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Short communication

Determination of dipyridamole in pharmaceutical preparations using square wave voltammetry

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Abstract

An analytical methodology using square wave voltammetry (SWV) at a hanging mercury drop electrode (HMDE) was developed for the quantitative determination of dipyridamole (DIP), a drug used for the treatment of several cardiovascular diseases, in pharmaceutical tablets and injections of Persantin® in phosphate buffer (pH 3.0; 0.1 M). After optimization of the parameters for SWV, analytical curves were obtained for application in the range of 1.28×10^{-6} M to 7.02×10^{-6} M. It was found a detection limit (DL) of 1.88×10^{-8} M (9.50 ng/ml). The repeatability and the reproducibility of the method were determinated by successive measurements of DIP solutions on the range of the analytical curve with a coefficient variation of 0.97% ($n = 5$) and 1.15%, respectively. The apparent recoveries were obtained by the IUPAC recommended procedure using the second reduction peak. Recoveries obtained by SWV were compared with the UV–vis spectrophotometric method. It was found that the determination of DIP in Persantin® tablets gave a mean value of 75.6 ± 0.4 mg (100.8%) and 68.9 ± 0.3 mg (91.8%) for SWV and UV–vis spectrophotometry, respectively. In the case of injections, it was found 10.4 ± 0.1 mg (103.4%) and 9.9 ± 0.2 mg (99.9%) for SWV and UV–vis spectrophotometry. Both apparent recoveries for the two types of formulations are in good accordance with the declared value of 75 mg (tablets) and 10 mg (injections).

Keywords: Dipyridamole; Square wave voltammetry; Analytical determination

1. Introduction

Dipyridamole (DIP), 2,6-bis(diethanolamino)-4,8-dipiperidinopyrimido-[5,4-*d*]pyrimidine, is used for the treatment of several cardiovascular diseases because of its vasodilating and antiplatelet properties [1].

Recent studies have reported that DIP exhibits a potent biological antioxidant activity behaving as an inhibitor of lipid peroxidation initiated by iron(II) ions or by thermolabile azo compounds [2,3]. In this way, the electrochemical oxidation of DIP has been extensively studied in our laboratory in order to bring better understanding about its electrochemical reactions [4–7].

The detection of DIP, in biological and tablets/injections samples, has been performed using various analytical methods including spectrophotometry [8], fluorescence spectrometry [9], high performance liquid chromatography [10–12], phosphorimetry [13] and polarography [14]. Other electroanalytical methodologies, including stripping techniques, have been employed for the determination of DIP in human serum when long accumulation times are used to improve sensitivity [15–17]. However, for some analytical applications including pharmaceutical preparations, which is the objective of the present paper, the determination can be carried out without the application of accumulation times precluding the interference of the excipients.

Considering these facts, the present work reports a more simple and rapid voltammetric procedure for routine quantification of DIP in pharmaceutical preparations such as tablets and injections, at a hanging mercury drop electrode (HMDE),

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using square wave voltammetry (SWV). Recoveries were performed to assess the accuracy of the results and these were compared to those provided by UV–vis spectrophotometry.

2. Experimental

2.1. Reagents

All reagents used in this work were of analytical grade from Aldrich (dipyridamole, USP (58-32-2)), Merck (di-sodiumhydrogen phosphate dihydrate), and Sigma (phosphoric acid). Water treated in a Milli-Q system (Millipore) was used to prepare the solutions. All chemicals were used without previous purification.

Phosphate buffered solution (pH 3.0; 0.10 M) was prepared by adding 0.1 M di-sodiumhydrogen phosphate dehydrate solution in a 0.10 M phosphoric acid solution until pH 3.0. Solutions of DIP ranging from 10^{-3} M to 10^{-4} M were prepared by dissolving appropriate amounts of the compound in 0.10 M phosphate buffer and purged with purified nitrogen, from White Martins (SS grade), for 5 min prior to the measurements to remove oxygen. Then, a continuous stream of nitrogen was passed over the solutions during the measurements.

