxacin · Biological sample · Glassy carbon paste electrode · Square-wave stripping voltammetry · Sensor

الخلاصة

لقد تم تطوير طريقة كهروتحليلية (electroanalytical method) بسيطة وسريعة وحساسة لتقدير المضاد الحيوي سيبروفلوكساسين (CPF) على سطح القطب المنشط كهربياً والمصنوع من عجينة الكربون الزجاجي (GCPE) الجديدة. حيث تسمح الطريقة المطورة بتقدير تركيز المضاد الحيوي سيبروفلوكساسين (CPF) في عينات البول البشري مباشرة الذي يجعلها مناسبة للتحليل في المختبرات الطبية. لقد تم اعتماد الطريقة الفولتامترية الكشطية مربعة الموجة (Square Wave Stripping voltammetry) والمعتمدة لتقييم العوامل التي تؤثر في قياس التراكيز الدقيقة من المضاد الحيوي سيبروفلوكساسين (CPF). وأظهرت الطريقة المستخدمة أفضل موجة لأكسدة السيبروفلوكساسين في وسط 0.1 مولاري من محلول الفوسفات المنظم برقم هيدروجيني 5.0 من بين جميع المحاليل المختبرة. علاوة على ذلك تم تحسين العوامل للطريقة الفولتامترية الكشطية مربعة الموجة عن طريق إجراء سلسلة من التجارب. وفي ظل الظروف المثلى تم الحصول على منحني معايرة في النطاق الديناميكي من تركيز 10 جزء في البليون إلى 750 جزء من البليون على سطح قطب قطره 1.6 ملم مع حد أدنى للتركيز قدره 10 جزء في البليون (25 نانومولار). إن القطب الجديد يمكن استنساخه بانحراف معياري نسبي (RSD) قدره 6.96% و 6.47% لسلسلة من خمسة قياسات متكررة على سطوح جديدة مختلفة من CPE على مستوى نفس التركيز وهو 0.5 جزء في المليون. وأخيراً تم بنجاح تطبيق الطريقة التي تم تطويرها لتحديد تركيز المادة الفعالة في أقراص فلوكساسين المتواجدة في سوق الدواء السعودي.

A.-N. Kawde (✉) · Md. A. Aziz · N. Odewunmi · N. Hassan ·
A. AlSharaa
Chemistry Department, King Fahd University of Petroleum
and Minerals, Dhahran 31261, Kingdom of Saudi Arabia
e-mail: akawde@kfupm.edu.sa

A.-N. Kawde
Chemistry Department, Faculty of Science, Assiut University,
Assiut 71516, Egypt

1 Introduction

Ciprofloxacin (CPF; 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid; Fig. 1) is a second-generation fluoroquinolone antibiotic with an expanded spectrum of activity against gram-positive and gram-negative bacteria through its inhibition of DNA gyrase,

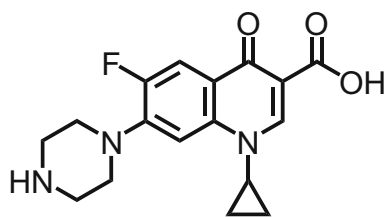


Fig. 1 Molecular structure of ciprofloxacin

an enzyme responsible for bacterial DNA replication [1,2]. Due to its high activity and comparatively low toxicity, CPF is used worldwide to treat bacterial infections in humans and animals [1,2]. It has also been approved by the United States Food and Drug Administration to prevent or slow anthrax after exposure [2,3].

Among the methods used to measure CPF concentrations for clinical and pharmaceutical quality control are those involving spectrophotometry [4,5], spectrofluorometry [6], flow injection chemiluminescence [7], capillary electrophoresis (CE) [8], and high-performance liquid chromatography (HPLC) coupled with various detectors [9–11]. Micro-emulsion electrokinetic chromatography (MEEKC) has been used for the simultaneous separation of seven fluoroquinolones (FQs) and to measure ciprofloxacin and lomefloxacin (LMF) concentrations in urine samples [12]. In addition, combinations of capillary electrophoresis (CE) and photodiode array detection [13,14], mass spectrometry, [15] and laser induced fluorescence (LIF) [16] have been used for analysis of CPF. All of these methods, however, have limitations for the routine analysis of CPF, including high cost, complex instrumentation, and/or long duration.

