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Electroanalytical Determination of Promethazine Hydrochloride in Pharmaceutical Formulations on Highly Boron-Doped Diamond Electrodes Using Square-Wave Adsorptive Voltammetry

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Abstract
The electrochemical oxidation of promethazine hydrochloride was made on highly boron-doped diamond electrodes. Cyclic voltammetry experiments showed that the oxidation mechanisms involved the formation of an adsorbed product that is more readily oxidized, producing a new peak with lower potential values whose intensity can be increased by applying the accumulation potential for given times. The parameters were optimized and the highest current intensities were obtained by applying +0.78 V for 30 seconds. The square-wave adsorptive voltammetry results obtained in BR buffer showed two well-defined peaks, dependent on the pH and on the voltammetric parameters. The best responses were obtained at pH 4.0, frequency of 50 s^{-1}, step of 2 mV, and amplitude of 50 mV. Under these conditions, linear responses were obtained for concentrations from 5.96×10^{-7} to 4.76×10^{-6} mol L^{-1}, and calculated detection limits of 2.66×10^{-8} mol L^{-1} (8.51 μg L^{-1}) for peak 1 and of 4.61×10^{-8} mol L^{-1} (14.77 μg L^{-1}) for peak 2. The precision and accuracy were evaluated by repeatability and reproducibility experiments, which yielded values of less than 5.00% for both voltammetric peaks. The applicability of this procedure was tested on commercial formulations of promethazine hydrochloride by observing the stability, specificity, recovery and precision of the procedure in complex samples. All results obtained were compared to recommended procedure by British Pharmacopeia. The voltammetric results indicate that the proposed procedure is stable and sensitive, with good reproducibility even when the accumulation steps involve short times. It is therefore very suitable for the development of the electroanalytical procedure, providing adequate sensitivity and a reliable method.

Keywords: Promethazine hydrochloride, Highly boron-doped diamond electrode, Square-wave adsorptive voltammetry, Pharmaceutical formulations

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1. Introduction

Promethazine hydrochloride (*N*,*N*-dimethyl-1-phenothiazin-10-yl-propan-2-amine hydrochloride), which belongs to the phenothiazine group, is a pharmaceutical compound widely used for its antihistaminic, sedative, antipsychotic, analgesic and anticholinergic properties [1]. However, promethazine hydrochloride can cause adverse effects in humans, such as endocrinal, cardiac and reproductive alterations. Therefore, its determination in commercial formulations is extremely important.

In practice, numerous procedures have been employed to determine this compound in pharmaceutical formulations, such as high performance liquid chromatography [2, 3], capillary zone electrophoresis [4], and spectrophotometry allied to flow-injection analysis [5–7]. The sensitivity achieved by all these procedures is highly satisfactory for the analytical quantification of pharmaceutical compounds. However, in some cases, a prior step is required before quantification, involving extraction from mixtures with other compounds or from complex samples, which is economically unfeasible in routine analyses.

In this context, electroanalytical techniques have proved to be excellent alternatives to determine this and other pharmaceutical compounds, since they are simple, cost little, and require relatively short analysis times, without the need for derivatizations or time-consuming extraction steps [8]. Moreover, these techniques are less sensitive than other analytical techniques to the effects of excipient substances in commercial formulations.

In addition to providing high precision in pharmaceutical analyses, electroanalytical techniques yield information about the kinetics and charge transfer mechanisms involved in a given reaction. This information is useful to evaluate the redox properties of these compounds and to supply information about the metabolic events and its redox and pharmaceutical properties in the human organism, since the reaction in humans is very similar to the redox process that occurs when electroanalytical techniques are employed [9].

As for the evaluation of the electrochemical behavior and quantification of promethazine hydrochloride by electroanalytical techniques, several electrodic surfaces such as glassy carbon [10–12], graphite powder [13], gold [5] and electrodes modified with deoxyribonucleic acid [14, 15] have already been used as working electrodes. All the works published in the literature state that this compound is a good electron donor, since it oxidizes easily, producing currents that are proportional to the analytical concentration. However, the major limitations of these electrodic surfaces have to do with the adsorption process of the reagents of the measuring solution or the resulting products of the electrochemical reactions, whose electrochemical responses sometimes display low sensitivity and poor reproducibility.

To solve this problem, boron-doped diamond electrodes have recently received increasing attention for application in the electrochemical determination of pharmaceutical compounds [16–21] due to their inertness in the adsorption of chemical species and their easy surface cleanup compared with other electrodic surfaces. Furthermore, boron-doped diamond electrodes present excellent electrochemical properties that include stable background currents, a wide potential window in aqueous media, long-term stability and high sensitivity for analytical purposes [22–26].

