Orbital: The Electronic Journal of Chemistry
journal homepage: www.orbital.ufms.br
ISSN 1984-6428

| Vol 8 | | No. 3 | | April-June 2016 |

Full Paper

Sensitive Voltammetric Determination of Ticlopidine in Pharmaceuticals Employing a Multi-Walled Carbon Nanotubes Paste Electrode

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Article history: Received: 14 December 2015; revised: 08 March 2016; accepted: 20 June 2016. Available online: 30 June 2016. DOI: http://dx.doi.org/10.17807/orbital.v8i3.831

Abstract: A sensitive analytical procedure has been developed for the determination of ticlopidine using a multi-walled carbon nanotubes paste electrode (MWCNTsPE). Direct oxidation of analyte was observed by cyclic voltammetry as evidenced by the presence of well-shaped irreversible peak at 1.05 V vs Ag/AgCl (3.0 mol L^{-1} KCl) in Britton-Robinson buffer solution (pH 5.0). The use of this electrode has been found to influence the electrochemical determination by presenting a higher intensity of oxidation current for ticlopidine and also a lower detection limit. Using differential pulse voltammetric modality, the obtained analytical curve was linear for ticlopidine concentration ranging from 0.75 to 20 µmol L^{-1}, with detection limit of 0.10 µmol L^{-1}. The proposed method was successfully used to determination of ticlopidine in pharmaceuticals, with satisfying results**.** In order to indicate that the method is of potential application in biological fluids adequate recovery results were obtained for the determination of ticlopidine in synthetic urine sample.

Keywords: ticlopidine determination; paste electrode; differential pulse voltammetry; urine sample

1. INTRODUCTION

Ticlopidine is an oral drug that inhibits the ability of platelets to clump and form blood clots. It works by thinning the blood, which helps reduce the risk of blood clots and stroke. It is used in patients in whom aspirin is not tolerated. This drug has a fast absorption and is metabolized in the liver. In two hours is almost completes the absorption of the ticlopidine and the most of 40% the orally administered drug is excreted in the urine. However, there is a major concern regarding the safety of ticlopidine, which is associated with severe and sometimes fatal blood dyscrasias. Inappropriate use of ticlopidine may cause changes in the blood, as a reduction of white blood cells or platelets. An overdose of ticlopidine can cause a very high risk of bleeding; in case of smaller doses of the drug, the antiplatelet effect will be greatly reduced [1, 2]. Therefore, the development of a sensitive and selective method for its determination in pharmaceuticals is highly desirable for both quality control purposes and clinical applications.

Several analytical methods for the determination of ticlopidine have been reported in the literature for pharmaceutical formulations and biological samples, such as gas chromatography [3], high performance liquid chromatography [4–6], ultra pressure liquid chromatography [7], near infrared reflectance spectroscopy [8] and spectrophotometry [9, 10].

A survey of the literature shows few studies describing electroanalytical methods for the determination of ticlopidine. Türköz and Onar [11] developed a polarographic method for ticlopidine determination in pharmaceutical preparations using a hanging mercury drop electrode (HMDE). This method presents a linear concentration range of 1.96 to 113 µmol L^{-1}, with detection limit of 0.517 µmol L^{-1} in 0.5 mol L^{-1} phosphate buffer (pH 5.0). Recently, square-wave voltammetric method for ticlopidine determination in pharmaceutical and human urine sample using a boron-doped diamond electrode (BDDE) was reported [12]. In this study, the obtained analytical curve was linear for the ticlopidine

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concentration range of 3.9 to 38.4 μmol L^{-1}, with a detection limit of 0.66 μmol L^{-1} in Britton-Robinson (BR) buffer solution (pH 5.0).

The determination of organic molecules in pharmaceutical dosage forms and biological samples using voltammetry has increased greatly over the last decades. The advantages of this method, that is, simplicity of operation, low-cost instrumentation, highly sensitivity, selectivity, economical, rapidity of data acquisition, and there is also the possibility of analysis of colored or solutions with suspended solids [13,14], are most significantly different from those of other conventional methods, such as chromatography and spectrophotometry. Some studies in the literature reports that the use of carbon nanotubes improves the performance of many electrodes for any applications, which reduces electrode surface fouling and presents a high surface area and a chemical stability [18, 15–21]. To the best of our knowledge, carbon nanotubes have not been used for determination of ticlopidine in pharmaceutical and biological samples. Additionally, in spite of previous publication by our research group on the ticlopidine determination using BDDE, the advantages of carbon nanotubes paste electrode here presented over BDDE are assigned to the higher magnitude of the peak current achieved by the nanomaterial, which in turn can improve the detectability of electrochemical sensor.

