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Development of an electrochemical method for the detection of quinolones: Application to cladoceran ecotoxicity studies

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ABSTRACT

This work presents the development of a sensitive and friendly environment electrochemical methodology with high analytical performance for quinolone detection. The method is based on square-wave anodic stripping voltammetry which is simple and reliable for determination of quinolone family on the bismuth film electrode (BiFE). The deposition potential, deposition time, buffer solution pH, voltammetry detection technique and bismuth concentration at fixed quinolone concentration (1 $\mu g\,L^{-1}$) were evaluated by fractionated factorial and central composite designs. The high cross-reactivity obtained in the evaluation of different quinolones showed that this methodology could be applied to quinolone family with limits of detection (LOD) close to 0.5 $ng\,L^{-1}$. Since is widely known that quinolones can reach aquatic ecosystems, the developed electroanalytical method was applied in the monitoring of moxifloxacin (MOXI) concentrations in ecotoxicity studies by means of acute toxicity test using two cladoceran species as biological models: *Daphnia magna* and *Ceriodaphnia dubia*. As a result of ecotoxicity, both species showed comparable sensitivities at MOXI. Toxicity studies of quinolones on aquatic organisms are very limited so the effects of MOXI to cladoceran species are highlighted. The interaction of both disciplines –Chemistry and Ecology- would allow knowing of global behavior of quinolones in natural environments, from an integrative perspective.

1. Introduction

Quinolones are a class of antibiotics commonly used in both human and veterinary medicine. These drugs inhibit key bacterial enzymes (DNA gyrase and topoisomerase IV) involved in unwinding the DNA helix for replication and transcription [1]. Quinolones are classified in generations where the newer quinolones have broad-spectrum bactericidal activity, excellent oral bioavailability, good tissue penetration and favorable safety and tolerability profiles. First-generation drugs (*e.g.*, nalidixic acid) achieve minimal serum levels. Second-generation quinolones (*e.g.*, ciprofloxacin) have increased gram-negative and systemic activity. Third-generation drugs (*e.g.*, levofloxacin) have expanded activity against gram-positive bacteria and atypical pathogens. Fourth-generation quinolone drugs (*e.g.*, MOXI) add significant activity against anaerobes [2,3]. Owing to the advantages of broad-spectrum antibacterial activity, high potency, non-cross resistance and low price, quinolone have been extensively used in recently years. However, due to the incomplete assimilation and metabolism in organism, a fraction of these drugs are discharged into the environment, reaching freshwater ecosystems and causing serious pollution to the aquatic systems [4,5]. Although the quinolones have been detected in wastewater influents and effluents as well as in surface waters in the $ng\,L^{-1}$ to $\mu g\,L^{-1}$ range [6,7], do not exist legislation for these contaminants in surface and human consumption waters nor for the protection of aquatic biota. Therefore, the development of analytical methods with high sensitivity is indispensable for its detection. Square-Wave Anodic Stripping Voltammetry (SWASV) is an extremely sensitive electrochemical technique for trace and ultra-trace determination of biologically active substances [8]. The conventional mercury electrodes are being replaced by environment friendly alternative electrodes. An alternative are the bismuth (Bi) electrodes that have good analytical performance and are less toxic than mercury ones. In 2000, Wang et al. [9], for the first time, reported a Bi-coated carbon electrode as a sensor for the stripping voltammetric analysis of lead, cadmium and zinc. The preparation process of Bi-bulk or Bi-film electrodes is chosen according to the analyte investigated. The formation of Bi-film on electrodes (BiFE) is carried out in two ways (*ex situ* or *in situ*). *Ex situ*, the electroplating of Bi film is carried out prior to transferring the electrode to the solution in which analyte is present. *In situ*, Bi ions are added directly into the solution to be analyzed, and the analyte is incorporated as the Bi film is

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formed [10].

In addition to detecting analytes at trace levels, their environmental monitoring using cladoceran species of different world distribution is of relevant importance. *D. magna* and *C. dubia* are very common planktonic invertebrate organisms inhabiting freshwater ecosystems such as rivers, streams, lakes and ponds [11]. Both are sensitive to various substances and can be easily cultured in laboratory conditions, therefore they are found to be very useful model organisms in ecotoxicology [12]. *D. magna* is of Holarctic geographical distribution –*e.g.* it is not present in southern hemisphere freshwater ecosystems- while *C. dubia* is of Neotropical distribution, thus the relevance of comparing the sensitivity of both cladoceran species. *C. dubia* is smaller with shorter life cycle, faster with erratic swimming habits, dense coloration and many morphological differences than *D. magna* [13].

