OXYTETRACYCLINE ELECTROCHEMICAL DETERMINATION AT DISPOSABLE PENCIL GRAPHITE ELECTRODE

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Oxytetracycline (OTC) is a broad-spectrum antibiotic that belongs to the tetracycline class. It is utilized in both human and veterinary medicine to treat a variety of bacterial infections and as a feed additive for animals. Therefore, there is an increased need in the development of simple, rapid and reliable methods for this antibiotic determination in various matrices. This work presents OTC voltammetric analysis at the disposable, cheap pencil graphite electrode (PGE).

The voltammetric response was investigated at different types of working electrodes emphasized that the highest sensitivity was exhibited by PGE using HB type pencil leads. Electrochemical pretreatment of the PGE did not bring any improvement in OTC oxidation signal. The effect of solution pH on the voltammetric behavior of OTC was examined using both cyclic voltammetry (CV) and differential pulse voltammetry (DPV) in Britton-Robinson Buffer (BRB) solutions with pH values ranging from 2.00 to 11.00. The oxidation was irreversible, resulting a diffusion-controlled process that was dependent on pH and involved an equal number of electrons and protons. The highest signal was observed at pH 4.56.

Applying DPV on HB PGE, the oxidation peak of the analyte increased linearly with its concentration in the range 1.00 × 10^{-6} to 3.60 × 10^{-4} mol/L. The method's limit of detection was calculated to be 6.80 × 10^{-7} mol /L OTC. The applicability of the developed DPV method using HB PGE was tested by quantification the antibiotic from veterinary pharmaceutical preparations. Employing the standard addition method, the obtained mean recovery was 103.12%.

Keywords: oxytetracycline, electroanalysis, disposable electrode, voltammetry

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1. Introduction

Oxytetracycline (OTC), (4S,4aR,5S,5aR,6S,12aR)-4-(dimethylamino)-1,5,6,10,11,12a-hexahydroxy-6-methyl-3,12-dioxo-4,4a,5,5a-tetrahydrotetracene-2-carboxamide (Figure 1) is a broad-spectrum antibiotic produced by a fermentation process induced by *Streptomyces Rimosus* bacteria and is widely used especially in farm animals to control intestinal and respiratory infections [1]. It acts by inhibiting protein synthesis from both a wide range of gram-positive and gram-negative bacteria and from microorganisms such as chlamydia, mycoplasma, rickettsia and parasitic protozoa [2].

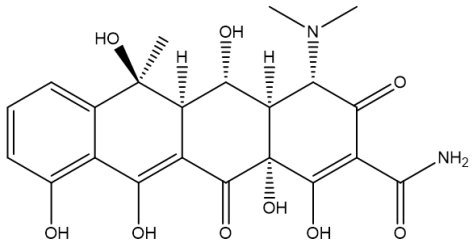


Fig. 1. Structural formula of OTC

In the past decades, numerous analytical methods have been developed for quantitative determination of tetracyclines (TCs.) Many techniques of tetracycline detection such as chromatography (liquid chromatography-mass spectrometry (LC-MS)[3][4], high-performance liquid chromatography (HPLC) [5]–[7], thin-layer chromatography (TLC) [8]–[11]), electrophoretic techniques (capillary electrophoresis (CE)[12][13], capillary electrophoresis-mass spectrometry (CE-MS) [14][15]), enzyme-linked immunosorbent assay (ELISA) [16]–[19], as well as numerous electrochemical techniques[20]–[23] including modified electrodes[24]–[27]. The advantage of electrochemical techniques is given by high sensitivity, simple instrumentation, facile sample preparation procedures and rapid response and analysis time. Having several functional groups (phenol, carbonyl, amide and amine) OTC can be oxidized or reduced electrochemically and therefore it can be determined voltametrically [28]. The aim of the study was to investigate the voltammetric behavior of OTC on PGE to develop a method for quantifying the drug in pharmaceutical samples.

2. Materials and methods

2.1. Reagents and solutions

A stock solution of OTC = 1.0×10^{-2} mol/L was prepared by dissolving 0.0496 g in double-distilled water. Working solutions, having a concentration of 5.0×10^{-5} mol/L (if not indicated otherwise) were obtained by dilution with a proper

supporting electrolyte. For the electroactivation of the working electrode 0.1 mol/L H_2SO_4, 1.0 mol/L KCl and 0.1 mol/L KOH were tested.