Taking these attributes into consideration, in this work a novel voltammetric method for ticlopidine determination using a multi-walled carbon nanotubes paste electrode (MWCNTsPE) is described. The proposed method was successfully applied in determination of ticlopidine in pharmaceutical formulations and the obtained results have been statistically compared with those obtained using spectrophotometry as comparative method [9]. Also, it was applied in the determination of ticlopidine in synthetic urine sample, with satisfying results, showing potential application of this method in biological fluids.

2. MATERIAL AND METHODS

2.1. Reagents and apparatus

Ticlopidine hydrochloride was obtained from Sigma-Aldrich. Boric acid, acetic acid, phosphoric acid and sodium hydroxide were obtained from Synth. Multi-walled carbon nanotubes (MWCNTs; of 10–40 nm in diameter and 5-20 μm in length; purit: 93%) was obtained from CNT Co. Ltd., Korea. Commercial pharmaceutical samples (250 mg of ticlopidine per tablet) were purchased from a local drugstore, city of Londrina, state of Paraná, in Brazil. All chemicals were of analytical reagent grade and solutions were prepared using ultra-purified water (resistivity > 18 MΩ cm) supplied by a Milli-Q system (Millipore®).

A BR buffer solution (pH 5.0) was chosen as supporting electrolyte. It was prepared by mixing of 0.040 mol L^{-1} of all necessary components (acetic acid, phosphoric acid and boric acid), with pH adjusted with a 2.0 mol L^{-1} NaOH solution.

A 10 mmol L^{-1} stock solution of ticlopidine was prepared before use in BR buffer solution (pH 5.0). Appropriate dilutions were made from this solution with supporting electrolyte.

A PalmSens potentiostat/galvanostat controlled with the PalmSens PC software was employed with the single-compartment glass cell containing three electrodes, MWCNTsPE (0.196 cm^2 exposed geometrical area) as working electrode, an Ag/AgCl (3.0 mol L^{-1} KCl) as reference electrode and a Pt wire as auxiliary electrode. Since dissolved oxygen did not interfere in anodic potential window, no deaeration of solution was needed. The glassy carbon electrode (GCE; 5 mm diameter) was used for comparison of the results. It was mechanically polished with 0.05 μm alumina powder and rinsed with doubly distilled water, sonicated for 5 min in absolute ethanol and then in ultrapure water; the polished GCE was dried at room temperature.

2.2. Preparation of MWCNTsPE

Multi-walled carbon nanotubes were purified with 2.0 mol L^{-1} HCl and then, treatment with mixture HNO_3:H_2SO_4 (3:1, v/v) for 12 h at room temperature. It promotes the partial destruction of carbon nanotubes and introduction of carboxyl groups at the ends or at the sidewall defects of the nanotubes structure. After this, the suspension was centrifuged, and the nanotubes was washed several times with ultrapure water until pH 6.5–7.0, and then dried at 120 °C for 6 h, as reported elsewhere [19].

The MWCNTsPE was prepared by mixing functionalized multi-walled carbon nanotubes (MWCNTs) and mineral oil (Nujol®) at a ratio of 30:70% (w/w). MWCNTs and mineral oil were carefully homogenized in a Petri dish with a stainless steel spatula for 10 minutes. The MWCNTs paste was

packed into an electrode body, consisting of a plastic cylindrical tube (60 × 6 mm) equipped with a stainless steel shaft serving as an external electric contact, which appropriate packing was achieved by pressing the electrode surface against a filter paper. This electrode presents an electroactive area of 0.0875 cm^2, which it was determined from the slope of plot I_{ap} vs $v^{1/2}$, according to Randles-Sevcik equation, using 5.0 mmol L^{-1} $K_3Fe(CN)_6$ in 0.10 mol L^{-1} KCl. The diffusion coefficient (D_o) for potassium hexacyanoferrate (III) of 6.2 × 10^{-6} cm^2 s^{-1} [22] was used in this study. The prior electrochemical activation was carried out by cyclic voltammetry, by cycling the potential between 0.0 and 1.3 (15 cycles) in BR buffer solution (pH 5.0), the supporting electrolyte. The carbon paste electrode has been widely used for sensor preparation and although they are manually prepared the reproducibility on the sensor preparation depends on this previous electrochemical activation as previously mentioned [18,20,21,25].

The pH measurements of a BR buffer solution were obtained using a combined glass electrode with an Ag/AgCl (3.0 mol L^{-1} KCl) external reference electrode connected to a pH-meter (Hanna Instruments, model HI-221).