Among the electroanalytical techniques currently available for use with boron-doped diamond electrodes and various electrodic surfaces, square-wave voltammetry [27, 28] has proved to be an extremely sensitive method for the detection of pharmaceutical compounds [29–31]. The analytical sensitivity of square-wave voltammetry (SWV) can be improved by using adsorptive steps, involving an initial accumulation step to preconcentrate the analyte into, or onto, the working electrode, which is then electrochemically oxidized or reduced in the current measurement step [32, 33].

The use of adsorptive steps allied to the square-wave voltammetry technique is well established. These steps provide high sensitivity and have proved useful in the determination of organic and inorganic compounds, since they are relatively simple, fast, and cause insignificant effects on the components of samples [34].

This study therefore investigated the electrochemical behavior of promethazine hydrochloride (PH) and developed an analytical procedure to quantify this compound in commercial formulations, employing highly boron-doped diamond (HBDD) electrodes and square-wave adsorptive voltammetry (SW-AdsV).

2. Experimental

2.1. Reagents and Equipment

All the voltammetric measurements were taken with a potentiostat (Autolab PGSTAT 30, Metrohm-Eco Chemie) controlled by a personal computer, using GPES version 4.9 software (General Purpose Electrochemical System, Metrohm-Eco Chemie). A Beckman Coulter model DU 640 spectrophotometer with 1 cm quartz cells was used for recording the ultraviolet-visible (UV-vis) measurements.

A Micronal B474 pH meter equipped with a 3.0 mol L^{-1} Ag/AgCl/KCl-glass combined electrode was used to adjust the pH values. All the solutions were prepared with water purified by a Milli-Q system (Millipore Corp.).

A conventional cell with a three-electrode system, consisting of an Ag/AgCl/Cl^- 3.0 mol L^{-1} electrode as the reference electrode, platinum wire as the auxiliary electrode, and a HBDD electrode as the working electrode, was used in all experiments.

The HBDD employed in the construction of the working electrode was produced by LAS-INPE, São Paulo, SP, Brazil. The boron-doped diamond film was grown on planar silicon substrate by chemical vapor deposition, under previously described conditions [25], and the boron-doping level used in this work was 1.5×10^{21} atoms cm^{-3}, which corresponds to about 20.000 ppm of the ratio of boron to carbon dissolved in methanol. This level of boron doping was adopted because preliminary work had shown that high density boron atoms can improve the conductivity of diamond films and, hence, the analytical sensitivity of organic compounds [18–35].

The working electrode was built according to a procedure similar to that used previously by Julião and co-workers [36, 37], whereby the silicon substrate with highly boron-doped diamond film was attached to a brass plate with silver paste to make electrical contact and further insulated with Teflon to protect areas other than the planar surface from contact with solutions.

A stock solution of 1.0×10^{-4} mol L^{-1} of USP-grade promethazine hydrochloride (PH) was prepared daily by dissolving an appropriate quantity of it in ultrapure water, which was then stored in a dark flask and kept in a refrigerator to prevent degradation.

0.1 mol L^{-1} of Britton–Robinson (BR) buffer, prepared as described in a previous paper [38], was used as the supporting electrolyte and the pH was adjusted to the desired value by adding appropriate amounts of 2.0 mol L^{-1} NaOH stock solution.

2.2. Working Procedure

In this work, all the electrochemical measurements were taken under ambient conditions. Prior to the experiments with the HBDD electrode, cyclic voltammetry (CV) experiments were conducted at 100 mV s^{-1} in the potential interval of +1.3 to −0.8 V in a cell containing 0.5 mol L^{-1} H_2SO_4 to

provide the stabilized electrochemical profile of the working electrode. After each measurement with PH, the solution was stirred thoroughly for 45 seconds to remove any possible residues adsorbed on the electrodic surface, thus ensuring the reproducibility of the all experiments.

The experimental and voltammetric parameters of maximum peak current and maximum selectivity (half-peak width) were optimized based on a systematic study of the experimental parameters that affect the responses, such as the pH of the medium, accumulation time (t_a) and potential (E_a), the potential pulse frequency (f), amplitude of the pulse (a) and height of the potential step (ΔE_s) or scan increment. All the parameters were duly optimized, since their values strongly affect the sensitivity of voltammetric analyses.

To this end, the appropriate solutions were transferred into the electrochemical cell containing the electrolyte support. The solutions were then stirred during the application of the accumulation potential for a given time. This accumulation potential and time were initially optimized. Following the accumulation steps, anodic scans were made in the interval of 0.1 V to 1.2 V vs. Ag/AgCl/Cl^- 3.0 mol L^{-1}, using the square-wave voltammetry technique.

After the optimization of the voltammetric parameters, analytical curves were obtained in pure electrolyte using the standard addition method. The standard deviation of the mean current measured in the oxidation potential of PH for ten blank voltammograms in pure electrolytes (S_b) and the slope of the straight line of the analytical curves (s) were employed for DL and QL determinations using $DL = 3S_b/s$ and $QL = 10S_b/s$ [39, 40].

