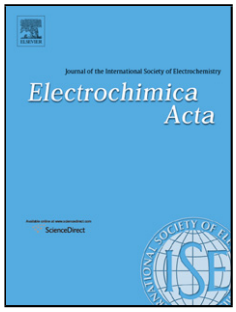


Title: Enrofloxacin behavior in presence of soil extracted organic matter: An electrochemical approach

Authors: Monica Antilén, Camila Valencia, Emilia Peralta, Camila Canales, Christian Espinosa-Bustos, Mauricio Escudey

PII: S0013-4686(17)31100-3
DOI: http://dx.doi.org/doi:10.1016/j.electacta.2017.05.104
Reference: EA 29539

To appear in: *Electrochimica Acta*

Received date: 18-1-2017
Revised date: 5-5-2017
Accepted date: 16-5-2017

Please cite this article as: Monica Antilén, Camila Valencia, Emilia Peralta, Camila Canales, Christian Espinosa-Bustos, Mauricio Escudey, Enrofloxacin behavior in presence of soil extracted organic matter: An electrochemical approach, Electrochimica Actahttp://dx.doi.org/10.1016/j.electacta.2017.05.104

Enrofloxacin behavior in presence of soil extracted organic matter: An electrochemical approach

Monica Antilén*[1,4], Camila Valencia[2], Emilia Peralta[2], Camila Canales[1], Christian Espinosa-Bustos[1], Mauricio Escudey[3,4]

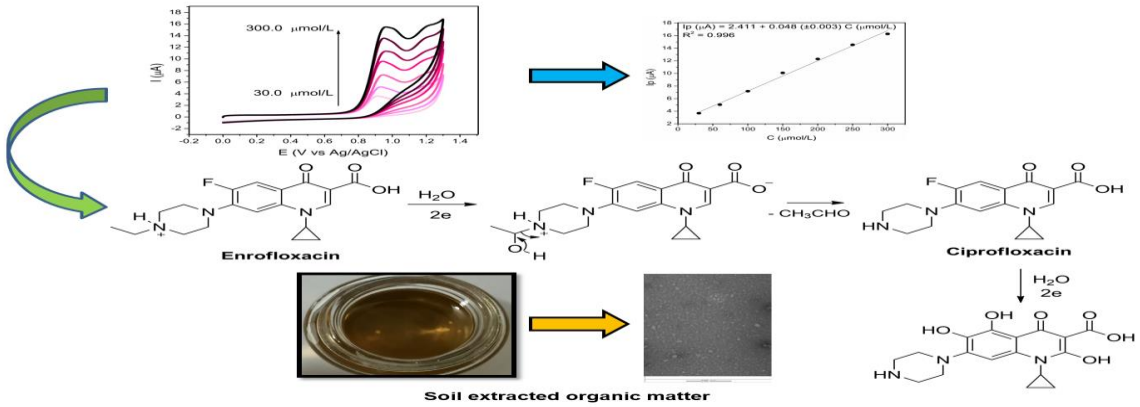
[1] *Pontificia Universidad Católica de Chile, Facultad de Química, Vicuña Mackenna 4860, 7820436, Santiago, Chile. +56226864745, mantilen@uc.cl*

[2] *Universidad Tecnológica Metropolitana, Facultad de Ciencias Naturales, Matemáticas y del Medioambiente, Av. José Pedro Alessandri 1242, Santiago, Chile.*

[3] *Universidad de Santiago de Chile USACH, Av. L.B. O`Higgins 3363, Estación Central, Chile Departamento de Química de los materiales, Facultad de Química y Biología,*

[4] *Centro de Desarrollo de Nanociencia y Nanotecnología, CEDENNA, Av. B. O'Higgins 3363, Santiago 7254758, Chile*

Graphical abstract



ABSTRACT

In this work, a novel and simple method aimed at determining and quantifying Enrofloxacin in presence of Natural Organic Matter (NOM) is proposed. The method was based on the electrochemical oxidation of Enrofloxacin by using cyclic voltammetry as technique. It was found that this analyte presents a good electroactivity, in absence and in presence of NOM. However, this electrochemical behavior is highly pH-dependent, since the reaction is more favorable when less acid the media is. At this point, different pH values were studied in order to corroborate this phenomenon. Additionally, kinetic studies were done to determine the control of the reaction, the number of transferred electrons in the entire process and the rate determining step of the reaction by analyzing the Tafel slope. With these antecedents, a mechanism was proposed and the final product of the reaction was corroborated by using LC-MS. Finally, analytical parameters were studied with the aim of proposing this new method as an electrochemical sensor of Enrofloxacin. It was found that the method is highly linear, precise and accurate. Moreover, this method is not only sensitive but also selective to Enrofloxacin in presence of NOM, in comparison to spectrophotometric methods previously reported.

KEYWORDS: Natural Organic Matter, Enrofloxacin electro-oxidation, volcanic soils, Selectivity

1. INTRODUCTION

Agricultural soil management is frequently associated to an agricultural practice to incorporate stabilizer amendments, as well as to the use of organic wastes as soil improvers. In this context, the massive use of antibiotics at veterinary level [1,2] and the application of animal waste to agricultural soils, impacts the role played by this kind of practices, becoming an incorporation process and environmental pollution. In this regard, unlike waste from wastewater treatment (biosolids), which have a pretreatment before its application to agricultural soils, animal waste (guano) is not treated previous to re-use, causing a clear incorporation of antimicrobials into agricultural soils. Reports in the 80's indicated that more than half of the antibiotics used in animals are excreted through the feces in their original form [3]. Subsequently it was shown that more than 90% of the drugs may be unloaded through animals feces and urine [4], being residues of these drugs redistributed by a series of migration and degradation processes in soils and groundwater. Therefore, the veterinary antibiotics are being incorporated into the environment, mainly through the disposal of animal manure and/or biosolids over agricultural soils. In this regard, recently the World Health Organization (WHO) has pointed out that the lack of antimicrobial antibiotics availability now it is a reality and that therefore research related to these drugs, even more at environmental level (environmental matrices), it is a critical and priority research area.

