Orbital: The Electronic Journal of Chemistry
journal homepage: www.orbital.ufms.br
e-ISSN 1984-6428

| Vol 10 | | No. 3 | | Special Issue May 2018 |

FULL PAPER

Optimization and Validation of a Methodology for the Quantification of Streptomycin Using Square Wave Voltammetry

Luciane Miranda*[a], Vanessa E. dos Anjos[b], Sueli P. Quináia[a]

[a]Departamento de Química, UNICENTRO, R. Simeão Varela de Sá, 03, Guarapuava (85040-080), Paraná, Brazil.
[b]Departamento de Química, UEPG, Av. General Carlos Cavalcanti, 4748, Ponta Grossa (84030-900), Paraná, Brazil

Article history: Received: 27 September 2017; revised: 21 November 2017; accepted: 30 November 2017. Available online: 03 May 2018. DOI: http://dx.doi.org/10.17807/orbital.v10i3.1091

Abstract:

The quantification of pharmaceuticals drugs is typically accomplished using chromatography, which requires several pretreatment steps of the sample, demanding time and supplies. Thus, the present work proposed the development and validation of a voltammetric method to quantify the antibiotic streptomycin (STP), in aqueous samples. Initially, the parameters of the technique were optimized applying square wave adsorptive cathodic stripping voltammetry (SW-AdsCSV), using the hanging mercury drop electrode (HMDE) as the working electrode. Then, the adequacy of the analytical method was evaluated using validation criteria, such as precision, selectivity, linearity, limits of detection and quantification. The linearity was evaluated by a standard addition curve from STP 8.09 to 210.4 μg L^{-1}. The limits of detection (LOD) and quantification (LOQ) were 0.18 and 0.60 μg L^{-1}, respectively. The accuracy and precision of each method were expressed as percentage of STP recovery in fortified solutions, resulting in deviation below 20%, acceptable for analytes at the trace level, indicating accuracy and precision of the methods. The voltammetric methods have advantages over others described in literature, since they do not require steps of pre-treatment of the samples, being, this way, fast and low cost analyzes. The method was applied in natural and effluent water; however, STP was not detected in a real sample, in three samplings.

Keywords: aminoglycoside antibiotic; natural water; streptomycin; voltammetry

1. Introduction

Antibiotics are natural (obtained by the action of microorganisms) or synthesized substances, which, used in small quantities, either prevent the growth of microorganisms or cause their death [1]. Penicillin was the precursor of antibiotics first described in literature, in 1940 [1, 2]. There are several classes of antibiotics, such as β-lactams, tetracyclines, cyclic peptides and aminoglycosides. Among the mechanisms of action of antibiotics on microorganisms, we can mention inhibition of bacterial protein synthesis, inhibition of RNA synthesis, inhibition of bacterial cell wall formation, or affecting bacterial membrane permeability [1, 3].

Streptomycin sulfate (STP) is an aminoglycoside antibiotic, which is composed of amino sugars connected by glycosidic bonds [4] (Figure 1). The STP has the molecular formula $C_{21}H_{34}N_7O_{12}$ .1 ½ H_2SO_4 and molecular weight 728.69 g mol^{-1}. STP is a white crystalline powder, odorless, very water-soluble and little alcohol-soluble. STP produces acidic or slightly acidic solutions with pH between 4.5 and 7.0, pKa (10.88 in strong acid, 11.9 in strong base) [5].

STP was synthesized in 1944 from the fungus *Streptomyces griseus*, and nowadays it is used to fight gram-negative bacteria, being considered one of the first effective antibiotics in use against many pathogenic bacteria, including *Mycobacterium tuberculosis* [6]. STP is applied in

*Corresponding author. E-mail: luciane_miranda@yahoo.com.br

the treatment of respiratory diseases in animals, such as genus Leptospira (*Leptospira spp.*) and pneumonia (*Pasteurella spp., Haemophilus spp*. and *Mycoplasma spp.*) [7]. The human use of STP occurs when other less toxic antibacterials are ineffective, or contraindicated for combating infections of the biliary tract, bones, articulations and infections of the central nervous system [8].

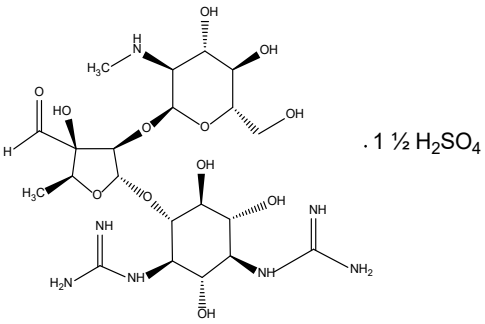

Figure 1. Chemical structure of streptomycin sulfate (STP).

Aminoglycoside antibiotics are also added in feed for growing animals. Thus, there is concern about the residues of antibiotics in food (e.g., milk and milk products) as they may cause intoxication and damage to human health. This fact shows the need of quality control of drugs as they establish the requirements of pharmacopoeias and estimation of the residual quantity in foods [6, 9]. Another fact that contributed to the need for studies related to antibiotics is the development of bacterial resistance genes, which may even impair the use of a given antibiotic or require the combination of these antibiotics to be effective [10, 11].

The sources of antibiotic input into the environment are diverse, occurring from human excretion, live-stock, and pets, use in agriculture (use of animal waste in crop fertilization), food additives in aquaculture, and, finally, the inappropriate disposal of expired drugs [12, 13]. As they reach the environment, these drugs can reach water bodies through rainfall in agricultural areas that receive animal waste, and release from treatment plants [14].