2.3. Analytical procedures

Cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square-wave voltammetry (SWV) were employed for investigation and determination of ticlopidine. SWV operating parameters (frequency (*f*), pulse amplitude (ΔE) and scan increment (ΔE_S)) and DPV operating parameters (pulse amplitude (ΔE), scan rate (v) and modulation time (t)) were optimized.

Analytical curves were obtained by addition of aliquots of the previously prepared ticlopidine standard solutions into the electrochemical cell containing 10.0 mL of the BR buffer solution (pH 5.0). Detection limit (LOD) was calculated as three times the standard deviation for 10 measurements of the blank solution divided by the slope of the respective analytical curve [23].

For sample preparation, 10 tablets containing ticlopidine were weighed and a suitable amount of the powder was transferred to 10.0 mL calibrated volumetric flasks containing BR buffer solution (pH 5.0). Then, 150 µL of this solution was transferred to 1.5 mL volumetric flask and this volume was completed with BR buffer solution (pH 5.0). For each sample, an aliquot of this solution (75 µL) was directly transferred to the electrochemical cell containing 10 mL of BR buffer solution (pH 5.0), after which the voltammograms were obtained. The ticlopidine concentration in each sample was determined using the regression equation of previously plotted analytical curve obtained with standard solutions of the ticlopidine.

The synthetic urine sample was prepared by dissolution of 0.731 g of NaCl, 0.275 g of $CaCl_2.2H_2O$, 0.400 g of KCl, 0.563 g of Na_2SO_4, 0.350 g of KH_2PO_4, 0.250 g of NH_4Cl, and 6.25 g of urea in a 250 mL volumetric flask and the volume was completed with water [24]. An aliquot volume of fresh synthetic urine (1.0 mL) was placed into the electrochemical cell containing 9.0 mL of BR buffer solution (pH 5.0). Considering the range of the analytical curve, this solution was suitable spiked with standard solution of ticlopidine to achieve a required concentration.

In order to compare the results obtained with the proposed method, a spectrophotometric method described by Kakde *et al.* [9] was employed, with minor modifications, as described elsewhere [12]. The absorbance was measured at 213.6 nm. The standard reference spectrophotometric ticlopidine determination was carried out using ThermoSpectronic spectrophotometer UV-visible, model Genesys, coupled to a computer, employing a 1 cm quartz cell.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of ticlopidine on MWCNTsPE

Figure 1 shows the cyclic voltammograms in the absence and presence of 0.10 mmol L^{-1} ticlopidine in BR buffer solution (pH 5.0) on the MWCNTsPE. It is evident that during the anodic scan, the single and distinct oxidation peak was observed at potential of 1.05 V *vs* Ag/AgCl (3.0 mol L^{-1} KCl), which the oxidation potential value is lower than observed by BDDE [12].

It is worthwhile to mention that the response obtained by MWCNTsPE was compared with the cyclic voltammetric response of GCE for 0.10 mmol L^{-1} ticlopidine in BR buffer solution (pH 5.0) (insert in Fig. 1). Despite of ticlopidine presents a higher oxidation potential value employing MWCNTsPE, a

better definition of peak current was observed, with an excellent repeatability (RSD < 1.0 %, for N = 6). Additionally, the peak current for ticlopidine obtained on the GCE remarkable decreases after second measurement, clearly indicating that the adsorption effects can be occurred at the GCE surface. After each measurement, there is necessity of regenerate the surface of GCE by polishing the same for recovery the current signal, which it unfeasible the analytical purposes. Using MWCNTsPE, these effects were not observed. Hence, further studies were carried out only with this electrode

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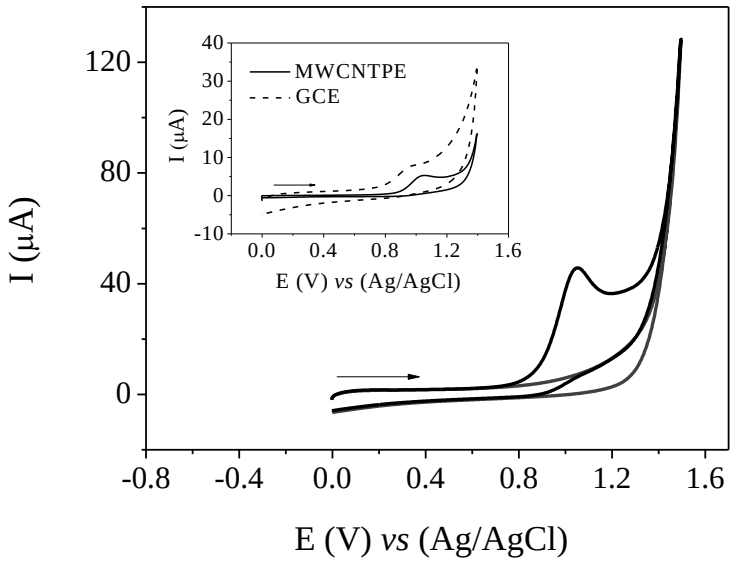

