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A highly improved method for sensitive determination of amitriptyline in pharmaceutical formulations using an unmodified carbon nanotube electrode in the presence of sulfuric acid

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ABSTRACT

The present paper describes a novel, simple and reliable differential pulse voltammetric method for determining amitriptyline (AMT) in pharmaceutical formulations. It has been described for many authors that this antidepressant is electrochemically inactive at carbon electrodes. However, the procedure proposed herein consisted in electrochemically oxidizing AMT at an unmodified carbon nanotube paste electrode in the presence of 0.1 mol L^{-1} sulfuric acid used as electrolyte. At such concentration, the acid facilitated the AMT electroxidation through one-electron transfer at 1.33 V vs. Ag/AgCl, as observed by the augmentation of peak current. Concerning optimized conditions (modulation time 5 ms, scan rate 90 mV s^{-1}, and pulse amplitude 120 mV) a linear calibration curve was constructed in the range of 0.0–30.0 μmol L^{-1}, with a correlation coefficient of 0.9991 and a limit of detection of 1.61 μmol L^{-1}. The procedure was successfully validated for intra- and inter-day precision and accuracy. Moreover, its feasibility was assessed through analysis of commercial pharmaceutical formulations and it has been compared to the UV–vis spectrophotometric method used as standard analytical technique recommended by the Brazilian Pharmacopoeia.

1. Introduction

Amitriptyline (AMT) (Fig. 1) is a tricyclic antidepressant that possesses antipsychotic, sedative and analgesic properties. This drug can be used in the treatment of depression, child bedwetting and psychomotor disorders such as aggression, hyperkinetic states and agitation [1–3]. On the other hand, even at therapeutic doses, it may provoke collateral effects related to drowsiness, sedation, confusion, dry mouth and blurred vision, whereas its overdoses may result in conditions that affect heart rhythms and changes in blood pressure. Therefore, quality control of pharmaceutical formulations is important nowadays [4]. The majority of methods reported for AMT quantification have been carried out by means of high-performance liquid chromatography [5], spectrofluorimetry [6] and UV–vis spectrophotometry [7], which are usually time-consuming and expensive. Besides, these methods present low sensitivity (especially for absorption in the UV–visible region) and require sample pretreatment via organic solvent extraction. Thus, it is highly desirable to search alternative procedures. In this respect, electroanalytical methods can be an excellent choice. However, even considering low electroactivity at conventional electrodes based on noble metal surfaces, such as platinum or gold, even using glassy carbon, only a few approaches based on electrode surface modification have been proposed for AMT determination. One of them was conducted by Turk et al. [8], who electrodeposited poly(thiophene) and poly(carbazole) on the reticulated surface of a glassy carbon electrode to quantify AMT. According to these authors, the presence of electron-donor

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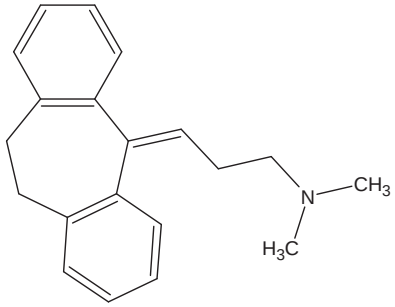

Fig. 1. Chemical structure of amitriptyline.

compounds that contain heteroatoms (e.g., sulfur (polythiophene) and nitrogen (polycarbazole)) enables the formation of radical cations which facilitate the electrooxidation of AMT and its voltammetric determination. In another study performed in a similar way, a carbon paste electrode modified with poly-(*N*-vinylimidazole) was developed [9]. Likewise, potentiometric measurements of AMT in a FIA system using an ion-selective electrode constructed with amitriptylinum phosphotungstate (Am-PTA) and amitriptylinum phosphomolybdate (Am-PMA) were carried out [10]. In another research, a carbon-polyurethane composite electrode (GPU) was employed to determine AMT by cyclic voltammetry [11]. However, in this study, the antidepressant was irreversibly oxidized and strongly adsorbed on the electrode surface with reagents and oxidation products. Another remark that should be pointed out relays on the fact that the majority of these electroanalytical methods have not been applied to real samples. Recently, our research group has developed a carbon paste electrode modified with DNA and inorganic matrix ($SiO_2/Al_2O_3/Nb_2O_5$) for quantifying AMT in pharmaceutical formulations [12].

