Article

Voltammetric Determination of Ethionamide in Pharmaceutical Formulations and Human Urine using a Boron-Doped Diamond Electrode

Bruno R. L. Ferraz,* Fernando R. F. Leite, Bruna L. Batista and Andréa R. Malagutti

Departamento de Farmácia, Universidade Federal dos Vales do Jequitinhonha e Mucuri,
39100-000 Diamantina-MG, Brazil

This work reports a simple and low cost voltammetric approach comprising a boron-doped diamond electrode (BDDE) to determine the antibiotic ethionamide (ETO). Cyclic voltammetry studies revealed that ETO exhibits an irreversible reduction peak at −0.95 V and an irreversible oxidation peak at +1.4 V onto BDDE (*vs.* saturated Ag/AgCl reference electrode) in Britton-Robinson buffer solution (pH 5.0, 0.1 mol L^{-1}). Different voltammetric scan rates (from 10 to 150 mV s^{-1}) suggested that the reduction of ETO on the BDDE surface is a diffusion-controlled process. Square wave voltammetry (SWV) optimized conditions showed a linear response to ETO from 1.00 to 80.0 μmol L^{-1} (R^2 = 0.998) with a limit of detection of 0.294 μmol L^{-1} and limit of quantification of 0.980 μmol L^{-1}. The developed square wave voltammetric method was successfully used in the determination of ETO in human urine and pharmaceutical formulation. The ETO quantification results in pharmaceutical tablets obtained by SWV-BDDE were comparable to those found by official analytical protocols.

Keywords: ethionamide, square wave voltammetry, boron-doped diamond electrode, antitubercular drugs

Introduction

Ethionamide (ETO, 2-ethylpyridine-4-carbothiamide; Figure 1) is an oral bacteriostatic antibiotic used for treatment of tuberculosis (TB).[1] It has been an important second line drug in the therapy of drug-resistance tuberculosis (MDR-TB) caused by *Mycobacterium tuberculosis*, which has become resistant to the first line anti-tuberculosis drugs, such as isoniazid and rifampicin.[2,3] ETO is almost fully metabolized by the liver, being only approximately 5% of the antibiotic excreted in an unaltered form in the urine.[4] However, the minimum dose of ETO to inhibit the growth of *M. tuberculosis* is sufficiently high to cause grave side effects, including gastrointestinal disorders, hepatotoxicity, neurotoxicity, cardiovascular effects, endocrine effects and skin reactions.[4] The duration of the treatment of MDR-TB is usually from two to four years, and requires the use of second-line drugs that are less effective and often poorly tolerated, especially by patients with a weakened immune system, leading to high recurrence rates and mortality.[3]
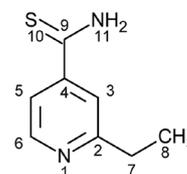

Figure 1. Chemical structure of ethionamide (ETO).

In this context, the development of a reproducible, sensitive and reliable analytical method to determine ETO in biological fluids and pharmaceutical formulations has been crucial to monitor the efficacy of the MDR-TB treatment and also to ascertain whether a drug has the proper content of active substance. To date, some analytical methodologies, such as spectrophotometry,[5,6] fluorimetry[7,8] and high-performance liquid chromatography (HPLC)[9-13] have been applied to quantify ETO in pharmaceutical formulations and body fluids or tissues after its intake.

Electrochemical techniques have also been applied for the determination of anti-tuberculosis drugs by using a variety of chemically modified electrodes.[14-16] Such techniques display good advantages for drug detection, for example high sensitivity, accuracy, precision, simplicity, low cost and elimination of laborious sample preparation steps. Nevertheless, only few works

*e-mail: brunoferraz96@hotmail.com

have reported the use of electrochemical methods for ETO detection.[17,18] Iwamoto and Osteryoung[17] used a rotating silver disc electrode to determine five thioamides (thiocetamide, thiourea, thionicotinamide, thiosemicarbazide and ethionamide) by cathodic stripping voltammetry. The ETO showed stripping reduction peak at ca. −0.77 V *vs.* saturated calomel electrode (SCE) in borate buffer solutions (pH 9.2, 0.1 mol L^{-1}). The authors demonstrated that the thioamides exhibit sharp stripping peaks in acetate buffer solution (pH 4.6, 0.1 mol L^{-1}), but the sensitivity of the disc electrode to these analytes decreased. They also showed that in alkaline solutions, thiomides are unstable and tend to suffer hydrolysis. A linear range for ETO from 0.09 to 60 μmol L^{-1} was obtained. In another study, Jain *et al.*[18] reported the use of a voltammetric sensor based on zirconia nanoparticles deposited onto glassy carbon electrodes for ETO quantification. The voltammetric behavior of ETO was investigated by cyclic voltammetry (CV) and square wave voltammetry (SWV) techniques, using sodium lauryl sulphate (0.1%) as an anionic surfactant. It was shown that the electrode process is controlled by diffusion. A linear range from 150 to 650 ng mL^{-1} and a limit of detection of 47.6 ng mL^{-1} were obtained in that study.