Figure 1. Cyclic voltammograms (40 mV s^{-1}) at BR buffer solution (pH 5.0) on MWCNTsPE in absence (dark gray solid line) and presence (black solid line) of 0.10 mmol L^{-1} ticlopidine. Insert: Comparison of MWCNTsPE with GCE in these same conditions.

The effects of mass ratio MWCNTs and Nujol on the paste was investigated in the analytical response. In this study, the following mass ratios (MWCNT:Nujol) of 20:80, 30:70 and 40:60 m/m for 0.10 mmol L^{-1} ticlopidine in BR buffer solution (pH 5.0) were used. A higher analytical signal was observed for the paste composition containing 40 % of nanotubes, which presents a loss of nanotubes during the measurements. Thereby, the mass ratio 30:70% was used in the paste composition for the ticlopidine determination in subsequent measurements. This composition makes possible to obtain a homogeneous paste and promotes a smoother electrode surface with good conductivity, an excellent repeatability and accuracy of analytical results, as also observed for other analytes [20, 21, 25].

3.2. Effect of different surfactants

The voltammetric behavior of 0.10 mmol L^{-1} ticlopidine in BR buffer solution (pH 5.0) was investigated in the absence and presence of some types of surfactants at concentration of 10 μmol L^{-1}, such as cetyltrimethylammonium bromide (CTAB), cetylpyridinium bromide (CPB) and sodium dodecyl sulfate (SDS). Ticlopidine molecule (pKa 7.31) [26] is partially protonated at pH 5.0 and electrode surface has a negative charges, due to introduction of polar hydrophilic surface groups after suitable acid treatment, mainly carboxyl group at the ends or at the sidewall defects of the nanotubes structure. So, in the presence of cationic surfactant (CTAB or CPB) a higher oxidation peak potential and similar anodic peak current were obtained when compared with ticlopidine in absence of both surfactants. Probably, there is competition between ticlopidine (positively charged) and CTAB or CPB (positively charged) in the active sites of the nanotubes, which its oxidation in the electrode surface is more difficult. On the other hand, in the presence of SDS (negatively charged) ticlopidine oxidizes in the same oxidation potential with a lower anodic peak current, probably due the repulsion of SDS and the electrode surface. No repeatability of analytical signals was obtained in the presence of surfactants. Additionally, no surfactant

was used for the development of the voltammetric method for ticlopidine determination using MWCNTsPE.

3.3. Effect of pH and supporting electrolyte

The CV was used to investigate the effect of pH (2.0 – 5.0) on the voltammetric response for 0.10 mmol L^{-1} ticlopidine with MWCNTsPE, using a BR buffer solution. Fig. 2 displayed the effect of pH on the oxidation peak current. Increasing the pH of the supporting electrolyte, the magnitude of this current was increased. In fact, for the measurements carried out at pH < pKa (7.31) [27], an electrostatic attraction between ticlopidine cation with the carboxyl groups of MWCNTsPE could be expected. The voltammetric responses for ticlopidine were characterized by well-defined oxidation peak and higher analytical signal with an excellent repeatability of analytical signals. In pH > 5.0 the solubility of ticlopidine decreases; thus the oxidation of ticlopidine was not investigated. On the other hand, the peak potential shifted to less positive values as the pH was increased from 2.0 to 5.0 (insert in Fig. 2), demonstrating proton participation in the electrode reaction of ticlopidine on the MWCNTsPE. This dependence is linear over this pH range, according to the following equation (Eq. 1):

$$E_{ap}\ (V) = 1.32 - 0.0535\ pH \qquad (r = 0.987)$$

The slope obtained for ticlopidine oxidation in Figure 2 (0.053 V pH^{-1}) is close to Nernstian theoretical value (0.059 V pH^{-1}). In this sense, we can interpret that the oxidation process can be assigned to oxidation of the thiofene group including two electrons and two protons [12, 28].