In this paper, the development of stripping voltammetric method for quinolone detection on BiFE was performed. Ciprofloxacin (CIPRO) was selected as quinolone model in the optimization stages due to the fact it is perhaps the most popular, and one of the most frequently prescribed medications in the world [14]. Also, this antibiotic is especially interesting because it is used therapeutically in many critical applications such as the treatment of anthrax infections, and it is a primary degradation product of enrofloxacin (ENR), which is used in aquaculture and agriculture [15]. Then, eight quinolones were analyzed by the methodology developed to check its usefulness in quinolone family detection. Thereafter, acute ecotoxicological test of MOXI –quinolone of the last generation- for two cladoceran species (*D. magna* and *C. dubia*) were performed. The monitoring of MOXI concentration during test was assessed by electrochemical method.

2. Materials and methods

2.1. Electrochemical method

2.1.1. Reagents

All reactive used are analytical grade. Bismuth nitrate and quinolones were purchased by Sigma-Aldrich. The stock solutions were prepared in high-purity water.

Britton-Robinson (BR) buffer solution (pH from 3.0 to 7.0) was prepared in the usual way from 0.04 mol L^{-1} acetic acid (99.5%, Cicarelli), 0.04 mol L^{-1} sodium borate (Carlo Erba) and 0.04 mol L^{-1} phosphoric acid (85%, Cicarelli) solutions. This solution was used in the optimization step. The acetic acid/acetate buffer 0.1 mol L^{-1} at pH 3.81 was utilized as working buffer. KCl 0.1 mol L^{-1} was added to BR and working buffer solutions as support electrolyte. The pHs of buffer solutions were adjusted by addition of 0.20 mol L^{-1} sodium hydroxide or phosphoric acid solutions.

2.1.2. Instrumentation

Electrochemical techniques were performed with a voltammetric analyzer Epsilon BAS, Bioanalytical Systems Inc. (West Lafayette Indiana, USA) with a three electrode system based on graphite–epoxy composite (GEC) as working electrodes [16]; platinum as auxiliary electrode and Ag/AgCl in 3 mol L^{-1} KCl solution as reference electrode (Eref) (Orion 92-02-00). All potentials are given *versus* Eref.

The effective area of GEC electrodes was determined by chronoamperometry using 2×10^{-3} mol L^{-1} $K_4[Fe(CN)_6]$ solution containing 1 mol L^{-1} KCl. The diffusion coefficient for ferrocyanide is 6.2×10^{-6} cm^2 s^{-1} [17]. The potential was stepped from 50 to 500 mV for 30 s. The effective area calculated using the Cottrell equation [18] was found to be 0.24 ± 0.03 cm^2 (% CV = 12.5%, N = 16).

The pH measurements were obtained using a combined glass electrode connected to a digital pH-meter (ORION, model 720A).

2.1.3. Analytical protocol

The GEC electrodes modified with bismuth film was plated *in situ* using the following procedure: the GEC, Eref, and Pt wire electrodes were immersed in an electrochemical cell containing working buffer (pH 3.81), 1 mg L^{-1} of bismuth, and the quinolone solutions. Then, bismuth and the quinolone were simultaneously deposited on the electrode surface by electrochemically reduction at −1.278 V *vs* Eref under stirring for 265 s. After deposition, the solution was left in quiescence for ~2 s. Square wave voltammetry was performed *via* a potential scan from −0.6 to 0.1 V with a frequency of 25.0 Hz, amplitude of 25.0 mV, pulse width of 50 ms, potential step of 5 mV, and pulse period of 0.2 s (without stirring).

2.2. Ecotoxicological studies

2.2.1. Test organisms and experimental conditions

Cladocerans (*D. magna* and *C. dubia*) were individually maintained in glass beakers with 30 mL of synthetic medium [19] comprising 2.4 g $MgSO_4$, 3.84 g $NaHCO_3$, 0.16 g KCl, and 2.4 g $CaSO_4{\cdot}2H_2O$ dissolved in 20 L of distilled water. The media were changed weekly and oxygenated by bubbling air for at least 24 h before using them. Temperature and photoperiod were maintained constant at (21 ± 1) °C and 16:8 (light:darkness), respectively; while pH and dissolved oxygen were measured at the beginning and at the end of each assay.

The pH measurements and dissolved oxygen (mg L^{-1}) were obtained using a combined glass electrode connected to a digital pH-meter (ORION, model 720A) and Trans Instruments Oximeter (HD3030), respectively. The cladoceran species were fed as proposed by Reno et al. [20] with 40 μL (absorbance = 1.5 λ, 650 nm) of *Chlorella vulgaris* (strain CLV2, taken by the CISECE, Mexico) per organism and maintained in cultivation chambers under controlled and constant conditions: photoperiod 16:8 (light:darkness) and temperature (20 ± 1) °C.

2.2.2. Acute toxicity of moxifloxacin to cladocerans

Acute (48- and 72-h) toxicity assays were started with five neonates (< 24 h) per triplicate of *D. magna* and *C. dubia* [21]. Between five and six MOXI concentrations plus the control (without MOXI) were used for both species: 0.1, 0.5, 2.5, 12.5, 62.5 and 312.5 mg L^{-1} for *D. magna* and 0.5, 2.5, 12.5, 62.5 and 187.5 mg L^{-1} for *C. dubia*. The assays were carried out using glass beakers with 30 mL of the culture medium. As indicative of the toxic effect, the authors considered the complete immobilization of the organisms. The number of live and dead organisms was recorded by naked eyes using a cold white light at 48 and 72 h. Results were considered acceptable when mortality in the control group was ≤10%. The effective concentration (EC50) or lethal concentration 50 (LC50) (*e.g.*, the dose required to kill half the members of the population tested) was determined at 48 and 72 h. These values and their 95% confidence limits were estimated using Probit analysis [22].