Although the mentioned works achieved such significant results, the effectiveness of the electrode sensors in detecting ETO in biological fluids and pharmaceutical formulations was not demonstrated. A comprehensive search in literature revealed that the use electrochemical sensors for quantification of ETO in pharmaceutical formulations and bodily fluids has not been reported yet. Thus, the objective of the present work was to investigate the electrochemical behavior of ETO on a boron-doped diamond electrode (BDDE) and to develop an electroanalytical methodology to quantify this antibiotic. The electroanalysis using BDDE is a fast, inexpensive and very sensitive procedure. BDDE is also particularly attractive for a wide range of electrochemical applications owing to its long-term stability, inert surface for absorption of reaction products, good resistance to passivation, high chemical stability, lower residual current and broad electrochemical window in aqueous medium, which allows the quantification of electroactive species without interference of water decomposition.[19-24]

Due to these advantages, BDDE have been successfully used in electroanalytical determinations of pharmaceutical compounds.[25-29] However, to the best of our knowledge, this is the first time that an electrochemical sensor based on BDDE is applied to the quantification of ETO in a pharmaceutical formulation and human urine.

Experimental

Reagents and solutions

Ethionamide was purchased from Sigma-Aldrich® (St. Louis, USA). A stock solution of ETO (1.0 mmol L^{-1}) in 10% ethanol aqueous solution (99.5%; Proquimios, Rio de Janeiro, Brazil) was prepared and stored in a refrigerator at 4-6 °C. The Britton-Robinson buffer solution (BRBS, 0.1 mol L^{-1}) was prepared by mixing equimolar amounts of phosphoric acid (85.0%; Dinamica, Diadema, Brazil), acetic acid (99.8 %; Proquimios, Rio de Janeiro, Brazil), and boric acid (99.5 %; Alphatec, Macaé, Brazil) and then its pH was adjusted with sodium hydroxide solution 1.0 mol L^{-1} (99.0%; Proquimios, Rio de Janeiro, Brazil). Ascorbic acid, uric acid and sodium carbonate were purchased from Isofar (Duque de Caxias, Brazil). Citric acid was purchased from Vetec Química Fina (Duque de Caxias, Brazil). Starch, magnesium stearate and microcrystalline cellulose were obtained from Valdequímica (São Paulo, Brazil). All chemicals were of analytical grade and all solutions were prepared with deionized water.

Apparatus

The voltammetric measurements were carried out on an Autolab PGSTAT 128 N (Metrohm Autolab B.V., Utrecht, The Netherlands) potentiostat/galvanostat controlled by NOVA 1.10.4 electrochemical software. The three-electrode electrochemical cell was set with BDDE as a working electrode, an Ag/AgCl 3 mol L^{-1} KCl electrode as a reference electrode, and a 1.0 cm^2 platinum foil as a counter electrode. The BDDE consisted of a thin film of boron-doped diamond deposited on a polycrystalline silicon wafer (Adamant Technologies SA, La Chaux-de-Fonds, Switzerland). The film thickness was approximately 1.2 μm and the exposed area was 0.35 cm^2. The content of boron in the BDDE was approximately 8000 ppm. Prior to the experiments, the BDDE was first rinsed with deionized water and cathodically treated by applying a potential of +3.0 V for 15 s followed by −3.0 V for 45 s in H$_2$SO$_4$ 0.5 mol L^{-1}.[30]

The pH measurements were done with a metrohm 827 pH lab pHmeter (Metrohm Autolab B.V., Utrecht, The Netherlands) calibrated with standard buffers at room temperature. The methodology used for comparison with the electrochemical method proposed in this study was performed according to the United States Pharmacopeia.[31] Thus, spectrophotometric analysis was carried out on a ultraviolet-visible (UV-Vis) 800 XI spectrophotometer (FEMTO, São Paulo, Brazil). UV detection was performed at a wavelength of 290 nm.

Analytical procedures

The electrochemical behavior of ethionamide on BDDE was first investigated using a CV. A volume of 10.0 mL of 0.1 mol L^{-1} BRBS (pH 5.0) containing 0.1 mmol L^{-1} of ETO was placed in the glass electrochemical cell and the electrochemical behavior of ETO at BDDE was investigated by CV at scan rate of 100 mV s^{-1} over the potential range from +1.6 to −1.4 V. Also, the scan rate was varied from 10 to 150 mV s^{-1} in a potential range of +0.1 to −1.4 V. The influence of the support electrolyte solution on the BDDE response for ETO was also examined by CV. The CV was registered for 0.1 mmol L^{-1} ETO in 10.0 mL of 0.1 mol L^{-1} acetate buffer (pH 5.0) and for 0.1 mmol L^{-1} ETO in 10.0 mL of 0.1 mol L^{-1} BRBS (pH 5.0) at scan rate of 100 mV s^{-1} over the potential range from +0.1 to −1.4 V.