On the other hand, fluoroquinolones are among the most persistent antibiotics in soil, remaining in soil for months, with average half-life > 60 days [5,6] after a process of fertilization with manure or biosolids, which represents a high risk of development of bacterial resistance [7]. Studies evaluating the sorption of fluoroquinolones (Enrofloxacin, Danofloxacin, Ciprofloxacin

and Norfloxacin) and sulfonamides (sulfadiazine, sulfachloropyridazine, sulfadimidine) in 13 Brazilian soils, demonstrated the existing influence on the adsorption process of the different physical, chemical and mineralogical properties of this matrix. In addition that sorption was higher for fluoroquinolones than for sulfonamides, where Norfloxacin showed the highest sorption, whereas the enrofloxacin showed the lowest. The researchers point out that no leaching exists, even in the worst-case scenario, i.e. a sandy and poor organic matter soil [8]. In addition, high concentration of residues of fluoroquinolones (Enrofloxacin was the most often detected compound) were detected in soils receiving organic waste from chickens, which again positions such waste as a source of environmental pollution and fluoroquinolones as compounds with high preference to remain in soils [9]. Fluoroquinolones have also been determined in activated and urban sludge (ciprofloxacin, 426 mg kg^{-1}); while norfloxacin and ciprofloxacin were found in compost samples with 22 and 20 mg kg^{-1}, respectively [10,11]. These studies and their results represent a warning signal for the use of such amendments, indicating the need of accomplishing a prior assessment regarding fluoroquinolones and establishing their behavior with the organic matter present in soils, where volcanic ash derived soils represent the best scenario for antimicrobial sorption, considering their high organic matter and clay content. Also due to the increased use of antibiotics both at international and national levels, as well as progress in the different analytical techniques, detection of antimicrobials has become more efficient in a variety of agro-ecosystems such as soils, waters, sediments [12-15]. In this analytical context, natural organic matter that is present in any of its forms (humic substances), in soil-solution samples, becomes an important interference, if we consider that spectroscopic techniques such as molecular absorbance, are highly used on the determination-quantification of this kind of pharmaceuticals in environmental samples. Additionally, electrochemical techniques, such as

cyclic voltammetry (CV), often offer advantages versus other methods, because in general, they are faster, cheaper, more secure and more sensitive than spectrometric methods. In this sense, a recent study described a new method that can establish the adsorption capacity of nanoparticles (or any other adsorbent), detected by an electrochemical technique, with the advantage that no separation of the adsorbent from the adsorbate is required [16]. This method was used for Ciprofloxacin [17], where analytical results of the electrochemical methodology were statistically precise and accurate. Considering progress in electrochemical determination of this kind of analytes, and the existence of these pharmaceuticals compounds in soil-solution samples, the present work describes the development of a new and simple analytical method to directly determine the enrofloxacin concentration in presence of natural organic matter, where the analytical parameters such as linearity, precision and accuracy were evaluated.

Also, we established the electrochemical behavior and the oxidation mechanism of enrofloxacin by using CV as technique. Furthermore, kinetic studies were done with the aim of calculate the number of transferred electrons in the entire process and to know if it is controlled by adsorption or diffusion; where Tafel plot was studied in order to propose a possible mechanism related to the electro-oxidation of this analyte and the rate determining step of the main mechanism. Finally, LC-MS studies were done to corroborate the final product of the reaction.

2. EXPERIMENTAL

2.1. Sample

Soils

Soil samples were collected from 0 to 0.20 m depth of uncultivated area of Frutillar (Fru) and Ralun (Rln) (Andisols) in southern Chile. The samples were obtained with a stratified random

system, where soil sample is taken from a field that has been divided into several subunits from which simple random cores are obtained. After, all samples were air dried and sifted through a 2-mm mesh sieve.

Humic Acids Purification

Briefly, the solid sample was equilibrated with 1 mol L^{-1} HCl, obtaining a suspension whose pH ranges between 1 and 2 at room temperature. The volume was then adjusted with 0.1 mol L^{-1} HCl to get a 10 mL liquid/1 g dry sample ratio. The suspension was centrifuged to separate the sediment (R1) from the supernatant (Fulvic Acid, FA-1). R1 was neutralized (pH = 7.0) with a 1 mol L^{-1} NaOH solution, and then a volume of 0.1 mol L^{-1} NaOH was added under N_2 atmosphere to give a 10:1 final extractant to soil ratio. The extraction was carried out under N_2 atmosphere with occasional stirring for a minimum of 4 h. The alkaline suspension was left overnight and then the supernatant was separated by centrifugation. Subsequently, the supernatant was acidified with 6 mol L^{-1} HCl under constant stirring to get pH 1.0 and then allowed to stand for 12-16 h. The precipitated Humic Acid (HA) fractions and FA (FA-2) supernatant were separated by centrifugation. The precipitated HA was redissolved in a minimum volume of 0.1 mol L^{-1} KOH under N_2; solid KCl was then added to attain a 0.3 mol L^{-1} K^+ concentration and then centrifuged to remove the suspended solid. The HA was re-precipitated by adding 6 mol L^{-1} HCl under constant stirring until a pH=1.0 was reached. The suspension was allowed to stand another 12 to 16 h, and then centrifuged, disregarding the supernatant. The HA precipitate was suspended into a 0.1 mol L^{-1} HCl and 0.3 mol L^{-1} HF mixture and stirred overnight at room temperature (if necessary, the acid treatment (HCl/HF) was repeat until the ash content be less than 1%). After centrifugation, the precipitate was transferred to a dialysis tube *(Visking, Co.*)

using distilled water until the dialysed water gives a negative test for chloride ($AgNO_3$). After freeze-drying, the HA was kept refrigerated [18,19].

2.2. Solutions

Natural organic matter solution

100 mg L^{-1} stock solution was prepared by dissolving the natural humic acid extracted from soils derived from volcanic materials (HA-Fru and HA-Rln) in 0.100 mol L^{-1} sodium hydroxide solution. The humic acid solution (30 mg L^{-1}) was prepared from the NOM stock solution by dilution with a previously prepared 0.01 mol L^{-1} phosphate buffer solution pH=7.0 (PBS).

Particle size distribution of NOM solution

To determine the nanoparticle size distribution the dynamic light scattering (DLS) analysis was used. The DLS data were collected on a Zeta sizer S-590 (Malvern Instrument, US). The scattering angle was 108º and the laser wavelength 690 nm. The autocorrelator delay time (τ) was 1 μs. A series of 120 scans were carried out on the sample, each one with a 1-s acquisition time.

Enrofloxacin solutions

10.0 mmol L^{-1} stock standard solution was prepared by dissolving the solid chemical (Sigma Aldrich) in 0.01 mol L^{-1} hydrochloric acid. This stock solution stored in dark place at 4°C was stable for at least 1 month. All Enrofloxacin solutions (0.200 mmol L^{-1}) were prepared from the Enrofloxacin stock standard solution by dilution with a previously prepared 0.01 mol L^{-1} phosphate buffer solution pH=7.0 (PBS). Using this solution, constant ionic strength and pH are maintained constant during the whole experiment.