High-performance liquid chromatography (HPLC) is the most commonly used technique for the quantification of aminoglycoside antibiotics [9], especially coupled with mass spectrometry (LC-MS) [15]. Bruijnsvoort et al. [16] proposed an HPLC-MS method for the determination of STP and dihydrostreptomycin in milk and honey. Pietro [17] developed a method with HPLC using liquid/liquid or solid phase extraction for quality control and analysis of residue of aminoglycosides in bovine milk (streptomycin, neomycin and gentamicin). Granados and Meza [18] optimized and validated a methodology by HPLC to identify and estimate streptomycin and streptidine in serum of streptomycin-treated patients. Streptidine is a metabolic derivative of STP with ototoxic potential (capable of causing damage to the ear).

Although chromatography is widely used in the quantification of drugs, this technique presents high equipment costs, use of high purity solvents and the need of highly trained operators, justifying the need to develop sensitive and lower-cost methodologies for the quantification of drugs in environmental samples. Thus, electrochemical detectors (with or without modification) offer the possibility to monitor drugs, as long as the molecule is electroactive, that is, with at least one active site capable of being reduced or oxidized electrochemically. Studies of the electroactivity of STP suggest that it adsorbs on the working electrode, and then it is reduced [6, 19]. Therefore, this work describes the optimization and validation of a cheap and fast analytical method for the quantification and monitoring of STP in aqueous samples. The results of the present study may contribute with data of STP in environmental samples.

2. Material and Methods

In the voltammetric measurements, a Metrohm 757 VA analyzer controlled by the VA Computrace software (Metrohm, Switzerland) was used. A 100 mL measuring cell with a hanging mercury drop electrode (HMDE) with approximately 0.30 mm^2 of surface area was used, as a working electrode, a platinum electrode as a counter electrode, and an Ag/AgCl reference electrode (KCl 3.0 mol L^{-1}) was employed. A purge system with ultrapure nitrogen (99.999%) was used to remove O_2.

Reagents with an analytical grade were used in the determinations, that comprise sodium hydroxide (Vetec, Brazil), sodium phosphate monobasic (Biotec, Brazil), ethanol (Biotec, Brazil) nitric acid (Biotec, Brazil), commercial humic acid (Sigma Aldrich, USA) and standard of

the sulfate of streptomycin (Sigma Aldrich, USA). Solutions were prepared using ultrapure water obtained from a TKA-GenPure purification system (USA). Measurements of pH were taken with a Hanna potentiometer (United Kingdom) with an Ag/AgCl glass electrode.

A stock standard solution of the antibiotic STP 7.3 mg L^{-1} was prepared in ultrapure water. Daily, work solutions of STP were prepared (1.0×10^{-5} or 1.0×10^{-6} mol L^{-1}). The supporting electrolyte was 0.01 mol L^{-1} NaOH with pH adjustment using 0.1 mol L^{-1} NaH_2PO_4, resulting in a buffer solution ($H_2PO_4^- \leftrightarrow HPO_4^{2-}$). The pH was evaluated from 7.0 to 10.0.

Solutions of commerical Humic Acid (HA) were prepared in order to evaluate the interference of the matrix. HA was considered to contain approximately 35% of dissolved organic carbon DOC [20].

Voltammetric Techniques

Cyclic voltammetry (CV) was used to evaluate the redox behavior of the STP molecule. The CV operating conditions were: accumulation potential (E_{ac}) of -1.2 V, accumulation time (t_{ac}) of 90 s, potential scan (ΔE) from -0.45 to -1.7 V and scan rate from 5 to 90 mV s^{-1}.

Previously, a study was carried out to select the voltammetric method (differential pulse or square wave) for quantitative analyses. In the assay with differential pulse adsorptive cathodic stripping voltammetry (DP-AdsCSV), a STP standard addition curve was prepared in the range from 365 to 11.1×103 µg L^{-1}. In the voltammetric cell, it was added 10.0 mL of the supporting electrolyte, at pH adjusted to 9.0. The DP-AdsCSV parameters were: E_{ac} (-1.2 V), sweep range (-1.0 to -1.4 V), t_{ac} (90 s), scan rate (40 mV s^{-1}), pulse time (40 ms), pulse amplitude (50 mV). To evaluate the square wave adsorptive cathodic stripping voltammetry (SW-AdsCSV), a curve of STP was constructed from 36.5 to 507.5 µg L^{-1}, using as supporting electrolyte (phosphate buffer solution) at pH 9.0. The parameters of SW-AdsCSV were: (-1.2 V), scanning range (-1.3 to -1.8 V), t_{ac} (60 s), scan rate (400 mV s^{-1}) and amplitude (50 mV).

In a quantitative analysis, the voltammetric method SW-AdsCSV was employed. In the optimization of the parameters, 61.42 µg L^{-1} STP was added in the cell for the optimization of the voltammetric parameters, except for t_{ac}, in which it was used 14.54 µg L^{-1}. The SW-AdsCSV parameters optimized for STP were: accumulation potential (-1.0 to -1.3 V); frequency (f) (60 to 140 Hz); scan increment (ΔE) (2 to 8); pulse amplitude (a) (20 to 50 mV); accumulation time (60 to 180 s) and equilibrium time (0 to 30 s).

In-House Validation

The performance of the voltammetric method was evaluated through an in-house validation. The parameters of the validation were performed using the parameters sensitivity, selectivity, linearity, detection and quantification limits, accuracy and precision, as well as other important aspects in the development of analytical methods according to validation guides [21-25].