Considering the aforementioned, to the best of our knowledge, the development of electroanalytical methods for the AMT determination using unmodified (bare) carbon nanotubes paste electrodes in sulfuric acid solutions and their application to real samples have not been reported until now. Several reports have demonstrated the advantages of carbon nanotubes in the sensor preparation, such as electrocatalytic effect towards oxidation/reduction of many compounds, thus reducing the overpotential, insignificant surface fouling of CNT-based electrodes, high surface area and high sensitivity [13–16]. Despite these features the authors emphasize the improvements of carbon nanotubes performance in the presence of a suitable electrochemical mediator. A survey of literature shows that electrochemical sensors based on carbon nanotubes and carbon electrodes modified with electrochemical mediator for determining neurotransmitters, amino acids, vitamins and drugs in biological, environmental and pharmaceutical samples have been extensively developed [17–26]. Thus, in order to obtain an innovative, simple and facile method we have employed an unmodified carbon nanotube electrode for AMT determination in pharmaceutical formulation by using measures in sulfuric acid medium. It is worth emphasizing that depending upon the electrode nature, the AMT electroxidation may take place through oxidation of alkylamine nitrogen atoms as a one-electron step leading to the formation of radical cations [27]. Moreover, taking into account that triethylamine radical cations may also be generated in sulfuric acid [28], it can be proven that the acid is able to improve the AMT electroxidation. Furthermore, strong adsorption of sulfate ions on the electrode surface forming a negatively charged film may enhance the electroxidation process of AMT positively charged in the acidic medium and decrease the over potential.

2. Experimental

2.1. Samples, solvents and reagents

Amitriptyline hydrochloride (99%; Sigma-Aldrich, St. Louis, MO, USA), sulfuric acid (95–97%; Merck, Darmstadt, Germany), orthophosphoric acid (85%; Merck), acetic acid ($\geq$ 99.8%, Sigma-Aldrich, Steinheim, Germany), boric acid ($\geq$ 99.5%; Sigma-Aldrich), sodium hydroxide (99%; Merck), hydrochloric acid (37%; Sigma-Aldrich), sodium sulfate ($\geq$ 99%; Sigma-Aldrich), perchloric acid ($\geq$ 69%; Sigma-Aldrich) and phosphate salt (99–102%; Merck) were dissolved in deionized water (resistivity 18.2 $M\Omega\,cm^{-1}$) from a Milli-Q purification system (Millipore, Billerica, MA, USA) to get their respective solutions and used without further purification. Multiwalled carbon nanotubes (MWCNTs) (93%, diameter 10–40 nm, and length 5–20 μm) were acquired from CNTsCo. Ltd. (Yeonsu-Gu, Incheon, South Korea), and mineral oil was supplied by Sigma-Aldrich. Graphite powder (purity 99.9%) was also purchased from Sigma-Aldrich, and black carbon (purity 99.5%) was obtained from Cabot Brasil Ind. Com. Ltda. (Paraiso, São Paulo, SP, Brazil). Pharmaceutical samples containing 25 mg AMT per tablet (Medley®, EMS®, Teuto®, Germed®, Eurofarma®) and 75 mg AMT per tablet (Amytril®) were bought at local drugstores. The inactive excipients used as possible interfering compounds magnesium stearate, talc, calcium phosphate, titanium dioxide, cellulose, lactose and silicon dioxide were supplied by local drugstores. A 0.1 mol L^{-1} phosphate solution was prepared by dissolving monobasic salt in 1.0 mol L^{-1}HCl, with further pH adjustment to the desired value. A 0.1 mol L^{-1} Britton–Robinson (BR) buffer solution was prepared from a mixture of acetic, orthophosphoric and boric acids dissolved in 1.0 mol L^{-1} NaOH, once more with further pH adjustment to the desired value. A 0.1 mol L^{-1} AMT stock solution was prepared from 0.1 mol L^{-1} sulfuric acid. All the solutions were stored at <5 °C and in absence of light.

2.2. Apparatus

Electrochemical measurements were performed with an Autolab PGSTAT-101 potentiostat/galvanostat (Eco Chemie B.V., Utrecht, The Netherlands) controlled by means of a GPES 4.9 (General Purpose Electrochemical System) software package (Eco Chemie B.V.). A conventional three-electrode electrochemical cell containing a reference electrode (Ag/AgCl, 3.0 mol L^{-1}), an auxiliary electrode (spiral platinum wire) and a working electrode (carbon nanotube paste) was used. The accuracy of the proposed method was checked by an analysis of pharmaceutical formulations using a Lambda-25 A UV–vis spectrophotometer (Perkin Elmer Inc., Waltham, MA, USA) at 239 nm, with a quartz cell (optical path 1 cm). For in-situ FTIR experiments, a single-compartment glass cell fitted with a 60° prismatic CaF_2 window in a thin electrolyte layer pattern was employed. FTIR spectra were recorded on a Nicolet Nexus 670 spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with an MCT detector.