The influence of pH on the voltametric behavior of OTC has been studied in the pH range 2.00-11.00, using as supporting electrolyte Britton-Robinson buffer (BRB).

2.2. Instrumentation

Electrochemical recordings were carried out using a standard voltammetric cell, which included a platinum wire as the auxiliary electrode, an Ag/AgCl (3.00 mol/L KCl) electrode as the reference, and a PGE as the working electrode. This setup was connected to a potentiostat/galvanostat (Autolab PGSTAT 12, Metrohm, The Netherlands), which was controlled by a PC running GPES 4.9 software. This software managed the applied potential to the working electrode, recorded voltammograms, and facilitated data acquisition and interpretation. The PGE, with a surface area of 15.86 mm² (0.50 mm diameter and 1.00 cm length exposed to the solution), was made from HB type graphite pencil leads, unless stated otherwise, and prepared as described subsequently. (section 2.3 Procedures).

2.3. Procedures

In this study, various working electrodes (pencil leads) with different hardness levels (HB, 2B, B, H, 2H, all from Rotring) were tested, along with a Pt electrode and a glassy carbon electrode (GCE) with a diameter of 3 mm and an active surface area of 7.1 mm². The PGE was prepared by cutting a 6.0 cm long pencil lead in half and inserting it into a Rotring Tikky mechanical pencil, leaving 1.5 cm of the lead exposed. To ensure a consistent active surface area during measurements, 1.0 cm of the electrode was immersed in the solution to be analyzed [29].

OTC powder (Romvac) containing 50% OTC (with the remainder being lactose) was used as the real sample. A stock solution with a theoretical concentration of 1.0×10^{-2} mol/L OTC in double-distilled water was prepared from this powder. This stock solution was then diluted to obtain an intermediate stock solution with a theoretical concentration of 1.0×10^{-4} mol/L OTC. The working solution used for voltammetric analysis had a theoretical concentration of 5.0×10^{-5} mol/L OTC in BRB at pH 4.56.

3. Results

3.1. The Influence of the Working Electrode Surface

PGE of different hardness (2B, B, HB, H, 2H), with the active surface of 15,7 mm^2, the Pt electrode and the GCE with the areas of the working surfaces of 7,1 mm^2 were tested. OTC showed two oxidation peaks: at about 0.850 V and 1.000 V, respectively. The highest anodic responses were obtained using HB type PGE, while on the Pt electrode, OTC showed a very wide signal located at much less

positive potentials compared to the peaks recorded on PGE. For the GCE a very small oxidation peak could be observed at approximately 0.900 V (Fig. 2).

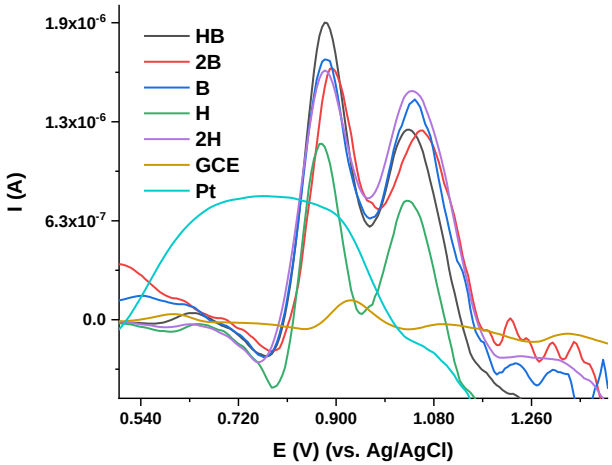


Fig. 2. Differential voltammograms recorded on different electrode materials for a 5.0×10^{-5} mol/L OTC in acetate buffer solution (ABS) pH = 4.00.

Considering that the intensity of a voltammetric signal depends on both the area of the working electrode's electroactive surface and the concentration of the analyte, it is necessary to characterize the electrochemical efficiency of the material by using a parameter, called sensitivity (S), defined as the ratio between the intensity of the peak current and the area and concentration unit, which is not affected by the above-mentioned factors.

