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Novel voltammetric investigation of dipyridamole at a disposable pencil graphite electrode

Iulia Gabriela DAVID*, Lorelei IORDACHE, Dana Elena POPA, Mihaela BULEANDRA, Victor DAVID, Emilia-Elena IORGULESCU

Department of Analytical Chemistry, Faculty of Chemistry, University of Bucharest, Bucharest, Romania

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Abstract: The present paper describes the voltammetric analysis of dipyridamole (DIP) at a cheap, disposable pencil graphite electrode (PGE). The working conditions were optimized with regard to the electrode material and the supporting electrolyte. Cyclic voltammetric investigations emphasized that DIP is irreversibly oxidized at the PGE. The electrode process is pH-dependent and controlled by both diffusion and adsorption. For DIP quantitative determination a differential pulse voltammetric (DPV) method in phosphate buffer solution pH 7.00 was developed. DIP's oxidation peak current varied linearly with the analyte concentration, presenting two linear ranges, namely 5.00×10^{-7}–2.50×10^{-5} M and 2.50×10^{-5}–2.50×10^{-4} M, with detection and quantification limits of 1.21×10^{-7} M and 4.03×10^{-7} M DIP, respectively. The newly developed DPV method using the inexpensive, disposable PGE was successfully applied for the simple and rapid determination of DIP from pharmaceutical formulations.

Key words: Dipyridamole, voltammetry, disposable electrode, pencil graphite electrode, pharmaceuticals

1. Introduction

Dipyridamole (DIP), known also as Persantin (2,6-bis (diethanolamino)-4,8-dipiperidinopyrimido [5,4-d] pyrimidine) (Figure 1), is a coronary vasodilator and antiplatelet drug introduced in 1959 in Germany [1]. To date, it has been used for the treatment of cardiovascular diseases like angina pectoris and myocardial infarction [2], its antioxidant properties being important for antithrombotic and vasodilatory activity. It was also shown that DIP behaves as an inhibitor of lipid peroxidation and of human tumor cell growth [3] and improves renal function [4,5]. Recent studies suggested that DIP improves the calvarial bone regeneration capacity of 3D-printed bioactive ceramic scaffolds [6] and may be used in the treatment of restless legs syndrome, having therapeutic effects on sensory and motors syndromes [7]. Fluorescence [8–10] and electrochemical [3,11] studies have been used to investigate some of DIP's biological properties. Due to the fact that DIP crystallization in solution is essential for the quality of drugs, a study regarding its solid–liquid equilibrium was recently reported [12].

In order to improve their performance, athletes have sometimes fraudulently consumed DIP, but the uncontrolled administration of this drug can have serious side effects [13]. DIP's effects in the body last a long time and its concentration in blood and urine is usually very low [14,15], but DIP plasma content remains unchanged over 2 weeks of storage [16]. Thus, the development of sensitive and selective methods for DIP assay in pharmaceutical and biological samples is required. As DIP (Figure 1) contains a stable heteroaromatic double

*Correspondence: i_g_david@yahoo.com

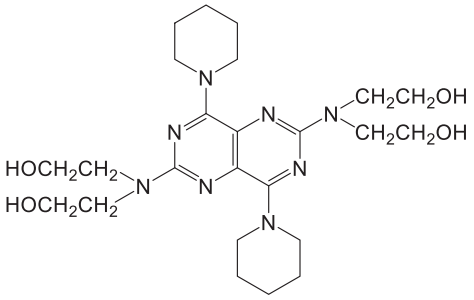

Figure 1. Dipyridamole's chemical structure.

central system responsible for its absorption and fluorescence characteristics [17], several analytical methods exploiting these properties [18–21], as well as other luminescence-based methods [13–22], have been developed for its quantification. On the other hand, several chromatographic methods with either spectrometric [23–25] or electrochemical [26] detection were reported for DIP analysis. Some of these are quite selective and sensitive, but usually they are time consuming and expensive, especially for the quantification of the major active component in pharmaceuticals. In this situation, voltammetric analysis, which is simple, rapid, and cost effective, presenting also sensitivity and selectivity, can be the ideal choice for drug quantification in pharmaceutical preparations.

Moreover, study of the voltammetric behavior of DIP in different conditions can give insight into similar redox processes taking place in living organisms explaining thus, at least partially, their action mechanism [3,11].

DIP's electrochemical (cathodic [1] and anodic [2,3,27,28]) behavior was investigated in aqueous [1,27], micellar [28], and nonaqueous media [2,3] at different electrodes, e.g., Pd [3], Pt [2,3,27], mercury-coated platinum microelectrode [1], glassy carbon, and graphite [28]. On the other hand, there are few literature reports related to voltammetric methods developed for DIP quantification in pharmaceutical [14,29–31] and biological samples [14,15,31,32].