A glassy carbon electrode (diameter 2.0 mm; Metrohm, Herisau, Switzerland) was also used as working electrode. It was carefully polished with a 0.5-μm alumina slurry on a flat surface, rinsed thoroughly with deionized water, and then sonicated immediately before using in deionized water for 2 min. pH values of the samples were measured with an 826 pH mobile digital pH meter (Metrohm).

2.3. Preparation of carbon nanotube paste electrode

The composition of carbon nanotube paste electrode was based on our previous publication [29,30] using a MWCNTs/mineral oil ratio equal to 22:78% (w/w). This composition was found to be

highly homogeneous; besides, it promoted a smoother electrode surface and good conductivity, even in the presence of high insulator amount. Therefore, it was used in the present study.

The paste was prepared by homogenizing the MWCNTs and mineral oil in a Petri dish with a metal spatula for 15 min until a completely homogeneous paste was obtained. After that, it was inserted into the bottom cavity of a glass tube (diameter 3 mm and depth 1 mm) in order to construct the electrode. Electrical connection was provided by a copper wire connected to the paste in the inner hole of the tube. The surface of the paste electrode was smoothed out and rinsed carefully with Milli-*Q* water. Before the analysis, the electrode was electrochemically activated in 0.1 mol L^{-1} sulfuric acid by consecutive cyclic voltammetry in a potential range of −1.0–1.0 V (30 cycles) at a scan rate of 100 mV s^{-1}.

2.4. Electroanalytical procedure for the AMT determination

Differential pulse voltammetry (DPV) was employed as electroanalytical technique to determine AMT, while cyclic voltammetry was employed to investigate the electrochemical behavior of drug. DPV measurements were carried out in 0.1 mol L^{-1} sulfuric acid (electrolyte) without removing oxygen under the following conditions: modulation time −5 ms, scan rate −90 mV s^{-1}, pulse amplitude −120 mV, and potential range −1.05–1.50 V.

2.5. Preparation of commercial pharmaceutical formulation

Six commercial pharmaceutical formulations were analyzed by the proposed method. Ten tablets from each formulation were pulverized into a fine powder using a mortar and a pestle. After weighing this powder, the amount corresponding to 19.6 mg of AMT was dissolved in 10.0 mL of 0.1 mol L^{-1} sulfuric acid. The solution was then sonicated for 2 min, agitated on vortex mixer again for 2 min followed by filtration through a high quality filter paper. Next, the obtained filtrate was filtered once more using a Chromafil ® polyester membrane (0.45 μm). The volume of the resulting solution was made up to 25.0 mL with 0.1 mol L^{-1} sulfuric acid to obtain the nominal AMT concentration of 2.5×10^{-3} mol L^{-1}.

2.6. Evaluation of excipients as possible interfering compounds

The selectivity of the proposed method was assessed by comparing the analytical response of AMT in the presence of inactive excipients compounds usually found in the pharmaceutical formulations. Excipients compounds, such magnesium stearate, talc, calcium phosphate, titanium dioxide, cellulose, lactose and silicon dioxide were added individually to standard solution of AMT at 25.0 μmol L^{-1} concentration. Different proportions AMT:excipient species (mol/mol): 1:1, 1:2 and 1:3 were investigated. The mixture was filtered when necessary and an adequate volume was *transferred* to electrochemical cell whose measurements were performed in the presence of 0.1 mol L^{-1} sulfuric acid.

3. Results and discussion

3.1. Electrochemical response of AMT at unmodified carbon nanotubes electrode in sulfuric acid solution

The cyclic voltammograms recorded for AMT at the unmodified carbon nanotube paste (CNTPE) and glassy carbon (GCE) electrodes in the presence of 0.1 mol L^{-1} sulfuric acid are shown in Fig. 2. It can be observed that AMT does not show any redox process at the GCE. It is important to emphasize that AMT does not show any redox process at GCE, even though in sulfuric acid solution as electrolyte. Moreover, a well-defined oxidation peak can be observed to CNTPE at 1.35 V vs Ag/AgCl with the absence of peak in the reverse scan. These results show that oxidation process for AMT was totally irreversible even in the potential range varying from −0.2 up to 1.50 V (data not shown).