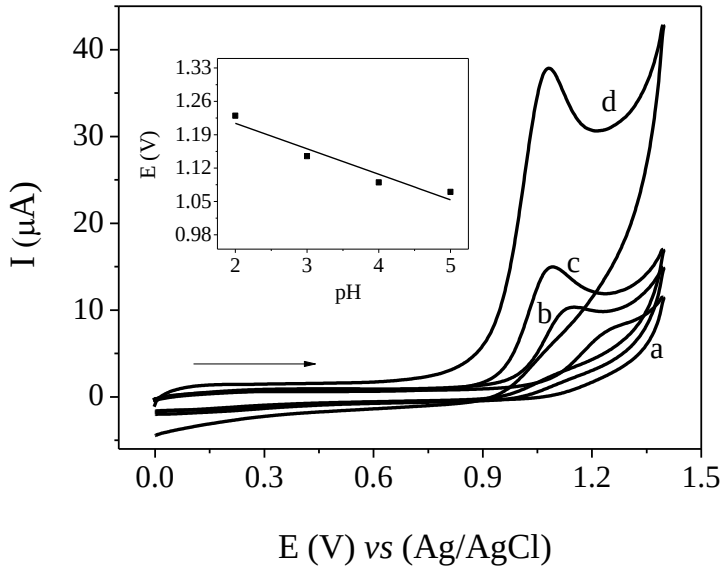

Figure 2. Cyclic voltammograms (40 mV s^{-1}) for the oxidation of 0.10 mmol L^{-1} ticlopidine in BR buffer solution employing MWCNTsPE at different pH values: (a) 2.0, (b) 3.0, (c) 4.0, (d) 5.0. Insert: effect of pH on the peak potential.

Moreover, the effect of the supporting electrolyte on the oxidation activity of ticlopidine was comparatively investigated for acetate, McIlvaine and BR buffer solutions at pH 5.0. It was found that the electrochemical behavior of ticlopidine in these supporting electrolytes was very similar, but the best repeatability for the oxidation signal of ticlopidine was obtained with the BR buffer solution. Therefore, this supporting electrolyte and pH was selected for the electroanalytical determination of ticlopidine in real samples.

3.4. Effect of scan rate

The effect of scan rate on the electrochemical behavior of 0.10 mmol L^{-1} ticlopidine in BR buffer solution (pH 5.0) has been investigated ranging from 5 to 250 mV s^{-1} by CV. As can be seen from Fig. 3, the oxidation peak shifted towards the positive direction with the increasing of scan rate, which was one of the characteristic features of the irreversible electrode reactions. The oxidation peak is linear with scan rate (inserted in Fig. 4), suggesting an adsorption-controlled mechanism [22] (Eq. 2):

I_{ap} (μA) = 7.29 + 0.455 ν (mV s^{-1}) (r = 0.995)

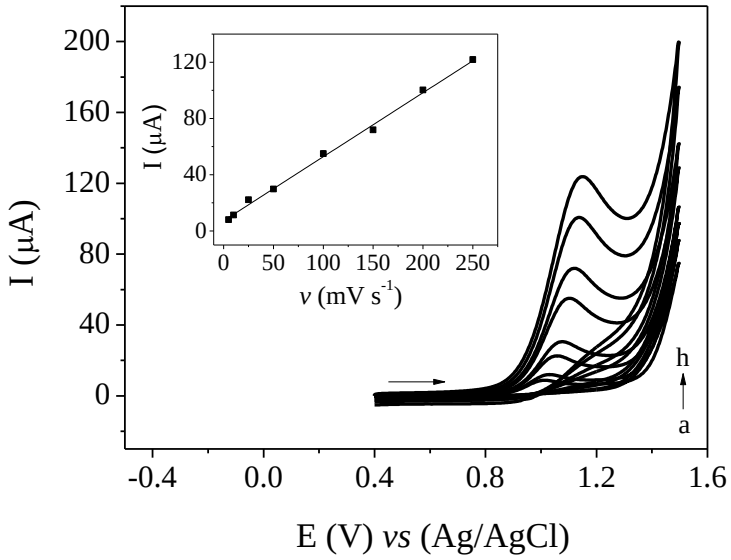

Figure 3. Cyclic voltammograms of 0.10 mmol L^{-1} ticlopidine in BR buffer solution (pH 5.0) obtained using MWCNTsPE at the following scan rates (ν): (a – h) 5 – 250 mV s^{-1}. Insert: linear relationship between the oxidation peak current and scan rate.

3.5. Optimization of operating parameters of SWV and DPV and analytical curves

The SWV parameters were optimized for 50 μmol L^{-1} ticlopidine in BR buffer solution (pH 5.0). This optimization was carried out in order to obtain current responses for the electrochemical oxidation of ticlopidine with highest magnitude and best peak shape. The ranges studied and the optimized values are shown in Table 1.