One major drawback of the voltammetric methods is related to the possible passivation of the working electrode's electroactive area and thus the necessity of surface cleaning before each recording in order to achieve reproducible measurements. This disadvantage can be avoided by using disposable working electrodes like those of the pencil graphite electrode (PGE) type. PGEs present good electrochemical characteristics and are cheap, easily commercially available, and simple to use. Thus, in recent years this type of electrode has gained even more applicability in voltammetric analysis of pharmaceutically [33–39] and biologically [40–43] important compounds.

To the best of our knowledge there is no report related to the voltammetric quantification of DIP at a PGE. Therefore, the present paper describes for the first time the cyclic voltammetric behavior of this compound at a disposable, commonly available and inexpensive PGE, suggesting a new oxidation mechanism for its electrooxidation. A novel sensitive and simple differential pulse voltammetric method for rapid DIP quantification in pharmaceutical samples using the cost-effective PGE is also presented.

2. Materials and methods

2.1. Reagents and solutions

DIP ($>$98% TLC powder), ethanol ($\geq$99.5%, ACS reagent), H_2SO_4 (98.0%, ACS reagent), $Na_2HPO_4\times 2H_2O$ and KH_2PO_4 (p.a., ACS reagent), CH_3COOH ($\geq$99.7%, ACS reagent), NaOH (pellets), H_3BO_3 (1 g per tablet), H_3PO_4 (85 wt% in H_2O), and KCl (99.0%–100.5%, ACS reagent) were purchased from Sigma-Aldrich.

Moreover, 0.1 M H_2SO_4 solution, acetate buffer solution (ABS) pH 4.00, phosphate buffer solution (PBS) pH 7.00, Britton–Robinson buffer (BRB) with pH values in the range 1.81–11.92, and 0.1 M KCl solution were used as supporting electrolytes in the voltammetric experiments. A 1.00×10^{-3} M DIP stock solution was daily prepared by dissolving the appropriately weighed amount of analyte in ethanol, under sonication, and kept in the refrigerator when not used. More diluted DIP working solutions were prepared, in 10-mL volumetric flasks, by (successive) dilution of the stock solution with the appropriate supporting electrolyte.

Pharmaceutical preparations of DIPIRIDAMOL tablets containing 25 mg of active principle per tablet, produced by S.C. Zentiva S.A., Romania, were purchased from a local pharmacy. Ten accurately weighed tablets of DIPIRIDAMOL 25 mg were ground with a pestle in a porcelain mortar in order to obtain a fine powder. An exactly weighed quantity of this powder, equivalent for the preparation of 50 mL of 1.00×10^{-3} M DIP solution, was dissolved in approximately 25 mL of ethanol, swirled and sonicated for 30 min, and finally filtered using Blue Ribbon Quantitative Whatman filter paper. For the complete recovery of the analyte, the filter paper was washed three times with ethanol. The filtrate and the washing solutions were collected in a 50-mL volumetric flask and brought to the mark with ethanol. Aliquots of 0.05 mL of this solution were further diluted with PBS pH 7.00 to a final volume of 10 mL, so that the DIP concentration in the tested solution fit within the linear range of the developed differential pulse voltammetric (DPV) method, and immediately analyzed. Thus, the composition of the diluted, voltammetrically tested DIPIRIDAMOL tablet sample solution was approximately 5×10^{-6} M DIP in 0.5% v/v ethanol in PBS pH 7.00.

Aliquots of this solution were further diluted with PBS pH 7.00, so that the DIP concentration in the tested solution fit within the linear range of the developed DPV method, and immediately analyzed.

2.2. Apparatus

Voltammetric measurements were carried out using a conventional three-electrode cell connected to an Autolab PGSTAT 12 electrochemical system (potentiostat/galvanostat). The voltammetric cell consisted of a Pt wire as auxiliary electrode, an Ag/AgCl (3.00 M KCl) reference electrode, and a working electrode. The working electrode was either a Pt disk electrode with a surface area of 3.14 mm^2 (2 mm diameter), a glassy carbon disk electrode (GCE) having a surface area of 7 mm^2 (3 mm diameter), or a PGE with a surface area of 15.86 mm^2 (0.5 mm diameter and 1.0 cm height immersed in 10 mL of solution to be analyzed).

In order to ensure a reproducible electroactive surface, the conventional solid electrodes (Pt and GCE) were polished with alumina powder, rinsed with distilled water, and dried before each recording.