A standard addition curve was built using the instrumental parameters optimized to assess linearity, LOD and LOQ. The STP concentration ranged from 8.09 to 210.4 µg L^{-1} with t_{ac} 180 s. The linearity of the calibration curve was estimated using a regression analysis with analysis of variance (ANOVA), as well as a lack-of-fit test at 95% confidence, using the Minitab software for Windows. The LOD and LOQ were estimated based on the expressions $3S_B/b$ and $10S_B/b$, respectively, where S_B is the standard deviation of 20 consecutive scans of the supporting electrolyte, and b is the slope of the curve [21].

The selectivity of the method was evaluated qualitatively from the analysis using commercial humic acid, since it is a substance of high molecular mass and considered abundant in natural water [26]. Thus, the interference effect was evaluated on the STP response (peak current) with additions of HA. A suspension with 0.015 g of HA was prepared in 10.0 mL with ultrapure water, and aliquots of HA were added in the cell containing the supporting electrolyte and STP. Additionally, the selectivity was evaluated by the effect of the matrix with natural river water. The STP response was measured in the absence of the matrix and in experiments containing 1.0 and 2.0 mL of natural water, respectively. In these tests, the standard additions of STP were from 10.2 to 40.6 µg L^{-1}.

The precision of the method was estimated based on repeatability and intermediate precision tests. Precision was expressed as relative standard deviation (RSD). In the repeatability tests, STP solutions at 35.0 μg L^{-1} were prepared in five independent replicates over 1 day. Intermediate precision was evaluated under the same conditions, but over 5 consecutive days. Accuracy tests of the method were assessed by STP recovery studies in ultrapure water fortified with 15.0 and 35.0 μg L^{-1} of the drug. In a sample of river water (dilution 1:10 mL) a standard addition curve was built in a sample fortified with 51.01 μg L^{-1}, with standard additions from 10.2 to 30.6 μg L^{-1} of STP.

In order to evaluate the presence of the method to quantify STP in samples of natural and effluent water, samples were collected at six points along the Paraná III Basin that covers Lake Itaipu. Three samplings were performed at approximately six-month intervals.

3. Results and Discussion

Cyclic voltammetry was performed to investigate the electrochemical behavior of the STP molecule with HMDE. The voltammogram cyclic in Figure 2-A shows the scan rate from 50 mV s^{-1} to STP, with an irreversible reduction peak at -1.1 V potential. Figure 2-B shows the response I_p as a function of the root of the scan ($v^{1/2}$) from 5 to 90 mV s^{-1}. Finally, in Figure 2-C, the relation between the *log* I_p versus *log* v.

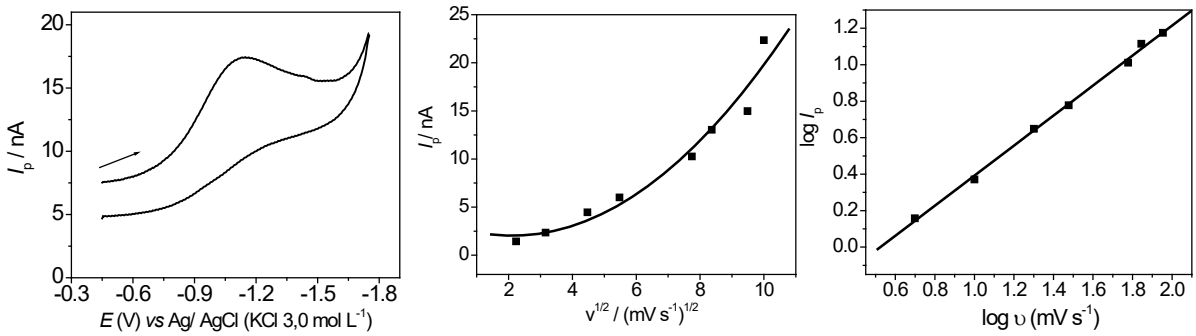

Figure 2. **A)** Cyclic voltammogram of STP at scan 50 mV s^{-1}, with HMDE. Conditions: C_{STP} (61.42 μg L^{-1}), supporting electrolyte 0.01 mol L^{-1} NaOH, pH 12.0; potential scan (-0.45 to -1.7 V), E_{ac} (-1.2) and t_{ac} (90 s); **B)** Response I_p as function of $v^{1/2}$; **C)** Dependence of the *log* I_p versus *log* v.

Figure 2-B presents the non-linear behavior of STP; this response suggested that the process is controlled by the adsorption of the species on the surface of the electrode. Also, Figure 2-C shows a linear curve with a slope of 0.82, showing that the adsorption process is more important than the diffusional one. According to literature, an angular coefficient (slope) close or equal to 1.0 suggests that the system is controlled by adsorption [27]. This behavior agreed with the work by Wang and Mahmoud, [19] who obtained a curve with a slope of 1.03. In the voltammetric measurements of STP, initially was used as supporting electrolyte 0.01 mol L^{-1} NaOH, based on literature [6,19]. Then, a pH study was performed as shown in Figure 3. Figure 3-A refers to the voltammetric signal for the supporting electrolyte at pH from 7.0 to 10.0, without baseline reduction. The voltammograms in Figure 3-B show the response to 30.0 μg L^{-1} STP at pH from 8.0 to 10.0, with baseline correction.

The voltammetric response of the supporting electrolyte at pH 7 in the Figure 3-A shows a peak in the potential next to the potential related to the STP, difficulty the quantification of STP, in this pH. Thus, the better response to STP was obtained at pH 9.0, as shown by the voltamograms in Figure 3-B. In the literature, it is suggested that in aqueous solutions STP is determined in pH between 9.0 and 9.5; in this medium, the STP molecule is stable like a cation with two charges [6].