During each toxicity test, MOXI concentrations in supernatant solutions at the beginning and end of the assay were measured.

3. Results and discussion

3.1. Electrochemical method

3.1.1. Electrochemical study of BiFE

The electrochemical characterization of BiFE was performed by cyclic voltammetry (CV) at different scans from 0.01 to 0.50 V s^{-1}. Bismuth was electrodeposited from Bi solutions with concentrations ranging from 0.6 to 1.4 mg L^{-1}. The Fig. i in Supplementary material shows the typical cyclic voltammograms of BiFE in 0.1 mol L^{-1} BR buffer solution with 0.1 mol L^{-1} KCl at pH 4.0 at different scan rates. The voltammograms displayed oxidation peaks (peak I) at −0.120 (± 0.025) V *vs* Eref and reduction peaks (peak II) at −0.629 (± 0.019) V *vs* Eref.

The peak current intensity for adsorbed molecules can be related directly to the surface coverage (Γ) and potential scan rate according Eq. (i) in Supplementary material [8]. Therefore, anodic and cathodic peak current intensities (CI_p) *vs* scan rate (v) were plotted (Fig. ii in

Supplementary material for surface coverage of 45 nmol cm^{-2}). This figure shows a linear relationship up to the scan rate of 0.1 V s^{-1}, indicating a diffusion-limited process, as expected for adsorbed electroactive species.

The surface concentration of electroactive species is an important parameter during the optimization, since this value affects the sensitivity of the analytical methodology and the kind of control (diffusional or kinetic) when the modified sensor interacts with the analyte. Therefore, the surface coverages (Γ) were estimated from the under peak areas, recorded during the cyclic voltammetries, using the following equation [8].

$$Q = n \times F \times A \times \Gamma \quad (1)$$

where Q is the charge under the peak, n the number of electrons (in our cases is n = 3), F the Faraday constant and A the effective area. Γ obtained were: (24 ± 3), (45 ± 5), (55 ± 8), (59 ± 12) and (62 ± 15) nmol cm^{-2} for the concentration Bi of 0.6, 0.8, 1.0, 1.2 and 1.4 mg L^{-1}, respectively. In spite of Γ continued to enhance as Bi concentration increases, its value was tended to plateau with higher variability.

Also, the heterogeneous electron transfer rate constant (k°) was calculated according to the method described by Laviron [23]. Anodic (α_a) and cathodic (α_c) transfer coefficients were calculated from the slopes and the intercepts of plots peak potentials (Ep) *vs* logarithms of scan rates [log (v)] for scan rates higher than 0.1 V s^{-1} (Fig. iii in Supplementary material). When this value is exceeded, the peak potential separation is increased upon increase of scan rate, indicating the limitation of charge transfer kinetics and therefore ΔEp > 200/n (mV) is fulfilled [23]. Both the equations for the calculations and values obtained are showed in Supplementary material (Eqs. (ii), (iii) and (iv), and Table i, respectively). The sum of transfer coefficients α_a and α_c is close to its normal value of 1, expect in the case of high surface coverage.

Similar values of k° from 8.3 to 12.8 s^{-1} (Table i in Supplementary material) were obtained for different surface coverages, achieving the higher values in the zone previous to plateau of Γ.

3.1.2. Voltammetric behavior of quinolone

The electrochemical behavior of CIPRO as model quinolone was first studied using CV. The voltammograms obtained using BiFE in presence and absence of CIPRO displayed the peaks I and II, where it was also demonstrated that the CIPRO detection on BiFE presented a higher current than that of the modified electrode, as shown in Fig. 1A. Thus, it was clear that the high current obtained in the presence of the analyte is due to CIPRO reaction to bismuth film favoring their oxidation.

Next, the electrochemical behavior of CIPRO on BiFE in SWASV was investigated. The SWASV of *in situ* reaction consisted of two stages: (I) deposition stage where the reduction from Bi^{3+} to Bi^0 through applied electronegative potential is occurred (Bi-film formation on electrode) and (II) resolution stage where the oxidation from Bi^0 to Bi^{+3} is achieved by the application of a potential scan from positive values (interaction with analyte).

In order to evaluate the repeatability of the BiFE, the electrochemical responses of blank electrode were measured in one day (intra-assay) and in ten different days (inter-assay) by SWASV, obtaining current intensities of (−51 ± 4) and (−57 ± 10) μA with variability coefficients of 8% (N = 3) and 18% (N = 30), respectively.