Electrochemical methods are frequently used in analytical chemistry due to their high sensitivity, low cost, fast response, simple instrumentation and portability. The poor electrocatalytic properties of bare conventional electrodes, however, limit their use in measuring CPF concentration [3,17]. These electrocatalytic properties can be improved by (1) modifying the electrode with a suitable electrocatalyst or electron mediator and (2) using a solution that enhances the electrochemical signal of the analyte, thus enhancing the electrochemical reaction. For example, a simple and rapid method based on multiwalled carbon nanotube (MWCNT)-modified glassy carbon electrodes (GCEs) was more sensitive than bare GCEs in measuring CPF using cyclic voltammetry and chronoamperometric techniques [17]. In addition, an electrochemical method using a carbon paste electrode (CPE) and based on the enhancing effect of an anionic surfactant (sodium dodecyl benzene sulfonate) was found to be effective in measuring CPF [3]. Use of an electrocatalyst or electron mediator or enhancer molecules to enhance the electrochemical reaction, however, increases the total cost of measurement, emphasizing the need to develop a simple, sensitive and cost-effective method of determining CPF concentrations in solution.

Interestingly, few reports have described the use of simple electrochemical methods to enhance the electrocatalytic properties of electrodes [18–20]. Applying a potential prior to analysis causes analyte to accumulate on the electrode surface, increasing the analyte signal, and reducing the limit of detection [21–23]. CPF contains many functional groups, including carboxylic, ketone, amine and aromatic groups, which are responsible for its adsorption onto the surfaces of carbon substrates such as activated carbon, carbon nanotubes and carbon xerogel (Fig. 1) [24]. Due to its increased sensitivity compared with bare GCEs, a MWCNT-modified GCE was used to develop a method for the determination of CPF [17]. Glassy carbon paste has a chemical composition similar to that of activated carbon and carbon nanotubes, as well as being inexpensive and easy to prepare. To our knowledge, GPCEs have not been used to measure CPF electrochemically.

The present report describes a novel, rapid and simple electroanalytical method using GCPE for the sensitive detection of CPF. To reduce the limits of detection, positive potential treatment, pH, amplitude, frequency, pulse width, accumulation time, and accumulation potential were optimized. The developed method resulted in a CPF limit of detection of 10 ppb (27 nM).

2 Materials and Methods

2.1 Reagents

Ciprofloxacin hydrochloride, uric acid, cytosine, L-alanine, L-phenylalanine, L-tryptophan, L-cysteine, L-tyrosine, Triton X-100, DL-methionine, dopamine, paracetamol, L-ascorbic acid, guanine, single stranded (ss) DNA, zinc sulfate, copper sulfate, ferric sulfate, lead sulfate, sodium sulfate, potassium sulfate, ammonium sulfate, graphite powder and mineral oil were obtained from Sigma-Aldrich (St. Louis, MO, USA). Glassy carbon spherical powders (2–12 μm) were obtained from Alfa Aesar (Ward Hill, MA, USA). Phosphate buffer (PB) solutions with different pHs were prepared from standard solutions of monosodium phosphate (NaH_2PO_4) and disodium phosphate (Na_2HPO_4). Floxacin tablets (drug) were purchased from a local pharmacy in Thuqba, Saudi Arabia. All solutions were prepared using doubly distilled water.

2.2 Working Electrode Preparation

The GCPE and CPE were prepared as described by us previously [23]. Briefly, 70 mg of 2–12 μm glassy carbon powder was suspended in 30 mg mineral oil. A portion of the resulting paste was packed firmly into the electrode cavity (1.6 mm diameter and 2.0 mm depth) of the polytetrafluoroethylene

(PTFE) sleeve. Electrical contact was established via a copper wire. The paste surface was smoothed with weighing paper and rinsed carefully with double distilled water prior to each measurement. The GCE was polished with 3.0 and 0.05 μm alumina slurries and washed with double distilled water prior to each use.