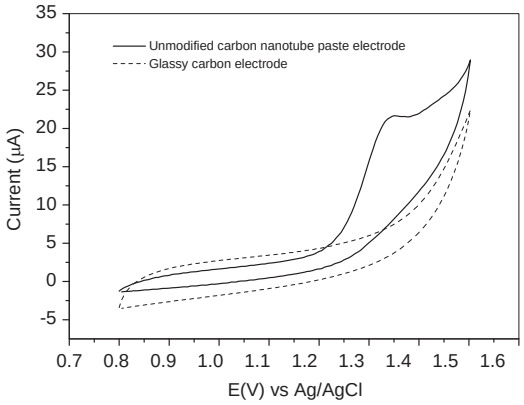

Fig. 2. Electrochemical responses of the unmodified carbon nanotube paste and glassy carbon electrodes. Conditions: AMT concentration (0.222 mmol L^{-1}), sulfuric acid concentration (0.1 mol L^{-1}), and scan rate (90 mV s^{-1}).

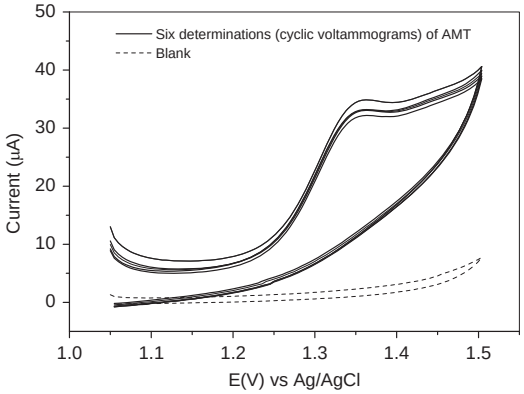

Fig. 3. Six cyclic voltammograms recorded for AMT at the unmodified carbon nanotube paste electrode. Conditions: AMT concentration (0.222 mmol L^{-1}), sulfuric acid concentration (0.1 mol L^{-1}), and scan rate (90 mV s^{-1}).

In order to evaluate possible strong adsorption of AMT or its oxidation products on the CNTPE electrode surface, which may preclude the development of a precise and accurate electroanalytical methodology, six consecutive cyclic voltammograms were recorded (Fig. 3). As can be observed, the anodic oxidation peak has a tendency to become stable for all the measurements. These results suggest the viability of the electroanalytical method for repetitive determinations when 0.1 mol L^{-1} sulfuric acid solution was used as electrolyte, without regeneration or renewal of the electrode surface.

With the aim to investigate the effect of acid concentration higher sulfuric acid concentration (0.5 mol L^{-1}) was also used. The increase in the electrolyte concentration provided a higher anodic peak current, as observed in Fig. 4A. Moreover, two anodic oxidation peaks can be observed at 1.21 and 1.29 V. A substantial increase in the anodic peak current for the first one, followed by a shift of the anodic peak potential (Epa) toward less positive values when compared to the measurements carried out in 0.1 mol L^{-1} sulfuric acid (Fig. 3). Under this condition, the second anodic peak at 1.29 V can be attributed to the oxidation products of AMT strongly adsorbed on the electrode, which may result in ineffective

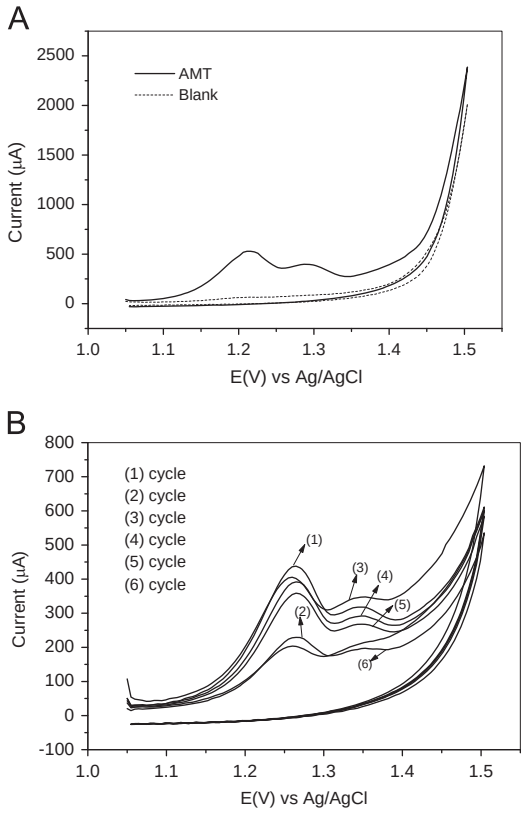

Fig. 4. AMT determination using the unmodified carbon nanotube paste electrode. (A) sulfuric acid concentration (0.5 mol L^{-1}); (B) Six cyclic voltammograms recorded for AMT in the presence of 0.1 mol L^{-1} sulfuric acid after using 0.5 mol L^{-1} sulfuric acid. Conditions: AMT concentration (0.222 mmol L^{-1}), and scan rate (90 mV s^{-1}).