Optimization of the voltammetric parameters

Previous voltammetric tests for STP were performed using DP-AdsCSV and SW-AdsCSV, to verify which would be the most sensitive. The analytical curve in Figure 4-A shows an analytical

curve from 365 to 11.1×10^3 µg L^{-1} of STP with I_p measured at potential -1.27 V, using the DP-AdsCSV. Figure 4-B shows the STP I_p response obtained by SW-AdsCSV at potential -1.6 V, in the range from 36.5 to 507.5 µg L^{-1}.

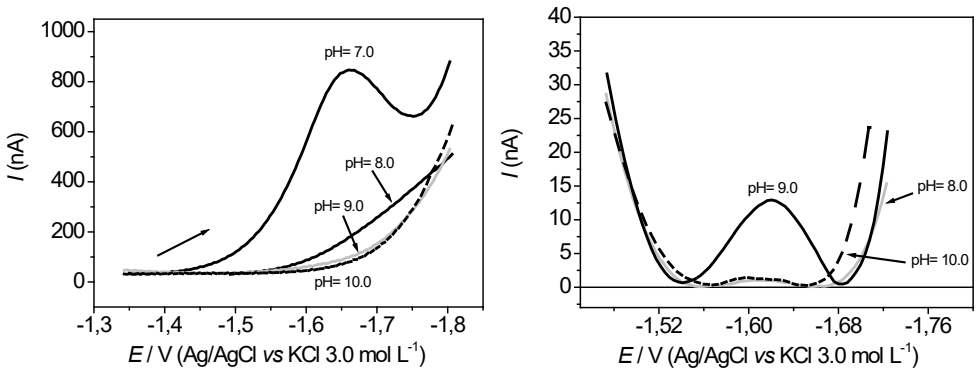

Figure 3. Voltammograms of SW-AdsCSV at scan 50 mV s^{-1} with HMDE, related to: **A)** Supporting electrolyte (phosphate buffer solution), pH from 7 to 10; potential scan (-1.45 to -1.7 V), E_{ac} (-1.2) and t_{ac} (90 s); **B)** 30.0 µg L^{-1} STP, in the pH from 8 to 10, with baseline reduction.

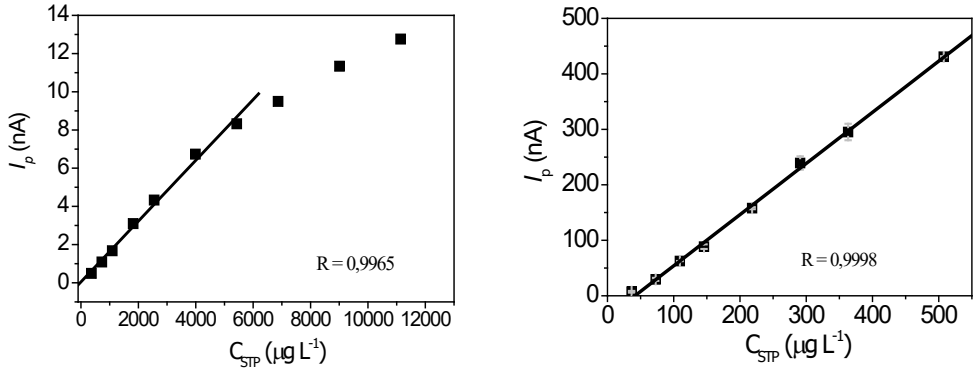

Figure 4. Standard addition curve for STP by using voltammetry: **A)** DP-AdsCSV range from 365 to 11.1×10^3 µg L^{-1}; **B)** SW-AdsCSV range from 36.5 to 507.5 µg L^{-1}.

The objective of this stage of the study was to evaluate the method of pulse application. Thus, with the application of differential pulse, it was possible to verify a response of the STP only above 365 µg L^{-1}, as shown the Figure 4.

The voltametric parameters were evaluated in a univariate way, by adding 61.42 µg L^{-1} of STP, except for the accumulation time, in which it was used 14.54 µg L^{-1}. The SW-VAdsRC parameters optimized for STP were: accumulation potential (-1.0 to -1.3 V); frequency (*f*) (60 to 140 Hz); potential step (2 to 8 mV); pulse amplitude (20 to 50 mV); accumulation times (60 to 180 s) and equilibrium (0 to 30 s).

The Square-wave voltammograms in Figure 5-A show the variation of the E_{ac} with an addition of 61.42 µg L^{-1} of STP in the cell, in Figure 5-B the baseline correction was applied to -1.1 and -1.2 V, with a better response.

The variation of the accumulation potential (Figure 5-B) shows the best voltammetric STP response at -1.2 V and agrees with Fedorchuk et al. [6], who evaluated this parameter and found a maximum response with E_{ac} from -1.2 to -1.3 V.

The frequency was evaluated from 60 to 140 Hz and the I_p ranged from 24.0 ± 1.1 to 60 Hz, until 30.0 ± 0.3 nA to 120 Hz that was the chosen parameter. The scan speed in square wave voltammetry is given by the product (f × ΔE). Thus, by increasing the scan increment, it is expected a better response of the peak current improving the sensitivity of the method [30]. The scan increment (ΔE) was evaluated from 2 to 8 mV, resulting in peak current of 20.0 ± 0.55 nA, based on the smaller deviations 4 mV was chosen, resulting in the scan speed 480 mV s^{-1}.

