Electrochemical behavior and voltammetric determination of pyrazinamide using a poly-histidine modified electrode

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A R T I C L E   I N F O

Article history:
Received 7 September 2012
Received in revised form 12 November 2012
Accepted 24 November 2012
Available online 20 December 2012

Keywords:
Screen printed electrode
Poly-histidine
Pyrazinamide determination

A B S T R A C T

Pyrazinamide (Pyrazinecarboxamide – PZA) is a drug that is used to treat tuberculosis. In the present work, the voltammetric behavior of PZA was studied using a screen-printed modified electrode (SPCE). The modified electrode was constructed using poly-histidine films, and it showed an electrocatalytic effect, thus promoting a decrease in PZA reduction potential and improving the voltammetric response. Cyclic voltammetry and electrochemical impedance spectroscopy techniques have been employed in order to elucidate the electrode reaction. The results allowed the proposal that in the PZA reduction, a further chemical reaction occurs that corresponds to a second-order process which is subsequent to the electrode reaction. In addition, a sensitive voltammetric method was developed, and it was successfully applied for PZA determination in human urine samples. The best response was found using SPCE modified with poly-histidine prepared by histidine monomer electropolymerization (SPCE/EPH). The electroanalytical performance of the SPCE/EPH was investigated by linear sweep (LSV), differential pulse (DPV), and square wave voltammetry (SWV). A linear relationship between peak current and PZA concentrations was obtained from 9.0 × 10−7 to 1.0 × 10−4 mol L−1 by using DPV. The limit of detection at 5.7 × 10−7 mol L−1 was estimated, and a relative standard deviation of the 5.0 × 10−6 mol L−1 of PZA of 10 measurement was 3.7%.

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1. Introduction

Pyrazinamide (Pyrazinecarboxamide-PZA) is one of the oldest drugs (1936) that is used to treat tuberculosis [1]. However, since 1985, its use has dramatically increased for the chemotherapy of tuberculosis and deserved special attention. Although the mechanism of the action of PZA is poorly understood [2], its use has shortened the time of therapy [3]. Nevertheless, side effects can be reported after use as liver injury, arthralgias, anorexia, nausea and vomiting, dysuria, malaise, fever, and sideroblastic anaemia. Therefore, monitoring the PZA level in human body fluids is vital for finding the lowest relative concentration that provides an effective therapeutic dosage and toxicity [1,3]. Given the importance of controlling the dosages of PZA in biological fluids to minimize unwanted effects, several analytical methodologies have been developed in the last two decades. The main methods are based on UV−vis using multivariate calibration methods [4], capillary electrophoresis [5] and several chromatographic methods [6,7]. A few papers are reported as employing electroanalytical methods for the determination of PZA in pharmaceutical preparations or biological fluids. In general, these reports focus on the development of analytical methods, and details about the electrochemical reactions are usually not provided. In addition, the electroanalytical methods that are mostly applied for PZA determination predominantly employ the mercury electrode. Alonso Lomillo et al. [8] described the use of differential pulse polarography (DPP) combined with the partial least-squares method for the determination of antituberculosis drugs in mixtures. The procedure was applied for the determination of PZA in pharmaceutical preparations and biological fluids. Maher and Youssef [9] described the simultaneous determination of PZA in drug mixtures using square-wave polarography based on non-parametric and chemometric peak convolution procedures. The performance of this method was found to be superior to the least-squares method, and it was applied for PZA determination of pharmaceutical preparations and biological fluids.

In recent years, there is a growing demand for rapid, reliable, and inexpensive sensors for the determination of different analytes in biomedical, environmental, and industrial samples [10–12]. Screen-printed electrodes can be produced with a wide range of geometries, and they can be used for printing whole electrodes array: reference, working, and counter electrodes. In addition, these device can be made both cheap and with a high degree of precision. Another advantage of screen-printed carbon electrodes (SPCEs) in comparison to conventional electrodes is that the problems of carry over and surface blocking are minimized, because they can be
used only once and then have to be discarded [10–12]. So, the development of simple electrochemical sensors based on the use of chemical modified screen-printed electrodes has shown significant progress in the electroanalytical chemistry in the last few years [10–12].

