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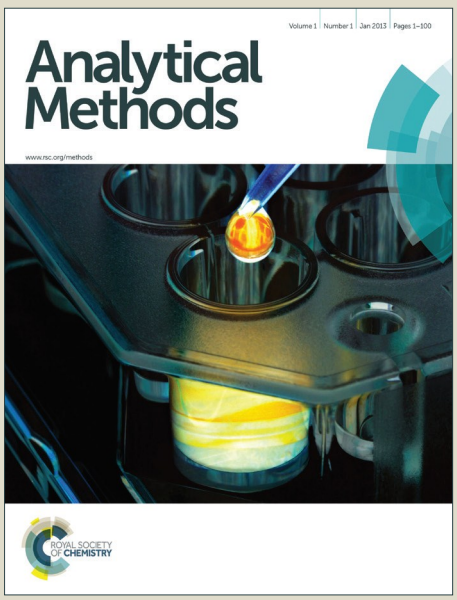

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Voltammetric determination of ciprofloxacin in urine samples and its interaction with dsDNA on a cathodically pretreated boron-doped diamond electrode

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Abstract

Voltammetric methods for the determination of the fluoroquinolone ciprofloxacin (CIP) were developed using a cathodically pretreated boron-doped diamond (BDD) electrode coupled with square-wave voltammetry (SWV) and differential pulse voltammetry (DPV). In cyclic voltammetric measurements, the CIP electrooxidation was an irreversible process controlled by diffusion of the analyte to the electrode surface. Analytical curves were obtained for CIP concentrations from 2.50 to 50.0 μmol L^{-1}, for SWV, and from 0.500 to 60.0 μmol L^{-1}, for DPV, with detection limits of 2.46 and 0.440 μmol L^{-1}, respectively. On the other hand, adequate recovery values were obtained for the determination of CIP in synthetic urine samples by DPV. On the other hand, SWV was employed to evaluate the interaction between CIP and double-stranded dsDNA (calf thymus in aqueous solution). From the obtained results, we inferred that CIP binds to dsDNA by intercalation, with a binding constant calculated as 5.91×10^5 L mol^{-1}. Thus, the cathodically pre-treated BDD electrode was successfully used for the determination of CIP in biological samples and for studies on the interaction of that fluoroquinolone with dsDNA.

Keywords: Ciprofloxacin electroanalytical determination, BDD electrode, Cathodic pretreatment, Square-wave voltammetry, Differential pulse voltammetry, DNA.

1. Introduction

Fluoroquinolones (FQs), third-generation quinolones characterized by fluorination at the C6 position, became very widely used because of their activity against both gram-negative and gram-positive bacteria in urinary tract infections, osteomyelitis, community-acquired pneumonia, and gastroenteritis.[1] It has also been found that FQs present activity against *M. tuberculosis*.[2] Concerning the mechanism of action, FQs interact with enzyme-bound DNA complexes and thus produce conformational changes that lead to inhibition of normal enzyme activity.[3] Consequently, the resulting drug-enzyme-DNA complex blocks progression of replication and hinders normal bacterial DNA synthesis, which leads to the rapid death of bacterial cells.3 Among FQs, ciprofloxacin (CIP – see Fig. 1) is extensively used and has an oral bioavailability of 70%, with a mean elimination half-life of 4 h in healthy humans.[4] The main excretion pathway of CIP is urinary with usual concentrations in the range of 100–200 mg L^{-1}. Adverse effects on the gastrointestinal tract and the central nervous system may be observed during therapy with this drug.[5]

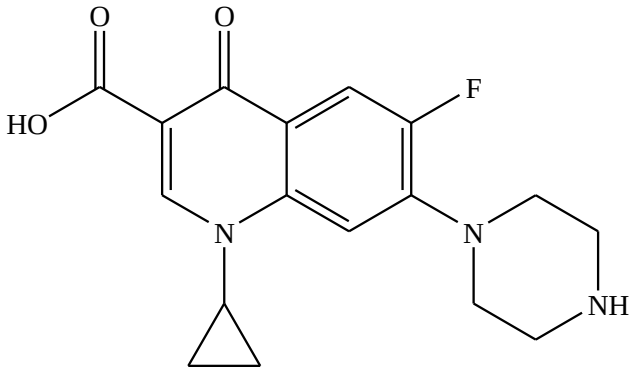

Fig. 1 Chemical structure of ciprofloxacin.

The electrochemical behavior and the quantification of CIP have been previously investigated using different electrode materials. Ni et al.[6] determined CIP using linear sweep stripping voltammetry (LSSV) and a mercury electrode. The obtained analytical curve was linear for the concentration range of about 97 to 870 µmol L^{-1}, with a limit of detection (LOD) value of 33 µmol L^{-1}. The authors also proposed the simultaneous determination of CIP, ofloxacin, and norfloxacin by LSSV and a chemometric analysis. Fotouhi et al.[7] determined CIP by amperometry coupled with a multi-wall carbon nanotubes film-modified GC (MWCNT/GC) electrode; the obtained analytical curve presented a linear concentration