Table 1

Sensitivities, peak potentials (E_p) and currents (I_p) of the OTC main DPV oxidation signal recorded in ABS pH = 4.00 at different working electrodes

Electrode	E_p (V)	I_p (A)	S (A×L/mol×cm^2)
2B	0.874	1.27×10^{-6}	0.162
B	0.869	1.31×10^{-6}	0.166
HB	**0.879**	**1.67×10^{-6}**	**0.213**
H	0.874	1.43×10^{-6}	0.182
2H	0.888	1.34×10^{-6}	0.171
GCE	0.927	1.84×10^{-7}	0.052
Pt	0.766	9.32×10^{-7}	0.262

From Table 1. one can observe that the highest sensitivity for OTC voltammetric determination was reached using HB graphite leads as electrode material. Therefore, HB PGE was used in further experiments.

3.2. The influence the solution pH on voltametric behavior of OTC

The OTC behavior in different pH solutions was tested by cyclic voltammetric using Britton-Robinson buffer solutions with pH values between 2.00 - 11.00 (Fig. 3 (a)). Cyclic voltammograms showed an oxidation peak (at pH = 6.00 and pH = 9.00 even two ill-defined anodic waves at about 0.800 V and 1.100 V, respectively, could be observed) and no reduction signal indicating that OTC oxidation on HB PGE was an irreversible process across the entire range of pH and regardless of the number of potential scans applied.

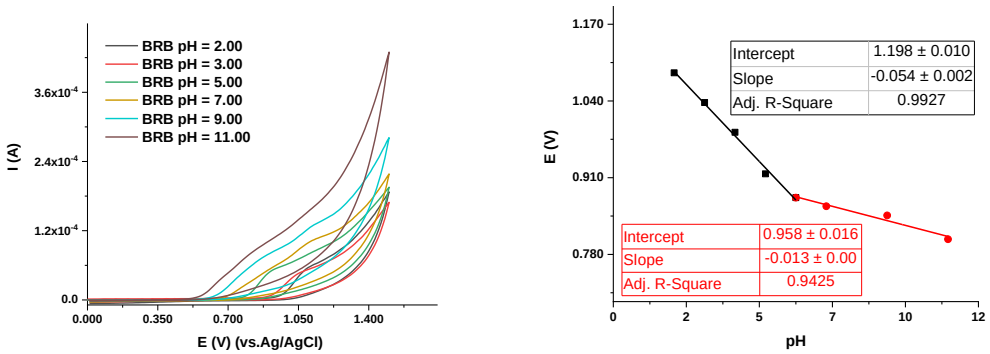
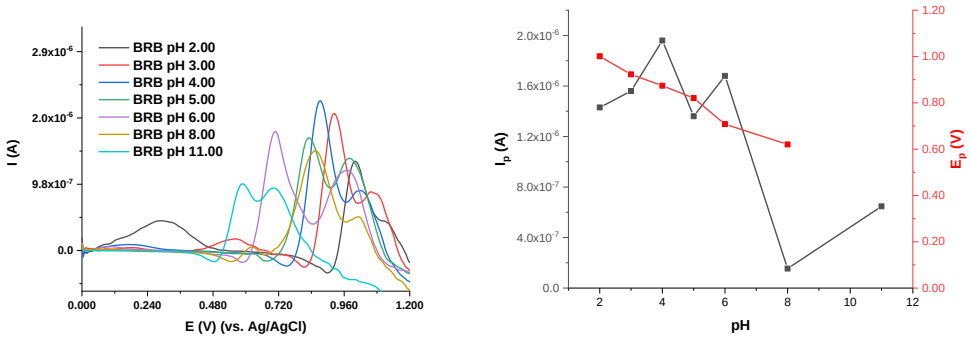


Fig. 3. (a) Cyclic voltammograms (selection) recorded at HB PGE for solutions with concentration 8.0×10^{-4} mol/L OTC in BRB with different pH; (b) variation of OTC oxidation peak potential (obtained by CV on PGE HB) with the solution pH.

As it can be observed from the voltammograms in Fig. 3 (a) the oxidation peak potential ($E_{p,CV}$) of OTC shifted to less positive values as the pH of the supporting electrolyte increased. This suggested that the electrochemical oxidation of OTC on HB PGE also involved protons.

The regression equation describing the $E_{p,CV}$ = f (pH) dependence for the first scan, for pH values lower than 6, was $E_{p,CV} = -0.0544 \times pH + 1.1984$ (R^2 = 0.9927) (Fig. 3 (b)). The value of its slope was close to the theoretical one (-0.059 n/z V/pH where n and z are the number of protons and electrons, respectively, involved in the electrode process) given by the Nernst relation. It resulted that the ratio between n and z participating in the OTC oxidation process was 1. Similar results were obtained also for the second and third cyclic voltammetric scan. Due to the fact that DPV is a more sensitive technique, which will be employed for the quantitative analysis, it was also applied to investigate the effect of pH on the voltammetric response of OTC on HB PGE using BRB solutions with different pHs. The obtained DP voltammograms indicated that both the OTC oxidation peak current and potential were pH dependent (Fig. 4 (a) and (b)).