The proposed procedure was compared to the ultraviolet-visible spectrophotometry measurements, according to procedure recommended by British Pharmacopeia [1], where the spectrum and the characteristic absorbance of the PH was evaluated in 249 nm. Analytical curves also constructed and the DL and QL values were calculated using the same relations employed in the voltammetric measures, but the S_b values used was determinate by the standard deviation of the y-intercept in the analytical curves.

The recovery experiments were carried out using the voltammetric and spectrophotometry procedures. For this, a known amount of pharmaceutical formulations were adding to the supporting electrolytes, followed by standard additions of the PH stock solutions, and plotting the resulting analytical curves. All the measurements were taken in triplicate.

The recovery efficiencies were calculated by relationships between the PH concentration found value, which refers to the concentration obtained by extrapolating the analytical curves of the corresponding spiked samples, and the PH concentration added value, that corresponds to the nominal concentration of the samples, multiplied by 100.

The precision of the proposed procedure was evaluated based on reproducibility experiments realized with different standard solutions of PH in different day (intraday). The accuracy was evaluated from experiments of the repeatability obtained in ten replicated determinations in the same solution of PH (interday).

The relative standard deviations (RSD) were calculated for reproducibility and repeatability measures, using the relationships between the standard deviation and the mean of the peak current values obtained.

2.3. Analysis of Commercial Formulations

After calculating the DL and QL for the determination of PH in the supporting electrolyte, it was studied the accuracy, reproducibility, precision of the procedure and the interference from excipients used in the commercial PH formulations. This was done by means of recovery experiments with three distinct commercial products purchased locally.

The first commercial formulation was Fenergan tablets, which contain 25 mg of the PH by tablet. The tablets were crushed into a power and a carefully weighed portion of the powder, sufficient to produce a final concentration of 1.0×10^{-4} mol L^{-1} of PH, was transferred into 10 mL volumetric flasks and diluted to volume with pure water. The mixture was sonicated for 10 minutes, after which the solution was filtered and an aliquot was transferred to an electrochemical cell containing the supporting electrolyte for evaluation of the analytical parameters. The commercial formulation was an Aventis Pharma (Brazil) product.

The second commercial formulation was injectable Fenergan, which contain 50 mg of PH by 2 mL ampoule. The solution was prepared taking up an appropriate amount of the contents of ampoule, placing it in a 10 mL volumetric flask and completing the volume with pure water to a final PH concentration of 1.0×10^{-4} mol L^{-1}. This formulation was also from Aventis Pharma (Brazil).

The third commercial formulation was injectable Lisador produced by Farmasa (Brazil), a pharmaceutical formulation containing 25 mg of PH, 750 mg of dipyrone, 25 mg of adiphenine hydrochloride and an undetermined concentration of propylene glycol in each 2 mL ampoule. This compound was utilized to evaluate the influence of the other pharmaceutical compounds on the proposed procedure. A sample of this formulation was prepared in a manner similar to that of the injectable Fenergan.

All the samples were used immediately after their preparation to prevent decomposition by light or heat.

3. Results and Discussion

3.1. Electrochemical Behavior

In the continuous cyclic voltammetry of PH on the HBDD electrode, the first anodic sweep, with a scan potential of -0.4 to 1.1 V, revealed an oxidation peak at around $+0.77$ V (peak a_1) and a cathodic peak at approximately $+0.40$ V (peak c_1) during the reverse scan. In the subsequent scan, the intensity of peak a_1 diminished and a very sharp anodic peak

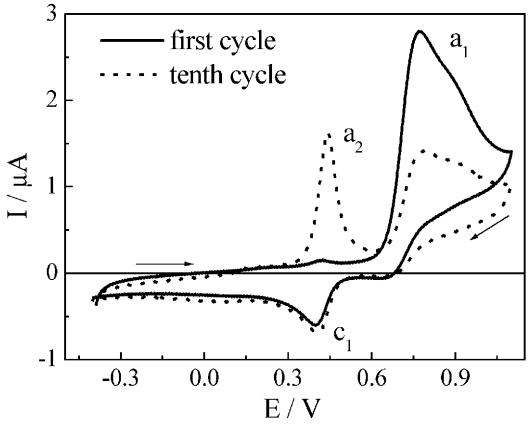

Fig. 1. Cyclic voltammograms for 2.91×10^{-5} mol L^{-1} of PH at HDDB electrode in BR buffer, pH 4.0, with scan potential ranging from -0.38 to $+1.4$ V vs. Ag/AgCl saturated with 3.0 mol. L^{-1}, at a scan rate of 100 mV s^{-1}.