range of 40 to 1000 μmol L^{-1}, with a LOD value of 6 μmol L^{-1}. Ensafi et al.[8] developed a method for the determination of CIP also at a MWCNT/GC electrode, but using linear sweep voltammetry; the obtained analytical curve presented a linear concentration range of 3.0 to 1200 μmol L^{-1}, with a LOD value of 0.9 μmol L^{-1}. In another paper of the same research group,[9] a sensor containing $MgFe_2O_4$ nanoparticles and multi-walled carbon nanotubes ($MgFe_2O_4$-MWCNTs) was prepared and used for the determination of CIP by cyclic voltammetry (CV); the obtained oxidation peak current was dependent on the analyte concentration, with a linear response for the concentration range of 0.10 to 1000 μmol L^{-1} and a LOD value of 0.01 μmol L^{-1}. Nejem et al.[10] developed a sensor based on polyvinyl chloride, where the electroactive compound was the complex between CIP and tetraphenylborate. A wider linear concentration range (19.7 μmol L^{-1} to 20.0 mmol L^{-1}) was obtained when dibutylphthalate or bis(2-ethylhexyl)sebacate was used as plasticizer, with LOD values of 3.70 or 7.36 μmol L^{-1}, respectively. Zhang et al.[11] constructed a sensor for the determination of FQs based on the polymerization of β-cyclodextrin and L-arginine on a carbon paste electrode. Using differential pulse voltammetry (DPV), for CIP they obtained a linear concentration range of 0.05 to 100 μmol L^{-1}, with a LOD value of 0.01 μmol L^{-1}. Recently, Diab et al.[12] investigated the voltammetric behavior and the determination of CIP using CV and differential pulse anodic stripping voltammetry (DPASV) coupled with a bare or DNA-modified GC electrode. For DPASV and the modified GC electrode, a linear response was obtained for the concentration range of 1.0 to 10.0 μmol L^{-1}, with a LOD value of 0.12 μmol L^{-1}. On the other hand, Kawde et al.[13] compared the CIP analytical signals obtained using square-wave adsorptive stripping voltammetry coupled with a GC, GC paste (GCP) or carbon paste electrode. With the GCP electrode, a linear response was obtained for the concentration range of 0.27 to 2.00 μmol L^{-1}, with a LOD value of 0.033 μmol L^{-1}. Additionally, Al-Ghamdiz et al.,[14] using square-wave voltammetry (SWV) and a hanging mercury drop electrode, obtained a linear analytical curve for the CIP concentration range of 0.30 to 2.00 μmol L^{-1}, with a LOD value of 0.007 μmol L^{-1}. Finally, Montes et al.[15] reported on the development of two analytical methods for the determination of CIP using a boron-doped diamond (BDD) electrode: batch-injection analysis with amperometric detection (BIA-AMP) and capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C^4D). For these methods, the obtained linear ranges were of 1 to 1000 μmol L^{-1} and 50 to 250 μmol L^{-1}, with LOD values of 0.3 and 5.0 μmol L^{-1}, respectively.

Several articles have reported that CIP interacts with the DNA molecule and that this interaction could be related with genotoxic activity.5,[16,17] DNA plays a key role in cell

proliferation, synthesis of proteins, and transcription of genetic information in living cells.[18] Damage in the DNA cells and/or in the double helix structure of biomolecule following interaction with toxic compounds may cause mutations that can result in the development of cancers.[19] As recalled by Arshad et al.,[20] the binding of drugs to DNA occurs covalently and non-covalently. In the latter case, it may involve binding *via* the minor groove of DNA (the negatively charged phosphates outside the DNA double helix) or intercalation between the unwound DNA base pair.

Electrochemical methods are excellent tools to evaluate the interactions of compounds with the DNA molecule using DNA-modified electrodes or assessing DNA in the solution phase.[21-26] In a modified electrode, changes in terms of current intensity and/or peak potential in the electrochemical response of DNA nitrogenous bases residues can reflect alterations in the double-stranded DNA (dsDNA) structure. In the solution phase, the interaction of compounds with dsDNA can be monitored by alterations in the electrochemical response of the target compound. dsDNA presents limited accessibility to the nitrogenous base residues contained in nucleotides within the rigid structure of the DNA double helix and, consequently, no oxidation peaks are detected.[27] This kind of evaluation considering the target compound has been performed for calcein,[28] gallic acid,[29] nicotine,[30] and magnolol.[31] Particularly, the interaction of CIP with DNA has been evaluated using mercury,[32] GC,[12,33] and MWCNT/GC electrodes.[34] To the best of our knowledge, the use of a BDD electrode to monitor the interaction between CIP and dsDNA has never been reported.

BDD is a type of sp^3 carbon material that presents very low and stable background current, a wide working potential window, mechanical robustness, compatibility with biological materials, and high data reproducibility.[35] Thus, an excellent performance of BDD electrodes has been observed in the determination or study of the redox behavior of many organic compounds.[36-40] An interesting aspect of BDD electrodes is that, for some analytes, the electrochemical response depends on the surface termination (hydrogen or oxygen), which can be tuned by cathodic or anodic electrochemical pretreatments, respectively.[41-45]

In this study, we report on the use of a cathodically pretreated BDD electrode to investigate the electrochemical behavior of CIP, using CV, to develop new analytical methods for the determination of this analyte in urine samples, using DPV and SWV, and to evaluate the interaction of this drug with dsDNA in aqueous solutions, using SWV.

2. Experimental

2.1. Reagents and solutions

All reagents, including CIP (Sigma-Aldrich, ≥98%), H_2SO_4 (Sigma-Aldrich, 95–98%), calf thymus dsDNA (Sigma-Aldrich), boric acid (Acros Organic, 99.5%), phosphoric acid (QHemis, 85% m/v), acetic acid (QHemis, 99.7% v/v), and sodium hydroxide (Synth), were used as received. A 10 mmol L^{-1} CIP stock solution was prepared in aqueous 0.5 mol L^{-1} H_2SO_4; the total dissolution of the compound was attained through vigorous shaking, followed by ultrasonication for 10 min. Before use, the aqueous 2.5 mg mL^{-1} stock solution of DNA was stored at 4 ^{o}C for 24 h to ensure complete dissolution of the nucleic acid. The nucleotide (monomer units) concentration was calculated based on the molar absorption coefficient at 260 nm (ϵ_{260} = 6600 L mol^{-1} cm^{-1}). The ratio of the DNA absorbance intensities at 260 and 280 nm was 1.88, indicating that the DNA was free from proteins.[46]