The PGE, consisting of a 1.5-cm-long graphite pencil lead (0.5 HB if not stated otherwise), 1.0 cm being exposed to the analyzed solution, was obtained as previously reported [44].

The pH values of the investigated solutions were measured with a combined pH-glass electrode connected to a Consort P901 Scientific Instrument pH/mV/°C-meter (Belgium).

2.3. Procedures

Electrode activation by electrochemical pretreatment was performed either by cycling ten times the potential from -500 mV to $+2000$ mV at a scan rate of 500 mV s^{-1} or potentiostatically by applying at the PGE a constant potential of $+2000$ mV for 60 s, using successively each of the following supporting electrolytes: 0.1 M H_2SO_4, acetate buffer solution (ABS) pH 4.00, and phosphate buffer solution (PBS) pH 7.00.

Cyclic voltammetric recordings were carried out in the potential range 0 to +1000 mV at a scan rate of 100 mV s^{-1} if not stated otherwise.

Differential pulse voltammograms were recorded from +100 to +1000 mV, applying the following optimized instrumental parameters: modulation amplitude 75 mV, step potential 4.94 mV, interval time 0.1 s, and modulation time 0.002 s. All the experiments were realized at room temperature (25.0 ± 0.2 °C).

The standard addition method was applied for recovery studies and for the evaluation of the content of the 25-mg DIPIRIDAMOL tablets. DPV responses were recorded at the PGE for 10 mL of diluted DIPIRIDAMOL tablet sample solution before and after 3 successive additions of 0.05 mL of 1.00 × 10^{-3} M DIP stock solution. The pH of the diluted DIPIRIDAMOL tablet sample solution tested voltammetrically was 7.00, the same as that of the buffer used, and it changed only with 0.02 pH units after the three additions of the (in total 0.15 mL) ethanolic DIP stock solution. The measured peak currents obtained for each of the 4 recordings were employed to calculate the % recoveries and the DIP content of the commercial 25-mg DIPIRIDAMOL tablets.

3. Results and discussion

3.1. Selection of the optimum working conditions

3.1.1. The influence of the working electrode

The electrochemical behavior of an analyte can be strongly affected by the electroactive surface material of the working electrode. Therefore, the first step of this study consisted of investigation of DIP voltammetric response at different working electrodes like Pt, GCE, and PGE. The DPV response recorded for DIP at the Pt electrode presents two large and less intense signals, whereas at the GCE one can observe an anodic peak at about 580 mV, somewhat higher than that recorded at Pt, but nevertheless much smaller (almost 10 times) than that obtained using the disposable PGE (Figure 2). These results could be explained by the linear dependence between the maximum peak current and the geometrical area of the electroactive surface of the working electrode, which is Pt (3.14 mm^2) < GCE (7 mm^2) < PGE (15.86 mm^2). Nevertheless, the obtained sensitivities (S, expressed as A/M × cm^2), i.e. Pt (0.57) < GCE (0.75) < PGE (3.06), suggest that the higher signal recorded at the PGE can be also attributed to the irregular morphology of the PGE surface, which can result in enhancement of the electrode electroactive surface area [45].

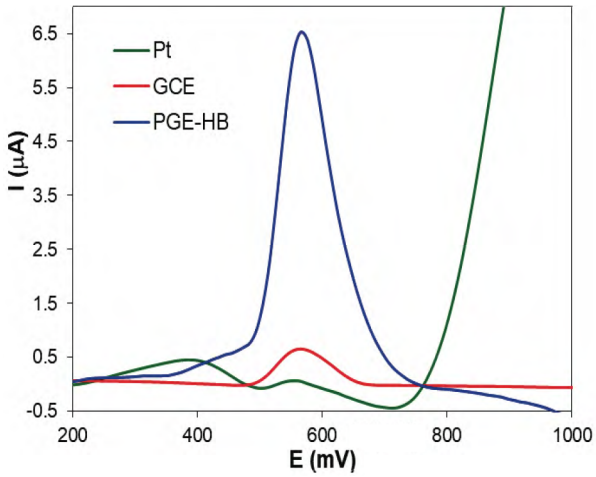

Figure 2. Differential pulse voltammograms recorded at different working electrodes for 1.25 × 10^{-5} M DIP in 0.1 M H_2SO_4 solution.

There are also various types of graphite pencil leads characterized by different hardness grades depending on their clay and graphite content and having, therefore, different properties; according to the European Letter Scale harder graphite pencil leads (noted with H) have higher clay content, whereas the softer ones (noted with B, derived from blackness) are characterized by higher graphite content [45,46]. The DPV oxidation signal of DIP was investigated using graphite pencil leads of different hardness degrees (from 2H to 2B). The highest peak was obtained at HB-type leads, which contain equal amounts of clay and graphite.