Table 1. Optimized DPV and SWV parameters for determination of ticlopidine in BR buffer solution (pH 5.0).

Parameters	Studied range	Optimum value
DPV		
Pulse amplitude (A) (mV)	10 – 125	75
Scan rate (ν) (mV s^{-1})	10 – 60	40
Modulation time (t) (ms)	5 – 20	7
SWV		
Square wave frequency (f) (Hz)	10 – 60	50
Pulse amplitude (A) (mV)	10 – 50	40
Scan increment (ΔEs) (mV)	1 – 4	4

These previously optimized DPV and SWV experimental parameters were used to record the analytical curves for ticlopidine in BR buffer solution (pH 5.0) using the MWCNTsPE. The analytical parameters associated to these curves are summarized in Table 2. According to these results, the best values for analytical parameters (broader linear range, slope and lower detection limit) were obtained using DPV. Hence, it was selected for the determination of ticlopidine in real samples. Fig. 4 and Fig. 5 shows the differential pulse and square-wave voltammograms and the respective analytical curve obtained after successive additions of the ticlopidine standard solution using MWCNTsPE. The anodic peak currents for ticlopidine increase linearly with their concentrations.

Table 2. Analytical parameters for the voltammetric determination of ticlopidine in BR buffer solution (pH 5.0) by DPV and SWV using MWCNTsPE.

	DPV	SWV
Peak potential (V)	1.04	1.03
Linear range (μmol L^{-1})	0.75 to 20	0.75 to 15
Correlation coefficient, r	0.995	0.989
Slope (μA mol^{-1} L)	3.7×10^5	2.0×10^5
Intercept (μA)	–0.048	–0.14
Detection limit (μmol L^{-1})	0.10	0.20

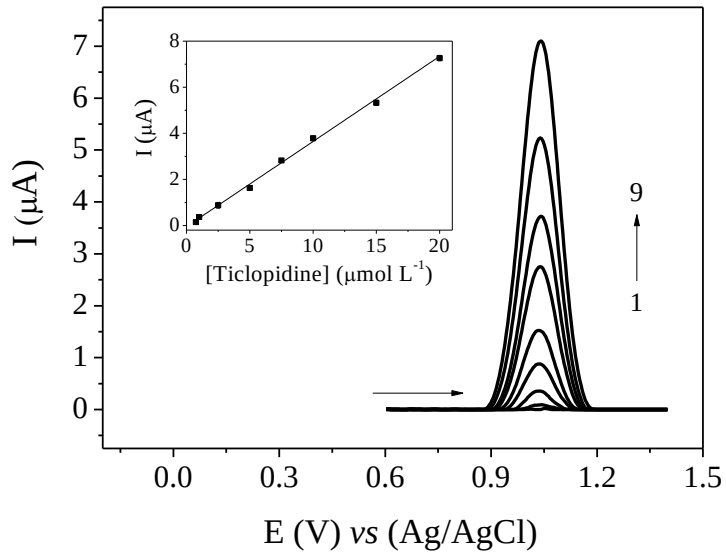

Figure 4. Differential pulse voltammograms obtained for the oxidation of ticlopidine in BR buffer solution (pH 5.0) using MWCNTsPE for the following concentrations of ticlopidine: (2 – 9): 0.75 – 20 μmol L^{-1}. Insert: Corresponding analytical curve for ticlopidine oxidation process. DPV conditions: A = 75 mV, ν = 40 mV s^{-1}, and t = 7 ms.