In order to study the effect of pH on the electrochemical behavior of ETO at BDDE, SWV were recorded for 0.1 mmol L^{-1} ETO in 0.1 mol L^{-1} BRBS with pH varying from 2.0 to 8.0 at step potential of 4.0 mV, frequency of 50 s^{-1} and potential pulse amplitude of 50 mV.

The analytical method was developed by SWV. The potential pulse amplitude (a), step potential (ΔE$_s$) and frequency (*f*) were considered as parameters to assess the optimum experimental performance for quantification of ETO using the BDDE. 10.0 mL of 0.1 mol L^{-1} BRBS (pH 5.0) containing 0.1 mmol L^{-1} ETO was placed in the glass electrochemical cell and potential pulse amplitude was varied from 10 to 70 mV (with a frequency and step potential fixed at 50 s^{-1} and 1 mV, respectively). The ΔE$_s$ was varied from 1 to 6 mV (with potential pulse amplitude and frequency fixed at 60 mV and 50 s^{-1}, respectively), and the frequency was varied from 10 to 90 s^{-1} (with potential pulse amplitude and the step potential fixed at 60 mV and 1 mV, respectively). The optimized SWV parameters were: a = 60 mV, ΔE$_s$ = 1 mV and *f* = 50 s^{-1}.

The best experimental condition for ETO analysis using the BDDE was obtained in 0.1 mol L^{-1} BRBS (pH 5.0) at a = 60 mV, ΔE$_s$ = 1 mV and *f* = 50 s^{-1}. The linearity of the method was evaluated by preparing twelve ETO solutions with concentrations varying from 1.0 to 80.0 µmol L^{-1} at three different days. The results were plotted as a calibration curve and the linear correlation coefficient was determined by linear fitting.

The limits of detection (LOD) and limits of quantification (LOQ) were determined using the ratio of 3σ/b and 10σ/b, respectively, where b is the slope of the calibration curve and σ is the standard deviation value from ten voltammograms of the blank determined, according to the IUPAC recommendations.[32]

Before each measurement, oxygen was removed by bubbling nitrogen through the solutions for 3 min. All the electrochemical experiments were carried out at room temperature.

After optimizing the experimental parameters, SWV of ETO reduction were recorded to quantify the antibiotic in pharmaceutical formulation and human urine.

Preparation and electrochemical analysis of pharmaceutical formulation and human urine

The developed voltammetric method was tested for determination of ETO in pharmaceutical formulations (tablets) and human urine. ETO tablets were purchased from a local drugstore. According to the manufacturer's information, each tablet contained 250 mg of ETO. Twenty tablets were powdered and then an amount of the powder equivalent to ca. 250 mg of ETO was accurately weighed, dissolved in 10% ethanol solution using magnetic stirring for 20 min and transferred to a 200 mL volumetric flask. After, it was diluted with 0.1 mol L^{-1} BRBS (pH 5.0) to the final volume of the volumetric flask. This solution was filtrated and an aliquot of 20 µL was transferred to a 25 mL volumetric flask and diluted with 0.1 mol L^{-1} BRBS (pH 5.0) to the final volume of the volumetric flask (this solution it was named test solution). The test solution was placed in an electrochemical cell and ETO concentration was determined by the standard addition method. The results obtained were compared with an official United States Pharmacopeia protocol.[31]

The suitability of the developed voltammetric method for analysis of ETO in biological fluids was also investigated by direct analysis of ETO in human urine. For this purpose, a sample of human urine (25 mL) was collected from a volunteer and stored at temperature of approximately 4 °C. The urine sample was fortified by adding an aliquot of 1250 µL of 1.0 mmol L^{-1} ETO standard stock solution and a final concentration of 50.0 µmol L^{-1} ETO was obtained. An aliquot of 2500 µL of the 50.0 µmol L^{-1} ETO solution was added to a 25 mL volumetric flask and the final volume was completed with 0.1 mol L^{-1} BRBS (pH 5.0). This solution was added to the electrochemical cell and the ETO concentration was determined by the standard addition method.