The parameter pulse amplitude (*a*) was investigated from 20 to 50 mV. The response

increased from 15 ± 0.4 to 20 ± 0.4 nA. This variation in response was not observed between 40 and 50 mV. Therefore, 50 mV was the amplitude chosen.

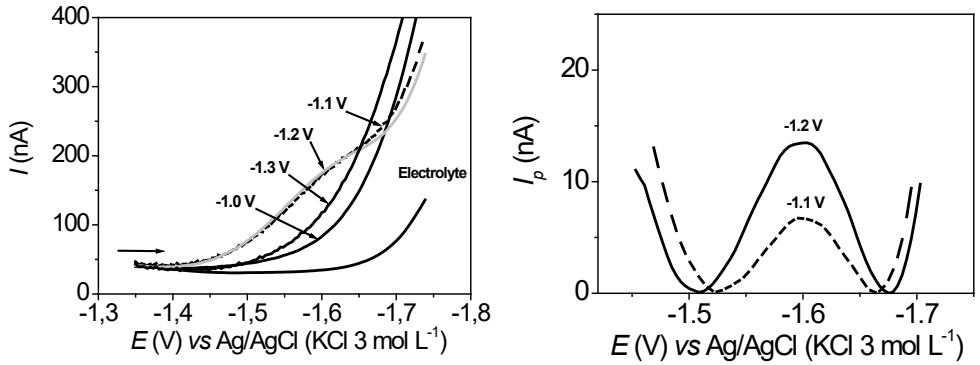

Figure 5. **A)** Square-wave voltammograms, accumulation potential variation, supporting electrolyte signal and STP addition of 61.42 µg L^{-1}; Variation E_{ac}: -1.0 to -1.3 V. Conditions: Frequency 100 Hz; amplitude 50 mV; potential step 4 mV and t_{ac} 120 s. **B)** voltammograms to E_{ac} (-1.1 and -1.2 V) with baseline correction.

The accumulation time was evaluated between 60 and 180 s, with an addition of 14.54 µg L^{-1} of STP in the voltammetric cell (Figure 6).

In the time interval evaluated (Figure 6) the increase in response up to 180 s is observed, however, the t_{ac} may vary depending on the sample, aiming at a better analytical response. Fedorchuk et al. [6], using the mercury film electrode, evaluated the STP peak up to 180 s in the concentration range of 5.0 to 50 µg L^{-1} and found that above 50 µg L^{-1}, the saturation of the electrode surface occurs when t_{ac} was applied over 30 s.

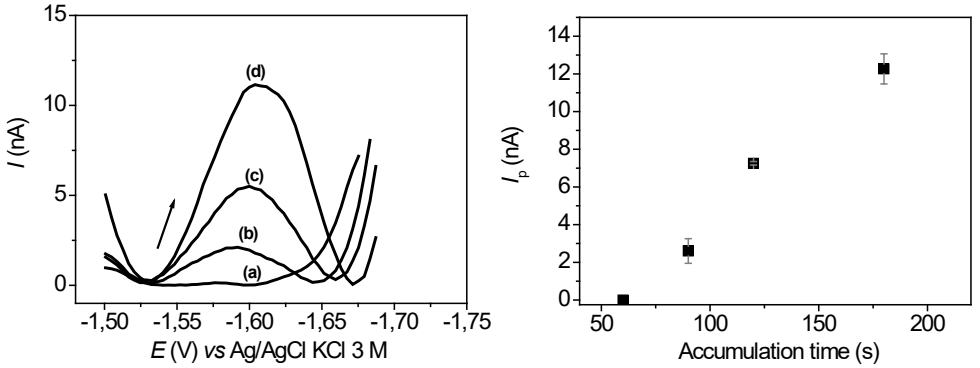

Figure 6: **A)** Square-wave voltammograms of STP at different t_{ac}: (a) 60, (b) 90, (c) 120 e (d) 180 s; C_{STP} of 14.54 µg L^{-1}. Conditions: E_{ac} -1.2 V, ΔE: 4 mV; a: 50 mV and f : 120 Hz. **B)** Correlation between the t_{ac} and I_p variation.

At the end of the accumulation step, the solution is left for a few seconds to equilibrate the chemical deposited on the surface of the electrode; this step requires a few seconds. In HMDE this time is about 15 to 20 s, for metal species [31]. The STP response I_p decreased with the equilibrium time after 2 s, so, this was the chosen time. This result showed that for organic molecules, such as the case of the drugs under study, the equilibrium time was much lower than that one recommended for metal analysis.

In order to evaluate the application of the proposes method to quantify STP in samples of natural and effluents water were collected at six points along the Paraná III Basin that covers Lake Itaipu. Three samplings were performed at approximately six-month intervals.

In-House validation of the voltammetric

method

Linearity is an important parameter in the validation for quantitative analyzes. A model is considered linear when the analytical signal increases linearly with the analyte concentration, allowing, thus, the construction of a calibration curve [24]. A standard addition analytical curve of STP was evaluated in 14 levels from 8.09 to 210.4 μg L^{-1}, as shown in Figure 7. The analytical response I_p to STP was measured with the reduction of the baseline in the voltammograms.

