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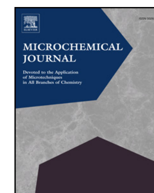

Development of an electrochemical method for the detection of quinolones: Application to cladoceran ecotoxicity studies

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A B S T R A C T

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 μg 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 μg 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

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