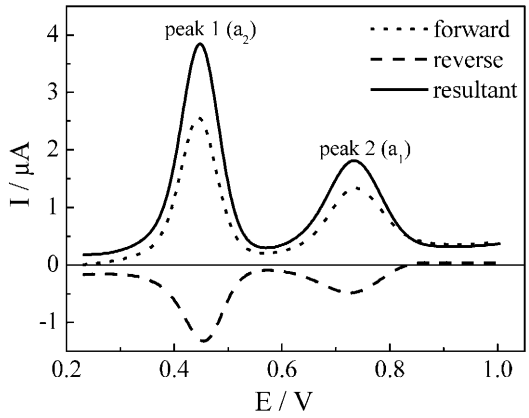

Fig. 2. Square-wave voltammograms for PH oxidation, with forward, reverse and resultant component of current, for 2.91×10^{-5} mol L^{-1} of PH in 0.10 mol L^{-1} BR, pH 4.0, on HDDB with $a = 50$ mV, $\Delta E_s = 2$ mV and $f = 10$ s^{-1}.

(peak a_2) appeared at around $+0.44$ V, while peak c_1 remained practically constant.

These results indicated that PH is oxidized in two steps, with peak a_1 corresponding to molecules generated by oxidation of PH in the first cycle, which are more readily oxidized than the initial molecule, thus producing a new voltammetric peak, a_2, at less anodic potential values. According to previous studies of PH and other compounds of the same chemical group (phenothiazines), the oxidation process occurs by the removal of one electron from the nitrogen atom, leading to the formation of a relatively stable cation radical whose oxidation results in promethazine sulfoxide [10, 11, 41].

Figure 1 illustrates cyclic voltammetric responses obtained for PH oxidation on HBDD electrode in BR buffer, pH 4.0, at a scan rate of 100 mV s^{-1}, indicating the first and tenth cycles of potential scans.

The influence of the scan rates on the PH peak potential and peak current values was evaluated in the range of 20 to 400 mV s^{-1}, considering only the anodic peaks. The results obtained for peaks a_1 ($I_p = -8.97 \times 10^{-7} + 3.96 \times 10^{-7}\ v^{1/2}$) and a_2 ($I_p = 8.52 \times 10^{-7} + 6.45 \times 10^{-8}\ v^{1/2}$) showed that there is a linear correlation between the square root of the scan rates and the peak current values, indicating a diffusion-controlled redox process. This conclusion was confirmed for peak a_1 by correlation between the logarithm of the peak currents and that of the scan rates ($\log I_p = -6.80 + 0.61 \log v$). For peak a_2, there is the similar correlation ($\log I_p = -6.24 + 0.22 \log v$) indicating that occurs a mixed control in the redox process.

SWV experiments showed that the PH presented two voltammetric peaks in the positive sweep direction, the first around 0.43 V (peak 1) and the second at approximately 0.74 V (peak 2) vs. Ag/AgCl/Cl^- 3.0 mol L^{-1}, which is in close agreement with the values obtained from the CV experiments, although the first peak showed low current intensity.

Similar to what was observed in the CV experiments, in the first scan, the current components obtained for PH presented a quasireversible behavior indicated by the presence of forward and reverse current components, with the latter presenting much lower values than those obtained for the forward component. After several experiments without cleaning the electrodic surfaces, the values of the current components increased, with a resulting stabilization of signal intensities. All the current components showed well-defined peaks, presenting the quasi-reversible redox process characterized by reverse current values nearly 50% lower than those of the forward current. The difference between the peak potential in forward and reverse scans is characteristic of the quasi-reversible redox process [27, 30]. Figure 2 shows the component currents obtained for PH on HBDD after the stabilization of the voltammetric responses, which occurred approximately in the tenth cycle of the scan of potential.

3.2. Effects of pH Values

In most analytical determinations of organic and inorganic compounds, the pH of the supporting electrolyte can affect the response. Therefore, this parameter was evaluated in the PH oxidation on HBDD, which showed the pH values that yield higher analytical signals (peak currents). The pH values of the BR buffer were changed from 2.0 to 8.0 and the experiments were carried out with the cyclic voltammetry technique.

Similar to previous studies [10], the PH responses revealed that peaks a_1 and c_1 underwent a shift in the potential to negative values and their intensity decreased as the pH increased; both peaks disappeared at pH above 6.0. The potential values of peak a_2 showed no displacement as a function of variations in the pH, but the peak currents increased up to pH 6.0, after which they declined rapidly. The increase in pH led to the displacement of peak c_2. The Figure 3 shows the relationships between peak current and