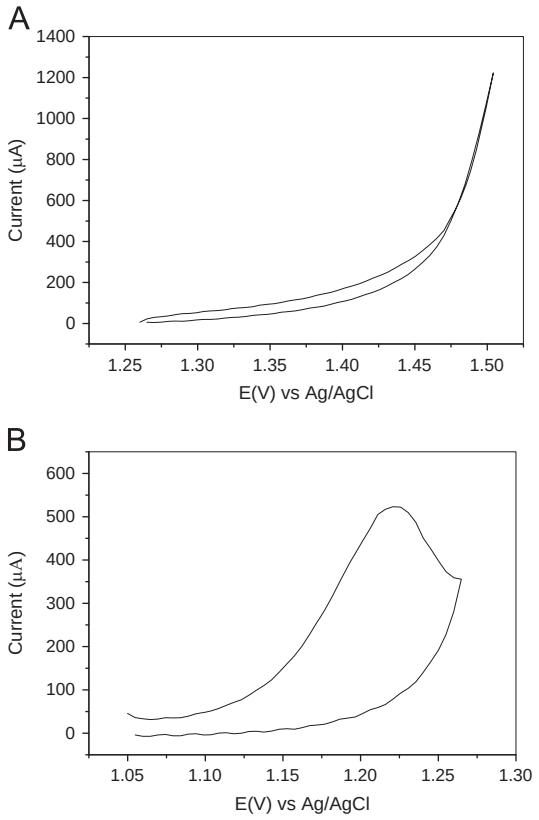

Fig. 5. Cyclic voltammograms recorded for AMT in the presence of 0.5 mol L^{-1} sulfuric acid. (A) Potential range (1.26–1.50 V); (B) Potential range (1.05–1.26 V). Conditions: AMT concentration (0.222 mmol L^{-1}), and scan rate (90 mV s^{-1}).

reproducibility of the results. This circumstance was confirmed by placing the electrode previously used in the 0.5 mol L^{-1} sulfuric acid into the 0.1 mol L^{-1} sulfuric acid. As observed in Fig. 4B, the reproducibility of consecutive cyclic voltammograms was badly affected. Furthermore, the second peak is very dependent on the first one, since no oxidation peak current was obtained in the potential range of 1.26–1.50 V (Fig. 5A). However, around 1.05–1.26 V a very well-defined oxidation peak can be observed (Fig. 5B). Besides, high concentration of sulfuric acid caused hardness of the paste with weak adhesive forces into the electrode cavity. Thus, the 0.5 mol L^{-1} sulfuric acid solution was avoided for analytical purposes.

Cyclic voltammograms were also recorded for AMT in the presence of 0.1 mol L^{-1} sodium sulfate solution at pH 1.0 adjusted using 0.1 mol L^{-1} perchloric acid. Similar electrochemical behavior of AMT was observed compared to the measurements performed in 0.1 mol L^{-1} sulfuric acid. These results indicate that the increase in the anodic peak current can also be explained by electrostatic interactions between the drug positively charged in the acidic medium ($pKa = 9.4$) and the sulfate strongly adsorbed on the electrode surface.

Fig. 6A and B show the pH effect on the electrochemical behavior of AMT on CNTPE electrode surface. It can be observed that AMT remained electrochemically inactive in the pH range of 4.0–6.0 (Fig. 6A). However, the more acidic medium facilitated the AMT electroxidation. Moreover, the results present a significant increase in the peak current and a shift of the Epa toward more negative values when sulfuric acid was employed (Fig. 6B), as reported before. The use of nitric acid as supporting electrolyte provides lower anodic peak current when compared with those obtained in presence of sulfuric acid. The results herein obtained differ from the study published by Toledo et al. [11], who employed a carbon-polyurethane composite electrode (GPU) to quantify AMT. According to described results, the peak current increased with the pH augmentation (among 4 up to 7), and the Epa shifts to less positive potential. The authors correlated the results with a proposed mechanism for AMT electrooxidation been pH-dependent, with one electron and one proton transfer. Electrochemical and quantum-chemical studies indicate that the oxidation process probably takes place through fission of the double bond between the external chain and the seven-carbon present in the ring.