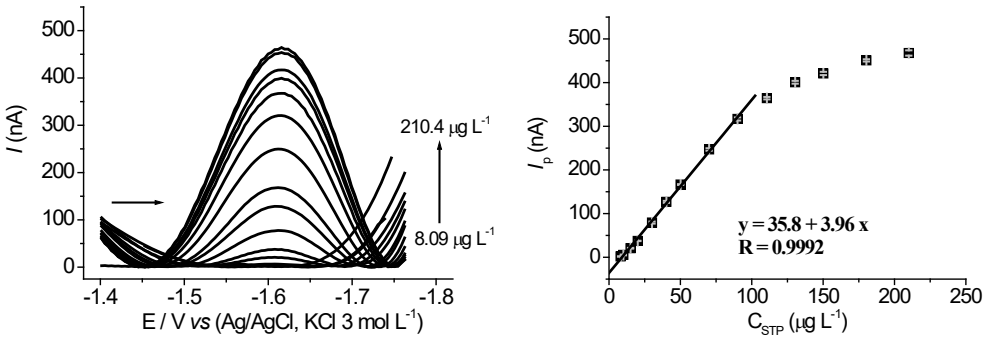

Figure 7. **A)** Calibration and linearity studies of voltammograms of SW-AdsCSV for STP, additions of 8.09–210.4 μg L^{-1}; with supporting electrolyte, at pH 9.0; **B)** Dependence on the peak current intensity as a function of concentration of STP with SD (n = 3).

The analytical curve in Figure 7-A shows the voltammograms of STP with maximum peak on potential -1.6 V. The concentration range from 8.09 to 90 μg L^{-1} of STP, apparently linear, was assessed by a regression analysis with a confidence level of 95%. The result of the linear model regression was [(F_{faj} (1.25); $F_{crictical}$ (4.25); p_{value} (0.00)], and for the quadratic model it was [F_{faj} (1.25); $F_{crictical}$ (3.39); p_{value} (0.00)]. However, it was found the lack of fit for linear models and values of regression analysis ($F_{faj} < F_{crictical}$, $p_{value} < 0.05$). In addition, this behavior was confirmed by the residual graphs (not shown) related to the adjustment of the linear and quadratic models; in both models there was a lack of homogeneity of the variance in the calibration data, which contradicts the hypothesis of the application of the technique [30]. Finally, the log function was applied to the I_p data, and the inverse function to the concentration ($1/C_{STP}$) and restriction of the data in the working range from 8.09 to 40.18 μg L^{-1} resulted in the adjustment of the model. The regression in the function was evaluated by *F* test at 95% confidence level. $F_{regression}$ was 5996.82 and $F_{critical}$ (4.49), indicating that $F_{regression} >> F_{critical}$ and the significance of the fitted curve. Adequacy-of-fit of the curve was evaluated by the lack-of-fit procedure of linear regression (F_{lof}). The F_{lof} value (2.47) was lower than $F_{critical}$ (3.26), showing no lack-of-fit in the model proposed [30].

The LOD and LOQ were calculated with the 20 measurements of the peak current in the supporting electrolyte medium at the reduction potential of STP, with standard deviation (S_B = 1.08 nA) and slope of the curve log I_p (nA) = 2.52 – 18.2 [$1/C_{STP}$ (mol L^{-1})]. The LOD and LOQ values were 0.18 and 0.60 μg L^{-1}, respectively, with t_{ac} 180 s. In Table 1 some works and their respective LOD calculated to STP by different techniques are shown, in order to compare them to the proposed method.

The LOD of the method for STP (Table 1) presents lower values than in literature, such as the voltammetric method developed by Wang and Mahmoud [19] for the quantification of STP in urine, as well as in relation to the chromatographic methods proposed for the quantification of STP in food [16,17]. In addition, in the samples, the LOD and LOQ were recalculated, considering the supporting electrolyte (phosphate buffer solution) solution prepared in a mixture of 1.0 mL of natural river water, diluted in 9.0 mL of ultrapure water. The response deviation (S_B) in this medium was 0.64 nA, and the slope of a standard addition curve in that medium (y = 21.64 + 0.42 x); based on these data, the LOD and LOQ values were 13.5 and 45.0 μg L^{-1}, respectively.

The influence of organic matter was evaluated with HA on the determination of the STP. The results indicated differences between the response of 30.5 μg L^{-1} STP in the absence of

organic material, comparing to the response with additions from 0.30 to 9.01 mg L^{-1} of DOC, present in the aliquots of HA. The response of STP decreased from (69.7 ± 2.28 nA) without HA to (14.3 ± 0.10 nA) in the presence of 9.01 mg L^{-1} of DOC. In natural water, the effect of interference was estimated in a river water sample (1.0 and 2.0 mL diluted in ultrapure water). The pH of the mixture with electrolyte was adjusted, as described in the optimization of the method. Figure 8 shows the voltammograms of the response for the analysis of the sample from 10.2 to 40.6 µg L^{-1} STP.

Table 1. The LOD of the proposed method in comparison with some works proposed for the quantification of STP in different matrices.

Technique or Method	Note	LOD (µg L^{-1})	Application (matrix)	Ref.
SW-AdsCSV	Phosphate buffer solution; HMDE	0.18	Natural and effluent water	This work
Differential Pulse Polarography	0.01 mol L^{-1} NaOH, MFE (mercury film electrode)	0.02	Pharmaceutical formulations and milk	[6]
ELISA method	-	20.0	Milk	[7]
HPLC-MS	-	1.0 and 2.0	Milk and honey	[16]
HPLC-MS	Liquid/ liquid or solid phase extraction	17.5	Milk	[17]
Diferential Pulse Polarography	0.01 mol L^{-1} NaOH; SMDE (Static Mercury Drop Electrode)	0.51	Urine	[19]
Molecular Fluorescence Spectroscopy	-	3.05	Pharmaceutical formulations and human plasma	[31]

As shown in Figure 8 in a natural water sample, the STP signal can be detected from the addition of 15.3 µg L^{-1} in the sample containing 1.0 mL of natural water, and 30.5 µg L^{-1} with 2.0 mL of the real sample. The interference effect raises with the increase of natural water. Thus, the mixture containing 1.0 mL of the natural sample was chosen for later studies.