Results and Discussion

Electrochemical behavior of ETO on BDDE

The electrochemical behavior of ETO in 0.1 mol L^{-1} BRBS (pH 5.0) was investigated by CV at 100 mV s^{-1} over

the potential range of +1.6 to −1.4 V. According to Figure 2, the ETO presents an oxidation peak at 1.4 V and also a reduction peak at −0.95 V. The reduction peak observed is more intense cathodic/anodic current peak (I_{pc}/I_{pa} ca. 4) and more defined than the oxidation peak. Therefore, the reduction peak was chosen for further studies.
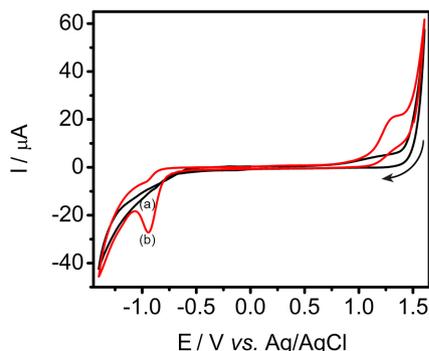

Figure 2. Cyclic voltammograms obtained with a boron-doped diamond electrode (BDDE) in 0.1 mol L^{-1} Britton-Robinson buffer solution (BRBS, pH 5.0) in the absence (a) and presence (b) of ethionamide (ETO, 0.1 mmol L^{-1}). Scan rate: 100 mV s^{-1}.

Effect of pH

The influence of solution pH on the ETO electrochemical reduction was investigated by SWV in 0.1 mol L^{-1} BRBS with pH varying from 2.0 to 8.0. It was observed that an increase in pH up to 5.0 increased significantly the current response. For higher pH values, however, the cathodic peak current decreased (Figure 3), probably due to the instability and tendency of thioamides to suffer hydrolysis in alkaline solutions.[17] Thus, the pH of 5.0 was chosen for the further analysis of ETO. It was also observed that the cathodic peak potential (E_{pc}) exhibited a dependence on the pH solution (Figure 3, insert).

The transference of protons in aqueous medium from or toward organic molecules is usually considered to be a fast
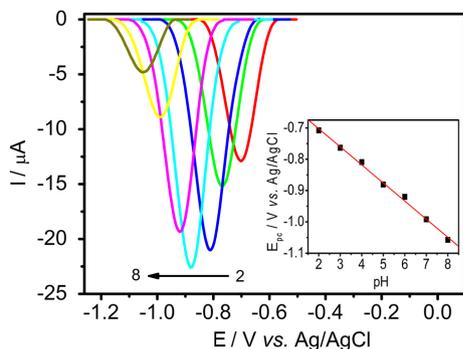

Figure 3. Square wave voltammograms of 0.1 mmol L^{-1} ethionamide (ETO) in 0.1 mol L^{-1} Britton-Robinson buffer solution (BRBS) in different pH values obtained with boron-doped diamond electrode (BDDE). Step potential = 4.0 mV, frequency = 50 s^{-1} and potential pulse amplitude = 50 mV. Insert: cathodic peak potential (E_{pc}) *vs.* pH plot.

process, meaning that protons are in equilibrium in solution near the electrode,[33] thereby justifying this investigation. A linear correlation was obtained from pH 2.0 to 8.0 in the E_{pc} *vs.* pH curve with a slope of 0.058 V/pH at 25 °C, according to the following equation (equation 1).

$$E_{pc} \text{ (V)} = -(0.59 \pm 0.01) - (0.058 \pm 0.001)\text{pH} \qquad (1)$$

The obtained slope is close to a Nernst system, where n (number of protons) = ne (number of electrons).[34] This result is in agreement with a previous report by Kane,[35] in which the reduction process of ETO is described as involving four electrons and four protons, according to the scheme shown in Figure 4.
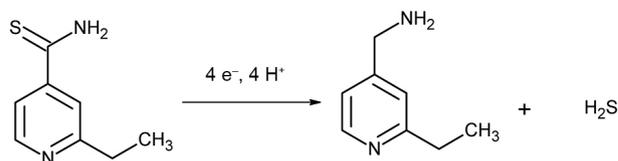

Figure 4. Reaction mechanism of the ethionamide (ETO) reduction as suggested by Kane.[35]

Influence of support electrolyte

In order to use the BDDE for ETO determination, the optimization of the voltammetric method was performed through a systematic study of the dependence of the analytical signal on the CV experimental parameters. In this sense, the influence of the buffer solution on the BDDE response was examined by testing two different buffer solutions (acetate and BRBS), which have maximum buffering capacity at pH 5.0 (Figure 5).
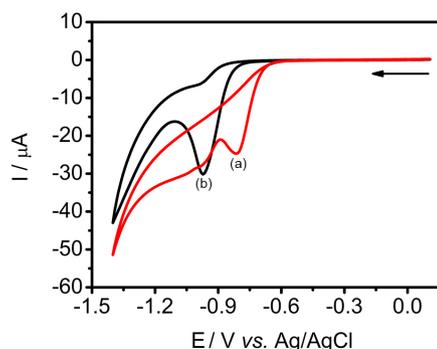

Figure. 5. Cyclic voltammograms of 0.1 mmol L^{-1} ethionamide (ETO) in (a) acetate buffer (pH 5.0, 0.1 mol L^{-1}) and (b) Britton-Robinson buffer solution (BRBS; pH 5.0; 0.1 mol L^{-1}) obtained with a boron-doped diamond electrode (BDDE). Scan rate = 100 mV s^{-1}.