In most electrochemical measurements, a pH 7.0 (0.1 mol L^{-1}) Britton-Robinson (BR) buffer solution (0.04 mol L^{-1} in acetic, phosphoric, and boric acids) was used as supporting electrolyte; its pH was adjusted to 7.0 with a 1.0 mol L^{-1} NaOH solution. Synthetic urine was also used, of which a sample (250 mL) was prepared by dissolving in water 0.73 g of NaCl (Acros Organic, 99.5%), 0.40 g of KCl (Acros Organic, 99%), 0.28 g of $CaCl_2.2H_2O$ (Nuclear, 99–107%), 0.56 g of Na_2SO_4 (Merck, 99%), 0.35 g of KH_2PO_4 (Acros Organic, 99%), 0.25 g of NH_4Cl (Mallinckrodt Baker 99.5%), and 6.25 g of urea (Reagen).[47]

2.2. Apparatus

All electrochemical measurements were performed using an Autolab PGSTAT-30 (Eco Chemie) potentiostat/galvanostat controlled with the GPES 4.9 software. The voltammetric studies were carried out using a three-electrode single-compartment Pyrex® glass cell (20 mL), with a BDD electrode (0.36 cm^2 exposed area) as the working electrode, a 1 cm^2 Pt foil as the counter electrode, and an Ag/AgCl (3.0 mol L^{-1} KCl) as the reference electrode, to which all electrode potentials hereinafter are referred to. The BDD electrode, which consisted of a BDD film (boron content of 8000 ppm) deposited by CVD on a p-silicon substrate, was acquired from Adamant Technologies, La Chaux-de-Fonds, Switzerland.

All the voltammograms obtained by DPV and SWV were baseline-corrected by application of the moving average method (included in the GPES 4.9 software), with a step

window of 5 mV. The BDD electrode surface was electrochemically activated by two procedures: (i) anodic pretreatment (APT): polarization at 0.5 A cm^{-2} for 10 s in a 0.5 mol L^{-1} H_2SO_4 solution, and (ii) anodic pretreatment followed by a cathodic pretreatment (CPT): polarization at 0.5 and –0.5 A cm^{-2} for 5 and 180 s, respectively, also in a 0.5 mol L^{-1} H_2SO_4 solution (the cathodic pretreatment was always preceded by a short anodic one to assure that the electrode surface was effectively clean). The voltammetric responses of CIP on the BDD electrode activated by these two different pretreatments were compared. The pH of the BR buffer solution was measured with an Orion pH meter (Expandable Ion Analyzer EA 940), which was recalibrated daily using commercial buffer solutions of pH 4.0, 7.0, and 10.0.

2.3. Electrochemical behavior and determination of CIP

CV was used to investigate the electrooxidation of CIP on the BDD electrode. DPV and SWV were used to develop the electroanalytical method to determine CIP, which was then applied to doped synthetic urine samples. After the optimization of the experimental parameters for these techniques, the respective analytical curves (triplicate for both SWV and DPV) were obtained by spiking the electrolyte with known quantities of the CIP stock solution. The limit of detection (*LOD*) for both techniques was calculated as equal to three times the standard deviation for the blank solution (n = 6) divided by the slope (average of three slopes) of the respective analytical curve.[38,43] The obtained *LOD* values were compared to choose the best performing voltammetric method. For both techniques, the repeatability of the CIP responses in the same solution and in different solutions was checked with intra-day (n = 10) and inter-day (n = 10) determinations for 50 µmol L^{-1} CIP, when relative standard deviations (*RSD*) were calculated.

The best performing voltammetric method was used to determine CIP in urine, which was used immediately after its preparation. For that, CIP at two different concentrations (6.0 and 40.0 µmol L^{-1}) was added to urine samples and these were directly analyzed in triplicate.

2.4. Interaction of CIP with dsDNA in solution

Square-wave voltammograms were obtained using the CPT BDD electrode for solutions containing 50.0 mg L^{-1} (124 µmol L^{-1}) dsDNA and different concentrations of CIP (from 5.0 to 100 µmol L^{-1}) in the 0.1 mol L^{-1} BR buffer solution (pH 7.0), after interaction

times of 10 s and 3 h. Analytical curves (n = 3) for CIP in the absence and presence of dsDNA were compared.

3. Results and Discussion

First, the voltammetric behavior of CIP was investigated in a 0.1 mol L^{-1} BR buffer solution (pH 7.0, a value in the physiological range[48]) using CV, SWV, and DPV. Then SWV was used to investigate the interaction of CIP with dsDNA, since this can provide valuable information on the genotoxicity of the drug.

3.1. Electrochemical pretreatment of the BDD electrode

To determine whether the predominant type of surface termination on the BDD electrode had any effect on its electrochemical activity towards CIP, square-wave voltammograms for 50 μmol L^{-1} CIP were obtained on an APT or CPT BDD electrode. As can be seen in Fig. 2, the electrooxidation of CIP is irreversible, but for both pretreatments of the BDD electrode, well-defined voltammetric shapes and intense responses for CIP were obtained: on the CPT BDD electrode, the oxidation peak current (I_p) and peak potential (E_p) for the drug were 37.0 μA and 1.04 V, respectively, whereas on the APT BDD electrode they 22.8 μA and 1.01 V. Thus, a ~62 % more-intense I_p value was obtained using a CPT BDD electrode (with a 30 mV more positive E_p value). Hence, the cathodic pretreatment of the BDD electrode was chosen for all additional measurements. The anodic peaks in the voltammogram shown in Fig. 2 are due to the two-electron oxidation of the CIP amine group to hydroxylamine (piperazine moiety).[12,34]