(a) (b)

Fig. 4. (a) Differential pulse voltammograms recorded on HB PGE for solutions of 5.0×10^{-5} mol/L OTC in BRB with different pH values and (b) variation of OTC oxidation peak potential and current with pH.

As can be observed from the voltammograms in Fig. 4 (a) the oxidation peak potential ($E_{p,DPV}$) of OTC shifted to less positive values as the pH of the supporting electrolyte increased. The regression equation describing the $E_{p,DPV}$ = f (pH) dependence was $E_{p,DPV} = -0.0645 \times pH + 1.1255$ ($R^2 = 0.9849$) (Fig. 4 (b)). From $E_{p,DPV}$ = f (pH) resulted a z/n ratio approximately equal to 1. As expected, this result was consistent with that achieved by CV. On the other hand, the highest OTC DPV oxidation signal was recorded in BRB solution pH = 4.00.

3.3. The electrolyte nature impact on the voltametric behavior of oxytetracycline

Besides the supporting electrolyte pH, its nature may also have an effect on the voltametric response of an analyte. Therefore, differential pulse voltammograms were recorded on HB PGE for 5.0×10^{-5} mol/L OTC in supporting electrolyte solutions with the following pHs: pH = 4,00, namely BRB with pH = 3.00, pH = 4.00, pH = 4.56 and pH = 5.00, acetate buffer solution (ABS), pH = 4.00. Comparing the intensities of OTC anodic peaks recorded in the various supporting electrolytes (Fig. 5) one can conclude that the best sensitivity for OTC DPV analysis can be obtained employing BRB pH = 4.56 as voltammetric environment.

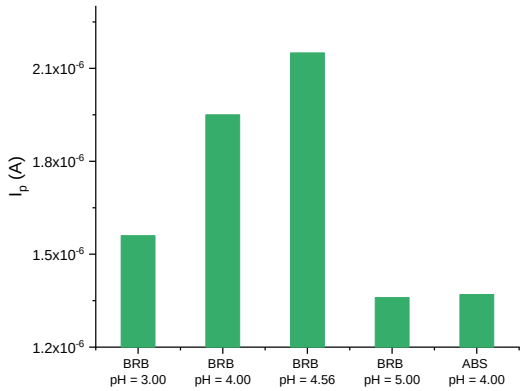


Fig. 5. OTC anodic peak currents recorded by DPV on HB PGE in different supporting electrolytes with close pH values.

3.5. The influence of the scan rate on the OTC voltametric process

The study of the voltametric behavior of OTC on HB PGE was carried out by cyclic voltammetry in BRB solution, pH = 4.56 by recording voltammograms at different scan rate (Fig. 6).

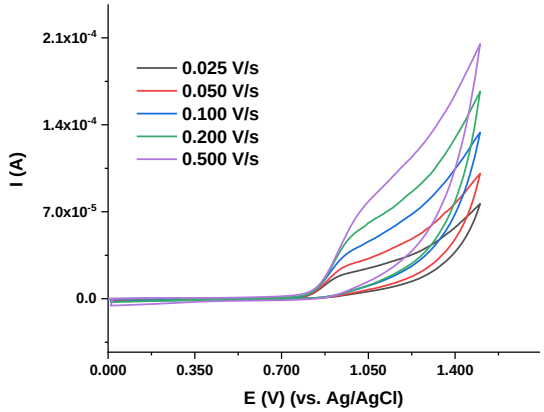


Fig. 6. Cyclic voltammograms recorded at different scan rates on HB PGE for 1.0×10^{-3} mol/L OTC in BRB solution pH = 4.56.

CV recorded on HB PGE in BRB solution pH = 4.56 at different scan rates have shown that OTC exhibited an anodic peak located around 0.900 V and no reduction peak, indicating that OTC was irreversibly oxidized on PGE. It was also observed that with the increase of the scan rate, the peak slightly moved to more positive potentials, which is characteristic for an irreversible electrode process. In order to establish whether OTC oxidation was limited by the analyte diffusion

towards the electrode or by its adsorption onto the PGE the $I_p = f(v)$, $I_p = f(v^{1/2})$ and $\log I_p = f(\log v)$ dependencies were assessed (Table 2).