2.3 Sample Preparation from Commercial Drugs

One tablet of commercial drug (Floxacin) was crushed in a mortar, dissolved in deionized water, and filtered. The filtrate was diluted to 100 mL with deionized water and 0.859 mL of the latter was diluted to 50.0 mL with deionized water. Aliquots of 4.0 μL of this solution were added to 2.0 mL PB (pH 5.0) or 2.0 mL PB (pH 5.0) containing 10 % fresh urine. Written informed consent was obtained from all subjects providing urine. Those individuals were healthy and did not take any CPF-containing drugs.

2.4 Electrochemistry

A CHI660C instrument was used for all electrochemical work. Square-wave adsorptive stripping voltammetry (SWASV) measurements were performed by pretreating the GCPE and GCE surfaces at +1.7 V [19,23] and the CPE surfaces at +1.5 V for 60 s each in a glass cell containing a blank solution (0.1 M PB, pH 5.0), followed by 60 s accumulation at +0.6 V potential, unless otherwise mentioned, in a separate glass cell containing specific CPF concentrations in buffer. Both solutions were stirred in the process. After a 5-s reset period (without stirring), positive SWASV was performed over a +0.4 to +1.4 V potential range. Unless mentioned otherwise, a newly pretreated GCPE surface was used for each measurement. Ag/AgCl (saturated KCl) and Pt electrodes were used as reference and counter electrodes, respectively.

3 Results and Discussion

The electrocatalytic properties of the GCPE toward the electrochemical oxidation of CPF were assessed by recording SWASVs in both the absence and presence of CPF and before and after pretreatment of the GCPE at +1.7 V (Fig. 2A). The peak potential of CPF oxidation appeared at nearly the same position under all conditions. To compare the performance of the GCPE with that of conventional carbon electrodes, SWASVs were recorded before and after electrochemical pretreatment of a GCE (Fig. 2B) and a CPE (Fig. 2C), with pretreatments at +1.7 and +1.5 V, respectively. All carbon electrodes showed enhanced CPF electro-oxidation signals after pretreatment, but the enhancement was greater for the GCPE than for the GCE and CPE, with

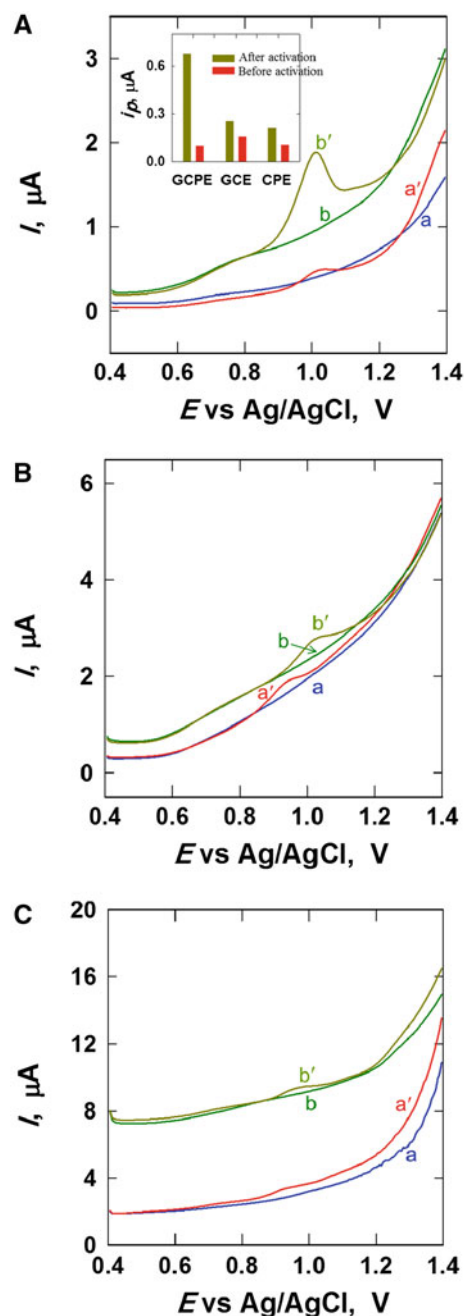


Fig. 2 Square-wave stripping voltammograms of GCPE (a), GCE (b) and CPF in 0.1 M PB, pH 5.0 before (a and a') and after (b and b') electrode pretreatment. a and b absence of CPF; a' and b' presence of 0.5 ppm CPF. Accumulation time, 60 s; accumulation potential, +0.6 V; pulse height (amplitude), 50 mV; pulse width (increment), 6 mV and frequency, 15 Hz. Inset in A shows the corresponding histogram

the highest signal observed for the pretreated GCPE (Fig. 2A, inset). Since a high electrochemical oxidation signal is essential for the fabrication of an ultra-sensitive electrochemical sensor, GCPE was chosen as a good transducer material for the electroanalytical determination of CPF.