For a better electrochemical comparison of the electrode material, the electrodes' sensitivities, which are independent of the geometrical surface area, were calculated. The results (Table 1) emphasized that DIP's most sensitive response is obtained at HB pencil leads, this type of lead being further used as electrode material.

Table 1. The peak potentials and sensitivities for the oxidation peaks obtained by DPV for DIP in 0.1 M H_2SO_4 solution at different types of graphite pencil leads.

Lead type	E_p (mV)	S (A/M × cm^2)
2H	585.9	2.18 ± 0.14
H	585.9	2.44 ± 0.18
HB	584.9	3.06 ± 0.09
B	584.9	2.52 ± 0.11
2B	606.1	1.49 ± 0.20

Numerous literature reports showed that the electrochemical pretreatment of carbon-based working electrodes leads to improvement of the selectivity [47] and/or sensitivity [48] of the voltammetric determinations of some analytes due to modification (increase in the electrode active area and/or formation of oxygenated groups) [46] of the electrode active surface. As these resulting properties depend on the electroactivation conditions (galvanostatic or potentiostatic, supporting electrolyte), the influence of the PGE surface electrochemical pretreatment on DIP's voltammetric response was studied at the HB-PGE activated either by cyclic voltammetry (CV) or potentiostatically in different electrolytes (0.1 N H_2SO_4, ABS pH 4.00, and PBS pH 7.00), according to the procedure described in section 2.3. Procedures. Unfortunately, none of the electroactivated PGE presented any improvement in the DPV oxidation signal of DIP (peak potential shift or peak current increase). Therefore, the nonactivated HB-PGE was used as the working electrode for further investigations.

The possibility of using the same pencil lead for several voltammetric measurements was tested by recording five repetitive cyclic voltammograms for DIP in PBS pH 7.00 using the same HB graphite lead. It was observed that the height of the DIP oxidation peak decreased in the second scan by about 63%, the decrease being less pronounced in the next scans (about 12% and 2% in the third and the next scans, respectively) (voltammograms not shown). This fact is probably due to electrode surface fouling by adsorption of the DIP oxidation products and hence a new pencil lead should be employed for each voltammetric recording. Due to the fact that the quality of graphite pencil leads is strictly controlled during their production process, the composition and surface of leads of the same type (the same hardness and the same manufacturer) are uniform and the analytes' voltammetric signals recorded on individual PGEs are very similar, being characterized by good electrode-to-electrode reproducibility [49].

3.1.2. The stability of the DIP stock solution

The stability of DIP stock solution, either aqueous or ethanolic, was investigated for 7 days after preparation. Each stock solution was divided in two parts, one part being stored in the refrigerator and the other under

ambient conditions. Differential pulse voltammograms of the DIP working solutions prepared from each stock solution were recorded after several time intervals, using a new PGE every time, and the intensities of the DIP oxidation peaks were compared (Figure 3).

It was observed that the peak intensity remains almost constant for the analyzed samples prepared from the ethanolic stock solution, independently of the storage conditions, whereas DPVs recorded on working solutions prepared from aqueous DIP stock solution present lower anodic peaks, indicating low stability. This observation leads to the conclusion that for the voltammetric analysis of DIP it is not necessary to prepare fresh stock solution if ethanol is the solvent, whereas aqueous DIP stock solutions have to be prepared daily.

3.1.3. The influence of pH and the nature of the supporting electrolyte

Besides the electrode material, other important factors affecting the electrochemical response of a compound are pH and the nature of the supporting electrolyte employed. Thus, the influence of the supporting electrolyte pH on DIP's voltammetric behavior at the HB-PGE was investigated in the pH range 1.81 to 11.92, using CV (voltammograms not shown) and DPV (Figure 4) in the universal BRB. Cyclic voltammetric recordings emphasized that DIP is irreversibly oxidized during the whole investigated pH range, presenting only anodic waves and no cathodic signal. By increasing the solution pH, the DIP anodic peak potential shifts towards less positive values (Figure 4), suggesting that the electrode process involves proton exchange. The ratio of the protons to electrons involved in the electrode process was estimated from the slope of the E_p = f (pH) plots described by the equations E_p = –26.082 × pH + 589.79 (R^2 = 0.9962) and E_p = –25.622 × pH + 587.69 (R^2 = 0.9942) for CV and DPV, respectively. Comparing the slopes of these equations with the theoretical value of 59.16x/n, where x represents the number of transferred protons and n that of the electrons, the x/n ratio was found to be 1/2, indicating that the exchange of each two electrons is accompanied by one proton.