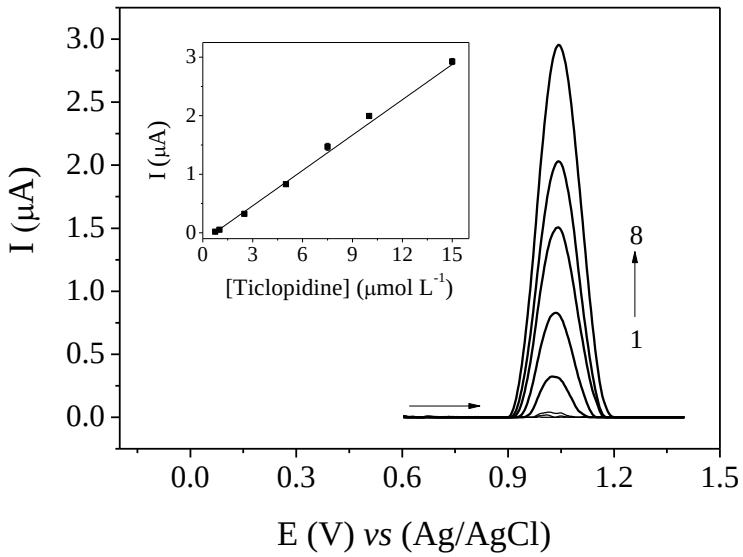

Figure 5. Square-wave voltammograms obtained for the oxidation of ticlopidine in BR buffer solution (pH 5.0) using MWCNTsPE for the following concentrations of ticlopidine: (2 – 8): 0.75 – 15 μmol L^{-1}. Insert: Corresponding analytical curve for ticlopidine oxidation process. SWV conditions: A = 40 mV, f = 50 mV s^{-1}, and ΔE_S = 4 mV.

Intra-day repeatability of the peak current magnitude was tested by 10 replicates of DPV measurements at 7.5 μmol L^{-1} in BR buffer solution (pH 5.0). The inter-day repeatability of magnitude of the peak current was evaluated by measuring the peak current for similar fresh solutions over a period of 5 days. A good RSD values were obtained: intra-day, 2.2% and inter-day, 1.7%, indicating that this electrode provide to be suitable electrochemical sensor for the precise determination of ticlopidine.

The fabrication reproducibility of the MWCNTsPE was assessed in three different pastes by

measuring the peak current of 7.5 μmol L^{-1} ticlopidine in BR buffer solution (pH 5.0), which were constructed independently by the same procedure. Relative standard deviation value of 2.4% was obtained among these three electrodes, confirming that the preparation of the paste is reproducible. Besides, the stability of the MWCNTsPE was investigated. After 60 measurements, the initial voltammetric response decrease in average 7.5%, which the renovation of surface was realized for achievement of reproducible results.

3.6. Comparison with other electroanalytical methods

The analytical characteristics resulting from our proposed novel method and those obtained with other electrodes are summarized in Table 3. These results reveal that the detection limit for ticlopidine obtained in this work is lower than those obtained using HMDE [11] and BDDE [12]. Moreover, this electrode provides simplicity of preparation and use, low cost, very high stability and thus can be alternatively used for the determination of ticlopidine in pharmaceutical formulations and biological samples. Although renewal of the electrode surface is required after 60 measurements, excellent repeatability and reproducibility were obtained; the electrode is mechanically robust. To conclude, MWCNTsPE represents a sensitive electrochemical sensor for ticlopidine determination, satisfying the demands of modern electroanalytical chemistry.

Table 3. Comparison of characteristics of the proposed method with the previously reported voltammetric methods for the determination of ticlopidine.

Electrode	Technique	Concentration range (μmol L^{-1})	LOD (μmol L^{-1})	Reference
HMDE	SWP	1.9 – 113	0.52	[11]
BDDE	DPV	3.9 – 38.4	0.66	[12]
MWCNTsPE	DPV	0.75 – 20	0.10	This work

3.7. Interference study

The selectivity of the proposed method was evaluated by the addition of possible interferents (commonly present in the analyzed pharmaceutical formulations and urine samples), such as starch, polyvinyl alcohol, citric acid, povidone, methylcellulose, and magnesium stearate, ascorbic acid, caffeine, epinephrine, dopamine, urea, and uric acid, to standard solution containing ticlopidine, at the concentrations ratios (standard solution:interferent compound) of 1:1, 1:10, and 10:1 (w/w). The corresponding oxidation peak currents were compared with those obtained in the absence of each interferent. The analysis of the obtained responses allowed concluding that these compound do not significantly interfere (< 5 %) in the determination of ticlopidine under the used working conditions. Fig. 6 shows the voltammetric response for any interferents commonly present in urine sample. According to this figure, a well-defined oxidation peak for ticlopidine appears at the potential around of 1.05 V and no oxidation peaks for the other analytes were observed in this potential value. No oxidation peak for caffeine or urea was observed employing MWCNTsPE in these conditions. Uric acid presents an oxidation peak at 0.45 V and its only presents a significant interference in concentration values above fifty times greater. Ascorbic acid presents one oxidation peak at 0.30 V. Thereby, these results revealed that the use of proposed method in this kind of analysis could be limited depending on the presence of particular excess of uric acid.