Fig. 3 **a** Square-wave stripping voltammograms of 0.5 ppm CPF in 0.1 M PB at *a* pH 4.5, *b* pH 5.0, *c* pH 6.0, *d* pH 7.0, and *e* pH 8.0 on GCPE after pretreatment at +1.7 V for 60 s. Other working conditions are described in the legend of Fig. 2. **b** Plots of pH vs. peak current (*a*) and peak potential (*b*)

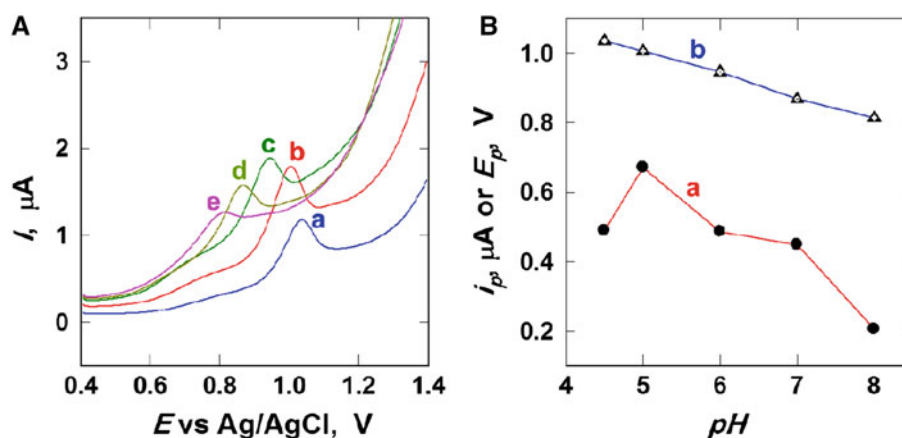


Table 1 Optimal parameters for ciprofloxacin determination by the square-wave stripping voltammetry method using a pretreated electrode at 1.7 V

Electrode	Sensing media (pH)	Amplitude (mV)	Frequency (Hz)	Potential increment (mV)	Accumulation potential (V)	Accumulation time (s)
GCPE	0.1 M PB (pH 5.0)	100	30	1.0	0.6	100

The pH of the aqueous medium and the SWASV parameters can greatly influence the detection limits of any analyte. Thus, the effects of pH and the optimization of SWASV parameters for CPF electro-oxidation on GCPE were analyzed.

3.1 Effect of pH

The pH dependence of 0.5 ppm CPF was analyzed systematically in 0.1 M PB at pH values ranging from 4.5 to 8.0 (Fig. 3). As the pH increased, the electro-oxidation peak potential (E_p) of CPF became less positive. The relationship between E_p and pH can be represented by the equation, E_p (V) = 1.3290–0.0648 pH. The slope of 64.8 mV/pH (Fig. 3B, b) indicates that equal numbers of protons and electrons are involved in the electrochemical reaction of CPF. This slope was close to the theoretical value of 59 mV/pH and was in agreement with results obtained using MWCNTs—GCE in a Britton–Robinson buffer solution (0.04 M H_3PO_3 , 0.04 M H_3PO_4 , and 0.04 M CH_3COOH , titrated to the desired pH with 0.2 M NaOH) for CPF [17]. Figure 3 shows that the highest electro-oxidation signal (Fig. 3B, a), with a peak potential around +1.0 V (Fig. 3A, a), was obtained at pH 5.0. Since CPF has two pK_a values (6.09 and 8.62), most CPF molecules at pH 5 are in the cationic state [25], resulting in an electrostatic attraction between CPF molecules and the anodic-activated GCPE at pH 5.0. Since pH 5.0 was the optimum pH, it was utilized in all further experiments.