As can be observed from inspection of Fig. 1B, only an oxidation peak at −0.03 (± 0.01) V *vs* Eref was obtained from SWASV. Again, the oxidation current of CIPRO in BiFE was higher than blank electrode. This would indicate than CIPRO favoring the oxidation from Bi^0 to Bi^{+3}in the resolution step. The proposed mechanism can be the complex formation between three molecules of CIPRO and one molecule of bismuth, according this structure: Bi $(C_{17}H_{17}FN_3O_3)_3 \times 2H_2O$, such as appears in the bibliography [24].

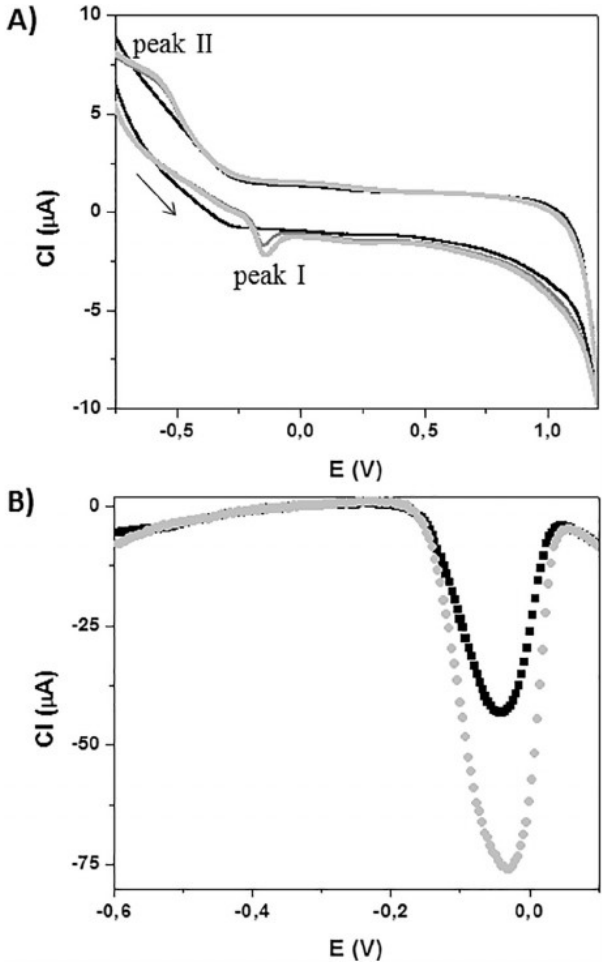

Fig. 1. A) Cyclic voltammetric behavior of naked GEC electrode (black line) and BiFE with and without CIPRO (light grey and grey lines, respectively) at 1×10^{-3} mol L^{-1}, v = 100 mV s^{-1}. B) SWASV responses of BiFE with and without CIPRO at 1 ppb (light grey and black lines, respectively). The potential range was from −0.8 to 1.2 V and the direction of potential sweep is indicated with an arrow. The assays were performed in 0.1 mol L^{-1} BR buffer solution with 0.1 mol L^{-1} KCl at pH 4.0. The potentials (E) were measured *versus* Eref.

The electrochemical behaviors on BiFE of others quinolones (ENR, danofloxacin = DAN, ofloxacin = OFL, flumequine = FLU, sarafloxacin = SAR, marbofloxacin = MAR, MOXI) were similar than CIPRO one (data not showed).

3.1.3. Optimization of experimental conditions

The systematic optimization procedure of five factors involved in the quinolone electrochemical detection was performed by using response surface methodologies through fractionated factorial and central composite designs by Design-Expert 7.1.6 (Table 1). The evaluated factors were A = deposition potential (from −0.9 to −1.3 V *vs* Eref), B = deposition time (from 180 to 240 s), C = pH (from 3.0 to 7.0, B-R solution buffer and KCl 0.1 mol L^{-1}), D = voltammetry detection technique (SWV and DPV) and E = bismuth concentration (from 0.9 to 1.3 mg L^{-1}). Both designs were performed at fixed level of ciprofloxacin (1 μg L^{-1}). On the one hand, the significance of five factors was evaluated by fractionated factorial design of 16 experiments at two levels. The analysis by ANOVA test was adjusted to factorial model (R^2 = 0.9299) where four factors resulted significant (p-value < 0.05): A, B, C and D. Although D factor was significant, SWV was selected as electrochemical detection technique because it is faster than DPV (few seconds *vs* 2 min). On the other hand, the optimal conditions of A, B and

Table 1
Factors, levels and results obtained by fractionated factorial and central composite designs.

Fractionated factorial design			Central composite design				
Factors[a]	Levels	Significance[b]	Levels	Optimal condition	Global desirability	Predicted response (μA)	Experimental response (μA)
A (V)	−0.9 and −1.3	Significant	−0.9 to −1.3	−1.278	0.70	−32	−39 ± 5
B (s)	180 and 240	Significant	180 to 600	298			
C	3.0 and 7.0	Significant	3.0 to 7.0	3.81			
D	SWV and DPV	Significant	SWV	SWV			
E (ppm)	0.9 and 1.3	Not significant	1.0	1.0			

[a] Factors: deposition potential (A), deposition time (B), buffer pH (C), electrochemical quinolone detection (D) and bismuth concentration (E).
[b] ANOVA test by Design Expert program (significant = p-value < 0.05).