As shown in Figure 5, the BRBS provides the best response with better defined cathodic peak and improved sensitivity in comparison with the acetate buffer.

Effect of scan rate

The effect of the potential scan rate on the BDDE electrochemical response was also investigate and a plot of the I_{pc} *vs.* square root of the potential scan rate ($v^{1/2}$) for 0.1 mmol L^{-1} ETO solution in 0.1 mol L^{-1} BRBS (pH 5.0) resulted in a straight line (Figure 6), suggesting that the electrochemical process is controlled by diffusion. Moreover, a linear correlation was obtained in the log I_{pc} *vs.* log v curve (Figure 6, insert) with a slope value of 0.53. This slope is very close to the theoretical values reported in literature for diffusion-controlled processes.[36,37]
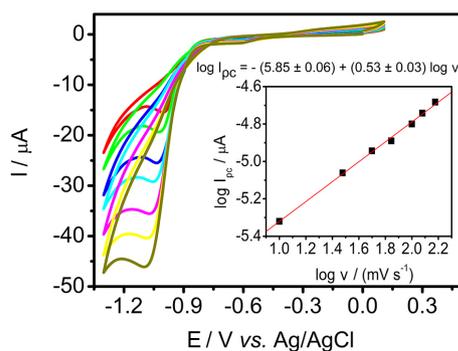

Figure. 6. Cyclic voltammograms of 0.1 mmol L^{-1} ethionamide (ETO) in 0.1 mol L^{-1} Britton-Robinson buffer solution (BRBS, pH 5.0) obtained with a boron-doped diamond electrode (BDDE). Potential scan rates = 10-150 mV s^{-1}. Insert: log cathodic current peak (I_{pc}) *vs.* log potential scan rate (v) plot.

Influence of the potential pulse amplitude, step potential and frequency

The effect of the potential pulse amplitude on the SWV response of the BDDE for 0.1 mmol L^{-1} ETO was verified maintaining the frequency and the step potential at 50 s^{-1} and 1 mV, respectively. The values of current peak displayed an increase with increasing potential pulse amplitude from 10 to 70 mV. On the other hand, when the potential pulse amplitude was higher than 60 mV, the increase of the current peak value was accompanied by a peak distortion effect. Therefore, the best voltammetric sensitivity was obtained with 60 mV, which was selected for further investigations.

The values of current peak were also found to vary with respect to the steps potential (1-6 mV) applied on SWV at constant potential pulse amplitude of 60 mV and frequency of 50 s^{-1}. The step potential of 1 mV was selected as it sets the best voltammetric profile owing higher sensitivity and selectivity for ETO determination.

The effect of the frequency from 10 to 90 s^{-1} on the SWV response of the BDDE for 0.1 mmol L^{-1} ETO was also evaluated, maintaining the potential pulse amplitude at 60 mV and the step potential at 1 mV. The values of

current peak presented an increase when the frequency was increased from 10 to 50 s^{-1}. Thus, the frequency value of 50 s^{-1} was chosen for further studies. A plot of the I_{pc} *vs.* the square root of the frequency was constructed and resulted in a straight line, indicating that the ETO reduction process is controlled by diffusion.[38]

Analytical characterization

In order to obtain an analytical curve for the determination of ETO, square wave voltammograms of ETO reduction were obtained for different concentrations of ETO in BRBS (pH 5.0, 0.1 mol L^{-1}), after optimization of the experimental parameters (Figure 7a).
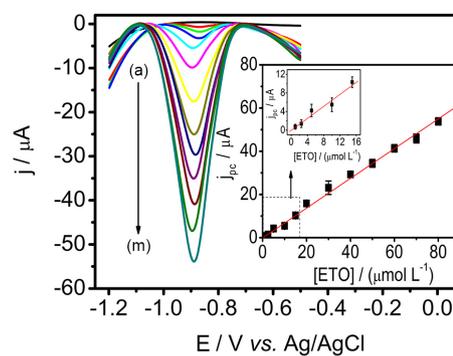

Figure. 7. Square wave voltammograms of ethionamide (ETO) electroreduction obtained with a boron-doped diamond electrode (BDDE) under optimized conditions. ETO concentrations: (a) 0.00, (b) 1.00, (c) 2.50, (d) 5.00, (e) 10.0, (f) 15.0, (g) 20.0, (h) 30.0, (i) 40.0, (j) 50.0, (k) 60.0, (l) 70.0 and (m) 80.0 µmol L^{-1}. Step potential = 1.0 mV, frequency = 50 s^{-1} and potential pulse amplitude = 60 mV. Insert: calibration plot.