3.2 Optimization of SWASV Parameters

All electrochemical SWASV parameters were analyzed systematically for 0.5 ppm CPF in 0.1 M PB (pH 5.0). The

optimal SWASV parameters are summarized in Table 1. As amplitude increased, peak potential decreased linearly (data are not shown), whereas a linear increase in frequency increments of the CPF electro-oxidation signal from 5 to 45 Hz had no effect on peak potential (data not shown). These results indicated that the electrochemical reaction followed an adsorption controlled process [26]. Due to the noises appearing on the SWASV at 45 Hz, 30 Hz was chosen as the optimum frequency.

In contrast, an increase in pulse width from 2 to 15 mV resulted in an increased CPF signal, whereas any further increase in pulse width resulted in a reduced signal (data not shown), indicating that the optimum pulse width was 15 mV.

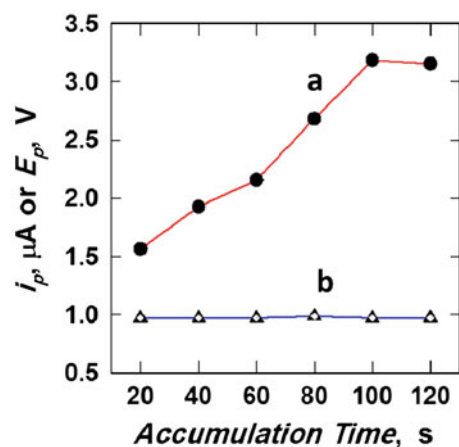


Fig. 4 Comparison of accumulation time vs. peak current (*a*) and peak potential (*b*) of 0.5 ppm CPF in 0.1 M PB, pH 5.0, at an accumulation potential of +0.5 V. Other working conditions are described in the legend of Fig. 3

Table 2 Effect on peak current of 0.5 ppm ciprofloxacin electro-oxidation at GCPE electrodes under optimum conditions in the presence of various contaminants (0.5 ppm)

Potential interference	Signal change (%)	Potential interference	Signal change (%)
Uric acid	-5.80	DL-methionine	+8.26
Cytosine	-10.22	Dopamine	+5.61
L-alanine	-7.44	Paracetamol	-1.42
L-phenylalanine	-9.07	L-ascorbic acid	+2.76
L-tryptophan	+3.03	Guanine	-5.14
L-cysteine	+8.59	ssDNA	-2.21
L-tyrosine	-5.93	Zn ²⁺ , Cu ²⁺ , Fe ³⁺ , Pb ²⁺ , Na ⁺ , K ⁺ , NH ₄ ⁺	-6.72
Triton X-100	-1.55		

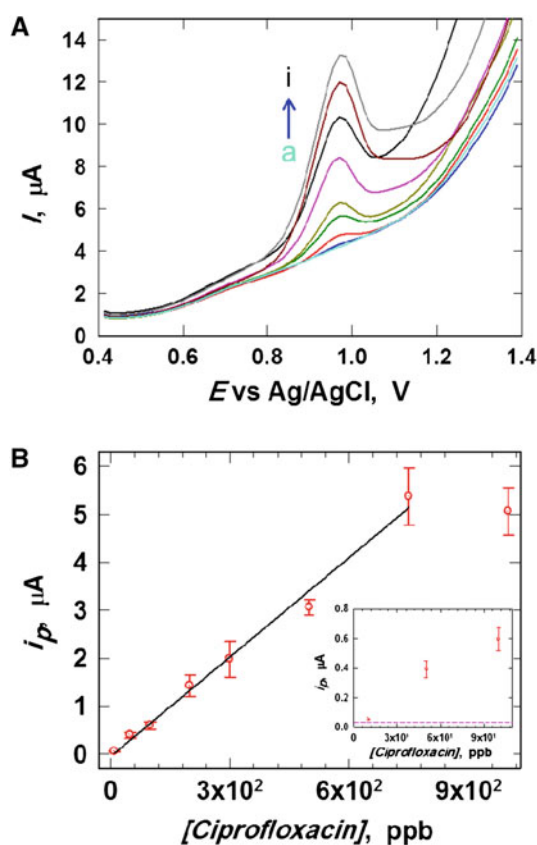


Fig. 5 **a** Square-wave stripping voltammograms of *a* 0.0, *b* 10, *c* 50, *d* 100, *e* 200, *f* 300, *g* 500, *h* 750 and *i* 1,000 ppb CPF on GCPE after pretreatment at +1.7 V for 60 s. Working conditions are described in Table 1. **b** Dependence of peak current on CPF concentration. The inset represents a magnified graph at low concentration. The dashed line corresponds to three times the standard deviation (SD) of peak current in the absence of CPF