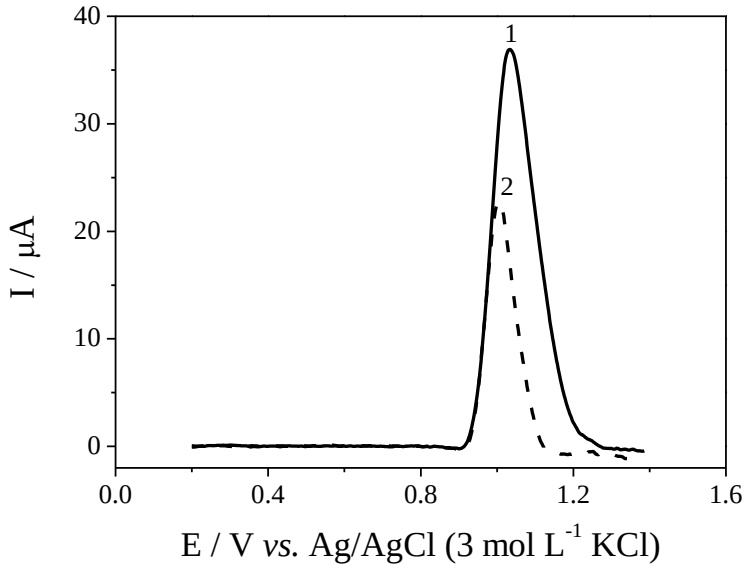

Fig. 2 Square-wave voltammograms obtained for 50 μmol L^{-1} CIP in a 0.1 mol L^{-1} BR buffer solution (pH 7.0) at a cathodically (1) or anodically (2) pretreated BDD electrode. SWV conditions: f = 100 Hz, a = 50 mV, and ΔE_s = 2 mV.

3.2. CIP voltammetric behavior

The electrochemical behavior of CIP was investigated by CV for 50 μmol L^{-1} CIP in a 0.1 mol L^{-1} BR buffer solution (pH 7.0). First, successive cyclic voltammograms at a scan rate (v) of 200 mV s^{-1} were obtained without any intermediate cleaning of the electrode surface; as can be seen in Fig. 3A, continuously diminishing values of the CIP current peak (voltammograms 1–5, peak I) were obtained due to the adsorption of the drug and/or its oxidation products on the electrode surface. Peak II, which is seen only in the first scan, might be attributed to sp^2-carbon impurities on the BDD surface.[49] After cleaning of the BDD surface with just squirts of deionized water, a response similar to that of the first voltammogram was obtained; thus, this simple cleaning procedure is sufficient to reactivate the BDD electrode surface. Since no cathodic peaks were observed during the reverse CV scan (see Fig. 3A), we conclude that the CIP electrooxidation process is irreversible, as previously observed by SWV (Fig. 2).

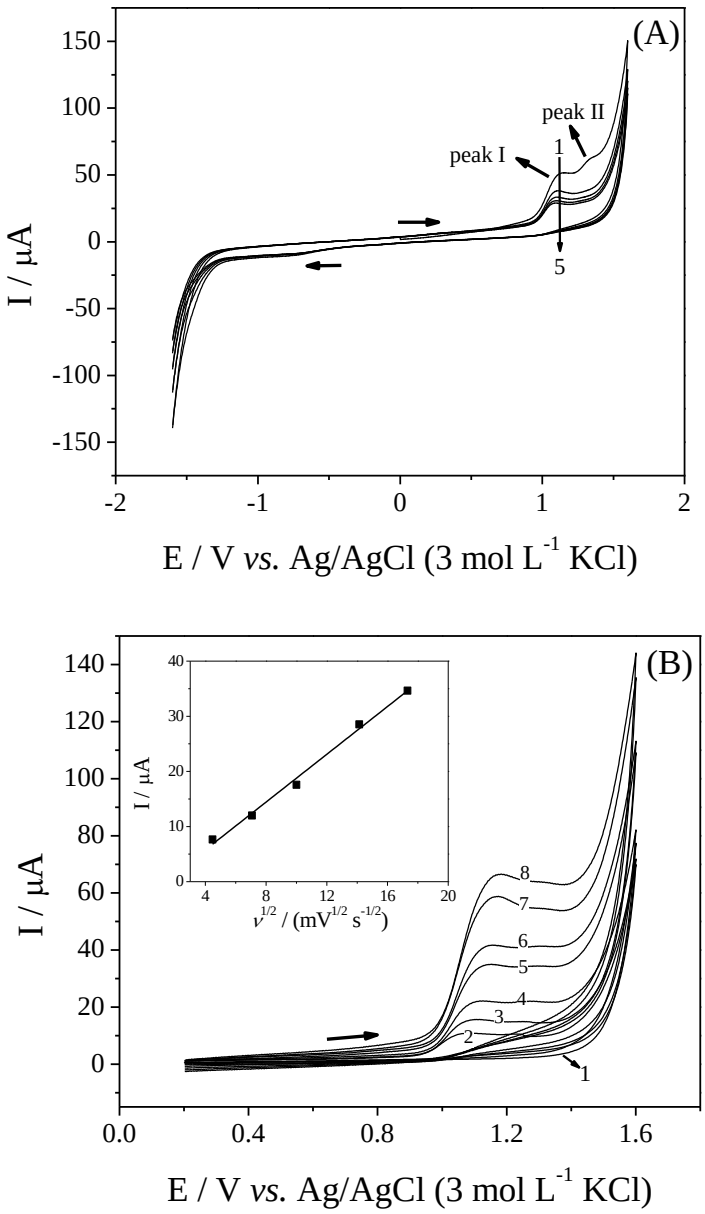

Fig. 3 (A) Successive cyclic voltammograms (1-5) obtained with a cathodically pretreated BDD electrode at a scan rate of 200 mV s^{-1} for 50 μmol L^{-1} CIP in a 0.1 mol L^{-1} BR buffer solution (pH 7.0). (B) Cyclic voltammograms for the blank solution (0.1 mol L^{-1} BR buffer solution (pH 7.0), 1) and for 50 μmol L^{-1} CIP in a 0.1 mol L^{-1} BR buffer solution (pH 7.0) using scan rates of 20 (1, 2), 50 (3), 100 (4), 200 (5), 300 (6), 400 (7), and 500 mV s^{-1} (8). Inset: respective oxidation peak currents *vs.* square root of the scan rate (linear from 20 to 300 mV s^{-1}, with r = 0.9923).