The BDDE showed a linear response range from 1.0 to 80.0 µmol L^{-1} (Figure 7b) as expressed by equation 2:

$$j_{pc} \text{ (µA)} = -(0.688 \pm 0.012) \text{ [ETO]} + (0.101 \pm 0.155) \quad (2)$$

The correlation coefficient was 0.998 (n = 12). A lower limit of detection of 0.294 µmol L^{-1} and the limit of quantification of 0.980 µmol L^{-1} were determined using a ratio of 3σ/slope and 10σ/slope, respectively, where σ is the standard deviation value from ten voltammograms of the blank determined according to the IUPAC recommendations.[32] The linear range and limit of detection of the proposed electroanalytical method were similar or better than those reported in earlier reports on electrochemical or other analytical techniques (Table 1). In addition, the reaction occur at the BDDE reached a dynamic equilibrium very rapidly upon addition of ETO, leading to a response time to reach 100% of signal shorter than 1 s. The advantages exhibited by the BDDE for ETO reduction are principally its high sensitivity, low limit of detection,

Table 1. Comparison of different analytical methods proposed for determination of ethionamide (ETO)

Technique	Linear range	Limit of detection / (μmol L^{-1})	Sample	Reference
Spectrophotometry	60.2-601.5	4.27	pharmaceuticals and serum	5
Fluorimetry	6.00-48.1	0.481	urine, plasma	7
HPLC-UV	1.50-60.1	0.300	plasma	8
HPLC-MS	0.154-36.8	0.046	plasma	11
HPLC	0.60-18.0	0.300	serum	12
Eletrochemical	0.09-60.0	data not showed	not applied	17
Electrochemical	0.90-3.91	0.283	not applied	18
This paper (electrochemical)	1.00-80.0	0.294	pharmaceutical and urine	–

HPLC: high-performance liquid chromatography; UV: ultraviolet; MS: mass spectrometry.

fast response time and simplicity, demonstrate that this electrode could be potentially used as a electrochemical detector for determination of ETO in HPLC and capillary electrophoresis systems, for example.

Intra-day and inter-day precision

The intra-day precision of the current peak magnitude was determined by successive measurements (n = 7) at 40.0, 60.0 and 70.0 μmol L^{-1} ETO solution in 0.1 mol L^{-1} BRBS (pH 5.0). When these repeated current peak values were compared with the initial values, relative standard deviations (RSD) of 3.30, 2.90 and 2.30% were obtained, respectively, indicating a good intra-day precision of the proposed voltammetric method. The inter-day precision for the current peak magnitude of the 40.0, 60.0 and 70.0 μmol L^{-1} ETO in 0.1 mol L^{-1} BRBS (pH 5.0) was evaluated over a period of 7 days. A good RSD values were also obtained: 4.80, 4.50 and 3.50%, respectively. Hence, it is possible to conclude that the SWV-BDDE approach for ETO determination provides results with adequate precision.

Interference study

Prior to analyzing the samples (pharmaceutical and human urine), the selectivity of the proposed method for ETO was tested by the assessment of the effect of possible interfering (commonly occurring in pharmaceutical formulations and human urine), such as magnesium stearate, starch, microcrystalline cellulose, uric acid, ascorbic acid and citric acid. Solutions of these compounds were freshly prepared at a ETO solution:interferent compound concentration ratio of 1:100 under the same conditions used for 10.0 μmol L^{-1} ETO in 0.1 mol L^{-1} BRBS (pH 5.0). The analytical response was monitored and compared with the signal obtained for the pure ETO solution (Table 2). The results revealed that the proposed method is highly selective for ETO, once the possible interfering did not affect the cathodic current of the antibiotic under the concentration tested.

Table 2. Effect of possible interfering compounds on the determination of ethionamide (ETO)[a]

Interfering compound[b]	Relative response / %
Magnesium stearate	98.0 ± 2.3
Starch	100.1 ± 3.3
Microcrystalline cellulose	96.9 ± 4.2
Uric acid	97.3 ± 1.3
Ascorbic acid	98.6 ± 3.1
Citric acid	98.5 ± 0.4

Concentration added: [a]10.0 μmol L^{-1}; [b]1.0 mmol L^{-1}.

Determination of ETO in pharmaceutical formulation and human urine, and recovery tests

The BDDE was applied to determine ETO in tablets and human urine. Each experiment was conducted in triplicate and using the standard addition method. For comparison purposes, the concentration of ETO in the tablets was also determined by the official spectrophotometric protocol.[31] The data were statistically compared through the paired *t*-test and F-test,[39] and the results are summarized in Table 3. It was possible to observe that there was no statistical difference between these two methods at a confidence level of 95%, indicating that the BDDE can be successfully used for voltammetric determinations of ETO in pharmaceutical formulations.