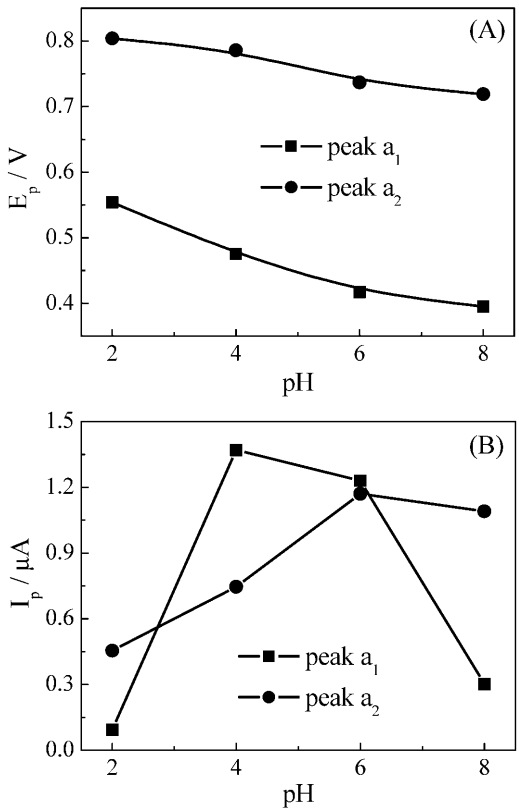

Fig. 3. Relationships between peak potentials (A) and pH, and peak currents (B) and pH, obtained from cyclic voltammograms for 2.91×10^{-5} mol L^{-1} PH in 0.10 mol L^{-1} BR at 100 mV s^{-1}. (■) peak a_1 and (●) peak a_2.

peak potential and the pH values obtained for two anodic peaks of the PH.

To continue the study of the electrochemical behavior and development of the electroanalytical procedure for the determination of PH on HBDD, BR buffer with pH adjusted to 4.0 was selected because of its greater analytical sensitivity for both anodic peaks.

3.3. Influence of Accumulation Time and Potential

As Figure 1 indicates, peak a_1 decreased considerably along the cycle, while the signal of peak a_2 increased. This behavior seems to indicate that the stepwise application of potential to generate the species that oxidize in less negative potential, peak a_2, can improve the analytical response of this peak. Thus, a study was made of the influence of the values of accumulation time and potential of the species on the values of the currents for both peaks. To this end, the accumulation potential was varied from 0.7 to 1.0 V, for periods of time ranging from 15 to 120 seconds, and the responses were evaluated by the SWV technique.

The peak potential values of both peaks hardly varied with the shift in the E_a and t_a. However, in the peak current values, the variation in E_a and t_a revealed that the responses presented different behaviors at two voltammetric peaks.

Thus, the height of peak 1, i.e., peak a_2 in cyclic voltammetry, increased for a given time according to the E_a applied. Beyond that time, the height of the peak declined. For peak 2, i.e., peak a_1 in cyclic voltammetry, the peak height showed a slight decrease up to 60 seconds, after which this height remained practically constant, considering all the E_a evaluated.

The study of t_a and E_a enabled us to define the optimal experimental condition to achieve the best reproducibility in the responses. Thus, the highest peak currents and analytical reproducibility for peak 1 were attained with 0.78 V as E_a for 30 seconds. As for peak 2, the same parameters were applied and, although they decreased the responses slightly, good reproducibility of the responses was nevertheless achieved.

In this work, we were able to use short times in the accumulation steps with good reproducibility, which proved satisfactory for the development of the electroanalytical procedure due to promote adequate sensitivity and a reliable methodology.

3.4. Voltammetric Parameters

The SWV parameters were studied to determine the optimal values providing the best analytical signal. The f, a, and ΔE_s were also evaluated considering both voltammetric peaks for the PH oxidation process on HBDD, using adsorptive voltammetry.

Frequency is a very important voltammetric parameter in this optimization, because variations in frequency modify the apparent reversibility of redox reactions. Peak currents usually show an increase as a function of the increase in the frequencies of pulse potential employed, regardless of the reversibility of the redox process, but the correlation between peak currents and frequency is not strictly linear [42], as Figure 4A shows for both voltammetric peaks of PH on HBDD.

The two voltammetric peaks illustrated in Figure 4A indicated that peak 1 and peak 2 presented different peak current behaviors as a function of the frequency variation. The response obtained for peak 1 was characteristic of the redox process, in which the reactant is weakly adsorbed on the electrodic surface. The peak current increased with the frequency, but this increase was not linear and no maximum was observed. On the other hand, the profile of the curve of peak 2 showed a very well-defined maximum which is characteristic of the quasi-reversible redox process with a strong adsorptive process and very slow electrochemical kinetics [42, 43].

Based on these findings, a low frequency pulse potential of 50 s^{-1} was selected for the voltammetric optimization. Peak 1 was selected for analytical purposes because of its low adsorption, allowing for greater reproducibility in analytical responses.