2.3. Electrochemical and LC-MS experiments.

All electrochemical experiments were conducted on a VoltaLabPGZ100 potentiostat system in a three-compartment/three-electrode glass cell, under a high purity argon atmosphere. Glassy carbon electrode (GCE) was prepared and used as working electrode. A Pt wire was used as counter electrode. All potentials quoted in this work are referred to an Ag/AgCl reference electrode (CHI111, *CHInstruments*, Inc.) The prepared solutions were subsequently deaerated with Ar at least for 20 min.

Enrofloxacin determination

The electrochemical determination of Enrofloxacin was conducted by cyclic voltammetry between 0.00 mV and +1300 mV at 100 mV s^{-1} scan rate in a glass cell with 10 mL of Enrofloxacin solution in PBS. The determination was carried out at pH 3.0; 5.0 and 7.0 in 10 mL of phosphate buffer solution, where the PBS themselves were measured as blanks respectively.

Electro-kinetics studies

The determination of the electro-oxidation mechanism of Enrofloxacin was based on an electrochemical study, which consisted on the analysis of the dependence of the current signal when the scan rate changes. At this point, a 0.200 mmol L^{-1} Enrofloxacin solution (in PBS, pH 7.0) was oxidized at different scan rates, from 5 mV s^{-1} to 250 mV s^{-1}.

LC-MS experiment

LC-MS experiment was carried out on an UHPLC Eksigent® coupled with MS detector ABSciex®, Triple Quad 4500 model equipment.

A sample was taken from the electrochemical cell after the electro-oxidation of Enrofloxacin. This sample was injected by using a syringe, and the data was collected in a range of 100.0 – 600.0 Da, at 200 Da s^{-1} and positive polarity.

2.4. Analytical Parameters.

Linearity

Using the above mentioned electrochemical system, a study was accomplished to establish the relationship between current peak and Enrofloxacin concentration within the $3.0x10^{-4}$ to $3.0x10^{-3}$ mmol L^{-1} range. The electrochemical determination of Enrofloxacin was conducted by cyclic voltammetry to test current peak vs. Enrofloxacin concentration linearity; six calibration curves were constructed. The current peak (Ip) (oxidation and reduction) was recorded at constant potential obtained from the respective voltammogram for each Enrofloxacin concentration. To establish the limit of detection (LOD) and limit of quantification (LOQ) of the method, the Enrofloxacin calibration curves were used. From these curves, the standard deviation of the lineal model regression was calculated ($s_{y/x}$), from which the LOD ($3s_{y/x}/m$) and LOQ ($10s_{y/x}/m$) were obtained.

Accuracy and Precision

Pharmaceutical Enrofloxacin samples (Rostrum®, Drag Pharma) were analyzed to determine accuracy through recovery experiments with water-PBS extraction, and precision of the electrochemical method. For tablets analyses, 10 tablets were ground to fine powder and a representative amount of Rostrum® Drag Pharma was dissolved into a previously prepared 0.01 mol L^{-1} hydrochloric acid solution. The relative error (Er), relative recoveries of the analyte (RR) and relative standard deviation (RSD) were calculated for five replicates.

Selectivity

Selectivity was determined by comparing six voltammograms of 0.300 mmol L^{-1} of enrofloxacin in PBS (pH=7.0) in presence or absence of natural organic matter solution (0.30 mg L^{-1}). Also, calibration curves of enrofloxacin in presence of natural organic matter were obtained at the same range of concentration aforementioned.

3. RESULTS AND DISCUSSION

In the current study, Enrofloxacin quantitation was studied by the development of a new and simple electrochemical method, using CV. This method allowed evaluating the NOM as a possible analytical interferent. Moreover, the proposal of a mechanism by studying the dependence of the enrofloxacin oxidation on scan rate was now feasible. Additionally, the control of the oxidation reaction of Enrofloxacin (adsorption or diffusion) and the number of transferred electrons of the total process could be determined.

3.1. Voltammetric studies

Voltammetric profile of enrofloxacin electro-oxidation

Figure 1 shows the electrochemical response of the enrofloxacin electro-oxidation, in comparison with the same solution in absence of this analyte. This voltammogram shows that on the anodic scan, two current peaks appear, while no signals showed up on the cathodic scan. This finding evidenced an irreversible reaction. The presence of two current peaks is indicative of two oxidation processes occurring at 900 mV and 1100 mV, respectively.

(FIGURE 1)

Influence of pH on enrofloxacin electro-oxidation

Enrofloxacin molecules may exist as cation (ENR^+), anion (ENR^-) or zwitterion ($ENR^\pm$), depending on the pH of the medium. For instance, this parameter is very important when the adsorption processes and the electro-oxidation of this analyte is studied. Figure 2 shows the voltammetric response towards the Enrofloxacin electro-oxidation at different pH. It is very noticeable how the acidity of the media affects the electrochemical behavior, since the oxidation peaks appear at different overpotentials. At this point, the reaction is more favorable when the reaction occurs at pH 7.0 as Enrofloxacin requires less energy to be oxidized. The trend demonstrated that the more acidic the medium, the less favorable the electro-oxidation of Enrofloxacin becomes, and the current peaks are shifted towards more positive potentials. This latter observation can be explained by understanding which species are present before the electrochemical reaction starts.

(FIGURE 2)

At pH 7.0, the molecule is found as its neutral form, which is more susceptible to be oxidized, since one proton is already removed. Then at pH 5.0, there is 80% of ENR^+ + 20% of $ENR^\pm$, which implies that most of the specie is in its protonated form, and more energy will be needed to oxidize the molecule, since a proton needs to be removed to let the oxidation to occur. Finally, at pH 3.0, the Enrofloxacin is totally protonated and as it was aforementioned, it will require more energy to generate the oxidation process. Additionally, it is important to notice that there is a double-signal when the voltammogram is recorded at pH 5.0. This phenomenon can be attributed to the existence of two main species in solution (ENR^+ and $ENR^\pm$), which are oxidized at different potentials. Then, the first peak can be related to the electro-oxidation of the

zwitterion, while the second current peak would correspond to the electro-oxidation of the protonated specie, which requires more energy (see inset Figure 2).

In terms of current, there is not a noticeable change on the magnitude of the signal, since the three studies are done at the same concentration. The low increment on the current while decreasing the pH, might be due to an increment on the capacitive current or solvent evolution. Since the response does not vary significantly, this parameter is practically independent on the variation of pH.