2.2. Instrumentation

The electrochemical measurements were carried out using a potentiostat from Voltalab model PGZ402 with a hanging mercury drop electrode model MDE150. A conventional three-electrode cell containing a platinum wire counter electrode and a Ag/AgCl(s) (3.0 M KCl) reference electrode were employed for the voltammetric application with HMDE. Experimental parameters that affect SWV response, like frequency (f), pulse amplitude (ΔE_a) and scan increment (ΔE_i), were optimized for electroanalytical purposes.

Optical absorption spectra were registered using a Hitachi U-2000 spectrophotometer coupled to a PC with the Spectracalc software from Galactic using 10.0 mm optical pass quartz cuvettes.

2.3. Dipyridamole assay in formulations

Dipyridamole determination was performed in commercial tablets and injections of Persantin® by using voltammetric and spectrophotometric techniques. Two tablets of the drug were grounded thoroughly until a fine powder is obtained. The samples to be analyzed were prepared by dissolving the powder with phosphate buffer solution (pH 3.0; 0.10 M) and stirred in an ultrasonic bath. No other sample preparation for injections was used except dilution with buffer solution. All concentrations used for voltammetry and spectrophotometry were adjusted to the calibration interval. The determination was performed by the standard addition method and the apparent recoveries were obtained according the procedure recorded by IUPAC [18].

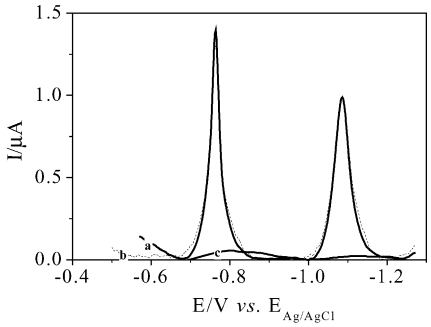

Fig. 1. SWV curves for DIP 8.85 μM in 0.10 M phosphate buffer solution (pH 3), using a HMDE ($A = 0.012$ cm^2). Currents: (a) total; (b) direct and (c) reverse; $f = 100$ s^{-1}; $\Delta E_a = 50$ mV and $\Delta E_i = 2$ mV.

3. Results and discussion

The electrochemical reduction of DIP over the HMDE, obtained by SWV, is showed in Fig. 1. The reduction is characterized by two successive peaks around −0.78 V and −1.10 V versus Ag/AgCl, presenting a typical behavior of an irreversible process.

3.1. Effect of pH

The influence of pH on the reduction peaks of DIP was investigated in the pH range of 1.5–7 where the compound is only slight soluble [19]. It can be observed in Fig. 2 that the peak potentials shifted linearly to less positive values with an increase of pH, indicating that the mechanism of the electrode reaction is dependent on pH. The highest peak current was observed at pH 3, which was chosen for the present analytical applications.

The pK_a of the compound (5.9), obtained by the interception of the E_p versus pH graph (insert of Fig. 2), was very close to the value reported in the literature (5.8) [20].

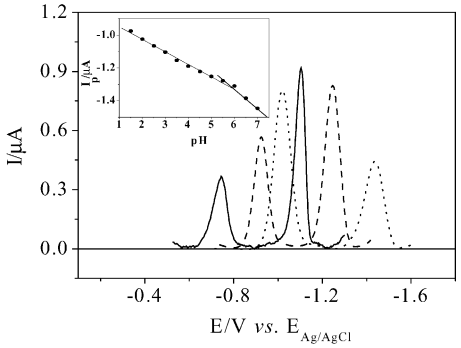

Fig. 2. SWV curves for DIP 5.50 μM in 0.10 M phosphate buffer solutions of different pHs, using a HMDE ($A = 0.012$ cm^2). pH: (—) 3.0; (- - -) 5.0 and (. . .) 7.0; $f = 100$ s^{-1}, $\Delta E_a = 50$ mV and $\Delta E_i = 2$ mV. Insert: E_p vs. pH curve.