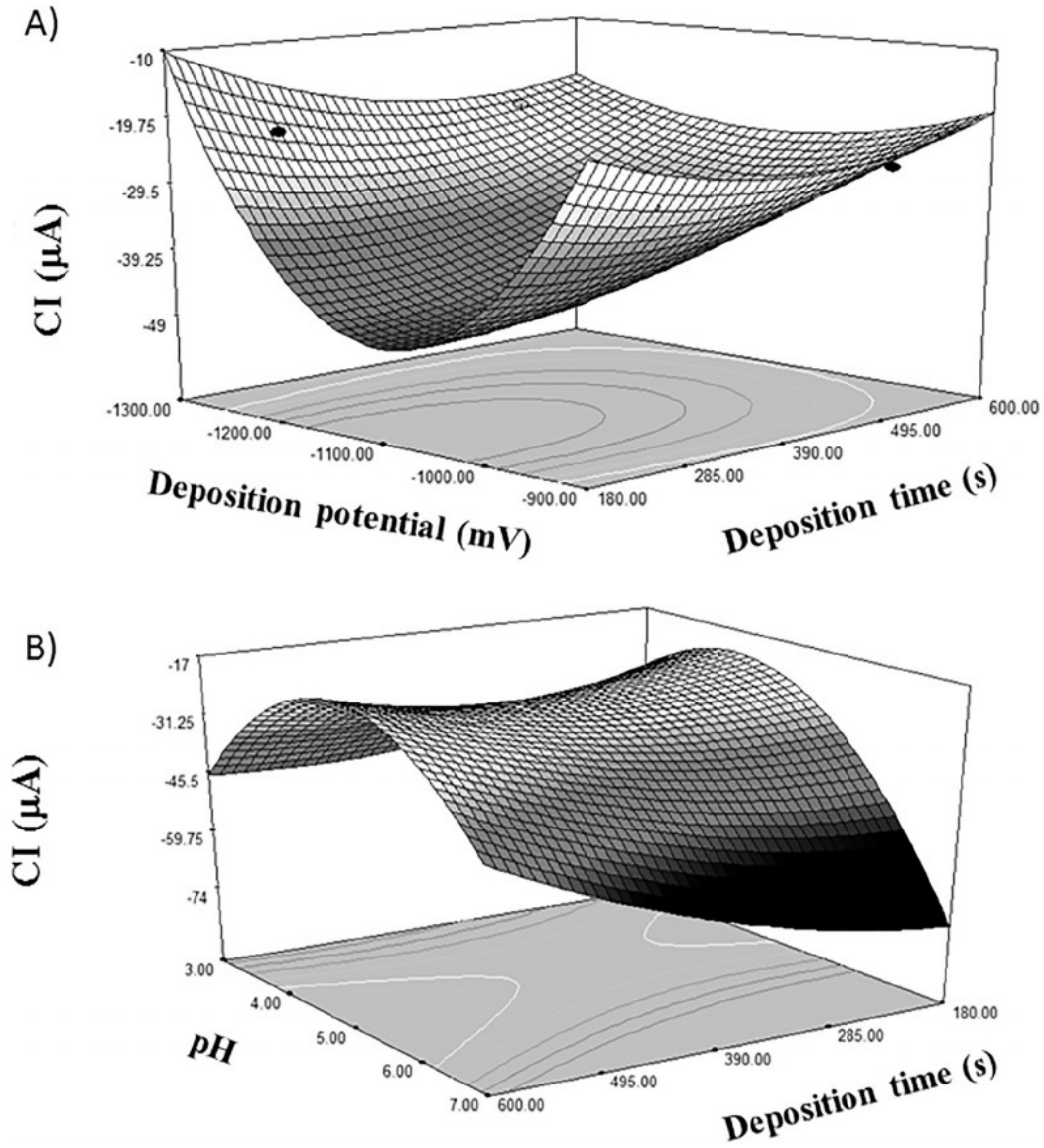

Fig. 2. Second-order response surface plot in the current intensity (y) for the quinolone detection. Dependence of y on the deposition potential, deposition time and pH is shown (CIPRO concentration = 1 μg L^{-1}).

C factors were evaluated by central composite design of 20 experiments at five levels with six central points. In this design, ANOVA test was applied to obtain a significant fitted model and not significant lack of fit (p-values must be minor and major to 0.05, respectively). In this case, the responses were adjusted by cubic model (R^2 = 0.9862). The following fitted regression model was used to quantitatively investigate the effects of A, B and C on the quinolone detection by:

$$y(CI) = -1253 - (2.1 \times A) - (2.2 \times B) + (453.8 \times C) + (0.7 \times A \times C) + (0.03 \times B \times C) - (6.5 \times C^2) \quad (2)$$

where CI is the electrochemical response in current intensity. Some quadratic terms and interactions of factors were not included in the Eq.