The Intermediate precision of the method was calculated in recovery assays performed on five consecutive days, in samples fortified with 35.0 µg L^{-1} of STP. The mean recovery values were 35.77 µg L^{-1}, corresponding to 102.0%, with RSD 8.11%. For repeatability, the recovered mean was 37.6 µg L^{-1}, and the recovery obtained was 106.5%, and RSD 8.51%. These values confirm the accuracy of the method for the determination of STP, deviations of up to 20% are acceptable for the determination of analytes at trace levels [32].

The accuracy of the method was evaluated by STP recovery in pure electrolyte by addition of standard in a solution fortified with 15.0 and 35.03 µg L^{-1} of STP. A natural sample was fortified with 51.01 µg L^{-1} STP and the concentrations recovered are shown in Table 2.

Table 2. Recovery tests of STP in ultrapure water and real sample (n = 3).

Sample	*C_{STP} Added (µg L^{-1})	C_{STP} Recovered (µg L^{-1})	Recovery (%)
Ultrapure water	15.0	12.74 ± 0,60	85.0
Ultrapure water	35.03	36.06 ± 1,50	103.03
Sample (1:10 v/v)	51.01	49.28 ± 2,43	96.63

*C_{STP}: concentration of streptomycin

The calculated STP recovery values show that the method can be considered validated, with recoveries higher than 80%. The AOAC [23] validation guide recommends recoveries between 60-115% (analyte level ≥ 1 µg L^{-1}). However, the interference study showed that the effect is high for aqueous samples, justifying the need for dilution of the samples.

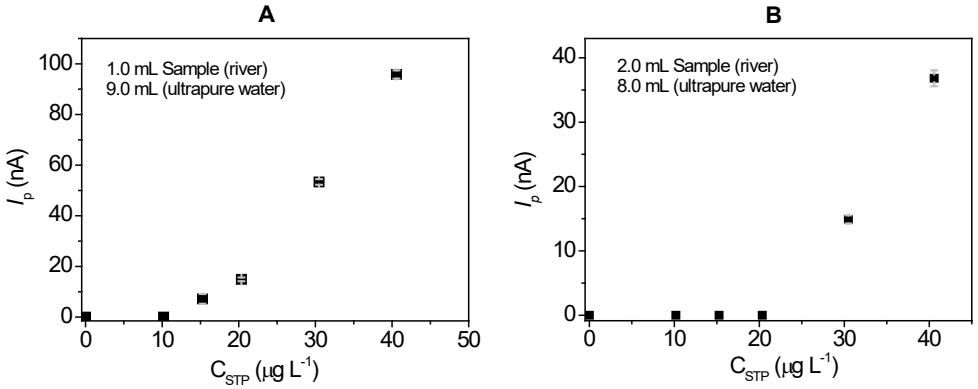

Figure 8. Study of matrix interference with natural water, showing the response I_p as a function of the addition of STP from 10.2 to 40.6 µg L^{-1}. **A)** 1.0 mL of the sample; **B**) 2.0 mL of the sample.

Then, the optimized and validated SW-AdsRCV method was applied to samples of natural water and sewage. Samples were collected from six points along the Paraná River Basin III (Paraná River) in three samplings. In these measurements, it was added 1.0 mL aliquot of filtered sample and 9.0 mL of ultrapure water. In the potential range evaluated (-1.4 to -1.8 V), there was no characteristic peak of STP, indicating that it has not been detected, in any samplings. In these measurements, an aliquot of 1.0 mL of filtered sample and 9.0 mL of ultrapure water was added, with the supporting electrolyte prepared in this medium. The results were interesting from the environmental point of view because it is a drug considered toxic, and because of the fact that some antibiotics are able to cause resistance in microorganisms, when used without proper control [10,11,33].

4. Conclusions

The parameters of validation from the proposed methods showed that they are validated and present precision and accuracy. In addition, voltammetric methods with analyte pre-concentration steps at the electrode allow detection limits of the order of magnitude of chromatographic methods, considered as standard methods for drug analyses. The results showed that the organic matter causes interference effects on the measurements. This effect was overcome with the dilution of the samples, allowing the quantification of the drug. The methodology was applied in the samples collected from six points of the region surrounding Lake Itaipu. STP was not detected in the samples.

Acknowledgments

Authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES), the Brazilian National Council for Scientific and Technological Development (CNPq), the Center-West State University of Paraná (UNICENTRO), the Araucária Foundation and the Itaipu Technological Park Foundation (FA/PTI) for the infrastructure and financial support.

References and Notes

[1] Guimarães, D. O.; Momesso, L. S.; Pupo, M. T. *Quím. Nova* **2010**, *33*, 667. [Crossref]

[2] Projan, S. J.; Shlaes, D. M. *Clin. Microbiol. Infec.* **2004**, *10*, 18. [PubMed]

[3] Patrick, G. L.; An Introduction to Medicinal Chemistry, Oxford University Press: New York, 2005, chapter 16.

[4] Hardman, J. G.; Goldam, L. E. As bases farmacológicas da terapêutica: fármacos antimicrobianos. 9th ed. Rio de Janeiro: McGraw-Hill, 1996.