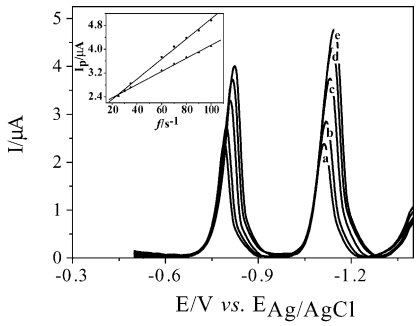

Fig. 3. SWV curves for DIP 0.10 mM in 0.10 M phosphate buffer solution (pH 3), using a HMDE ($A = 0.012\ cm^2$); *f*: (a) 25 s^{-1}; (b) 35 s^{-1}; (c) 60 s^{-1}; (d) 80 s^{-1} and (e) 100 s^{-1}. $\Delta E_a = 50$ mV and $\Delta E_i = 2$ mV. Insert: I_p vs. *f* curve: (▲) first peak ($R = 0.9984$) and (●) second peak ($R = 0.9981$).

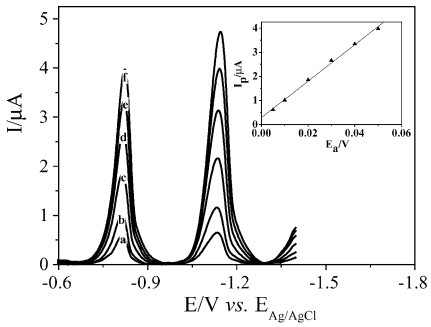

Fig. 4. SWV curves for DIP 0.10 mM in 0.10 M phosphate buffer solution (pH 3), using a HMDE ($A = 0.012\ cm^2$). ΔE_a: (a) 5 mV; (b) 10 mV; (c) 20 mV; (d) 30 mV; (e) 40 mV and (f) 50 mV; $f = 100\ s^{-1}$ and $\Delta E_i = 2$ mV. Insert: I_p vs. ΔE_a curve ($R = 0.9984$).

3.2. Effect of square wave frequency

The frequency (*f*), in SWV experiments, is an important parameter since it allows to obtain important information about the reversibility degree, the number of electrons for a reversible process and to estimated the *nα* value for an irreversible process. Moreover, the frequency is a parameter that determines the signal intensity and thus the sensibility of the analytical methodology. Fig. 3 shows square wave voltammograms obtained for different frequency values in the range of 10 s^{-1} to 100 s^{-1}. Frequencies values over 100 s^{-1} resulted in distortion of the voltammograms. The linearity between peak current (I_p) versus *f*, showed in the insert of Fig. 3, confirms the irreversibility of the reduction process. Another criterion that describes the irreversibility of the system in SWV is the relationship existed between the potential peak and the logarithm of the frequency, as described by Eq. (1) [21].

$$\frac{\Delta E_p}{\Delta \log f} = \frac{2.3RT}{n\alpha F} \quad (1)$$

where α is the transfer coefficient, *n* the number of electrons involved in the reaction and the other terms have their usual meaning.

From the slopes of the graphs E_p versus log(*f*), the values of *nα* for the first (1.11) and the second (1.09) peaks were calculated according to Eq. (1). As the number of electrons transferred in each step is known ($n = 2$) [22], the transfer coefficient for both stages could be estimated as being equal to 0.56 and 0.55.

3.3. Effect of pulse amplitude

Another important parameter involved in SWV is the pulse amplitude (ΔE_a). Fig. 4 shows the square wave voltammograms for both peaks obtained by applying pulse amplitude values in the range of 10–50 mV. For analytical applications, a value of 50 mV was chosen for ΔE_a. The peak currents registered for different values of ΔE_a allow to calculate the surface coverage (Γ) by adsorbed DIP molecules according to Eq. (2) [23].

$$\frac{\partial I_p}{\partial \Delta E_a} = 500An\alpha n^2 Ff\Delta E_i\Gamma \quad (2)$$

where $\partial I_p/\partial \Delta E_a$ is the slope of the graph I_p versus ΔE_a, *A* (0.012 cm^2) is the electrode area and the other terms have their usual meaning.

From Eq. (2), the quantity of DIP adsorbed on the electrode surface was estimated to be 3.08×10^{-10} mol cm^{-2}.

3.4. Effect of scan increment

The last parameter investigated was the scan increment (ΔE_i), which was varied from 1 mV to 7 mV. Fig. 5 shows the square wave voltammograms for both peaks, suggesting that an increase in the scan increment is responsible for a better sensibility in the analysis. However, it can be seen that as the scan increment increases, the peaks turn to be wider and this fact implies loss of selectivity. In this way, a scan increment of 2 mV was chosen for the analytical applications.