3.2. Kinetic studies

In order to determine if this oxidation process is diffusion-controlled or adsorption-controlled, the slope obtained from the logarithm of the current peak (log Ip) versus the logarithm of the scan rate (log v) was evaluated. With this information, it was possible to calculate the number of transferred electrons (n). Additionally, Tafel plot was studied in order to determine the rate determining step of the entire process.

Influence of the scan rate on enrofloxacin electro-oxidation

Figure 3 shows the value of the logarithm of the peak current (Ip) associated to the oxidation response of Enrofloxacin towards the variation of the scan rate (logarithmic base), which is studied between 5 mV s^{-1} and 250 mV s^{-1}. As it is possible to observe, while the scan rate is higher, the oxidation response has an increment as well. This latter behavior can be explained by the interpretation of the Nernst – Planck equation (eq.1), where *J* corresponds to the matter flux which has different contributions: first of all, there is a diffusion term, where ($\partial c/\partial x$) is the concentration gradient at a distance *x* and time *t*. Secondly, there is a migration component due to the electrical field, where *z* and *c* are the charge and the concentration of the specie, and ($\partial ø/\partial x$)

is the potential gradient. Finally, there is a convection term, where $V(x,t)$ is the hydrodynamic rate. F and D correspond to Faraday's constant and the diffusion coefficient of the analyte, while R is the gas constant and T the temperature. Then, at higher the scan rate, the oxidation of the species that are reacting is being produced on sites that are closer to the electrode surface ($x \rightarrow 0$). At these circumstances, the ($\partial c/\partial x$) value is each time higher, which results on the obtainment of higher current peaks (i) [20], as it is observed on equation 1.1, where A is the electrode area and n the number of transferred electrons.

$$J\ (x,t) = \ -D\frac{\partial C(x,t)}{\partial x} - \left(\frac{zFDC}{RT}\right)\frac{\partial \emptyset\ (x,t)}{\partial x} + C\ (x,t)V\ (x,t) \qquad (1)$$

$$i = \ -nFAJ(x,t) \qquad (1.1)$$

As it is observed in figure 3a, the slope value is 0.466 (≈0.5), which implies that the process is diffusion-controlled. In order to corroborate this observation, figure 3b shows the relation between the peak current versus the square root of the scan rate, giving a linear correlation coefficient (R^2) close to the unity. This result gives evidence that, effectively, the system is controlled by diffusional processes related to the migration of the analyte towards the electrodic surface and vice versa. At this point, there are no considerable surface reactions (adsorption control) in the range of the studied scan rates [21].

(FIGURE 3a and 3b)

Furthermore, the peak potential (Ep) is also dependent on the variation of the scan rate, where the shifts on the peak potentials confirm the irreversibility of the reaction (160 mV). With this information, the equation (2) (R^2 = 0.98, n =8) can be obtained, which is consistent with an

electrochemical-chemical (EC) reaction mechanism that indicates that there is a charge transfer process before a chemical step, which gives the irreversible nature to the reaction [22].

$E_{p\text{-Enrofloxacin}}$ (V) = (0.080 ± 0.001)log v + 1.05 (2)

Then, it is possible to corroborate that the process is irreversible and diffusion-controlled.

Among these results, it is possible to calculate the number of transferred electrons by using the Randles-Sevcik equation (3), which is associated to irreversible processes that are diffusion-controlled [23,24].

$$Ip = 2.99 \times 10^5 n[(1-\propto)n_\propto]^{1/2} A C_b D^{1/2} v^{1/2} \quad (3)$$

In equation (3), A is the electrode area (0.07 cm^2), n and n_α the number of transferred electrons in the global process and in the determining step respectively, C_b corresponds to the analyte concentration (2.0 x 10^{-4} mol L^{-1} = 2.0 x 10^{-7} mol cm^{-3}) and D, the diffusional coefficient associated to this kind of fluoroquinolones [25,26] (≈ 4 x 10^{-6} cm^2 s^{-1}).

Moreover, since it is necessary to know the value of [(1-∝) $n_\propto$], an approximation based on the relationship between the peak potential (Ep) and the half-peak potential ($E_{p/2}$) is used (equation 4).

$$[(1-\propto)n_\propto] = \frac{47.7\ \text{mV}}{E_p - E_{\frac{p}{2}}} \quad (4)$$

For instance, in this case:

$$[(1-\propto)n_\propto]^{1/2} = 0.089 \quad (4.1)$$

So, the number of electrons involved in the global reaction, n, is 4 electrons (3.98 calculated).

Determination of the mechanism

Additionally, the Tafel plot has a value close to 120 mV per decade, which means that the rate determining step in the global reaction corresponds to an electrochemical step (E) before or after a chemical step (intramolecular rearrangement).

Finally, the proposed mechanism is shown in scheme 1.

(SCHEME 1)

The electro-oxidation of enrofloxacin is favorable due to the existence of an ethyl group on the piperazine nitrogen, where its alpha carbon atom is more susceptible to be oxidized, which would imply, in a further step, the release of one equivalent of acetaldehyde as subproduct and the formation of one equivalent of ciprofloxacin (fast chemical step). This latter observation was corroborated by LC-MS (m/z = 332.7 Da) shown in figure 4. Then, there is a second oxidation process that involves 2 electrons and the formation of a trihydroxilated derivative [27], which is confirmed by mass spectrometry as well (m/z = 359.8 Da)

(FIGURE 4)

3.3. Analytical parameters

To study the analytical parameters of the CV-enrofloxacin assay, we estimated the linearity, precision, and accuracy of the electrochemical method.

Linearity

From Figure 5a, it is seen that as the analyte concentration increases, the height of the anodic peak increases, producing a linear relationship with enrofloxacin concentration. The system linearity (I_p = 2.411 + 0.048xC) with regard to enrofloxacin concentration (Fig. 5b), makes possible the use of glassy carbon electrodes as a prospective enrofloxacin amperometric sensor, with detection (LOD) and quantification (LOQ) limits of $28x10^{-3} \pm 4x10^{-3}$ and $95x10^{-3} \pm 14x10^{-3}$ mmol L^{-1}. Precision results (RSD) (n=6) showed 15% for LOD and LOQ. These data show a good correlation between the peak current and the concentration of Enrofloxacin (R^2=0.996; n=6).

(FIGURE 5a and 5b)

Accuracy and precision

The voltammetric response (I_p) of the commercial product in comparison with standards can be observed in Table 1, evidencing the absence of a matrix effect once the drug has been dissolved in PBS. With these values of I_p it is evident that signals of both standard enrofloxacin (*Sigma-Aldrich*) and commercial drug are equivalent.