The effect of different accumulation potentials (+0.2, +0.4, +0.6, +0.8 and +1.0 V vs. Ag/AgCl Sat. KCl) was also studied (data are not shown). Initially, there was an increase in signal as the potential increased, with a significant reduction

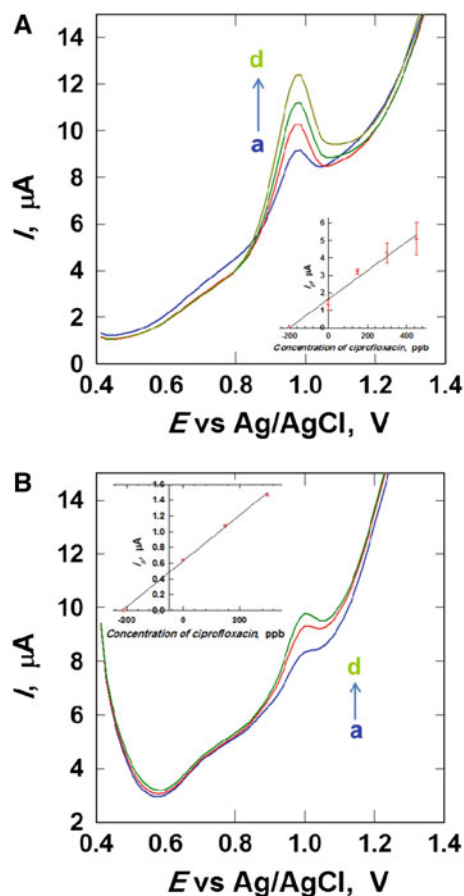


Fig. 6 **a** Square-wave stripping voltammograms of CPF in 0.1 M PB, pH 5.0 *a* without, and with *b* 150, *c* 300, *d* and 450 ppb CPF. Other working conditions are described in Table 4. **b** Square-wave stripping voltammograms for CPF in 0.1 M PB, pH 5.0 containing 10 % fresh urine *a* without, and with *b* 150 and *c* 300 ppb CPF. Other working conditions are described in the legend of Fig. 5. The respective insets show plots of peak current vs. added CPF concentration (ppb)

at potentials greater than +0.6 V, indicating that the optimum accumulation potential was +0.6 V.

Using these optimum electrochemical parameters, the influence of the accumulation time from 20 to 100 s on the CPF SWASV signal was assessed (Fig. 4). The signal increased in a nearly linear fashion up to 100 s and then leveled off or decreased within the range of the relative standard deviation (RSD) (Fig. 4a). Changing the accumulation time did not affect the peak potential (Fig. 4b). All subsequent experiments were performed using an accumulation time of 100 s.

3.3 Reproducibility

To test for intraday reproducibility, five SWASVs of 0.5 ppm CPF were assessed at five new GCPE surfaces under the optimum working conditions (data not shown). The mean ± standard deviation peak current was 3.0 ± 0.2 μA, with

Table 3 Electrochemical determination of ciprofloxacin at GCPE in pharmaceutical formulations in different media

Product name	Labeled amount (mg)	Detection media (pH)	Found amount (mg)	Recovery (%)
Floxacin	500.0	0.1 M PB (5.0)	500.0	100.0
Floxacin	500.0	0.1 M PB (5.0) containing 10.0 % Fresh human urine (v/v)	525.0	105.0