The use of polymeric film-modified electrodes based on polyaninoacids has presented many desirable features as follows: electrocatalysis, pre-concentration properties, low cost, and good stability [13–15]. Poly-histidine is a synthetic polyanimocid-bearing imidazole group ($p_{K_a} = 6.0$) that can be used as voltammetric sensors of several interesting analytes [14–19]. Reactions of electrochemical reduction of myoglobin [16], NAD$^+$ [17] and oxidation of cytochrome [16], ascorbic acid and dopamine [18] could be improved using modified electrodes with the aid of a poly-histidine film. An alternative enzyme-less biosensor for the L-ascorbate determination employing films of the poly-histidine-copper complex was related by Hasebe et al. [19]. Recently, Bergamini et al. [14,15,20] have shown that a chemically modified electrode with poly-histidine exhibits strong dependence with the procedure adopted for film formation. For instance, films formed by a direct deposition of PH have exhibited polyelectrolytic properties, and they can be used for anion pre-concentrations such as chromium VI [15] and tetrachloraurates complex [14]. Films of poly-histidine obtained by electrochemical oxidation of the histidine as monomer [20] exhibited an effective catalytic activity on the reduction of the isoniazid.

The aim of the present work is to explore the potentials of using a screen-printed carbon electrode (SPCE) [21–23] modified with poly-histidine for the determination of PZA using different voltammetric techniques. The purpose is to use the electrocatalytic properties of the PH film to promote a decrease in the PZA reduction potential. A voltammetric sensor was optimized testing different methods of electrode coating, and the best results were the use of poly-histidine for PZA determination in human urine sample without any pretreatment.

2. Experimental

2.1. Apparatus

Voltammetric measurements were carried out using a μAUTO-LAB Type III (EcoChemie) connected to a microcomputer controlled by GPES 4.9 software for data acquisition and experimental control. The electrochemical impedance measurements were carried out using a FRA software. The measurements were performed in a conventional electrochemical cell of 10.0 mL, where the screen-printed carbon electrode (SPCE) was coupled. The arrangement of electrodes is based on an alumina ceramic base that is 45-mm long, 10-mm wide, and 0.8-mm thick, where working, reference, and auxiliary electrodes are exposed on the ceramic surface. All the electrodes are made of carbon-conducting ink. An electrical contact area is placed at the end, which is connected with the active part of each electrode via internally conducting carbon parts, which are covered by a protective dielectric layer. The sensor is connected via a cable to the potentiosstat and used without any pretreatment.

2.2. Screen-printed modified electrode preparation

The screen-printed carbon electrode (Oxley Developments, UK) modified with poly-histidine (PH) was prepared using three different procedures.

2.2.1. Procedure 1 (SPCE/PH)

An aliquot of 10.0 μL of a PH solution (1%, m/v) was placed on the screen-printed carbon electrode surface and then submitted to heating for 5 min at 80 °C. A transformation of the PH structural conformation is expected, improving its adherence to the electrode surface.

2.2.2. Procedure 2 (SPCE/Glu-PH)

The PH and glutaraldehyde were prepared using a mixture of PH (1%, m/v) and glutaraldehyde (0.05%, m/v) solutions. An aliquot of 1.0 μL of glutaraldehyde solution was placed onto the screen-printed electrode surface, where 9.0 μL of PH solution was subsequently added. After mixing, the electrode was placed in a drying oven at 80 °C for 5 min.

2.2.3. Procedure 3 (SPCE/EPH)

The screen-printed carbon electrode was placed in a 0.1 mol L$^{-1}$ phosphate buffer solution (pH 9.0) containing 0.02 mol L$^{-1}$ histidine (monomer), which was previously deaerated with nitrogen for 10 min. The electrode was submitted to six potential cycles between −0.8 and +2.0 V (vs. Ag/AgCl) at a scan rate of 100 mV s$^{-1}$, adopting a methodology previously described in literature [14,20]. The voltammetric curves present a peak at +1.3 V due to oxidation and deposition of the histidine monomers.

2.3. Analysis of PZA in human urine

An aliquot (10.0 mL) of human urine sample was spiked to PZA in order to reach a final concentration of 1.0 × 10$^{-5}$ mol L$^{-1}$. An aliquot of 100 μL of the urine sample was diluted with 10 mL of phosphate buffer 0.1 mol L$^{-1}$ (pH = 1.0) and submitted to electroanalysis after an earlier 10 min of deaeration with nitrogen. The PZA content in these samples was determined by four successive additions of the PZA standard solution.