Next, cyclic voltammograms were recorded for a 50 μmol L^{-1} CIP solution at different scan rates in the range of 20 to 500 mV s^{-1} (Fig. 3B); a clean CPT BDD electrode surface was used to record each voltammogram. Again, no cathodic peaks were observed, which confirms the irreversibility of the CIP electrooxidation process. From the slope of the obtained linear

plot of I_p *vs.* v (r = 0.9875), the surface concentration (Γ) of the electroactive species was estimated as 77 pmol cm^{-2}, according to the following equation:[34]

$$I = \frac{n^2F^2vA\Gamma}{4RT} \quad (1)$$

where n is the number of electrons involved in reaction (assuming that n = 2), F the Faraday constant (96485 C mol^{-1}), A the electrode surface area (0.36 cm^2), R the universal gas constant (8.314 J mol^{-1} K^{-1}), and T the thermodynamic temperature (298 K).

As can be seen in the inset of Fig. 3B, the peak current for peak I (after baseline correction) increased proportionally to the square root of the scan rate (linearly from 20 to 300 mV s^{-1}, with r = 0.9923); this behavior confirms the irreversibility of the electrooxidation process.[50,51] On the other hand, the good linearity of the I_p *vs.* v and I_p *vs.* $v^{1/2}$ plots is an indication that the reaction is proceeding via diffusion of the analyte to the BDD surface and involves adsorption/desorption of reactants and products, as previously observed for estrone.[52] Additionally, the linear relationship between log I_p and log v for the CIP oxidation peak could be described by the following equation: log I_p = 0.0242 + 0.630 log v, with r = 0.9836 (linear for the v range of 20 to 500 mV s^{-1}). In this case, a slope equal to 0.5 is related to a process that is controlled by semi-infinite linear diffusion, while a slope equal to 1 is related to adsorption control;[52,53] thus, from the slope value of 0.630, we infer that adsorption is not strong for the CIP oxidation on the BDD electrode and that the reaction is limited by diffusion of the analyte to the electrode surface.

As can be perceived from Fig. 3B, the value of E_p for CIP electrooxidation shifted to more positive values as the scan rate increased, which is another indication that the oxidation reaction is associated to an irreversible electron-transfer kinetics.[50] For a totally irreversible reaction, the transfer coefficient (α) and the standard heterogeneous rate constant (k_s) can be calculated using the following equation:[50,54,55]

$$I_p = 0.227nFAC_0^*k_S\, exp\left[(\alpha n_\alpha F/RT)\left(E_p - E^0\right)\right] \quad (2)$$

where C_0^* is the CIP concentration and E^0 the formal redox potential. Indeed, a plot of ln I_p *vs.* ($E_p - E^0$) for the different scan rates yielded a straight line, whose slope and intercept are proportional to $\alpha n_\alpha F/RT$ and k_s, respectively: ln I_p = –11.4 + 21.8 ($E_p - E^0$), with r = 0.9963. Considering that a value of E^0 = 1.10 V was obtained from the intercept of a plot of E_p *vs.* v,

values of 0.56 and 1.35×10^{-2} cm s^{-1} were calculated for αn_α and k_s. Thus, assuming $n = 2$, α can be estimated as equal to 0.28, a reasonable value since the transfer coefficient can range from zero to one.[50] From the obtained k_s value, we can conclude that the electron-transfer kinetics is quite fast, despite being an irreversible process.

3.3. CIP determination

The determination of CIP using SWV or DPV was comparatively investigated to obtain the best electroanalytical method for subsequent studies. For both cases, the effect of the respective experimental parameters on E_p and I_p for 50 µmol L^{-1} CIP in a 0.1 mol L^{-1} BR buffer solution (pH 7.0) was investigated. For SWV, the investigated parameter values were: 10–200 Hz, for the square-wave frequency (*f*); only 50 mV, for the pulse amplitude (*a*); 2–10 mV, for the scan increment (ΔE_s). The obtained optimized values were f = 100 Hz and ΔE_s = 2 mV. For DPV, the investigated parameter values were: 10–200 mV, for the pulse amplitude (*a*); 2.0–20 mV s^{-1}, for the scan rate (*v*); 2.5–15 ms, for the modulation time (*t*). The selected optimized values were a = 80 mV, v = 10 mV s^{-1}, and t = 7.5 ms. Then, using the optimized parameter values of each technique, the respective analytical curves were obtained (in triplicate) for different concentrations ranges: from 2.50 to 50.0 µmol L^{-1}, for SWV, and from 0.500 to 60.0 µmol L^{-1}, for DPV (see Fig. 4). The obtained *LOD* values using SWV and DPV were 2.46 and 0.440 µmol L^{-1}, respectively. All the analytical parameters associated to these curves are listed in Table 1.