(TABLE 1)

A 95.6 ± 0.8 % recovery indicates the good accuracy of the method (reported 52.2±0.4 mg of enrofloxacin for a sample of 50 mg) that corresponds to 4.4± 0.8 % relative error, RSD = 18.1%, which is an acceptable accuracy.

Selectivity

Natural organic matter from soils constitutes an important interferent, especially in analytical determinations using spectrophotometric techniques. Furthermore, the linearity of the method is very poor. This is confirmed by analyzing the absorbance vs. Enrofloxacin concentration, where in the absence of NOM a $R^2 = 0.998$ ($y = 0.014 \times C + 0.012$, $n = 7$) was obtained, while in the presence of NOM (30 mg L^{-1}), the linear correlation decreases to $R^2 = 0.985$, with cut-off values on the y-axis much higher than those obtained in the absence of this interferent. However, the observed sensitivity reduction is more significative, with slopes decreasing by half ($y = 0.007xC + 0.399$, $n=7$) that causes loss of the analytical quality of the obtained results. No other electroanalytical techniques reported measure this kind antibiotic in presence of NOM.

The development of a new approach to overcome the existence of this interferent constitutes a contribution to obtain information concerning the presence of antibiotics for veterinary use, such as enrofloxacin in aqueous samples.

The voltammetric response of Enrofloxacin (300.0 μmol L^{-1}) in the absence and presence of the same type of NOM mentioned as interferent, is compared (Table 1) where the values of peak current (I_p), analytical signal evaluated, are not modified more than 5%, indicating that the presence of NOM does not constitute an interferent for the developed method. This was corroborated by analyzing calibration curves in the absence ($y = 0.052xC + 3.836$, $R^2 = 0.997$, $n= 7$) and presence of NOM ($y = 0.048xC + 3.302$, $R^2 = 0.998$, $n = 7$), with similarity in terms of sensitivity and linear correlation coefficient.

Thus, it is interesting to know the type of NOM that interferes in spectroscopic methods but not in the novel developed method. These humic substances have a very similar total acidity, ca. 8.9

mEq g^{-1}, composed of more than 80% by carboxylic groups and about 20% of phenolic groups. The above can be complemented with the sequence of relative abundance of functional groups, obtained by solid state NMR, wherein O/N alkyl > Alkyl C > Aromatic> Carboxylic (data not published). However, the particle size distribution indicates that this OM is constituted, under the measurement conditions (pH 7.0; PBS), mainly of particles with a mean diameter of 90 nm (64%) and 1 nm (36%). This characterization results corroborate the interferent is formed by nanoparticles that in the presence of the established electrochemical disturbance, do not record a response that may mean changes in the observed responses towards the analyte of interest.

4. CONCLUSIONS

A new and novel method was developed to determine and quantify Enrofloxacin in presence of NOM. This method is highly pH-dependent, since Enrofloxacin may exist as different species. At this point, at pH 7.0 the reaction is more favorable since the oxidation requires less energy to be accomplished, and it is attributed to the existence of Enrofloxacin as zwitterion.

Additionally, kinetic studies were done. It was found that the electro-oxidation of Enrofloxacin at pH 7.0 is diffusional-controlled and 4 electrons are transferred in the entire process. Also, the rate determining step of the reaction corresponds to an electrochemical step associated to an electronic transfer previous or after a chemical step. This latter result was corroborated by studying the Tafel slope, which has a value of 120 mV per decade. Parallel to this, LC-MS studies were done to corroborate the final product of the reaction, being the formation of a trihydroxilated derivative. With these data, a mechanism involving the formation of ciprofloxacin was proposed.

Analytical parameters were studied from this new electroanalytical method, which can be considered accurate and precise, showing good linearity response, (n=6; R^2 = 0.996) with

detection and quantification limits of $28x10^{-3}$ mmol L^{-1} and $95x10^{-3}$ mmol L^{-1} respectively. Also recovery results (96% of recovery) and precision (RSD=18%) for a commercial product (*Rostrum*®) were acceptable. In relation with selectivity, the new method show the same electrochemical response in presence or absence of soil extracted organic matter, composed mainly by nanoparticles around 90 nm with an important presence of carboxylic acidity, which is not considered as an analytical interference. With this advance this method would allow us to determine this analyte in environmental aqueous samples.

5. ACKNOWLEDGMENTS

Support from Fondecyt project 1130094 and Basal Funding for Scientific and Technological Centers of Excellence FB0807 CEDENNA are kindly acknowledged.

6. REFERENCES

[1] A.K. Sarmah, M.T. Mayer, A.B. Boxall, A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (Vas) in the environment, Chemosphere 65 (2006) 725.

[2] A. Millanao, M. Barrientos, C. Gomez, A. Tomova, A. Buschmann, H. Dölz, F. Cabello, Uso inadecuado y excesivo de antibióticos: Salud pública y salmonicultura en Chile, Rev. Med. Chile 139 (2011) 107.

[3] J.B. Addison, Antibiotics in sediments and run-off water from feedlots, Resid. Rev. 92 (1984) 1.

[4] R. Kroker, Aspekte zur ausscheidung animikrobiel wirksamer subsanzen nach der chemoterapeutischem von nutztieren, *Wiss. Umwelt* 4 (1983) 305.

[5] E.M. Golet, I. Xifra, H. Siegrist, A.C. Alder, W. Giger, Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil, Environ. Sci. Technol. 37 (2003) 3243.

[6] A.B. Boxall, P. Johnson, E.J. Smith, C.J. Sinclair, E. Stutt, L.S. Levy, Uptake of veterinary medicines from soils into plants, J. Agric. Food Chem. 54 (2006) 2288.

[7] A. Ernervik, A risk analysis of the potential harm on the soil environment caused by antibiotics in biosolids. Bachelor D. Dissertation, Lund University, Sweden, 2011.

[8] R.M.P. Leal, L.R. Alleoni, V.L. Tornisielo, J.B. Regitano, Sorption of fluoroquinolones and sulfonamides in 13 Brazilian soils, Chemosphere 92 (2013) 979.

[9] R.M. Leal, R.F. Figueira, V.L. Tornisielo, J.B. Regitano, Occurrence and sorption of fluoroquinolones in poultry litters and soils from São Paulo State. Braz, Sci. Total Environ. 432 (**2012**) 344.

[10] A. Gobel, A. Thomsen, C. McArdell, A. Joss, W. Giger, Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment, Environ. Sci. Technol. 39 (2005) 3981.

[11] M. Lillenberg, S. Yurchenko, K. Kipper, Presence of fluoroquinolones and sulfonamides in urban sewage sludge and their degradation as a result of composting, Int. J. Environ. Sci. Tech. 7 (2010) 307.