3. Results

3.1. Electrochemical investigations

Fig. 1 exhibits the cyclic voltammograms recorded for 2.0 × 10$^{-5}$ mol L$^{-1}$ PZA in 0.1 mol L$^{-1}$ phosphate buffer pH 1.0 using the screen-printed electrodes: unmodified (SPCE) (curve b); modified by electropolymerization of histidine (SPCE/EPH) in phosphate buffer pH 7.0 (curve a); and a direct addition of poly-histidine (SPCE/PH) solution (curve c) and a mixture of poly-histidine and glutaraldehyde (SPCE/Glu-PH) (curve d).
On the unmodified electrode (Fig. 1, curve b), the PZA exhibits a anodic and cathodic peak at −0.44 V/−0.33 V (vs. Ag/AgCl) that are attributed to 1,4-hidropirazinium [24]. The $I_{pa}/I_{pc} = 0.86$ and $E_{pa}/E_{pc} = 110$ mV. The magnitude of the anodic and cathodic peak currents remains constant after successive cycling, suggesting that the product of the electrode reaction is not adsorbed on the electrode surface [25].

On SPCE/PH and SPCE/Glu–PH electrodes (Fig. 1, curves c and d), PZA is reduced at a more negative potential −0.51 V/−0.25 V vs. Ag/AgCl, and cathodic peak current decreases by 32% and 50% in comparison to SPCE without modification respectively. The $I_{pa}/I_{pc} = 0.68$ and 0.73 with values of $E_{pa}/E_{pc}/2 = 240$ mV, respectively. These results suggest that the PH film obtained by the direct chemical reaction following a reversible charge-transfer step (EC). The diagnostic criteria just mentioned can be applied to an irreversible electrochemical reaction.

Nevertheless, in cyclic voltammograms obtained on modified electrodes by electropolymerization of the monomer (SPCE/EPH), the reduction occurs at −0.25 V and the peak current is 12% higher. The ratio of $I_{pa}/I_{pc} = 0.78$ and $E_{pa}/E_{pc}/2 = 16$ mV [25] indicates that two electrons are involved in the process. Comparing these results with those obtained with SPCE unmodified, it is clear that the PH films formed by electropolymerization facilitate the reduction of pyrazinamide and also assist in stabilizing the generated product, as there is an increase in $I_{pa}/I_{pc}$ ratio and a decrease in $E_{pc}/E_{pa}/2$ values. The change in potential and current peak observed for PZA reactions in these films could be attributed to the different accessibility of the analyte though the film on the electrode surface. Films formed by electropolymerization could be formed by oriented monomers on the electrode surface, favoring the reactant diffusion within the inner domains to reach the electrode surface [26].

In order to clarify the relevance of film formation, electrochemical impedance spectroscopy (EIS) was carried out as a simple and effective way in order to measure the charge-transfer resistance ($R_{ct}$) of the electrochemical reactions [25]. The EIS measurements were performed in a phosphate buffer solution at a pH 1.0 content $1.0 \times 10^{-4} \text{mol L}^{-1}$ of PZA using the SPCE modified with PH films and SPCE unmodification (Fig. 2, curve B) and modified by SPCE/PH (Fig. 2, curve D), SPCE/Glu–PH (Fig. 2, curve C), and SPCE/EPH (Fig. 2, curve A). All the curves exhibit a semicircular and a linear portion. The first semicircle corresponds to the charge-transfer process through the film at a high frequency range, whereas the second one is due to the diffusion process in the low frequency range. The diameter of the semicircle represents the magnitude of $R_{ct}$ at the electrode surface. The measured EIS data were fitted with an equivalent circuit, as shown in Fig. 2 (detail). This equivalent circuit (Randles circuit) consists of the ohmic resistance ($R_{in}$) of the electrolyte solution, the double layer capacitance ($C_{dl}$), electron-transfer resistance ($R_{ct}$), and the Warburg impedance ($Z_w$) resulting from the diffusion of ions from the bulk of the electrolyte to the interface. This equivalent circuit was used to fit the impedance spectra and extract the values of $C_{dl}$ and $R_{ct}$. The fitted values of $R_{ct}$ for the SPCE, SPCE/PH, SPCE/Glu–PH, and SPCE/EPH were 20.14 kΩ, 24.89 kΩ, 26.31 kΩ, and 9.04 kΩ, respectively. The $R_{ct}$ for the SPCE, SPCE/PH, and SPCE/Glu–PH is much higher than the $R_{ct}$ values observed for SPCE/EPH. This low $R_{ct}$ value for the SPCE/EPH implies that the charge-transfer process is relatively fast compared with the other screen-printed electrodes. Thus, it can be concluded that the SPCE/EPH presented an improvement in the effective electron-transfer rate [20,27].