Table 4 Ciprofloxacin determination by several electrochemical methods

Method	Sensing materials	Sensing media (pH)	Linear range (μM)	Limit of detection (μM)	Limit of quantification (μM)	Ref.
Square-wave stripping voltammetry	GCPE	0.1 M PB (pH 5.0)	027–2.0	0.033	0.027	Present work
Differential pulse adsorptive voltammetry	CPE with Cetyltrimethylammonium bromide	0.1 M PB (7.0)	0.1–20.0	50.0	–	[28]
Amperometry	Carbon nanotube (CNT) composite film on GCE	–	40.0–1,000.0	6.0	–	[17]
Cyclic voltammetry	MgFe ₂ O ₄ NPs-CNTs-modified GCE	0.1 M PB(3.0)	0.10–1,000.0	0.01	0.08	[29]
Potentiometry	Molecularly imprinted network receptors	0.05 M PB(4.5)	50–1,000.0	10.0	50.0	[30]
Potentiometry	Molecularly imprinted polymer	0.01 M HEPES (pH 4)	–	10.0	–	[31]
Potentiometry	CPF nanocomposite of CP, and PVC membrane	Water	1.0–100,000.0	1.0	–	[27]

an RSD of 6.96 %. Similarly, the mean \pm standard deviation interday reproducibility was $3.1 \pm 0.2 \mu\text{A}$, with an RSD of 6.47 %. Thus, this electrode showed quite reproducible behavior.

3.4 Interference from Other Compounds

The effects of organic and inorganic compounds commonly present in pharmaceuticals and biological samples on the determination of 0.5 ppm CPF were analyzed using the optimal parameters of activated GCPE. At the same concentration as CPF, uric acid, cytosine, L-alanine, L-phenylalanine, L-tryptophan, L-cysteine, L-tyrosine, Triton X-100, DL-methionine, dopamine, paracetamol, L-ascorbic acid, guanine, deoxyribonucleic acid, Zn²⁺, Cu²⁺, Fe³⁺, Pb²⁺, Na⁺, K⁺, and NH₄⁺ (Table 2) had almost no effect on the peak current response of CPF, with signal changes $\leq 10\%$.

3.5 Concentration Dependence

Concentration-dependent SWASVs of GPCE were obtained under optimum conditions, and the calibration plot was constructed after subtracting the mean of the corresponding zero

CPF response (Fig. 5). The curve (Fig. 5B) showed a linear dynamic range from 10 to 750 ppb, as the peak current increased linearly with increasing CPF concentration ($R^2 = 0.97$). The calibration plot obeyed the linear regression equation, $y = 0.0069x - 0.0483$, where y and x are the peak current and concentration of CPF, respectively. The peak current did not increase further at CPF concentrations higher than 750 ppb. The net peak current at 10 ppb CPF was greater than three standard deviations of the current in the absence of CPF (Fig. 5B, inset), making the limit of quantification of the sensor 10 ppb (27 nM) CPF. The calculated limit of detection at 3σ was 12.2 ppb (33 nM) CPF.

3.6 Analytical Determination of CPF in Commercial Drugs (Floxacin) in Buffer with and Without 10 % Urine

The concentration of CPF in Floxacin solution was determined in PB and in PB containing 10 % urine by the standard addition method, in which increments of standard CPF were successively introduced into a single measured volume (4.0 μL) of the unknown commercial sample (Fig. 6A, B), with SWASVs determined for the original sample (Fig. 6A, a) and

after each addition (Fig. 6A, b–d). Increasing the CPF concentration increased the CPF recorded peak current gradually in both media (Fig. 6A, B, insets).

The calculated percentage of CPF recovered from Floxacin in buffer was about 100 % (Fig. 6A, inset), whereas the calculated percentage of CPF recovered from Floxacin in buffer containing 10 % fresh urine was 105 % (Fig. 6B, inset). The analyses of CPF in both media are summarized in Table 3.

4 Conclusions

GCPE was used to measure CPF in 0.1 M PB, pH 5.0, as it showed the highest signal among the tested electrodes. The limit of detection of this sensor was 10 ppb, without enhancement of the analyte. The sensor benefited from the high catalytic properties of activated GCPE, the accumulation of CPF, proper buffer selection and optimal SWASV parameters. This low limit of detection was comparable to that of the previously described electrochemical CPF sensor (Table 4). The sensor described here may be useful in analyzing CPF in pure form and in pharmaceutical products, either in buffer or buffer containing biological samples. This procedure is therefore suitable for the accurate and precise routine determination of CPF in pharmaceuticals, making it suitable for routine analysis in clinical laboratories.

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