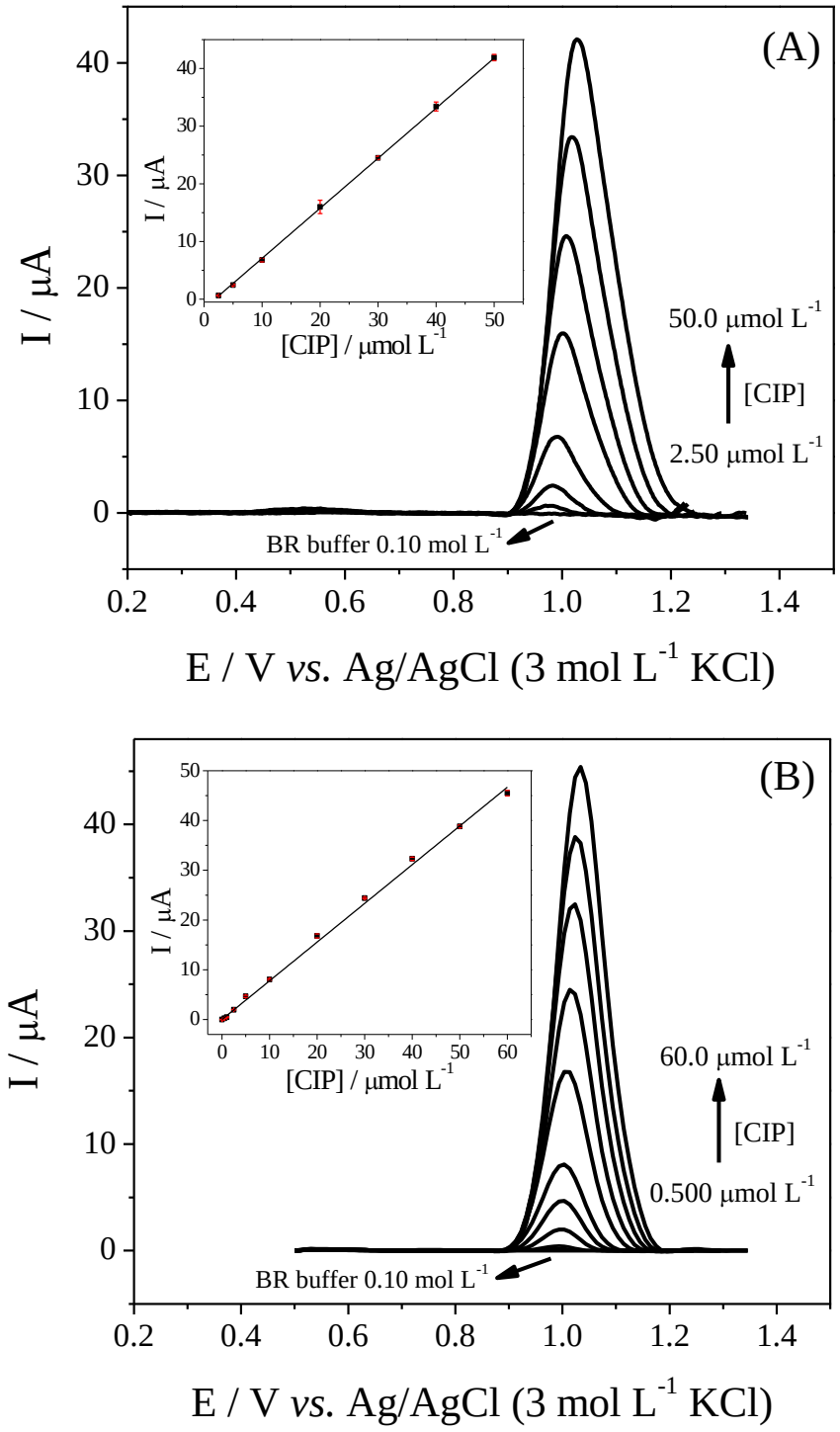

Fig. 4 (A) Square-wave (f = 100 Hz, a = 50 mV, and ΔE_s = 2 mV) and (B) differential pulse (a = 80 mV, v = 10 mV s^{-1}, and t = 7.5 ms) voltammetric curves obtained for the oxidation of CIP at different concentrations in a 0.1 mol L^{-1} BR buffer solution (pH 7.0) using a cathodically pretreated BDD electrode. CIP concentrations: (A) 2.50, 5.00, 10.0, 20.0, 30.0, 40.0, and 50.0 μmol L^{-1}; (B) 0.500, 1.00, 2.50, 5.00, 10.0, 20.0, 30.0, 40.0, 50.0, and 60.0 μmol L^{-1}. Insets: respective analytical curves.

Table 1 Analytical results obtained with the SWV and DPV techniques in the determination of CIP in a 0.1 mol L^{-1} BR buffer solution (pH 7) using a cathodically pretreated BDD electrode.

Parameters	SWV	DPV
Intercept (y_B) / μA	–2.0 ± 0.3	0.5 ± 0.1
Slope / (μA L μmol^{-1})	0.89 ± 0.01	0.767 ± 0.006
Limit of detection (μmol L^{-1})	2.46	0.440
Linearity range (μmol L^{-1})	2.50 – 50.0	0.500 – 60.0
Correlation coefficient (r)	0.998	0.997

Parameters values used in each technique: SWV – f = 100 Hz, a = 50 mV, and ΔE_s = 2 mV; DPV – a = 80 mV, v = 10 mV s^{-1}, and t = 7.5 ms.

For SWV, the repeatability of the CIP responses for intra-day (n = 10) and inter-day (n = 10) determinations of a 50 μmol L^{-1} CIP solution resulted in *RSD* values of 3.32% and 6.86%, respectively. For DPV, these values were 1.35% and 5.30%, respectively. Hence, from these obtained values we conclude that the proposed voltammetric methods are adequately precise. However, according to the results obtained for the determination of CIP using both voltammetric techniques (Table 1), a lower *LOD* value was attained when DPV was used. Thus, this technique was chosen for subsequent investigation on the determination of CIP in urine samples.