In the present work, it was assumed that the oxidation of alkylamine nitrogen atoms involves one electron transfer, which leads to the formation of radical cations (Fig. 7). This process is facilitated by sulfuric acid, since it is known to be an oxidizing agent for obtaining triethylamine radical cations [28]. Moreover, such mechanism has also been observed for the electrochemical oxidation of aliphatic amines [31]. In order to notice changes in the C–N stretching of AMT in the region of 1250–1020 cm^{-1} and confirm the formation of the radical cations (as depicted in Fig.7), the in-situ FTIR experiments were carried out under the following conditions: potential applied 1.30 V during a 10 min period, 0.222 mmol L^{-1} AMT, and 0.5 mol L^{-1} sulfuric acid. As can be observed from the results shown in Fig. 8, the C–N stretching signal decreased over time due to the formation of the radical cations, thereby corroborating with significant data reported in the literature [28,31]. The signals at 1650 cm^{-1} and the bands in the region of 3000–3500 cm^{-1} can be attributed to the OH deformation and the OH stretch vibration of adsorbed water molecules, respectively [32].

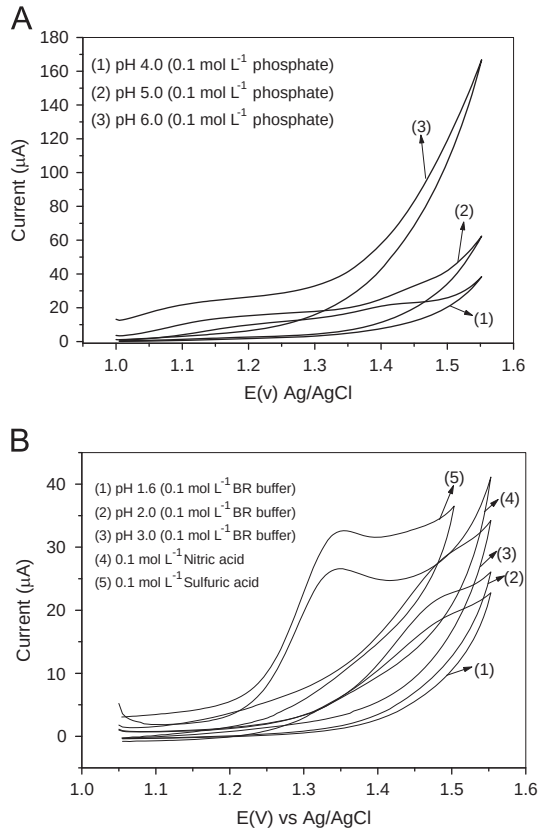

Fig. 6. Cyclic voltammogramns recorded for AMT at different pH values. (A) pH 4.0, 5.0, and 6.0; (B) pH 1.6, 2.0, 3.0 (0.1 mol L^{-1} BR buffer solutions), nitric and sulfuric acid at 0.1 mol L^{-1} concentration Conditions: AMT concentration (0.222 mmol L^{-1}), and scan rate (90 mV s^{-1}).

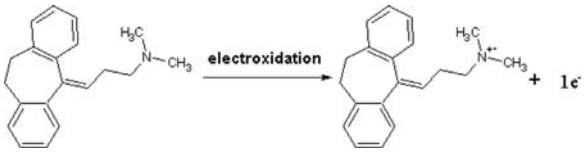

Fig. 7. Proposed eletroxidation mechanism for AMT at the unmodified carbon nanotube paste electrode in the sulfuric acid medium.

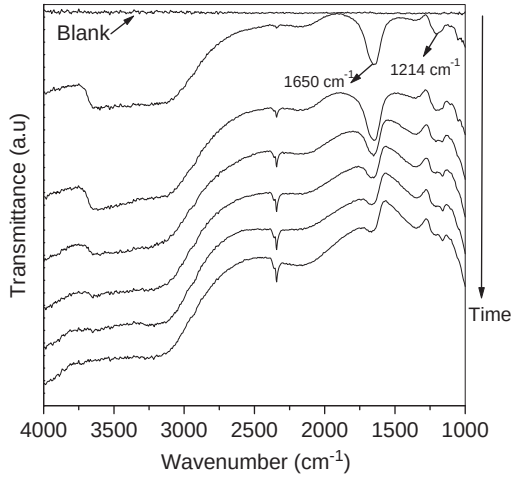

Fig. 8. in situ FTIR spectra displaying the AMT electroxidation at the electrode. Conditions: AMT concentration (0.222 mmol L^{-1}), sulfuric acid concentration (0.5 mol L^{-1}), potential applied (1.30 V), and time (10 min).