Furthermore, the proposed method presents some advantages when compared to the official spectrophotometric protocol: sample preparation of the proposed method is simple because it does not require analyte extraction steps using large amounts of organic solvent, besides showing a higher sensitivity and a large linear range.

Table 3. Ethionamide (ETO) determination results for pharmaceutical samples according to the proposed electroanalytical square wave voltammetry boron-doped diamond electrode (SWV-BDDE) method and the official spectrophotometric protocol[31]

Sample	Label value / (mg *per* tablet)	By proposed method / (mg *per* tablet)[a]	By official method / (mg *per* tablet)[a]
Tablet	250	252.4 ± 9.7	247.8 ± 7.7

[a]n = 3.

The accuracy of the SWV-BDDE method and the possibility of matrix interferences were further checked by performing analytical recovery experiments. Precise amounts of ETO were added to pharmaceutical tablet sample and the recovery percentage values were calculated from the actual and added ETO concentrations (Table 4). It can be clearly observed that there was no influence of the matrix on the response obtained by SWV-BDDE.

Table 4. Results of addition-recovery experiments using boron-doped diamond electrode (BDDE) for determination of ethionamide (ETO) in urine and pharmaceutical samples

Concentration of ETO added / (μmol L^{-1})	Concentration of ETO expected / (μmol L^{-1})	Concentration of ETO found[a] / (μmol L^{-1})	Recovery[a] / %
Human urine sample			
0.0	–	4.97 ± 0.37	–
5.0	9.97	10.36 ± 0.42	103.9 ± 4.4
15.0	19.97	20.99 ± 0.22	105.1 ± 0.3
20.0	24.97	25.30 ± 0.60	101.3 ± 2.4
Pharmaceuticals			
0.0	–	4.52 ± 0.18	–
5.0	9.52	9.93 ± 0.04	104.4 ± 2.4
15.0	19.52	20.21 ± 0.22	101.9 ± 3.2
20.0	24.52	24.71 ± 0.30	100.8 ± 0.6

[a]n = 3.

Conclusions

This work demonstrated that a SWV-BDDE method can be used to quantify ETO in human urine and pharmaceutical formulations. Under optimized experimental conditions, the cathodic current peak was linear for ETO concentrations from 1.00 to 80.0 μmol L^{-1} with a limit of detection of 0.294 μmol L^{-1}, which was similar or better than many reported analytical methods for ETO determination. Additionally, the concentrations of ETO found in pharmaceutical tables

by SWV were equivalent to those attained by UV-Vis spectrophotometry at a confidence level of 95%. Satisfactory recovery results were obtained in the determination of ETO in human urine, indicating that the BDDE was also successfully applied in this kind of sample. The SWV involving BDDE is a simple, sensitive, precise, accurate and environmentally-friendly approach that does not need sophisticated instruments or any separation step, allowing the analysis of ETO without laborious and time-consuming procedures. The analytical frequency of the proposed method is approximately 10 determinations *per* hour.

Acknowledgements

References

1. Brunton, L. L.; Chabner, B. A.; Knollmann, B. C.; *As Bases Farmacológicas da Terapêutica de Goodman e Gilman*, Artmed: Porto Alegre, 2012.

2. World Health Organization (WHO); *Guidelines for the Programmatic Management of Drug-Resistant Tuberculosis*; WHO: Geneva, Swiss, 2008.

3. Lemos, A. C. M.; Matos, E. D.; *Braz. J. Infect. Dis.* **2013**, *17*, 239.

4. Arbex, M. A.; Varella, M. D. C. L.; de Siqueira, H. R.; de Mello, F. A. F.; *J. Bras. Pneumol.* **2010**, *36*, 641.

5. Sikorska-Tomicka, H.; *Mikrochim. Acta* **1985**, *3*, 151.

6. Walash, M. I.; Metwally, M. E. S.; El-Brashy, A. M.; Abdelal, A. A.; *Il Farmaco* **2003**, *58*, 1325.

7. Walash, M. I.; El-Brashy, A. M.; Metwally, M. E. S.; Abdelal, A. A.; *Acta Chim. Slov.* **2004**, *51*, 283.

8. Walash, M. I.; El-Brashy, A. M.; Metwally, M. E. S.; Abdelal, A. A.; *J. Chin. Chem. Soc.* **2004**, *51*, 1059.

9. Hemanth Kumar, A. K.; Sudha, V.; Ramachandran, G.; *Asian J. Biomed. Pharm. Sci.* **2014**, *38*, 1.

10. Gennaro, M. C.; Calvino, R.; Abrigo, C.; *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2001**, *754*, 477.

11. Conte, J. E.; Wang, G.; Lin, E. T.; Zurlinden, E.; *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2001**, *753*, 343.