The *LOD* value obtained by DPV (0.440 μmol L^{-1}) is lower than the values previously reported by Fotouhi et al.7 (6 μmol L^{-1}) using MWCNT/GCE and amperometry, Ensafi et al.8 (0.9 μmol L^{-1}) also using MWCNT/GCE with linear sweep voltammetry, and by Nawaz et al.[33] (9.0 μmol L^{-1}) using DPV with a DNA-modified GC electrode. Furthermore, the here-obtained *LOD* value is close to or lower than those obtained by Montes et al.[15] using a BDD electrode with BIA-AMP (0.3 μmol L^{-1}) or with CE-C^4D (5.0 μmol L^{-1}), respectively. This analytical aspect attests the good performance of the BDD electrode attained after a simple and rapid electrochemical pretreatment in comparison to that attained with a modified GC electrode.

3.4. Application of the DPV method to urine samples

Since the main excretion pathway of CIP is urinary, the proposed DPV method was applied to determine two different concentrations of CIP (6.0 and 40.0 μmol L^{-1}) in urine samples, when excellent recoveries were obtained (see Table 2). Hence, we conclude that the DPV method can be used for the determination of CIP in urine samples, since there is no considerable interference from the urine matrix.

Table 2 Results for the recovery of CIP from urine samples using the DPV method with a cathodically pretreated BDD electrode (n = 3).

Sample	CIP / (μmol L^{-1})		Average recovery (%)
	Added	Found	
A	6.00	6.06 ± 0.09	101
B	40.0	39.8 ± 0.9	99.5

3.5. Interaction of CIP with DNA

Calf thymus DNA, consisting of 41.9 mol % of guanine-cytosine and 58.1 mol % of adenine-thymine, is a natural DNA that is widely used in studies on the binding of drugs or pollutants that affect the structure and function of DNA. Recently, Garbellini et al.[56] evaluated the interaction of some organophosphorus pesticides (chlorpyrifos, metamidophos, and monocrotophos) with calf thymus dsDNA using a BDD electrode, using different DNA-pesticide interaction times and pesticide concentrations.

In the present work, SW voltammograms were obtained for 50.0 mg L^{-1} (124 μmol L^{-1}) dsDNA and different concentrations of CIP (from 5.0 to 100 μmol L^{-1}) in the 0.1 mol L^{-1} BR buffer solution (pH 7.0), after interaction times of 10 s and 3 h, using the CPT BDD electrode (see Fig. 5).

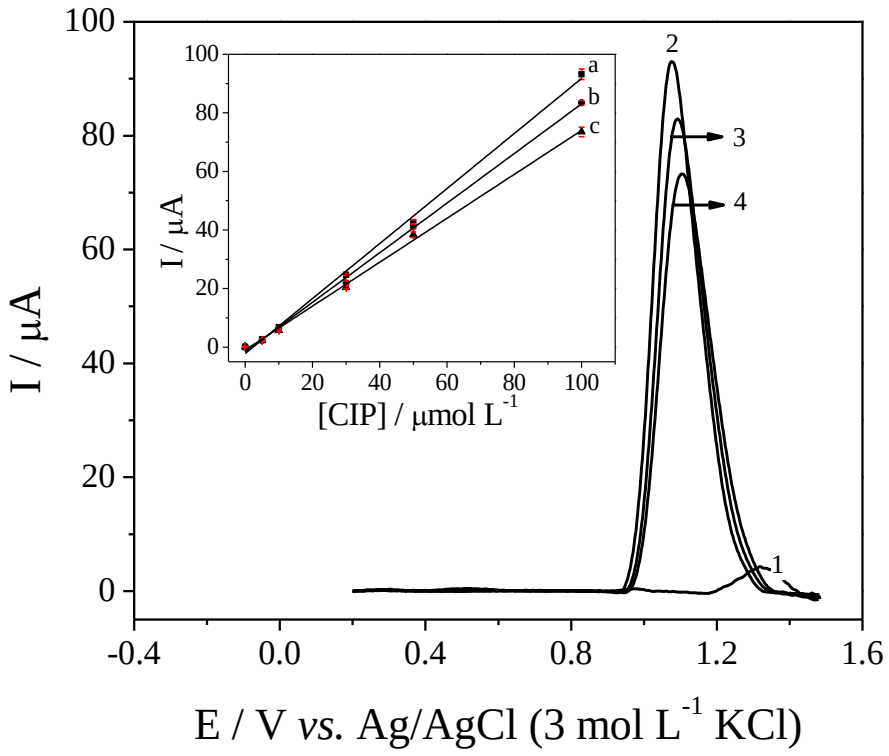

Fig. 5 Square-wave voltammetric curves obtained using a cathodically pretreated BDD electrode and a 0.1 mol L^{-1} BR buffer solution (pH 7.0) containing: 124 μmol L^{-1} dsDNA (1); 100 μmol L^{-1} CIP (2); 124 μmol L^{-1} dsDNA + 100 μmol L^{-1} CIP with interaction time of 10 s (3) or 3 h (4). Inset: Analytical curves (n = 3) for individual CIP (a) and for CIP in the presence of 124 μmol L^{-1} dsDNA with interaction time of 10 s (b) or 3 h (c).

The interaction between the two species was monitored through alterations in the CIP voltammetric signal. Throughout this study the ionic strength was kept at 0.0758 mol L^{-1}, as reported by Mongay and Cerda[57] for the Britton-Robinson buffer at pH 7.0. Thus, in the presence of dsDNA, a decrease in the CIP oxidation I_p and a positive shift in the CIP peak potential E_p were observed (see Fig. 5), which are indications of the occurrence of CIP interaction with dsDNA. The decrease of the I_p value of 11% or 21% for CIP in the presence of dsDNA for interaction times of 10 s or 3 h, respectively, when compared to the CIP peak in the absence of the nucleic acid, can be attributed to a decrease of the free drug concentration due to the formation of a slow-diffusion CIP-DNA complex. With the addition of dsDNA, the E_p value is positively shifted, from 1.076 V (without dsDNA) to 1.097 V (interaction time of 10 s) or 1.108 V (interaction time of 3 h); from this, we infer that the CIP interaction with DNA possibly occurs by intercalation, as previously reported by Fotouhi et al.,[34] who used CV and UV-Vis spectroscopy, and Radi et al.,[58] who used DPV to analyze the interaction of

some FQs with DNA in solution. Radi et al.[58] also suggest that the piperazine moiety of FQs plays an important role in binding to dsDNA.