3.2. Scan rate effect on the electrochemical behavior of AMT

The scan rate (30–150 mV s^{-1}) influence on the electrode response was evaluated. The relationship of the anodic peak current (*I*pa) with the square root of the scan rate ($\nu^{1/2}$) and the scan rate (ν) makes it possible to define whether the electron transfer process is diffusion or adsorption controlled, respectively. A better correlation coefficient was obtained for the *I*pa *vs.*$\nu^{1/2}$ dependence, thus suggesting the diffusion-controlled AMT oxidation, which was confirmed by the slope of 0.543 calculated from the log(*I*pa) vs. log(ν) plot (r=0.9987).

From cyclic voltammogram (Fig. 6B) of solution containing AMT in the presence of 0.1 mol L^{-1} sulfuric acid, the number of electron (n) transferred in the oxidation process has been estimated according to equation $E_p - E_{p/2} = 47.7/\alpha n$ [33], where α, E_p and $E_{p/2}$ are the electron transfer coefficient, peak potential and half-peak potential, respectively. For an irreversible process α=0.5. Thus, the n was found to be 1.19, therefore indicating that the number of electron transferred in the rate-determining step should be equals to 1.0.

The effective electroactive area of the electrode (0.066 cm^2) was determined based on the Randles–Sevcik equation [33] from the *I*p vs. $\nu^{1/2}$ plot (r=0.997) using 5.0 mmol L^{-1} $K_4Fe(CN)_6$ as probe in 1.0 mol L^{-1}KCl as supporting electrolyte. After that, the coefficient of the AMT diffusion in the sulfuric acid medium was measured chronoamperometrically. The potential was fixed at 1.35 V vs Ag/AgCl and current was recorded for 60 s in different AMT concentration: 10–70 μmol L^{-1}. Furthermore, four linear *I* vs. $t^{-1/2}$ plots were constructed according to the Cottrell equation [33], and the coefficient of the AMT diffusion, estimated from the average slope. The diffusion coefficient was found to be equals to 3.60×10^{-5} cm^2 s^{-1}.

3.3. Choice of electroanalytical technique

Two electroanalytical techniques: differential pulse voltammetry (DPV) and square wave voltammetry (SWV) were applied for determining AMT. Table 1 shows the technique parameters studied, optimum values obtained, as well as sensitivity and determination coefficients. The assays were performed using a 30.0 μg L^{-1} AMT standard solution added in 0.1 mol L^{-1} sulfuric acid used as supporting electrolyte. As can be observed, the sensitivity of DPV was found to be 10 times higher than that one achieved for SWV, with a better correlation coefficient obtained (r=0.9991). Therefore, DPV was employed under the optimized conditions to validate the method.

3.4. Comparison between carbon paste electrodes made of different carbonaceous materials

Graphite and black carbon paste electrodes responses were compared to the CNTPE electrode. The composition of the black carbon/mineral oil paste was similar to carbon nanotubes paste (22:78% (w/w)), whereas the graphite/mineral oil paste was prepared in the ratio of 65:35 (w/w). These proportions made it possible to achieve homogeneity and good conductivity of the paste, with a smoother surface and easy to handle. Differential pulse voltammograms were recorded, and the results are presented in Table 2. A higher peak current and a slight shift of peak potential toward less positive value was observed for the CNTPE, it is important to emphasize the great advantages over the other carbon electrodes.

3.5. Validation and application of the proposed method

The validation results for the proposed method are shown in Table 3. The plot of current as a function of theoretical

Table 1
SWV and DPV optimized performances for the AMT determination.

Tecnhique	Studied interval	Optimum value	Linear range (μmol L^{-1})	Sensitivity (μA μmol $^{-1}$ L)	r
Square wave voltammetry (SWV)	Pulse amplitude (5.0–70.0 mV) Frequency (10–290 Hz)	35 mV 180 Hz	30.0–105.0	0.1368	0.9938
Differential pulse voltammetry (DPV)	Modulation time (5–95 ms) Potential step (3–19.5 mV) Pulse amplitude (30–130 mV)	5 ms 13.5 mV 120 mV	5.0–30.0	1.088	0.9991

Table 2
Comparative study of optimized performances of the carbon nanotube paste, graphite paste and black carbon paste electrodes. Condition: 25 μmol L^{-1} AMT solution.