As can be observed from Figure 4, the best shaped and most intense peak for DIP oxidation was obtained

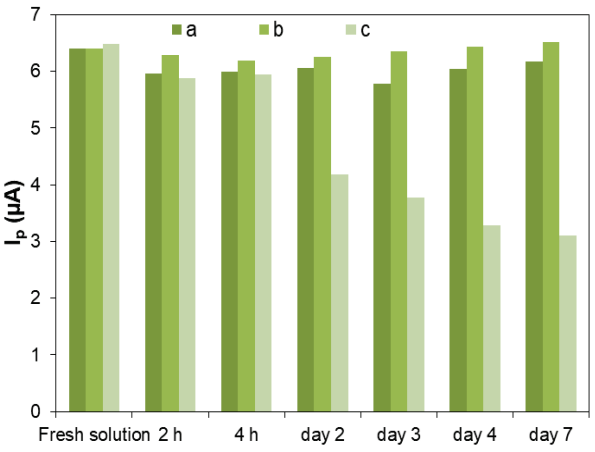

Figure 3. DPV peak currents recorded at the HB-PGE for 1.25 × 10^{-5} M DIP in 0.1 M H_2SO_4 solution prepared at different time intervals from: a) 1.00 × 10^{-3} M DIP ethanolic stock solution stored under ambient conditions; b) 1.00 × 10^{-3} M DIP ethanolic stock solution stored in the refrigerator; and c) 5.00 × 10^{-4} M DIP aqueous stock solution stored in the refrigerator.

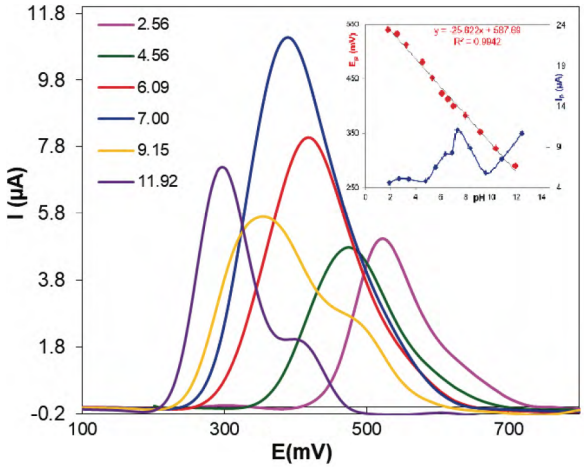

Figure 4. Differential pulse voltammograms recorded at the HB-PGE for 1.50 × 10^{-5} M DIP in BRB with different pH values (inset: variation in DIP oxidation peak potential and current with pH).

at pH 7.00. Therefore, the influence of some supporting electrolytes with similar pH values on DIP's oxidation peak was investigated by DPV (Figure 5). As the highest signal was recorded in PBS pH 7.00 this electrolyte was used for further DIP voltammetric investigations.

3.2. Electrochemical behavior of dipyridamole

In order to establish the nature of the DIP oxidation process at the HB-PGE, cyclic voltammograms were recorded at different scan rates (Figure 6). The linear variation of the DIP anodic signal according to the equation I_p (μA) = 0.0221 × v (mV/s) + 0.4003 (R^2 = 0.9990) suggests an adsorption controlled electrode process. This fact is also supported by the nonlinear $I_p = f(v^{1/2})$ dependence. Moreover, the log I_p vs. log (v) plot is linear, being described by the equation log I_p = 0.785 × log v − 1.083 (R^2 = 0.9865), where μA and mV/s are the units of I_p and scan rate (v), respectively. The slope value of this dependence is situated between 0.500, which is the value characteristic for a diffusion controlled process, and 1.000, which stands for an adsorption governed process. Thus, one can conclude that DIP electrooxidation at the HB-PGE is a mixed electrode process, being controlled by both the analyte diffusion toward the electrode and the analyte adsorption at the HB-PGE surface [50]. This observation is in accordance with that reported for DIP oxidation in PBS pH 7.4 at a GCE [51].