Table 2
Comparison of stripping voltammetry technique in the different analyte detection.

Technique[a]	Working electrode[b]	Analyte	LOD (ng L^{-1})	Original applications	Ref
SWAdSV	Cu (II)-HMDE	ENR	151	Pharmaceutical and human plasma	[28]
SWAdSV	HMDE	MOXI	440	Human urine and commercial formulations	[29]
AdSV	HMDE	Nalidixic acid	766	Pharmaceutical, human urine and serum	[30]
AdSV	HgE	OFL	1000	Pharmaceutical formulation	[31]
AdSWASV	GC	Levofloxacin	1800	Human urine	[32]
DPASV	HMDE	Levofloxacin, gatifloxacin and lomefloxacin	2380, 3200 and 1600	Commercial food	[33]
AdSV	HMDE	Rufloxacin	3340	Tablets, human plasma and urine	[34]
ASV	Cd (II)-graphene	CIPRO	19,500	Pharmaceutical and human urine	[35]
DPAdSV	Zn (II)-BiFE	Cr (VI)	0.0005	Reference material and river water	[36]
DPASV	Bi bulk	Tl (I)	1	Water	[37]
DPASV	BiFE	Cd (II) and Pb (II)	1500 and 50	Tap water	[38]
DPAdSV	BiFE	Methotrexate	409	Pharmaceutical	[39]
SWCSV	Antimony film	Tetracycline	66,600	Honey	[40]
DPAdSV	Ag/Cu-GRPE	Chlorpyrifos	1.4	Well water and soil sample	[41]
SWAdSV	Hg(Ag)FE	Chlornitrofen	9540	River water	[42]
SWASV	BiFE	Quinolone	0.5	Cladoceran cultures	This work

[a] SWAdSV: square-wave adsorptive stripping voltammetry; AdSV: adsorptive stripping voltammetry; AdSWASV: adsorptive square-wave anodic stripping voltammetry; DPASV: differential pulse anodic stripping voltammetry; ASV: anodic stripping voltammetry; DPAdSV: differential pulse adsorptive stripping voltammetry; SWCSV: square wave cathodic stripping voltammetry; SWASV: square wave anodic stripping voltammetry.
[b] HMDE: hanging mercury drop electrode; HgE: mercury drop electrode; GC: glassy carbon electrode; BiFE: bismuth film electrode; Ag/Cu-GRPE: Ag/Cu alloy nanoparticles and graphene nanocomposite paste electrode; Hg(Ag)FE: silver amalgam film on mercury electrode.

Table 3
Precision assays in the CIPRO and MOXI detection on BiFE with SWASV.

Statistics		[CIPRO] (ng L^{-1})		[MOXI] (ng L^{-1})	
		0.5	10	0.4	10
Intra-assay[a]	Mean	0.4	9	0.4	12
	% CV[c]	21	16	20	12
	% recovery	80	92	100	120
Inter-assay[b]	Mean	0.4	11	0.3	11
	% CV	25	18	30	24
	% recovery	80	110	75	110

[a] The intra-assay test was evaluated by multiple analyses (N = 3) in one assay.
[b] The inter-assay was assessed by analysis in three separate analytical runs (N = 9).
[c] Coefficient variation percentage.

(2) due to their effects were smaller than principal factor ones.

Fig. 2A and B shows response surface plot of electrochemical signal in function of evaluated factors. The higher response was obtained to extreme values of deposition potential and pH and longer deposition time.

Then, the factor combination that provides the best "values of desirable response" was investigated. In order to do that, minimize both deposition time and response (oxidative current intensity) was searched in the optimization stage in order to reduce the analysis time and obtain the higher response in absolute terms. The global desirability function, calculated from importance and weight criteria for all factors and responses, was ranged between 0 (where the combinations does not fulfil any requirement) and 1 (where all responses have a simultaneously desirable value) [25]. Table 1 shows the factor optimal conditions with a global desirability function of 0.70. Also, the predicted and experimentally obtained responses are showed which were not significantly different when were compared by a mean comparison test with alpha level of 0.05 [26].

3.1.4. Analytical performance

3.1.4.1. Calibration curves of quinolones. The calibration curves for determination of the quinolone concentration by SWASV were performed in the range from 0.1 to 500 ng L^{-1} of CIPRO and MOXI as model quinolones of second- and fourth-generations, respectively. The curves were built as CI *vs* quinolone concentration. Eqs. (3) and (4) were fitted for CIPRO and MOXI, respectively:

$$CI(\mu A) = -1.20(\pm 0.06)[CIPRO](ngL^{-1})^{-1} - 2.8(\pm 0.8)(N = 20, R^2 = 0.9832) \quad (3)$$

$$CI(\mu A) = -1.22(\pm 0.03)[MOXI](ngL^{-1})^{-1} - 3.8(\pm 0.5)(N = 20, R^2 = 0.9922) \quad (4)$$

The curves for CIPRO and MOXI had similar sensitivities according the slope values. LOD and limit of quantification (LOQ) were calculated as 3.3 and 10 times the standard deviation of blank samples obtained from the calibration curves, respectively [27]. LODs of 0.5 and 0.4 ng L^{-1} were obtained for CIPRO and MOXI, respectively, while LOQs were 1.5 and 1.2 ng L^{-1}, respectively. The linear ranges were from 1.5 to 25 ng L^{-1} and from 1.2 to 35 ng L^{-1}, respectively.