Considering the responses obtained for peak currents as a function of the variation in pulse amplitude for the oxidation of PH on the HBDD electrode, the results obtained for both

peaks demonstrated that an increase in the values of *a* caused a linear increase in the peak current values, as shown in Figure 4B. As expected from SWV theory [27, 28], the values of I_p show an almost linear variation with the pulse amplitude for values of *a* ranging from 5 to 50 mV, while, in practice, the E_p shows no variation as a function of *a*. Therefore, for the purpose of analysis, a value of 50 mV was chosen for *a*.

The response of peak currents to increasing scan increments was also evaluated. The results obtained for the two peaks indicate that the increase in ΔE_s did not affect the peak potentials. In this case, an increase in ΔE_s resulted in a decrease in I_p for both voltammetric peaks, as depicted in Figure 4C, which may indicate very slow electrochemical kinetics [28, 42]. Therefore, a value of $\Delta E_s = 2$ mV was adopted in subsequent experiments.

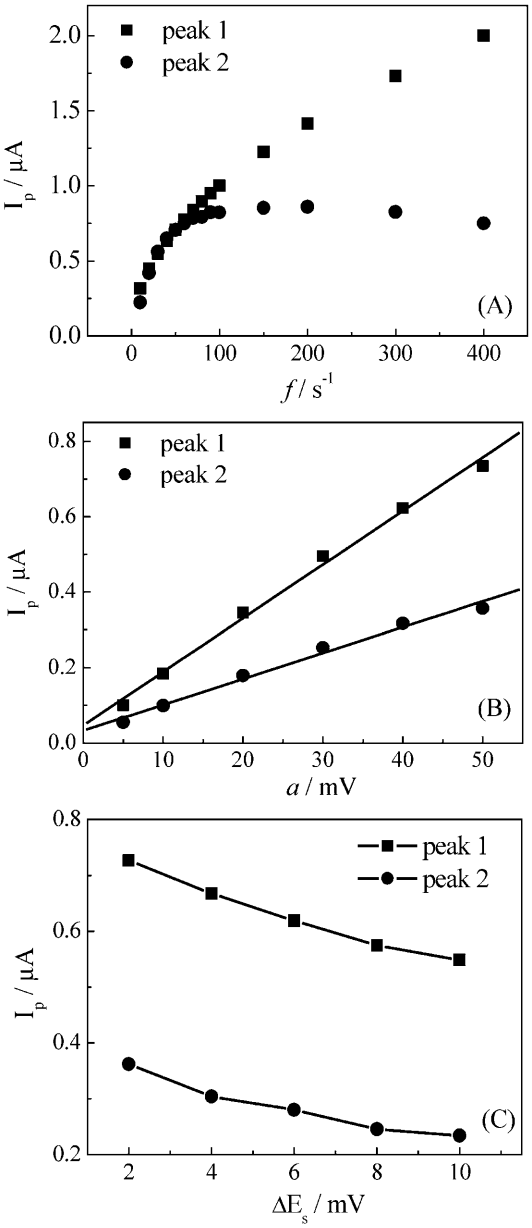

Fig. 4. Correlation between the peak currents and frequency of pulse potential (A), pulse amplitude (B), and scan increment (C) obtained from square-wave voltammograms of 2.91×10^{-5} mol L^{-1} PH in 0.10 mol L^{-1} BR pH 4.0, with $t_a = 30$ s, $E_a = 0.78$ V, on HBDD with variations of each parameter.

3.5. Analytical Curves

All the aforementioned parameters were employed not only to draw the analytical curves for the PH oxidation process on HBDD in a supporting electrolyte medium but also to determine the sensitivity of the proposed procedure and for other applications in commercial PH formulations.

The analytical curves were drawn as described in Sec. 2 for concentrations ranging from 5.96×10^{-7} to 4.76×10^{-6} mol L^{-1}, with aliquots from stock PH solution added consecutively to the electrochemical cell. The SW-AdsV responses were recorded with $t_a = 30$ s, $E_a = 0.78$ V, $f = 50$ s^{-1}, $a = 50$ mV, and $\Delta E_s = 2$ mV.

The analytical curves indicated a linear increase in the responses as a function of the increase in the analytical concentration of PH. Figure 5 shows the voltammograms and the linear correlation between peak currents and added concentrations for both voltammetric peaks.

The *DL* and *QL* values obtained by the proposed procedure for the two peaks were determined according to described in Sec. 2 as recommended by IUPAC [39]. These values were compared to similar results obtained by use of UV-vis spectrophotometric method, as recommended in British Pharmacopeia [1].