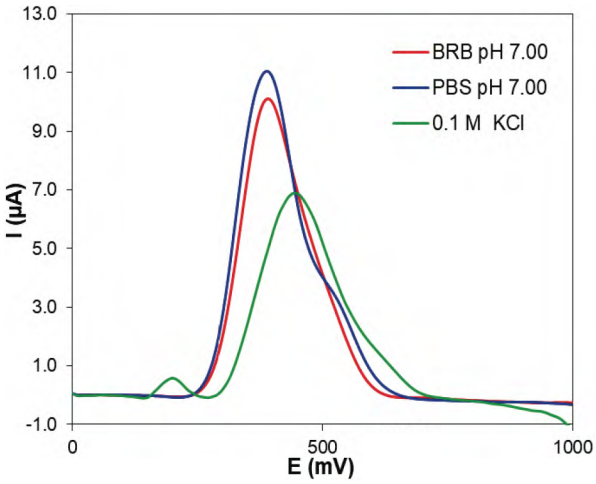

Figure 5. Differential pulse voltammograms recorded at the HB-PGE for 1.50 × 10^{-5} M DIP solutions in different electrolytes.

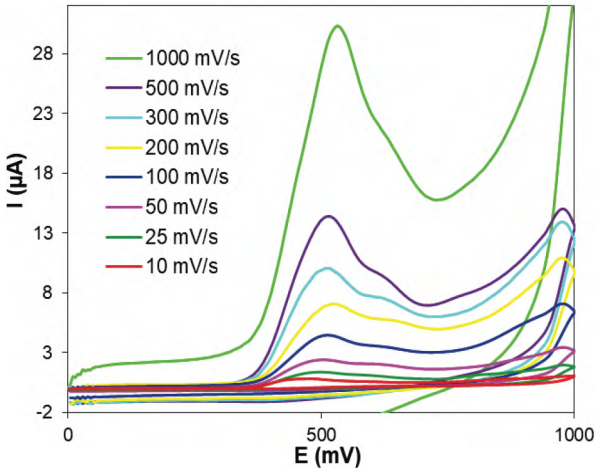

Figure 6. Cyclic voltammograms recorded at the HB-PGE for 1.50 × 10^{-5} M DIP in PBS pH 7.00 at different scan rates.

When the scan rate was increased, the DIP anodic peak potential (E_p) shifted towards more positive values, confirming thus the irreversibility of the oxidation process. Taking into consideration that for an irreversible electrode process the electron transfer coefficient (α) is 0.5 and that the slope of the $E_p = f(\log(v))$ plot is ($2.303RT/\alpha nF$) [52], the electron number was calculated (n = 4). R, T, and F have their usual meanings (T = 298 K, R = 8.314 J K^{-1} mol, and F = 96480 C mol^{-1}).

It must be mentioned here that our results are quite different from those already published for DIP voltammetric oxidation in organic solvents, in micellar media, or in acidic aqueous solution that consider either two consecutive one-electron diffusion-controlled processes or one-step oxidation involving two electrons, respectively [53]. Nevertheless, Castilho et al. [27] suggested that in the presence of H_2O_2, in acidic media, the

piperidine ring of DIP is first cleaved (with the generation of an aldehyde) and after that an N-oxide derivative is formed in the pyrimidino-pyrimidine ring. Keeping in mind this approach, we consider that, in the present conditions, DIP electrooxidation takes place also at the N atom from the piperidine ring. As there is no chemical oxidant (i.e. H_2O_2) we suggest that DIP oxidation at the HB-PGE leads to the formation of a double bond with the vicinal carbon instead of the N-oxide derivative. Based on these conclusions the following reaction mechanism was proposed (Figure 7).

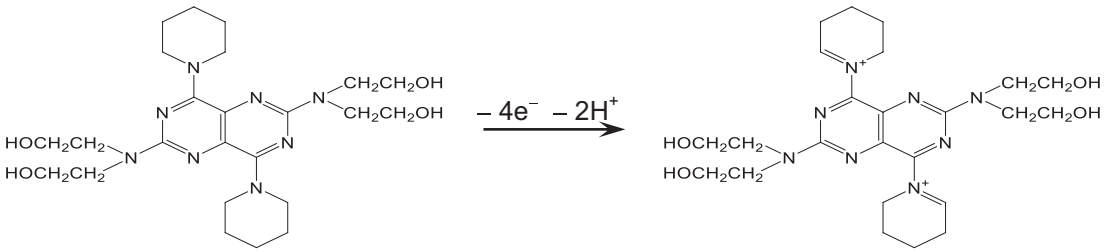

Figure 7. Tentative mechanism of the DIP electrochemical oxidation reaction.

3.3. Voltammetric determination of dipyridamole

Due to the fact that CV has limited sensitivity, the more sensitive DPV technique was further employed for the determination of DIP.