The effect of potential scan rate on the voltammetric response at a concentration of $2.0 \times 10^{-3} \text{mol L}^{-1}$ PZA reduction in the SCPE/EPH was investigated between 10 and 200 mV s$^{-1}$. The cathodic peak current varied linearly with the square root of the scan rate ($I_{pc} = 0.15 + 2.4 \times 10^{-1} v^{1/2}$ (mV s$^{-1}$)$^{1/2}$), suggesting that PZA reduction follows a diffusion-controlled mechanism. The dependence of the $I_{pa}/I_{pc}$ ratio on the scan rate is different from unity, and this ratio increases as the scan rate is increased. The diagnostic criteria just mentioned can be applied to an irreversible chemical reaction following a reversible charge-transfer step (EC). From these results, a scan rate of 50 mV s$^{-1}$ was chosen for further studies.

The effect of pH on cyclic voltammograms of $2.0 \times 10^{-3} \text{mol L}^{-1}$ PZA was compared in Fig. 3 using pH 1.0 that was adjusted to 11.0. Influences of the pH on the peak current showed that a peak current of PZA was maximum at pH 1.0, where a lower reduction potential and a higher current signal ($I_{pc}$) are observed. Although PZA presented a well-defined peak at more acidic conditions, this pH was avoided, as the electrode material was not stable. However, for values of pH > 9 (Fig. 3, detail), there are no significant variations of peak potentials, indicating that the electrode mechanism occurs without the participation of protons. From 2 < pH < 9, the results showed a shift to a more negative potential and a linear relationship (insert of Fig. 3). A slope of $-79.0 \text{mV pH}^{-1}$ suggests that the electron–proton transfer mechanism occurs in this pH

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**Fig. 2.** Complex plane plots of impedance spectra, $E = −0.32 \text{V (vs. Ag/AgCl)}$, obtained for $1.0 \times 10^{-4} \text{mol L}^{-1}$ PZA in phosphate solution (pH 1.0) for SPCE unmodified (curve B) and modified: SPCE/EPH (curve A), SPCE/Glu–PH (curve C) and SPCE/PH (curve D). Equivalent electrical circuit used to fit the impedance data obtained for SPCE modified and without modification was shown in detail.

**Fig. 3.** Effect of pH on the cyclic voltammograms recorded for a $2.0 \times 10^{-3} \text{mol L}^{-1}$ concentration solution of PZA at scan rate of 50 mV s$^{-1}$, pH values: (a) 1.0, (b) 3.0, (c) 5.0, (d) 7.0, (e) 9.0, and (f) 11.0. Plot of variations of $E_{pc}$ vs. pH was shown in detail.
According to the equation \( \Delta E / \Delta pH = (59.1 \text{ mV/n}) \times C^N_3 \) and using the slope obtained, we can suggest that the number of protons \( (N^+_{\text{H}}) \) transfer may be three. These results agree with the electrochemical behavior of pyrazine in the mercury electrode \([24,26]\). Polarographic behavior of pyrazine and several substituted pyrazines in aqueous acid were related by Swartz et al. \([26]\); these authors related that the stability of the product formed is dependent on the characteristics of the substituent. For pyrazine compounds containing electron-withdrawing ligands, a reversible behavior was observed, after two electrons and three protons.

The tendency of an electrochemically generated species to undergo the following chemical reactions is reflected by the \( I_{\text{pa}}/I_{\text{pc}} \) ratio \([25,28]\). Thus, in the absence of all coupled reactions, it equals unity, but it decreases if the reduction product reacts further (there is a decline in the return wave). Furthermore, the cathodic peak potential depends on both the PZA concentration and the sweep rate. We have obtained a \( dE/d\text{log}c \) (Fig. 4B) value of 23.82 mV and a \( dE/d\text{log}v \) value of 20.93 mV from the second plot. Both these values are in agreement with the theoretical value of 19.5 mV for an EC \([29,30]\), a process in which the chemical step follows second-order kinetics.

In order to check the order of the following chemical reaction, we investigated the dependence of the \( I_{\text{pa}}/I_{\text{pc}} \) ratio on the PZA concentration (Fig. 4C). If the chemical reaction followed first-order kinetics, then the \( I_{\text{pa}}/I_{\text{pc}} \) would be constant, regardless of the PZA concentration. However, results obtained show that the \( I_{\text{pa}}/I_{\text{pc}} \) depends on the PZA concentration; this observation is concordant with the studies conducted by Olmstead et al. \([31]\). We believe that the redox reaction of the PZA leads to 1,4-hidropirazinium ion, as shown in Scheme 1. In addition, the product formed can be consumed by an irreversible second-order chemical reaction that is coupled to the electroodic process.