3.5. Analytical determination of DIP in commercial formulations

After the optimization of pH and SWV parameters, analytical curves were obtained in the range of 1.28 μM–7.02 μM

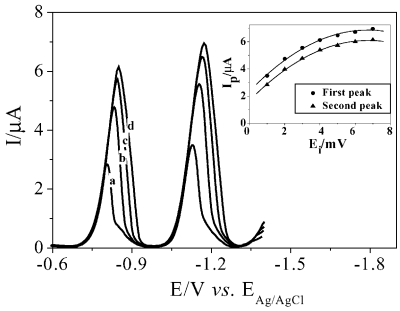

Fig. 5. SWV curves for DIP 0.10 mM in 0.10 M phosphate buffer solution (pH 3), using a HMDE ($A = 0.012\ cm^2$). ΔE_i: (a) 2 mV; (b) 4 mV; (c) 6 mV and (d) 7 mV; $f = 100\ s^{-1}$ and $\Delta E_a = 50$ mV. Insert: I_p vs. ΔE_i curves.

Table 1
Mean values for the determination of DIP ($n = 5$) in tablets and injections of Persantin® using SWV and UV–vis spectrophotometry

Technique	Tablets			Injections		
	DIP added (mg)	DIP found (mg)	Apparent recovery (%)	DIP added (mg)	DIP found (mg)	Apparent recovery (%)
SWV	75.0	75.6 ± 0.4	100.8 ± 0.5	10.0	10.4 ± 0.1	103.4 ± 0.7
UV–vis	75.0	68.9 ± 0.3	91.8 ± 0.4	10.0	9.9 ± 0.1	99.9 ± 0.7

by the standard addition method. Fig. 6 showed the square wave voltammograms for the second peak and in the insert the analytical curve ($n = 3$). The detection limit (DL) was calculated by the relation DL = $3\sigma/\theta$, where σ is the standard mean deviation for ten voltammograms registered for the blank and θ, the slope of the analytical curve. It was found a DL of 1.88×10^{-8} M (9.50 ng/ml) for the optimized conditions. The repeatability and the reproducibility of the method were determinated by successive measurements of DIP solutions with concentration in the range of the analytical curve with a variation coefficient of 0.97% ($n = 5$) and 1.15%, respectively.

3.6. Apparent recovery studies

The apparent recoveries were performed as recommended by IUPAC [18] using the currents for the second peak. Table 1 shows the mean obtained recovery percentages for tablets and injections of Persantin®.

In order to validate the methodology and confirm its potential in routine monitoring of DIP, given the simplicity as well as the time and cost-saving of the present proposed method of analysis, the same samples were subjected to a procedure using UV–vis spectrophotometry, and the apparent recoveries were performed at 280 nm.

For both techniques, five aliquots of the solution were analyzed by the standard addition method. The determination of DIP in Persantin® tablets gave a mean value of 75.6 ± 0.4 mg (100.8%) and 68.9 ± 0.3 mg (91.8%) for SWV and UV–vis spectrophotometry, respectively. In the case of injections it was found 10.4 ± 0.1 mg (103.4%) and 9.9 ± 0.2 mg (99.9%) for SWV and UV–vis spectrophotometry. Both apparent recoveries obtained for the proposed method for the two types of formulations are in good accordance with the declared value of 75 mg (tablets) and 10 mg (injections).

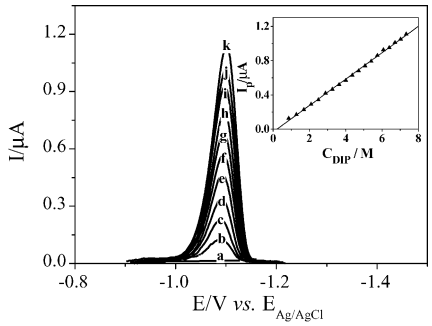

Fig. 6. SWV curves for DIP stock solution 4.40×10^{-5} M in 0.10 M phosphate buffer solution (pH 3.0), using a HMDE ($A = 0.012$ cm^2). Concentrations: (a) 0.43 μM; (b) 1.28 μM; (c) 2.09 μM; (d) 2.87 μM; (e) 3.63 μM; (f) 4.36 μM; (g) 5.06 μM; (h) 5.73 μM; (i) 6.39 μM; (j) 7.02 μM and (k) 7.33 μM, second peak; $f = 100\ s^{-1}$. $\Delta E_a = 50$ mV and $\Delta E_i = 2$ mV. Insert: analytical curve ($R = 0.9991$).