Also, other quinolones such as DAN, OFL, NOR, SAR, ENR and MAR had comparable slope values by their calibration curves (data not showed). Therefore, cross reactivities (% CR) close to 100%, calculated as the ratio between quinolone studied and ciprofloxacin slopes, were obtained. This fact demonstrated that the interaction of quinolone with bismuth film does not depend on substituents in basic structure. As a result, the high cross-reactivity obtained to quinolone studied showed that this methodology could be applied to quinolone family detection.

To compare the analytical performance of this methodology, other developments for different analyte detection by stripping voltammetry are summarized in Table 2. It should be noted that the LOD obtained in this work was very lower than typical values declared by bibliographies [28–42]. Moreover, several methods employing mercury electrode had been used for quinolone detection but all had higher LOD and were less friendly environment than this work [28–35]. Notably, stripping methods applied to analyte detection in cladoceran cultures have not been found.

3.1.4.2. Precision assay. The precision of methodology in CIPRO and MOXI detection was evaluated by intra- e inter-assays, whose results are presented in Table 3. Intra-assay and inter-assay precision as % CV were ranged from 12% to 21% and from 18% to 30%, respectively, with recoveries higher than 75% in all case. These values are considered acceptable according to recommendations for analytical method validation about precision requirements in the phase of method development [43].

3.2. Ecotoxicological studies

3.2.1. Acute toxicity of moxifloxacin to cladocerans

Hu et al. [44] had demonstrated that the genotoxic potential of the new generation quinolones is higher than earliest one. Moreover, Li et al. [5] evaluated acute toxicity of 21 quinolones by monitoring of photobacterium *Vibrio fischeri* and concluded that MOXI exhibited a notable higher toxic effect than the others. Therefore, MOXI was the quinolone selected to be used in the testing and their toxicity values was expressed as LC50 at 48 and 72 h. Cladocerans evaluated were *D. magna* and *C. dubia* because the former is a classical model used in the world and the latter is a native species in our geographic region.

In the control groups, there was not mortality for both species. Levels of pH and dissolved oxygen were ranged from 7.07 to 8.07 and 4.85 to 6.13 mg L^{-1}, respectively. Consequently, the conditions of the validity of the test were fulfilled [21].

For *D. magna*, 48 h and 72 h LC50 were 14.2 (6.4–33.8) and 3.4 (1.4–7.5) mg L^{-1}, respectively; while for *C. dubia*, 48 h and 72 h LC50 were 29.2 (13.6–72.8) and 5.4 (2.1–11.9) mg L^{-1}, respectively. As can be seen, the LC50 were higher than the relevant concentrations in aquatic environment, indicating limited ecotoxicity to MOXI exposure.

No studies were found to compare MOXI ecotoxicity with *D. magna* and *C. dubia*. However, these results are consistent with the EC50 values of 60 and 4.6 mg L^{-1} reported for *D. magna* with CIPRO and oxalic acid, respectively [45,46]. Moreover, Robinson et al. [47] studied the ecotoxicity of CIPRO, lomefloxacin, OFL, levofloxacin, ENR and flumequine which caused < 10% mortality in *D. magna* at a concentration of 10 mg L^{-1}. LC50 of 7.9, 7.2, 8.7, 34.0 and 5.3 mg L^{-1} for ENR, CIPRO, NOR, DAN and MAR, respectively had been obtained for *D. magna* in preliminary results of our group (data in publication process) [48]. Studies on other aquatic organisms demonstrated low ecotoxicity to quinolones, such is the example of flumequine for *Artemia salina* with 48 h EC50 of 308 mg L^{-1} [49]. Conversely, *Lemna minor* was more sensitive to ENR and CIPRO with EC50 of 0.107 and 0.0625 mg L^{-1}, respectively [50].

On the other hand, on the best of our knowledge, there no available data of the quinolone toxicity effects on *C. dubia*. In this work, *C. dubia* was approximately twice less sensitive than *D. magna* for acute toxicity test. However, the 72 h LC50 values for both species are within the same order of magnitude. Since two species reacted similarly to MOXI effect, could be proposed to replace the globally used biological model –*D. magna*- by the cladoceran of Neotropical distribution –*C. dubia*- as test organism for ecotoxicity assay of quinolone in southern hemisphere. The importance of using Neotropical species is based on the easy obtaining of test organisms in regional wetlands, the lower cost associated to bioassays as well as the environment and regional relevance of the results obtained.