Table 2

The different dependencies and their regression equations of the OTC anodic peak currents (I_p, A) obtained by cyclic voltammetry on the potential scan rate (v, V/s).

Dependence	Regression equation
$I_p = f(v)$	$I_p = = 1.2157\times 10^{-5} \times v + 6.7046$ ($R^2 = 0.9442$)
$I_p = f(v^{1/2})$	$I_p = = 1.1105\times 10^{-5} \times v^{1/2} + 4.7181\times 10^{-6}$ (R^2=0.9918)
$\log I_p = f(\log v)$	$\log I_p = 0.228 \times \log v - 4.843$ ($R^2 = 0.9910$)

It is known that a linear variation of the peak current with the scan rate $I_p = f(v)$ indicates an adsorption-controlled electrode process, while the linear variation of the peak current with the square root of the scan rate $I_p = f(v^{1/2})$ define a diffusion-limited process. Also, the values of the slope of the dependency $I_p = (\log v)$ is an important landmark for discerning the nature of the electrode mechanism. A value achieving 0.500 describe a mass-transfer process, while a value between 0.500 and 1.000 define a diffusion-controlled process. The results obtained were non-linear for $I_p = f(v)$ and linear for $I_p = f(v^{1/2})$ dependencies coupled with and the slope of the $I_p = (\log v)$ dependence of 0.228 indicated that OTC oxidation on HB PGE was a mass transfer-controlled process.

3.6. The influence of the number of potential cycles on the voltametric respomse of OTC on HB PGE

Despite the fact that PGE is a cheap, disposable working electrode, the possibility of using the same pencil lead as electrode material for several voltammetric measurements has been studied by recording five cyclic voltametric cycles on the same HB PGE for a solution of OTC in BRB pH = 4,56 (Fig. 7).

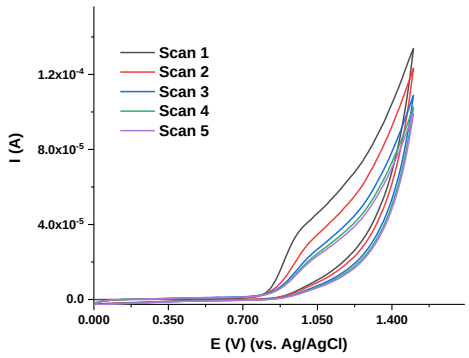
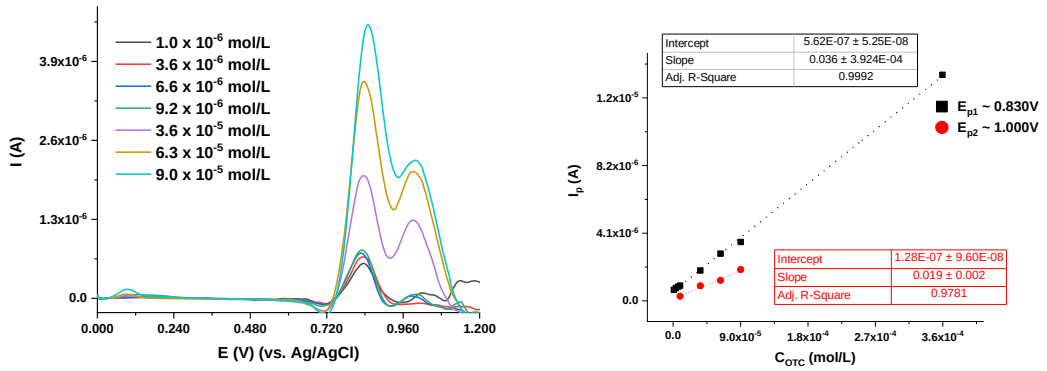


Fig. 7. Repetitive cyclic voltammograms recorded on the HB PGE for 1.0×10^{-3} mol/L OTC in BRB solution pH = 4.56; at 0.100V/s scan rate

Moreover, this kind of investigation can give further insights into the electrode process and the influence of the reaction intermediates or products. Following the cyclic voltammograms, it was observed that with the increase in the number of cycles, the anodic peak current decreased, the biggest decrease being highlighted between the first two cycles, stabilizing after the third potential scan. This leaded to the hypothesis that the surface of the working electrode was passivated, limiting the transfer of electrons and thus the oxidation of the analyte at the electrode surface. Probably, this passivation occurred because of the formation of a non-conductive polymer from the OTC oxidation product(s), which deposited on the HB PGE surface. Therefore, it was necessary to use a new pencil mine for each voltammetric recording to achieve reproducible results.