3.3.1. Linear range and detection limit

The influence of the DIP concentration on the anodic peak current obtained by DPV at the HB-PGE in PBS pH 7.00 was studied in the concentration range 5.00 × 10^{-7}–7.50 × 10^{-4} M DIP (Figure 8). The oxidation peak current (I_p) varied linearly with DIP concentration from 5.00 × 10^{-7} to 2.50 × 10^{-4} M, presenting two linear ranges, namely 5.00 × 10^{-7} to 2.50 × 10^{-5} M and 2.50 × 10^{-5} to 2.50 × 10^{-4} M DIP, described by the equations: I_p (μA) = 7.18 × 10^5× C_{DIP} (M) – 0.16 (R^2 = 0.9990) and I_p (μA) = 1.21 × 10^4× C_{DIP} (M) + 14.76 (R^2 = 0.9964), respectively.

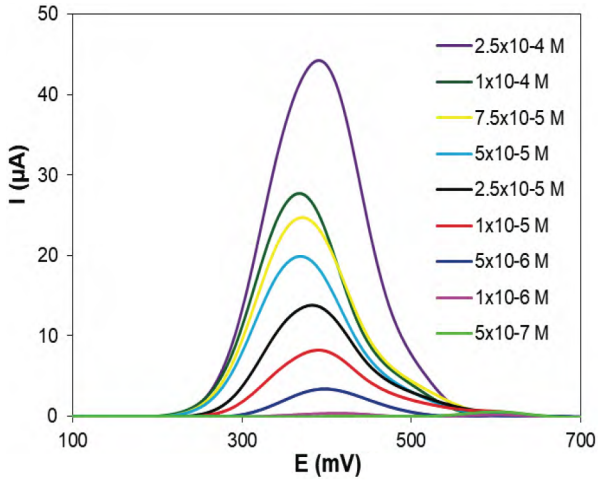

Figure 8. Differential pulse voltammograms recorded at the HB-PGE for different DIP concentrations in PBS pH 7.00.

The limit of detection (LOD) of 1.21 $\times$ 10^{-7} M DIP and the limit of quantification (LOQ) of 4.03 $\times$ 10^{-7} M DIP of this method were evaluated as LOD = 3 $\times$ s_{c_min}/b and LOQ = 10 $\times$ s_{c_min}/b, where s_{c_min} is the standard deviation of the lowest concentration from the calibration curve and b is the slope of the calibration curve obtained for the lower linearity range [54].

When compared with data previously published in the literature regarding the voltammetric determination of DIP (Table 2), the DPV at the PGE method developed in the present study does not have a very low LOD, but this aspect is not so important when the method is applied for the determination of the active principle in pharmaceutical formulations. Nevertheless, the present voltammetric method described for DIP analysis has one of the largest dynamic ranges (of three orders of magnitude) and the most important feature is related to the fact that it employs a nontoxic (compared to HMDE) and bare (unmodified) working electrode. This means a more rapid and cheaper method of analysis.

Table 2. Performance characteristics of DIP voltammetric analysis methods previously reported in the literature.

Technique	Electrode	Linear range (M)	Detection limit (M)	Sample	Ref
SWV	HMDE	1.28×10^{-6}–7.02×10^{-6}	1.88×10^{-8}	tablets	[29]
AdSV	HMDE	not given	1.00×10^{-9}	urine, tablets, injections	[14]
CAdSSWV	HMDE	9.00×10^{-9}–5.00×10^{-6}	4.00×10^{-11}	serum	[15]
DPAdSV	MIP-CPE	1.98×10^{-9}–1.98×10^{-7}	9.91×10^{-11}	tablets, human serum	[30]
LSV	CPE/presence of CTAB	5.94×10^{-8}–2.38×10^{-7}	1.98×10^{-8}	tablets	[31]
ASV	Nafion-GCE	1.00×10^{-9}–8.00×10^{-8}	8.00×10^{-11}	human serum	[32]
SWV	SCPE	8.00×10^{-8}–3.00×10^{-5}	2.00×10^{-8}	pharmaceuticals	[55]
DPV	PGE	5.00×10^{-7}–2.50×10^{-4}	1.21×10^{-7}	tablets	this study

SWV: square wave voltammetry; HMDE: hanging mercury drop electrode; AdSV: adsorptive stripping voltammetry; CAdSSWV: cathodic adsorptive square wave stripping voltammetry; DPAdSV: differential pulse adsorptive stripping voltammetry; MIP-CPE: molecularly imprinted carbon paste electrode; LSV: linear sweep voltammetry; CPE: carbon paste electrode; CTAB: cetyl trimethyl ammonium bromide; ASV: anodic stripping voltammetry; CGE: glassy carbon electrode; DPV: differential pulse voltammetry.