Table 1 shows the figure of merit obtained by use of the proposed and the recommended procedures. The linearity range (*LR*), the equation of the analytical curves, correlation coefficients (*r*), which determines the degree of linearity of the correlation between the concentration of PH and peak currents, as well as the standard errors of the intercept (SE_a), the standard errors of the slope (SE_b),

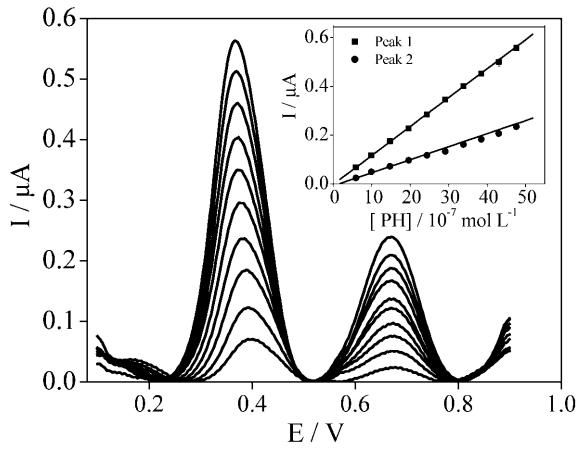

Fig. 5. Square-wave voltammograms for PH in 0.10 mol L^{-1} BR pH 4.0 at HBDD, with $t_a = 30$ s, $E_a = 0.78$ V, $f = 50$ s^{-1}, $a = 50$ mV, $\Delta E_s = 2$ mV, and concentrations of 5.96×10^{-7} to 4.76×10^{-6} mol L^{-1} of PH. The insert shows the average current obtained from three analytical curves for both voltammetric peaks.

Table 1. Analytical parameters obtained for the determination of PH using SW-AdsV and UV-vis spectrophotometry procedures. *LR:* linearity range; *r:* correlation coefficient; SE_a: standard error of the intercept; SE_b: standard error of the slope; S_b: standard deviation of the arithmetic mean of ten blank solutions; *DL:* detection limit; *QL:* quantification limit; *RSD:* relative standard deviation.

Parameter	SW-AdsV		UV-vis spectrophotometry
	Peak 1	Peak 2	
LR	5.96×10^{-7} to 4.76×10^{-6} (mol L^{-1})		
Equation curve	$I_p = -2.09 \times 10^{-9} + 0.12$ [PH]	$I_p = -7.62 \times 10^{-9} + 0.05$ [PH]	$Abs = 6.8 \times 10^{-3} + 0.08$ [PH]
r	0.9998	0.9994	0.9982
SE_a	1.38×10^{-9} (A)	7.30×10^{-10} (A)	2.12×10^{-3}
SE_b	5.48×10^{-4} (A/mol L^{-1})	4.34×10^{-4} (A/mol L^{-1})	6.76×10^{-9} (1/mol L^{-1})
S_b	1.03×10^{-8} (A/mol L^{-1})	7.17×10^{-9} (A/mol L^{-1})	8.82×10^{-9} (1/mol L^{-1})
DL (mol L^{-1})	2.66×10^{-8} (8.51 μg L^{-1})	4.61×10^{-8} (14.77 μg L^{-1})	3.50×10^{-7} (112.00 μg L^{-1})
QL (mol L^{-1})	8.86×10^{-8} (28.40 μg L^{-1})	1.54×10^{-7} (49.19 μg L^{-1})	1.17×10^{-6} (374.00 μg L^{-1})
RSD (repeatability)	4.93% ($n = 12$)	2.46% ($n = 12$)	2.54% ($n = 12$)
RSD (reproducibility)	3.87% ($n = 7$)	7.69% ($n = 7$)	1.75% ($n = 7$)

standard deviation of the arithmetic mean of ten blank solutions (S_b), the detection limits (*DL*), the quantification limits (*QL*), the repeatability (*RSD*%), and the reproducibility (*RSD*%) are presented. All the data were obtained in triplicate and the results reported here represent the average of the values obtained.

The *DL* and *QL* values of both peaks calculated by the proposed procedure were very close to values published previously using DNA-modified electrodes [14, 15], but lower than values obtained on glassy carbon electrodes [10] and graphite powder electrodes modified by ionic liquid [15]. Hence, the SW-AdsV proved to be a good choice for determining PH, since it allows electrodic surfaces to be analyzed without modifications or complex electrochemical pretreatments, simplifying the analytical procedure, lowering costs and providing reliable sensitivity for analytical purposes.

Besides, the proposed procedure presented much better sensibility than obtained by recommended procedure, indicating, this way, that the use of the HBDD allied to SW-AdsV is suitable tool for determination of the PH in different types of the samples.

The precision and accuracy were evaluated using the SW-AdsV procedure considering two voltammetric peaks, and the UV-vis spectrophotometric procedure, according to described in Sec. 2. For these, standard solutions containing 9.90×10^{-7} mol L^{-1} PH were employed for reproducibility experiments involving seven different measurements, and for repeatability experiments involving twelve replicated measurements. The RSD values for reproducibility and repeatability experiments were calculate and are showed in Table 1.

The above results allow us to conclude that good repeatability and reproducibility of analytical measurements can be obtained using HBDD and SW-AdsV with no electrochemical cleaning or pretreatment of the electrodic surface. The working electrode was cleaned by a few seconds of agitation, a fast, simple procedure that ensures reproducibility and sensitivity.