3.7. The influence of OTC concentration on its voltammetric signal

The influence of OTC concentration on the intensity the anodic peak currents was investigated by recording differential pulse voltammograms on HB PGE for BRB pH = 4.56 solutions with different OTC concentrations comprised between 1.0×10^{-6} – 3.6×10^{-4} mol/L (Fig. 8 (a)).



(a) (b)

Fig. 8. (a) Differential pulse voltammograms recorded on HB PGE for solutions with different OTC concentrations in BRB pH = 4.56; (b) variation of the peak currents with OTC concentration.

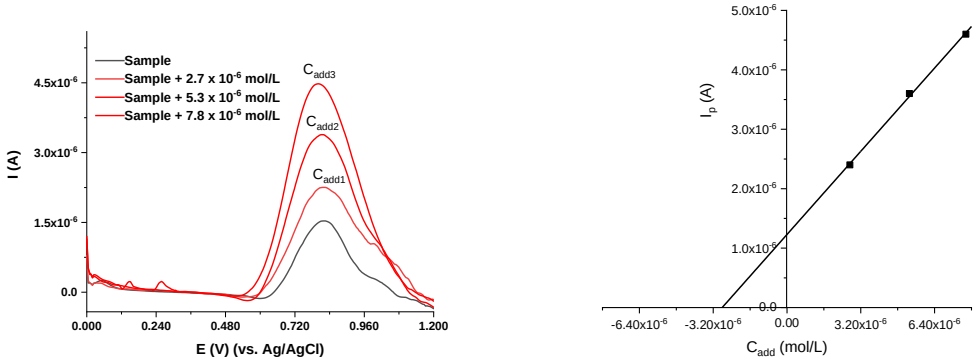
The first anodic peak of OTC showed a linearity range from 1.0×10^{-6} to 3.6×10^{-4} mol/L and for the second oxidation peak the linearity range was obtained from 9.2×10^{-6} to 9.0×10^{-5} mol/L (Fig. 87 (b)).

3.8. The limit of detection and quantification

The limit of detection and quantification of this method were expressed as LoD = 3Sa/B and LoQ =10Sa/B[30], where Sa is the standard deviation of the intersection and B is the slope of the obtained calibration line for the linearity range 1.0×10^{-6} mol/L – 3.6×10^{-4} mol/L. The calculated LoD and LoQ were calculated 6.8×10^{-7} mol/L and 2.3×10^{-6} mol/L, respectively.

3.9. The application of the DPV method for OTC quantification in pharmaceutical samples

Differential pulse voltammograms were recorded for the solution obtained from OTC powder 50% for the sample alone and after the three additions of OTC standard solution (Fig. 9 (a)).



(a) (b)

Fig. 9. (a) Differential pulse voltammograms recorded at HB PGE for a sample of OTC 50% powder dissolved in BRB pH = 4.56, for the sample only and after each addition of 0.03 mL of 1.0 × 10^{-4} mol/L OTC standard solution; (b) the dependence of the peak current on the added OTC concentration.

The voltammogram of the sample solution showed a single oxidation signal whose intensity increased linearly (Fig. 9 (b)) and facilitated its quantification. The OTC mean recovery from the sample was 103.12%.

4. Conclusions

The votammetric behavior of OTC was investigated by optimizing the electrode type (PGE, GCE or Pt electrode) used and afterwards the PGE lead type (2B, B, HB, H, 2H). The supporting electrolyte, along with the pH influence on voltammetric process study emphasized that OTC was oxidized in an irreversible, pH-dependent electrode process. CV and DPV results pointed out that in OTC oxidation the number of given electrons was equal to that of lost protons.

The optimized simple and rapid method was applied with good results for the OTC determination from a veterinary pharmaceutical containing 50% OTC with a mean recovery of 103.12%. The perspective for the next work is to study the possible interferent molecules found in other OTC veterinary preparations and to test the method in more complex matrices.

The study was also a preview for understanding the electrode mechanism of OTC oxidation in order to develop different electrodes based on molecularly imprinted polymers for antibiotic detection.

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