12. Deshpande, A. Y.; Gurav, S.; Punde, R.; Zambre, V.; Kulkarni, R.; Pandey, S.; Mungantiwar, A.; Mullangi, R.; *Biomed. Chromatogr.* **2011**, *25*, 985.

13. Bhanushali, C. J.; Zidan, A. S.; Rahman, Z.; Habib, M. J.; *AAPS PharmSciTech* **2013**, *14*, 1313.

14. Bergamini, M. F.; Santos, D. P.; Zanoni, M. V. B.; *J. Electroanal. Chem.* **2013**, *690*, 47.

15. Cheemalapati, S.; Devadas, B.; Chen, S. M.; Ali, M. A.; Al-Hemaid, F. M. A.; *Anal. Methods* **2014**, *6*, 6774.

16. Thapliyal, N.; Karpoormath, R. V.; Goyal, R. N.; *Anal. Chim. Acta* **2015**, *853*, 59.

17. Iwamoto, M.; Osteryoung, R. A.; *J. Electroanal. Chem.* **1984**, *169*, 181.

18. Jain, R.; Tiwari, D. C.; Pandey, P.; *J. Mol. Liq*. **2014**, *198*, 364.

19. Pleskov, Y. V.; *Russ. J. Electrochem.* **2002**, *38*, 1275.

20. Compton, R. G.; Foord, J. S.; Marken, F.; *Electroanalysis* **2003**, *15*, 1349.

21. Panizza, M. C.; Cerisola, G.; *Electrochim. Acta* **2005**, *51*, 191.

22. Rao, T. N.; Loo, B. H.; Sarada, B. V.; Terashima, C.; Fujishima, A.; *Anal. Chem.* **2003**, *75*, 1564.

23. Pedrosa, V. A.; Malagutti, A. R.; Mazo, L. H.; Avaca, L. A.; *Anal. Lett.* **2006**, *39*, 2737.

24. Codognoto, L.; Tanimoto, S. T.; Pedrosa, V. A.; Suffredini, H. B.; Machado, S. A. S.; Avaca, L. A.; *Electroanalysis* **2006**, *18*, 253.

25. Boonsong, K.; Chuanuwatanakul, S.; Wangfuengkanagul, N.; Chailapakul, O.; *Sens. Actuators, B* **2005**, *108*, 627.

26. Faria, E. O.; Lopes Junior, A. C. V.; Souto, D. E. P.; Leite, F. R. F.; Damos, F. S.; Luz, R. D. C. S.; dos Santos, A. S.; Franco, D. L.; dos Santos, W. T. P.; *Electroanalysis* **2012**, *24*, 1141.

27. Ardila, J. A.; Sartori, E. R.; Rocha-Filho, R. C.; Fatibello-Filho, O.; *Talanta* **2013**, *103*, 201.

28. Sartori, E. R.; Trench, A. B.; Rocha-Filho, R. C; Fatibello-Filho, O.; *J. Braz. Chem. Soc.* **2013**, *24*, 1504.

29. Santos, S. B.; Valezi, C. F.; Scremin, J.; Salamanca-Neto, C. A. R.; Dall'Antonia, L. H.; Sartori, E. R.; *Quim. Nova* **2014**, *37*, 1579.

30. Suffredini, H. B.; Pedrosa, V. A.; Codognoto, L.; Machado, S. A. S.; Rocha-filho, R. C.; Avaca, L. A.; *Electrochim. Acta* **2004**, *49*, 4021.

31. United States Pharmacopeia Convention; *US Pharmacopeia 38*, *National Formulary 33*, United States Pharmacopeia Convention, Rockville, 2015.

32. Analytical Methods Committee; *Analyst* **1987**, *112*, 199.

33. Laviron, E.; *J. Electroanal. Chem. Interfacial Electrochem*. **1981**, *124*, 1.

34. Papouchado, L.; Sandford, R. W.; Petrie, G.; Adams, R. N.; *J. Electroanal. Chem. Interfacial Electrochem.* **1975**, *65*, 275.

35. Kane, P. O.; *J. Electroanal. Chem.* **1961**, *2*, 152.

36. Gosser, D. K.; *Cyclic Voltammetry: Simulation and Analysis of Reaction Mechanisms*, VCH Publishers: New York, 1994.

37. Bard, A. J.; Faulkner, L. R.; *Electrochemical Methods: Fundamentals and Applications*; 2ⁿᵈ ed.; John Wiley & Sons: New York, 2001.

38. Lovric, M.; Lovric, S. K.; *J. Electroanal. Chem. Interfacial Electrochem.* **1988**, *248*, 239.

39. Triola, M. F.; *Introdução à Estatística*, 11ª ed.; LTC: Rio de Janeiro, 2013.