[12] D. Aga, S. O'Connor, S. Ensley, J.O. Payero, D. Snow, D. Tarkalson, Determination of the persistence of tetracycline antibiotics and their degradates in manure-amended soil using enzyme-linked immunosorbent assay and liquid chromatography-mass spectrometry, J. Agric. Food Chem. 53 (**2005**) 7165.

[13] A. Batt, D. Snow, D. Aga, Occurrence of sulfonamide antimicrobials in private water wells in Washington County, Idaho, USA, Chemosphere 64 (2006) 1963.

[14] A. Pruden, M. Arabi, H. Storteboom, Correlation between upstream human activities and riverine antibiotic resistance genes, Environ. Sci. Technol. 46 (2012) 11541.

[15] Y. Zhang, C. Zhang, D. Parker, D. Snow, Z. Zhou, X. Li, Occurrence of antimicrobials and antimicrobial resistance genes in beef cattle storage ponds and swine treatment lagoons, Sci.Total Environ. 463-464 (2013) 631.

[16] M. Antilen, F. Amiama, M. Otaiza, F. Armijo, M. Escudey, C. Pizarro, N. Arancibia-Miranda, A New Methodology to Evaluate Adsorption on Nanomaterials, J. Nano. Res. 17 (2015) 212.

[17] M. Antilen, O. Bustos, G. Ramirez, C. Canales, M. Faundez, M. Escudey, C. Pizarro, Electrochemical Evaluation of Ciprofloxacin Adsorption on Soil Organic Matter, New J. Chem. 40 (2016) 7132.

[18] M. de Nobili, G. Bragato, J. Alcaniz, A. Puigbo, L. Comellas, Characterization of electrophoretic fractions of humic substances with different electrofocusing behavior. Soil Sci. 150 (1990) 763.

[19] A. Watanabe, S. Kuwatsuka, Fractionation of soil fulvic acids using polyvinylpyrrolidone and their ionization difference spectra. Soil Sci. Plant Nutr. 37 (1991) 611.

[20] F. Santamaría, Diffusion Equation, in: D. Jaeger, R. Jung (Eds.), Encyclopedia of Computational Neuroscience, Springer, New York, 2015, p. 1018.

[21] N. Erk, Voltammetric behaviour and determination of moxifloxacin in pharmaceutical products and human plasma, Anal. Bioanal. Chem. 378 (2004) 1351.

[22] B. Uslu, B. Bozal, M. Kuscu, Anodic Voltammetry of Ciprofloxacin and its Analytical Applications, Open Chem. Biomed. Methods J. 3 (2010) 108.

[23] A. Bard, L. Faulkner, Electrochemical Methods: Fundamentals and Applications, Wiley, New York, U.S.A., 2000.

[24] R. Ríos, A. Marín, G. Ramírez, Nitrite electro-oxidation mediated by Co(II)- [tetra(4-aminophenyl) porphyrin]-modified electrodes: behavior as an amperometric sensor, J. Coord. Chem. 63 (2010) 1283.

[25] M. Rizk, F. Belal, F. Ibrahim, S. Ahmed, N.M. EL-Enany, Voltammetric analysis of certain 4-quinolones in pharmaceuticals and biological fluids, J. Pharm. Biomed. Anal. 24 (2000) 211.

[26] P.A. Suci, M.W. Mittelman, F.P. Yu, G.G. Geesey, Investigation of Ciprofloxacin Penetration into Pseudomonas aeruginosa Biofilms, Antimicrob. Agents Chemother. 38 (1994) 2125.

[27] V.S. Antonin, M.C. Santos, S. Garcia-Segura, E. Brillas, Electrochemical incineration of the antibiotic ciprofloxacin in sulfate medium and synthetic urine matrix, Water Res. 83 (2015) 31.

Figures

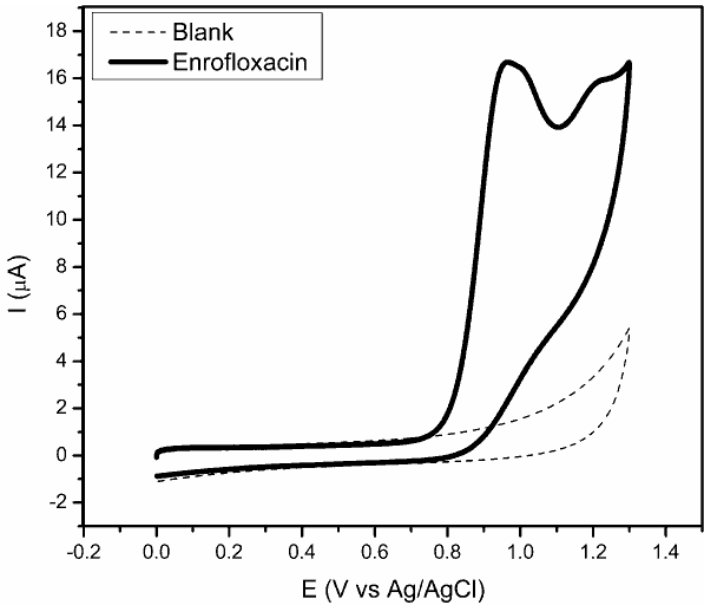


Figure 1. Voltammetric response of 300 μmol L^{-1} Enrofloxacin in buffer phosphate solution, pH 7.0, purged in Ar. Scan rate 100 mV s^{-1}

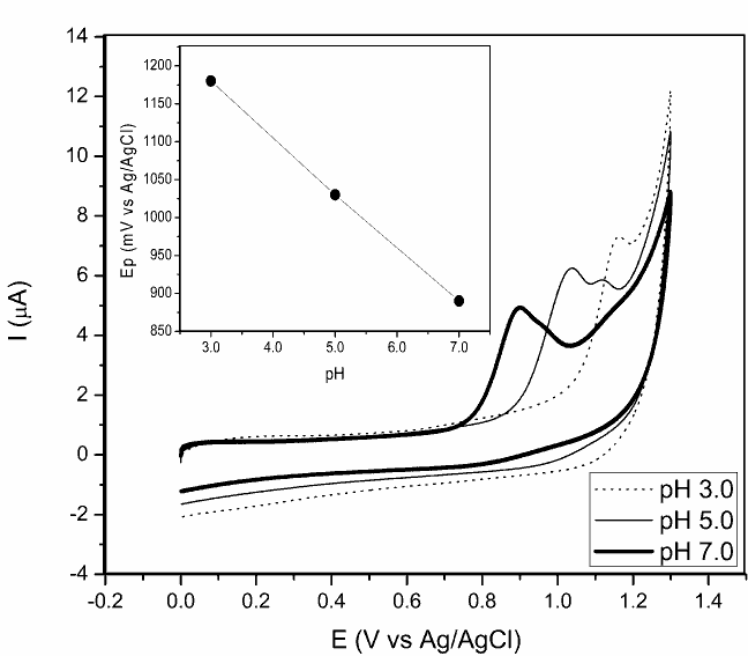


Figure 2. Voltammetric response of 200 μmol L^{-1} Enrofloxacin in buffer solutions, pH 7.0, 5.0 and 3.0, purged in Ar. Scan rate 100 mV s^{-1}. In inset, the pH dependence of Ep values for Enrofloxacin detection.