Analytical curves (n = 3) for CIP (from 5.0 to 100 μmol L^{-1}) in the absence and presence of 124 μmol L^{-1} dsDNA were compared, as presented in the inset of Fig. 5 (the *RSD* values for all the concentration levels in curves a, b, and c were lower than 7.0%, 7.6%, and 4.6%, respectively). Clearly, the slopes obtained for the CIP curves in the presence of dsDNA (0.848 μA L μmol^{-1}, for interaction time of 10 s, and 0.750 μA L μmol^{-1}, for that of 3 h) were lower than the value obtained for the CIP curve in the absence of the biomolecule (0.939 μA L μmol^{-1}). Moreover, the CIP – dsDNA interaction is time dependent, since it was clearly more significant after 3 h of interaction.

It is assumed that CIP binds to dsDNA to form a type of cooperative complex. To determine the binding ratio (m) and the binding constant (K_b) for this complex, the following equation can be used:[30,59,60]

$$\log [\Delta I / (\Delta I_{max} - \Delta I)] = \log K_b + m \log [CIP] \quad (3)$$

where ΔI is the difference of oxidation peak current for CIP in the absence and presence of dsDNA after 3 h of interaction; the maximum value of ΔI is designated as ΔI_{max}. As expected, CIP and dsDNA form a single complex; thus, the plot log [ΔI / (ΔI_{max} – ΔI)] *vs.* log [CIP] is a straight line (r = 0.9997). From the slope and intercept of this straight line, the experimental values of m and K_b could be obtained as 1.39 and 5.91×10^5 L mol^{-1}, respectively. Hence, considering the obtained value of m, we propose that a stable 1:1 complex (dsDNA:CIP) is formed. On the other hand, we conclude that the CIP – dsDNA interactions occur via intercalation, since the obtained value of K_b is in the range of 10^5 to 10^{11} L mol^{-1}.[23] Furthermore, it should be noted that the obtained value of K_b is almost identical to that reported by Dogan-Topal et al.[26] (6.03×10^5 L mol^{-1}) for the DNA cooperative complex with the anticancer drug lapatinib, which also intercalates into the nucleic acid.

One additional point that should be discussed in Fig. 5 is voltammogram 1, which was obtained for a solution containing 50 mg L^{-1} dsDNA and presents a small current peak at 1.32 V. Nevertheless, this current peak cannot be associated to any DNA oxidation process, being most probably due to the oxidation of sp^2 carbon impurities[49] on the BDD electrode surface. Besides that, as noted above, dsDNA presents limited accessibility to the nitrogenous base residues contained in nucleotides and, consequently, no oxidation peaks are commonly detected.[27] Oliveira and Oliveira-Brett[61] detected oxidation peaks for 200 mg L^{-1} dsDNA in

aqueous solution using a BDD electrode after it was submitted to drastic cathodic (–3.0 V, for 30 min) and anodic (3.0 V, for 30 min) pretreatments in a 0.1 mol L^{-1} acetate buffer solution (pH 4.5). In the present work, the dsDNA oxidation peaks were not detected possibly due to the different solution conditions and less drastic pretreatment of the BDD surface.

Finally, it should be recalled that the occurrence of oxidative lesions in dsDNA caused by active compounds commonly leads to the breaking of hydrogen bonds and the opening of the double helix, causing the bases to come into contact with the electrode surface.[62] Consequently, the electrochemical detection of this oxidative damage could be accomplished by monitoring the oxidation of the bases.[62] However, no voltammetric peaks were detected in our studies of the CIP-dsDNA interaction associated to oxidative damage, double helix opening or dsDNA unwinding. Thus we conclude that CPI did not cause oxidative lesions in dsDNA even after an interaction time of 3 h.

4. Conclusions

The electroanalytical performance of the BDD electrode for the detection of CIP was improved by a cathodic pretreatment. Then, the obtained results demonstrate the viability of using DPV and a cathodically pretreated BDD electrode for the determination of CIP in urine samples. The novel voltammetric procedure here reported yielded a lower *LOD* value (0.44 μmol L^{-1}) than those reported by Montes et al.[15] (5.0 μmol L^{-1}, using a BDD electrode and CE-C^4D), Fotouhi et al.7 (6 μmol L^{-1}), Ensafi et al.8 (0.9 μmol L^{-1}), and Nawaz et al.[33] (9.0 μmol L^{-1}) using modified GC electrodes. Moreover, excellent results were obtained using the DPV method in the analysis of CIP at two concentrations (6.0 and 40.0 μmol L^{-1}) in urine samples.

The results obtained on the interaction between CIP and dsDNA in aqueous solution by SWV with a cathodically pretreated BDD electrode allow us to suggest that the CIP interaction with dsDNA is by intercalation, as previously reported by Fotouhi et al.[34] and Radi et al.[58] The intercalation of compounds into DNA as an initial step leading to the formation of covalent adducts is an especially undesirable feature, since they can lead to mutations and carcinogenesis.[23] Additionally, this interaction is time-dependent, as clearly indicated by the fact that the slope of the CIP analytical curve diminishes for the longer interaction time.

Clearly, the coupling of a cathodically pretreated BDD electrode with voltammetric techniques is an adequate way to attain the determination of CIP in biological samples or to evaluate the interaction of this drug with dsDNA.

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