3.3.2. Repeatability

The repeatability of the HB-PGE response was evaluated at the lowest (5.00 $\times$ 10^{-7} M) and highest (2.50 $\times$ 10^{-4} M) concentrations of the linear range and also at an intermediate concentration (2.50 $\times$ 10^{-6} M) of DIP in PBS pH 7.00. Ten measurements were carried out at each concentration, using a new pencil lead every time. The repeatability was expressed as percentage relative standard deviation (RSD%). RSD% values of 9.41, 6.38, and 3.64 were obtained for 5.00 $\times$ 10^{-7} M, 2.50 $\times$ 10^{-6} M, and 2.50 $\times$ 10^{-4} M DIP, respectively. All RSD% values are within the accepted limits for the corresponding concentration levels [56].

3.3.3. Recovery studies on and analytical applications to pharmaceutical preparations

The practical applicability of the developed DPV at the HB-PGE method for DIP assay was tested on real samples, namely DIP tablets (DIPIRIDAMOL 25 mg, Zentiva), commonly available in Romanian pharmacies. The samples to be voltammetrically analyzed were simply and rapidly prepared as described in the Reagents and solutions section. Five replicate samples were analyzed. The DIP content of the pharmaceutical tablets was evaluated using the standard addition method. The differential pulse voltammogram recorded always at a new electrode for the DIP tablets solution properly diluted with PBS pH 7.00 used as supporting electrolyte

presents only the well-defined DIP characteristic oxidation peak (Figure 9) situated at about 400 mV, indicating that there is no interference from the other components of the tablets. The intensity of this peak increased linearly with successive additions of DIP stock solution, enabling thus quantitative determination of the drug.

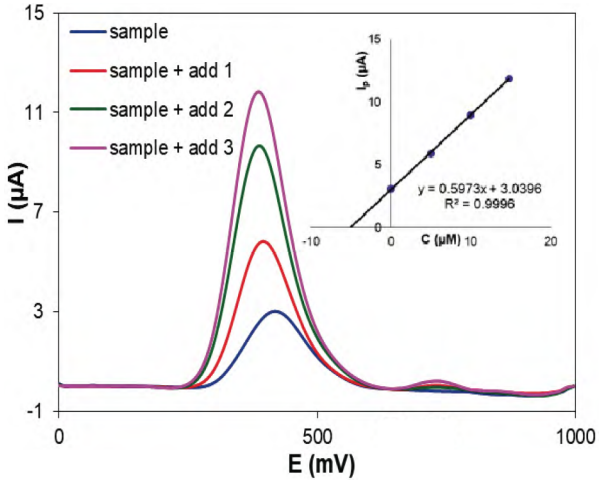

Figure 9. Differential pulse voltammograms recorded at the HB-PGE for 10 mL of sample solution before and after three successive additions of 0.05 mL of 1.00 $\times$ 10^{-3} M DIP stock solution; supporting electrolyte PBS pH 7.00.

The currents corresponding to the peaks recorded before and after each addition of DIP stock solution were measured (Figure 9) and used to calculate the DIP content of the DIPIRIDAMOL 25 mg Zentiva and the % recovery values, taking into consideration all the dilutions performed during the sample preparation (Table 3). It can be seen that the obtained results are very close to the DIP content claimed by the producer.

Table 3. Results of the DPV at PGE determination of DIP content from pharmaceutical tablets and the corresponding recoveries (n = 5); n = number of replicates; SD – standard deviation; RSD – relative standard deviation; R – recovery.

Claimed DIP content (mg)	25.00
Found DIP content by DPV $\pm$ SD (mg) RSD, %	25.17 $\pm$ 0.48 1.89
Average R $\pm$ SD, %	100.67 $\pm$ 1.90
RSD, %	1.89

In conclusion, the DIP voltammetric behavior investigated for the first time at the cheap disposable PGE emphasized that DIP is irreversibly oxidized at the HB-PGE in a complex (diffusion and adsorption controlled) pH dependent electrode process, involving one proton for each two electrons. Keeping in mind DIP's complex structure, its exact oxidation mechanism at the PGE necessitates deeper and further investigations, which will be the topic of another publication.

Based on DIP oxidation at the HB-PGE a new DPV method using this working electrode was developed for drug quantitative determination from pharmaceutical preparations. This method presents certain advantages like a wide linear range (almost three orders of magnitude), simplicity, rapidity, and low cost as it uses the unmodified PGE. This type of working electrode is commonly available worldwide commercially as a writing instrument, is environmentally friendly (nontoxic and does not involve any chemicals for its modification), and is cost effective (one PGE is more than 1000 times cheaper than a GCE) [41].

The applicability of the DPV method described here was successfully tested on DIP-containing tablets. The results obtained by the new method were in good agreement with the pharmaceutical's DIP content claimed by the manufacturer.

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