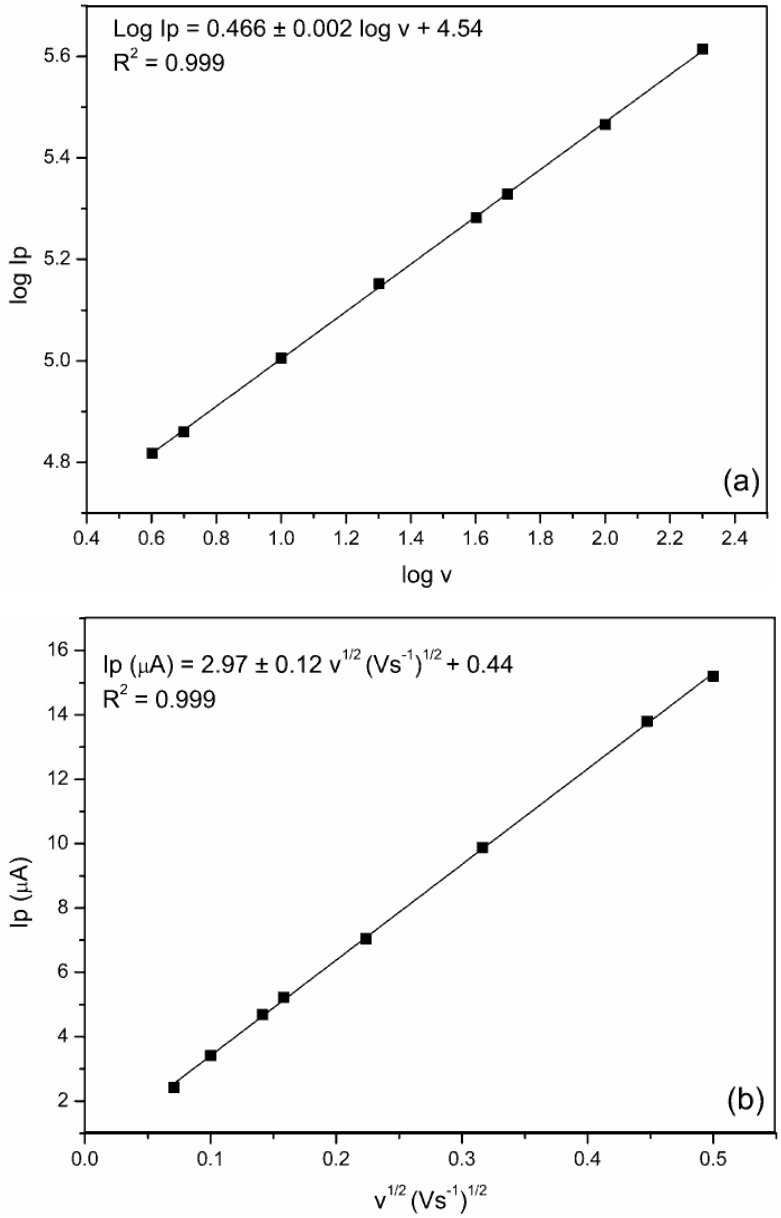


Figure 3. (a) Logarithm of the peak current (Ip) versus logarithm of the scan rate (v) and (b) variation of the peak current (Ip) versus the square root of the scan rate ($v^{1/2}$). 2.0 x 10^{-4} mol L^{-1} of Enrofloxacin in buffer phosphate solution (H_2PO^{4-}/HPO_4^{2-}) pH 7.00, purged in Ar.

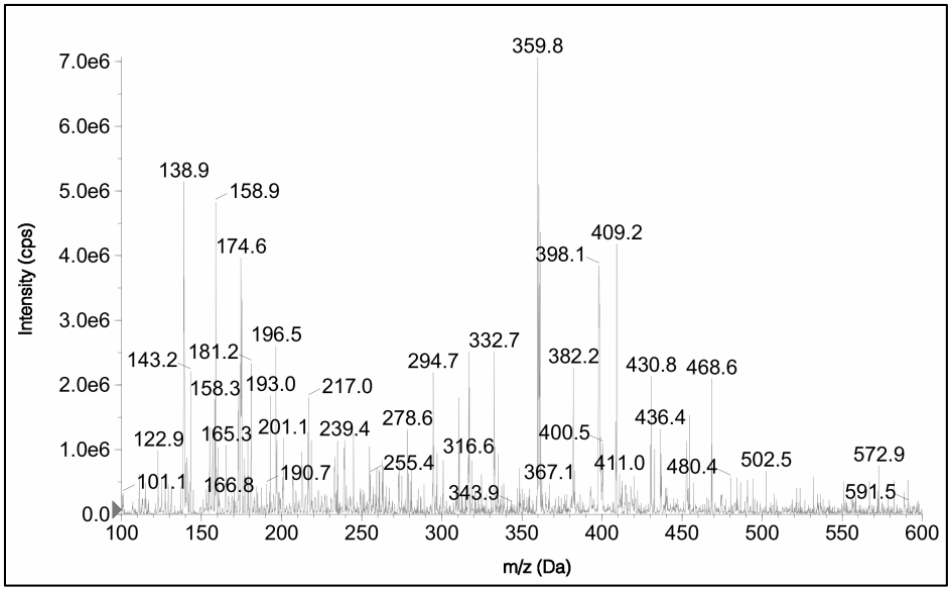


Figure 4. Mass spectrum of enrofloxacin after the oxidation process.