[5] BRASIL. *Farmacopeia Brasileira*, volume 2/Agência Nacional de Vigilância Sanitária. Brasília: ANVISA, 2010. 904 p., 2v/il. Available in: http://www.anvisa.gov.br/hotsite/cd_farmacopeia/pdf/volume2.pdf. Available: [Link]

[6] Fedorchuk, V. A.; Puchkovskaya, E. S.; Anisimova, L. S.; Slepchenko, G. B. *J. Anal. Chem.* **2005**, *60*, 518. [Crossref]

[7] ANVISA, Agência Nacional de Vigilância Sanitária; Programa de Análise de Resíduos de Medicamentos Veterinários em Alimentos de Origem Animal. Relatório 2006-2007, Monitoramento de Resíduos em Leite Exposto ao Consumo (5º e 6º anos de atividade). Jun. 2009. [Link]

[8] MEDICINANET. Available from: http://www.medicinanet.com.br/conteudos/medicamentos_injetaveis/3452/estreptomicina.htm. [Link]

[9] Stead, D.A. *J. Chromatogr. B* **2000**, *747*, 69. [Crossref]

[10] Varaldo, P. E. *J. Antimicrob. Chemother* **2002**, *50*, 1. [Crossref]

[11] Silveira, G. P.; Nome, F.; Gesser, J. C.; Sá, M. M.; Terenzi, H. *Quím. Nova* **2006**, *29*, 844. [Crossref]

[12] Boxall, A. B. A.; Kolpin, D. W.; Halling-Sørensen, B.; Tolls, J. *Environ. Sci. Technol* **2003**, *37*, 286A. [Crossref] [PubMed]

[13] Thiele-Bruhn, S. *J. Plant Nutr. Soil Sci*., **2003**, *166*, 145. [Crossref]

[14] Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zuagg, S. D., Barber, L. B.; Buxton, H. T. *Environ. Sci. Technol* **2002**, *36*, 1202 [PubMed] [Link]

[15] Wang X, Lu X, Chen J. *Trends Environ. Anal. Chem.* **2014**, *2*, 25. [Crossref]

[16] Bruijnsvoort, M. V., Ottink, S. J. M., Jonker, K. M. E Boer, E. *J. Chromatogr. A*, **2004**, *1058*, 137. [Crossref]

[17] Pietro, A. C., Desenvolvimento de métodos cromatográficos para o controle de qualidade e para a análise de resíduos em leite bovino de medicamentos veterinários. [Doctoral dissertation.] São Carlos, Brazil: Universidade Federal de São Carlos, 2008. Available: [Link]

[18] Granados, O.; Meza, G. *J. Pharm. Biomed. Anal.* **2007**, *43*, 625. [Crossref]

[19] Wang, J.; Mahmoud, J. S. *Anal. Chim. Acta* **1986**, *186*, 31. [CrossRef]

[20] Sodré, F. F. Especiação do cobre em águas naturais: Influência de fatores associados à urbanização. Tese [Doctoral dissertation] Curitiba, Brazil: Universidade Federal do Paraná, 2005. Available: [Link]

[21] ICH Q2A – Guideline for industry - Text on validation of analytical procedures: Definitions and terminology (March 1995). Available in: https://www.fda.gov/downloads/Drugs/.../Guidances/ucm073381.pdf. Available: [Link]

[22] EURACHEM-GUIDE. The fitness purpose of analytical methods: a laboratory guide to method validation and related topics. Teddington: LGC, 2014. Available: [Link]

[23] AOAC, Association of Oficial Analyitical Chemists; Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals, **2002**, 38 p. Available: [Link]

[24] Agência Nacional de Vigilância Sanitária (ANVISA); Resolução RE nº 899; de 29/05/2003. Available: [Link]

[25] IMENTRO, Instituto Nacional de Metrologia, Qualidade e Tecnologia (Brasil) – DOQ-CGCRE-008 – Orientações sobre validação de Métodos Analíticos. Jul. 2011. 20 p. Available: [Link]

[26] Wang, M.; Liao, L.; Zhang, X.; Li, Z. *Appl. Clay Sci.* **2012**, *67*, 164. [Crossref]

[27] Grosser-Jr., D. K. Cyclic voltametry: simulation and analysis of reaction mechanisms. New York: [s.n.], 1993.

[28] De-Souza, D.; Codognoto, L.; Malagutti, A. R.; Toledo, R. A.; Pedrosa, R. T. S.; Mazo, L. H.; Avaca, L. A.; Machado, S. A. S. *Quím. Nova* **2004***, 27*, 790. [Crossref]

[29] Aleixo, M. L. Voltametria: Conceitos e Técnicas. Universidade Estadual de Campinas, Instituto de Química São Paulo - SP, 2003. Available in: <http://www.chemkeys.com>. Available: [Link]

[30] Barros-Neto, B.; Scarminio, I. E.; BRUNS, R. E. *Como fazer experimentos: aplicações na ciência e na indústria*. 4th. Porto Alegre: Bookman, 2010.

[31] Belal, F., El-Ashry, S. M., El-Kerdawy, M. M, El-Wasseef, D. R. *J. Pharm. Biomed. Anal.* **2001**, *26*, 435. [Crossref]

[32] Ribani, M.; Bottoli, C. B. G.; Collins C. H.; Jardim, I. C. S. F.; Melo, L. F. C.; *Quím. Nova* **2004**, *27*, 771. [Crossref]

[33] Regitano, J. B.; Leal, R. M. P. *R. Bras. Ci. Solo* **2010**, *34*, 601. [Crossref]