3.8. Application of the proposed method for the determination of ticlopidine in pharmaceutical and urine samples

Commercial pharmaceutical tablets containing ticlopidine (250 mg per tablet) were analyzed by from previously plotted calibration curves in order to evaluate the validity of the herein proposed method. The results obtained employing the proposed method as well as the spectrophotometric method [9] are presented in Table 4. The obtained average values and standard deviations are also presented in this table. As can be seen, no significant differences were observed between the values found for the ticlopidine amounts using the proposed and the comparative method. Moreover, applying a paired *t*-test to the results obtained by both methods, the resulting *t* value (1.11) is smaller than the critical value (12.7 α = 0.05); indicating that there is no difference between the obtained results, at a confidence level of 95% [29]. Recovery experiment yielded sufficient values of 94.0

and 103% indicating that the results obtained with the proposed procedure is not statistically different from the comparative spectrophotometric method, at a 95% confidence level.

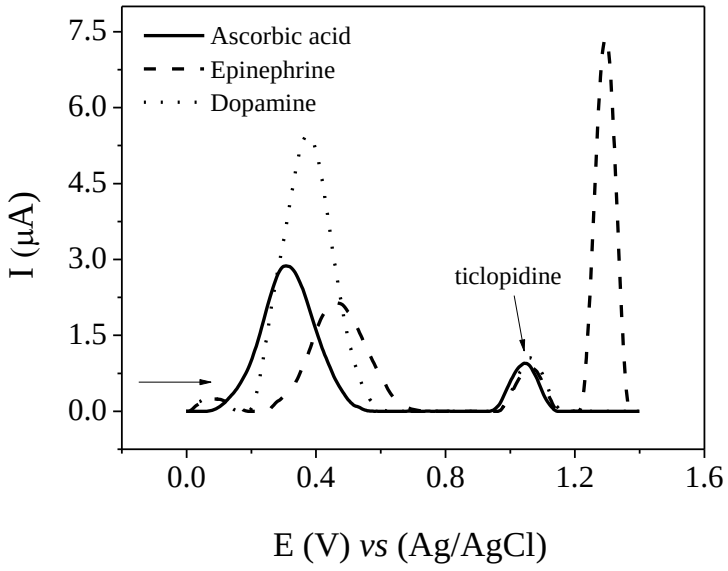

Figure 6. Differential pulse voltammograms obtained for the oxidation of ascorbic acid, epinephrine and dopamine at 75 μmol L^{-1} concentration in BR buffer solution (pH 5.0) containing 7.5 μmol L^{-1} ticlopidine. DPV conditions are the same as indicated in Fig. 5.

Table 4. Analysis of pharmaceutical tablets with declared amount of ticlopidine using proposed and reference methods.

Samples	ticlopidine (mg/tablet)			Relative error[b] (%)
	Label value	Spectrophotometric method[a]	DPV method[a]	
A	250	253 ± 2	258 ± 1	2.0
B	250	256 ± 4	240 ± 4	–6.2
C	250	256 ± 4	252 ± 3	–1.5

[a]Average of 3 measurements.
[b]Relative error (%) = 100 × (DPV method – Spectrophotometric method / Spectrophotometric method).

Subsequently, the proposed method was applied to determine ticlopidine in spiked synthetic urine samples employing the standard addition method under the optimized experimental conditions. Two different ticlopidine concentrations were spiked in one sample of the synthetic urine: 5.0 and 7.5 μmol L^{-1}. The recoveries obtained were between 92.8 ± 3% and 99.8 ± 4%, respectively. These results indicate that the proposed method could be efficiently used for the determination of the ticlopidine in complex matrices, as in urine sample, has also done by Scremin *et al.* [12].

4. CONCLUSION

The obtained results showed that the MWCNTsPE can be used in conjunction with a DPV technique for electrochemical behavior study and simple, rapid and precise determination of ticlopidine. Compared with GCE, BDDE and HMDE, the MWCNTsPE, used for the first time as sensor for the detection of ticlopidine, showed a higher sensitivity and analytical response. The oxidation of ticlopidine has been found to be pH-dependent and involves two electrons and two protons. Under optimized conditions, the MWCNTsPE showed a wide linear range from 0.75 – 20 μmol L^{-1} in BR buffer solution (pH 5.0), with low LOD of 0.10 μmol L^{-1}. Practical applicability of the proposed method was demonstrated on the analysis of the ticlopidine in pharmaceutical and urine samples, with satisfying results. This electrode can easily be produced and it has fast response, stability, practical surface renewal and good lifetime. Moreover, this procedure is simple, rapid and sensitive for quantitative determination of ticlopidine.

5. ACKNOWLEDMENTS

Financial support and scholarships from the Brazilian funding agencies Fundação Araucária, CNPq, and CAPES are gratefully acknowledged. The authors would like to express their especial acknowledgments to Prof. Dr. Luiz H. Dall'Antonia for the availability of use of potentiostat.

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