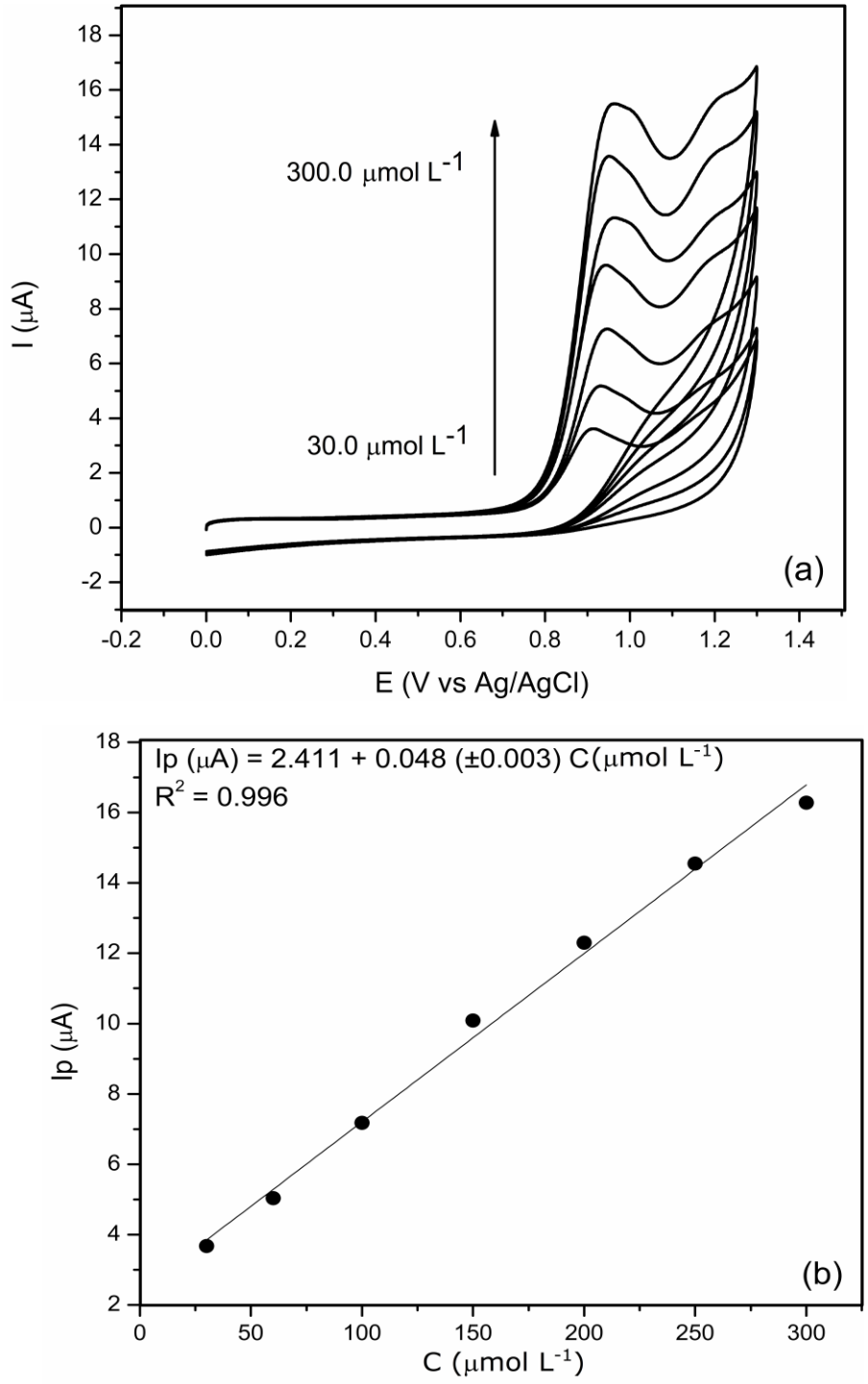
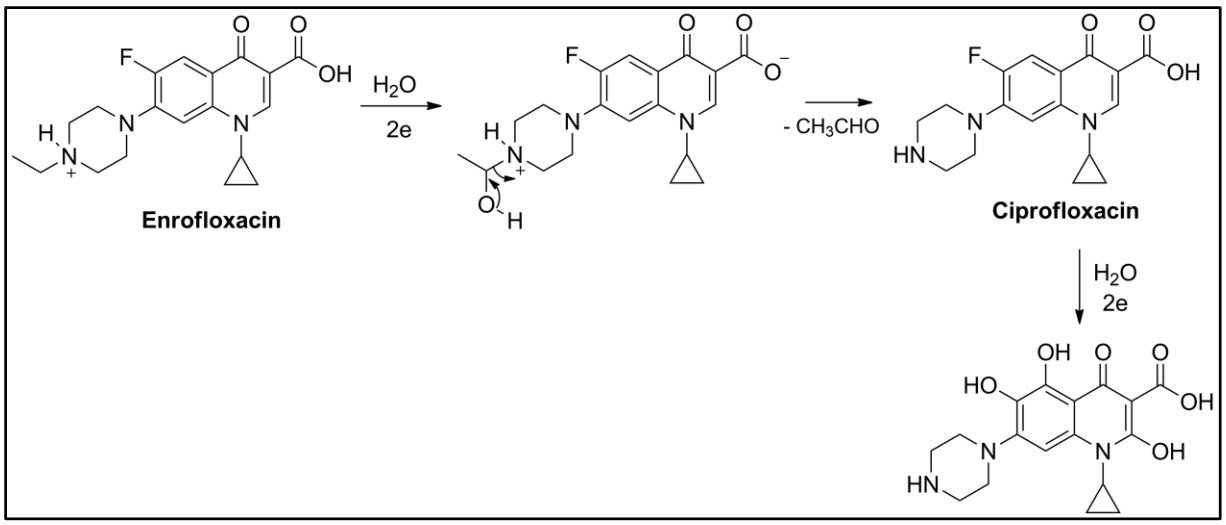


Figure 5. (a) GCE voltammetric response towards enrofloxacin concentration between 0.0V and 1.3V. v = 100 mV s-1 in a cell containing 10 mL of BPS-enrofloxacin; (b) Linear relationship between current anodic peak (Iap) and Enrofloxacin concentration (30-300μmol L^{-1}) (y=0.048xC + 2.411, R^2= 0.996; n=6).



Scheme 1. Proposed mechanism for enrofloxacin electro-oxidation

Table 1.- Peak potentials (Ep) and peak currents (Ip) corresponding to accuracy and precision tests associated to Enrofloxacin present in commercial drugs (Sigma – Aldrich and Drag – Pharma) and its response in presence of different humic acids (HA)

Accuracy and precision tests	Assay	Ep (V)	Ip (µA)
Commercial drugs	Sigma – Aldrich	0.90	5.0
	Drag – Pharma	0.90	5.0
ENR + different HA	ENR	0.97	19.0
	ENR + HA (commercial)	1.0	18.5
	ENR + HA (FRU)	0.95	17.7
	ENR + HA (RLN)	0.95	17.7