### 3.2. Analytical performance of different voltammetric techniques for the determination of PZA using the SPCE/EPH

The electrochemical behavior of the modified electrode is further evaluated in the direct detection of PZA using linear sweep, differential pulse, and square wave voltammetry. Using linear sweep voltammetry (LSV) under optimization conditions (pH = 1.0 and \( v = 50 \text{ mV s}^{-1} \)), an analytical curve was obtained (Fig. 4A) and a linear relationship \( I_{\text{pc}} \) versus \( [\text{PZA}] \) (Fig. 4A) was obtained in the concentration range of \( 7.5 \times 10^{-6} - 1.5 \times 10^{-4} \text{ mol L}^{-1} \) of PZA. The detection limit \([32]\) of \( 2.0 \times 10^{-6} \text{ mol L}^{-1} \) (three times the standard deviation of the intercept/slope) was obtained.

The voltammetric response of the SPCE/EPH electrode to the PZA concentration was also investigated using differential pulse voltammetry (DPV). At the proposed electrode, the reduction peak at \(-0.3 \text{ V (vs. Ag/AgCl)} \) was monitored and presented higher currents and a better voltammetric profile at a scan rate of \( 10 \text{ mV s}^{-1} \), a pulse amplitude of 75 mV, and a time of pulse duration of 5 ms. After optimizing the operating conditions for the SPCE/EPH, differential pulse voltammetric measurements were carried out in solutions containing different PZA concentrations. The differential pulse voltamogram and the analytical curve are represented in

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**Scheme 1.** Proposed electrochemical reaction of PZA at SPCE/EPH.
A study was carried out to determine the matrix effect of the voltammetry method to the determination of PZA in urine. Adequate voltammetric responses were found using a dilution ratio of 1:100 (sample (without treatment)/electrolyte support) and it was chosen as the best experimental condition for determination of the PZA.

An aliquot of 100 µL of urine samples spiked in order to obtain a 5.0 × 10⁻⁵ mol L⁻¹ of PZA was added directly in an electrochemical cell containing 10 mL of the phosphate buffer, and differential pulse voltammograms were recorded as described in the Experimental section. The results obtained after the standard addition method are presented in Table 1. Recoveries from 96% to 104% of PZA were obtained for urine samples (n = 3 repetitions). This is evidence of the accuracy of the proposed procedure. The statistical calculations for the results suggested good precision for the voltammetric method. According to the t-test, there were no significant differences between the recovery and added values at the 95% confidence level [32] and within an acceptable range of error. This result demonstrated that the SPCE/EPH can be successfully employed for the reliable determination of PZA in real urine samples.

4. Conclusions

In this study, three different procedures for the modification of SPCE with poly-histidine (PH) were characterized by cyclic voltammetry and electrochemical impedance spectroscopy. The modifications of the SPCE by monomeric histidine electropolymerization (SPCE/EPH), evaporation of poly-histidine (SPCE/PH), and evaporation of a mixture of poly-histidine/glutaraldehyde (SPCE/Glu-PU) lead to the formation of films with different electrochemical behavior. The results clearly show that the SPCE/EPH present a polymeric network on its surface, allowing the decrease of the PZA reduction potential without the adsorption of PZA. The SPCE/EPH led to a lower detection limit for PZA than the bare SPCE; thus, this methodology shows good possibilities of constructing a stable electrochemical sensor.

Acknowledgment

The authors thank financial support from FAPESP [process nos. 04/00111-8 (MFB) and 03/06598-3 (DPS)], CNPq and CAPES.

Table 1 Determination of PZA in a urine sample by DPV using a SPCE/EPH. E: relative error = added vs. found using a voltammetric procedure proposed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>PZA Added (mmol L⁻¹)</th>
<th>PZA Found (mmol L⁻¹)</th>
<th>E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.50</td>
<td>0.52</td>
<td>+4.0</td>
</tr>
<tr>
<td>2</td>
<td>0.50</td>
<td>0.51</td>
<td>+2.0</td>
</tr>
<tr>
<td>3</td>
<td>0.50</td>
<td>0.48</td>
<td>−4.0</td>
</tr>
</tbody>
</table>

References