3.6. Application of the Procedure with Commercial Formulations

Stability, recovery, specificity and precision data of commercial formulations were determined to evaluate the applicability of the proposed procedure to complex samples. To this end, three different forms of PH commercially available in Brazil (tablets and injectable PH) were used, which were prepared as described in Sec. 2.

The recovery curves were built by the standard addition method and the recovery percentage was identified graphically, with the abscissa axis referring to the concentration of PH in the electrochemical cell. Extrapolating the curve along this axis yields the sample concentration, allowing for the calculation of the recovery values. All the curves were built in triplicate, considering the use of the SW-AdsV and UV-vis procedures. Table 2 shows the recovery curve data obtained from commercial formulations, the nominal dosage, found dosage, found amount for recovery (%), relative standard deviation (*RSD*%), and Bias% for two analytical procedures employed here.

By use of the HDBB allied to SW-AdsV, the application in commercial formulations was realized considering only peak 1, which presented the highest analytical sensitivity and the lowest adsorption on the electrodic surfaces, diminishing this way, the possible loss in analytical sensitivities caused to adsorption of the components of the samples in electrodic surfaces.

For all recovery curves, the found concentration of PH ($[PH]_{found}$) are presented coupling to the values of confidence intervals of the mean values calculated by $\mu = \overline{x} \pm ts/n^{-1/2}$, where μ is the confidence interval, x is the mean of the concentrations PH found, t (coefficient of Student's t-distribution) is 4.3 for a 95% confidence level, s is a standard deviation of the PH concentrations calculated e n is the number of determinations.

Table 2 shows the calculated values, which fell within in a suitable range for analytical purposes, i.e., from 70% to 130% [44, 45], indicating that the proposed procedure is

Table 2. Determination of PH in commercial formulations, using HBDD electrode allied to SW-AdsV and UV-vis spectrophotometry. Artificially spiked samples with 9.90×10^{-7} mol L^{-1} of PH.

Electrolyte	Tablet	Injectable	Lisador	
SW-AdsV				
Nominal dosage	–	25 mg/tablet	50 mg/2 mL	5 mg/2 mL
$[PH]_{found}$/mol L^{-1}	8.69×10^{-7}	9.56×10^{-7}	9.42×10^{-7}	7.92×10^{-7}
Confidence interval	$\pm 1.23 \times 10^{-7}$	$\pm 0.83 \times 10^{-7}$	$\pm 2.31 \times 10^{-7}$	$\pm 0.46 \times 10^{-7}$
Recovery (%)	87.76	96.60	86.67	79.99
RSD (%)	5.00	3.37	2.58	1.89
Bias (%)	−12.22	−3.43	−4.85	−20.00
UV-vis				
Nominal dosage	–	25 mg/tablet	50 mg/2 mL	5 mg/2 mL
$[PH]_{found}$/mol L^{-1}	$8.65 \times 10^{-7} \pm 9.30$	–	–	$8.26 \times 10^{-7} \pm 0.03$
Recovery (%)	87.24	–	–	83.30
RSD (%)	6.61	–	–	0.02
Bias (%)	−12.63	–	–	−16.56

appropriate for the quantification of PH in complex samples such as pharmaceutical compounds.

The recovery curves obtained from the Lisador sample can also be used to evaluate the specificity of the proposed procedure, since this product contains other pharmaceutical compounds (dipyrone and adiphenine hydrochloride), which may interfere in the analytical response of PH on HBDD. Despite the presence of other compounds, however, the percentages of recovery in these samples proved suitable for analytical purposes, indicating that the procedure can be considered specific for PH, with little interference from other compounds.

In all recovery curves, the concentration used for artificially spiked formulations samples was 9.90×10^{-7} mol L^{-1} of PH, which was considered as reference values. This way, the percentage differences between the concentrations considered as reference values and the concentration calculated by recovery curves were determined as bias measurements and showed how much the PH has been measured by proposed procedures, indicating this way, the effects of the interferences of the samples in the analytical responses and consequently the efficiency of the procedures.

4. Conclusions

The results of this work led us to conclude that the PH oxidation mechanism on HBDD involves the formation of an adsorbed product that oxidizes more readily, producing a new peak with lower potential values. The intensity of both voltammetric peaks can be increased by applying +0.78 V for 30 seconds, causing the PH to present a pair of well-defined peaks with characteristics of a quasireversible redox process, with peak 1 being diffusion-controlled and peak 2 corresponding to an adsorptive process.

The use of square-wave voltammetry under optimized conditions revealed a good linear correlation between peak currents and PH concentration in a wide range of concentrations. The proposed procedure, which provides great sensitivity and specificity, as well as good accuracy and precision, is very simple and does not require complex preparation or renovation of electrodic surfaces.

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