4. Conclusions

In this work, we have described the quantitative determination of Dipyridamole (DIP) using SWV and UV–vis spectrophotometry in phosphate buffer solution (pH 3.0; 0.1 M). The SWV methodology is rapid and simple for the determination proposed in pharmaceutical formulations in relation to other conventional methods, such as UV–vis spectrophotometry. The sample preparation procedure is very simple since there is no need to eliminate the excipients, thus it results an increase in sensitivity and accuracy. The satisfactory results, small coefficients of variation and good recuperation obtained allow to recommend the procedure for the quality control of DIP in pharmaceutical preparations.

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References

[1] D. Darbar, N. Gillespie, G. Main, A.B. Bridges, N.S.J. Kennedy, T.H. Pringle, G.P. Mcneill, Am. J. Cardiol. 78 (1996) 736–740.
[2] J.P. de La Cruz, T. Carrasco, G. Ortega, F. Sánches de la Cuesta, Lipids 27 (1992) 192–194.
[3] L. Iuliano, J.Z. Pedersen, G. Rotilio, D. Ferro, F. Violi, Free Radical Biol. Med. 18 (1995) 239–247.
[4] M. Castilho, L.E. Almeida, L.H. Mazo, M. Tabak, Anal. Chim. Acta 375 (1998) 223–231.
[5] M. Castilho, L.E. Almeida, M. Tabak, L.H. Mazo, Electrochim. Acta 46 (2000) 67–75.
[6] M. Castilho, L.E. Almeida, M. Tabak, L.H. Mazo, J. Braz. Chem. Soc. 11 (2000) 148–153.
[7] M. Castilho, A.M.P. Almeida, L.E. Almeida, M. Tabak, L.H. Mazo, J. Electroanal. Chem. 374 (2002) 289–293.
[8] A.S. Issa, M.S. Mahrous, M.A. Salam, Talanta 34 (1987) 670–672.
[9] J.A.M. Pulgarín, A.A. Molina, P.F. López, Anal. Chim. Acta 370 (1998) 9–18.
[10] C. Deballon, M. Guernet, J. Pharm. Biomed. Anal. 6 (1987) 1045–1048.
[11] M.B. Heyob, J.L. Merlin, L. Pons, M. Calco, B. Weber, J. Liq. Chromatogr. 17 (1994) 1837–1848.
[12] J. Zhang, R.B. Miller, S. Russell, R. Jacobus, J. Liq. Chromatogr. Related Technol. 20 (1997) 2109–2121.

[13] J.A.M. Pulgarín, A.A. Molina, P.F. López, Analyst 122 (1997) 253–258.
[14] M. Tunçel, Y. Yazan, D. Dogrukol, Z. Atkosar, Anal. Lett. 24 (1991) 1837–1846.
[15] X. Zeng, S. Lin, N. Hu, Talanta 40 (1993) 1183–1187.
[16] Z. Wang, H. Zhang, S. Zhou, Talanta 44 (1997) 621–626.
[17] M.M. Ghoneim, A. Tawfik, A. Radi, Anal. Bioanal. Chem. 374 (2002) 289–293.
[18] D.T. Burns, K. Danzer, A. Townshend, IUPAC Recomm. (2001) 1–5.
[19] I.E. Borissevitch, C.P.F. Borges, G.P. Borissevitch, V.E. Yushmanov, S.R.W. Louro, M. Tabak, Z. Naturforsch. 51C (1996) 578–590.
[20] I.E. Borissevitch, C.P.F. Borges, V.E. Yushmanov, M. Tabak, Biochim. Biophys. Acta 1238 (1995) 57–62.
[21] M. Lovric, J. Electroanal. Chem. 248 (1988) 239–253.
[22] R.A. de Toledo, M. Castilho, L.H. Mazo, J. Braz. Chem. Soc. 15 (2004) 407–412.
[23] M. Lovric, S.K. Lovric, R.W. Murray, Electrochim. Acta 33 (1988) 739–744.