3.2.2. Electrochemical method

In order to study the evolution of the quinolones during each toxicity test, the developed method was applied for MOXI detection. This was useful to ensure accurate initial dosing and to determine the stability of the compounds during the test. With the finality to do that, the initial concentration for each test solution was confirmed and the final concentration of the solution after testing was analyzed. Aqueous samples before and after testing were diluted in working buffer solution at 10 ng L^{-1} concentration and were analyzed by SWASV technique in the optimal conditions. The initial concentrations were measured with developed method and calculated the % of expected values (see Table 4A). The initial concentrations were according to expected values from 80 to 106%. The number of alive organisms at 72 h and the % recoveries between initial and final concentrations are displaying on Table 4A. At the higher MOXI concentration, the lower recoveries and the lower number of alive organisms for both species, were obtained. These results could indicate a relation between quinolone degradation and toxic effect of MOXI to *D. magna* and *C. dubia* since recoveries lower than 80% were obtained at concentrations higher than 72 h LC50. Therefore, additional assays in absence of cladoceran were performed

Table 4
A) Analytical data of MOXI ecotoxicity test for Daphnia magna and Ceriodaphnia dubia and B) Analytical data of MOXI stability during ecotoxicity test.

A) Analytical data of MOXI ecotoxicity test for *Daphnia magna* and *Ceriodaphnia dubia*

Species	[MOXI] added (mg L^{-1})	No. organisms remain (at 72 h)	% of expected value[a]	% recovery[b]
D. magna	0	15	–	–
	0.1	13	98 (% CV = 10%)[c]	100 (% CV = 10%)
	0.5	12	101 (% CV = 14%)	99 (% CV = 12%)
	2.5	9	102 (% CV = 10%)	93 (% CV = 10%)
	12.5	6	102 (% CV = 9%)	78 (% CV = 8%)
	62.5	1	106 (% CV = 9%)	59 (% CV = 10%)
	312.5	0	81 (% CV = 10%)	46 (% CV = 9%)
C. dubia	0	15	–	–
	0.5	12	100 (% CV = 11%)	104 (% CV = 9%)
	2.5	10	101 (% CV = 10%)	103 (% CV = 10%)
	12.5	6	104 (% CV = 8%)	76 (% CV = 9%)
	62.5	3	80 (% CV = 9%)	62 (% CV = 9%)
	187.5	0	100 (% CV = 6%)	59 (% CV = 8%)

B) Analytical data of MOXI stability during ecotoxicity test

[MOXI] added (mg L^{-1})	% of expected value[a]	% recovery[b]
0.1	101 (% CV = 8%)	100 (% CV = 10%)
0.5	95 (% CV = 10%)	96 (% CV = 12%)
2.5	103 (% CV = 8%)	85 (% CV = 5%)
12.5	101 (% CV = 9%)	78 (% CV = 6%)
62.5	100 (% CV = 6%)	69 (% CV = 8%)
187.5	99 (% CV = 10%)	52 (% CV = 9%)
312.5	89 (% CV = 5%)	49 (% CV = 7%)

[a] % of expected value is the concentrations that were analyzed at the beginning of the test.
[b] % recovery is the concentration analyzed at the end of the test (72 h).
[c] Coefficient variation percentage.

for estimating the possible influence of organisms in the decrease of final quinolone concentration (see Table 4B). As result, there were not significant differences ($p > 0.05$) between recovery assays in presence and absence of cladoceran whereby the quinolone decrease could be only due to their photodegradation or other physicochemical facts related with the experimental conditions (*e.g.* adsorption of active principles in the glass beakers). This evidence would agree with previous studies where the photodegradation of quinolone was demonstrated with short half-lives in aqueous systems [51]. Also, Ebert et al. [50] obtained % recoveries of ENR and CIPRO similar than ours but for other species (*Lemna minor* and *Myriophyllum spicatum*) and attributed the decrease to light exposure.

4. Conclusion

A sensitive and friendly environment electrochemical methodology with high analytical performance was developed for quinolone detection. LODs of 0.5 and 0.4 ng L^{-1} were obtained for CIPRO and MOXI, respectively. Due to the fact that high cross reactivity was obtained for eight quinolones of different generations, this methodology would be useful for quinolone family detection in very low concentrations such as reported in environmental matrixes (ng L^{-1} to μg L^{-1}). On the order hand, ecotoxicity studies of quinolones on aquatic organisms is very limited so that great contribution about acute toxicity test of MOXI to two cladoceran species with different geographical distribution was done in this paper. Comparable sensitivity at this quinolone was achieved by both species. Electrochemical method was applied for the first time in the monitoring of MOXI concentrations during the ecotoxicological test, showing an adequate quinolone initial dose and degradation effect within 72 h of assays. The interaction of both disciplines would allow knowing both chemical and ecotoxicological effects of quinolones simultaneously.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2018.05.039.

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