Electrode	Oxidation peak current (μA)	Oxidation peak potential (Epa)	RSD (%)
Carbon nanotube paste	30	1.35	3.8
Graphite paste	20	1.36	4.0
Black carbon paste	18	1.40	3.7

Results taken as the mean of triplicate values ($n=3$).

Table 3
Linear ranges, limits of detection (LOD) and quantification (LOQ) obtained for the method of the AMT determination.

Parameter	Day	
Linear equation	1	$y(\mu A)=1.088$ [AMT μmol L^{-1}]+0.5126
	2	$y(\mu A)=1.048$ [AMT μmol L^{-1}]+0.7684
Correlation coefficient	1	0.9991
	2	0.9987
Analytical curve (μmol L^{-1})	1	0.0–30.0
	2	0.0–30.0
LOD (μmol L^{-1})	1	1.61
	2	1.55
LOQ (μmol L^{-1})	1	4.89
	2	4.71

concentration was constructed using a linear scale at different concentrations 0.0, 5.0, 10.0, 15.0, 20.0, 25.0, and 30.0 μg L^{-1}; in triplicate for each concentration. As can be observed, the sensitivity (μA μmol $^{-1}$ L) in intra-day was very similar presenting differences around 4.0%. The limits of detection (1.61 μmol L^{-1}) and quantification (4.89 μmol L^{-1}) were calculated respectively based on $3s_b/b$ and $10s_b/b$, according to the IUPAC rules [34], where s_b is the standard deviation of ten blank measurements, and b is the sensitivity. The intra- and inter-day precision (two consecutive working days), or repeatability, was assessed by analyzing ($n=6$) standard solutions containing 5.20 μg L^{-1} (low level), 15.00 μg L^{-1} (medium level) and 25.00 μg L^{-1} (high level) AMT (Table 4). The relative standard deviation (RSD) for the intra-day precision varied from 3.30 to 6.46%, whereas for the inter-day precision it ranged from 1.39 to 1.96%. The results obtained for the intra- and inter-day accuracy (Table 4) can also be considered satisfactory. The stability of the unmodified CNTPE for several voltammetric determinations using the 30.0 μg L^{-1} AMT solution was also evaluated. Reasonable precision (RSD=3.88%) can be achieved for sixty measurements, without losses electrode response. However, it is worth emphasizing that when the peak current decreases by about 10%, easy electrochemical procedure to clean the surface can be carried out by applying a potential of −1.0 V during 30 s. With this procedure, the peak current returns at its initial value.

Table 4
Precision and accuracy achieved for the method of the AMT determination.

Parameter	Day	Concentrations (μmol L^{-1})		
Nominal concentration		5.20	15.00	25.00
Analyzed concentration ($n=6$)	1	5.00	15.04	24.80
	2	5.10	14.60	24.10
Precision (relative standard deviation, %)	1	6.46	5.51	5.70
	2	4.88	3.30	5.75
Accuracy (relative error, %)	1	−3.85	0.27	−0.80
	2	−1.92	−2.66	−3.60
Inter-day ($n=2$)				
Analyzed concentration		5.05	14.82	24.50
Precision (relative standard deviation, %)		5.52	4.63	5.65
Accuracy (relative error, %)		−2.89	−1.19	−2.21

The comparison of the proposed method for AMT determination and previously reported ones is shown in Table 5. As can be observed, despites the simplicity of electrode preparation compared to those previous published methods, the procedure described herein provided a satisfactory limit of detection. As described in the Section 2.6, the selectivity of the method was evaluated by comparing the oxidation peak current of AMT in the absence and presence of excipient compounds. From the chosen AMT:excipient compounds molar concentration tested (1:1, 1:2 and 1:3), no significant signal variation (±7%) for AMT was observed when comparing a standard solution of AMT with the ones containing AMT and the excipient compounds.

The feasibility of the proposed method was checked by the analysis of pharmaceutical formulations and UV–vis spectrophotometry was employed as reference technique according to the Brazilian Pharmacopoeia [36].

The results obtained for different commercial brands of pharmaceutical formulations are presented in Table 6. As can be observed, the precision of both methods was not statistically different (Fisher (F-test at 95% confidence level), the same was accurate to average results (paired t-test at 95% confidence level), with low relative errors. Therefore, the proposed method also presented good accuracy and precision for the AMT determination in real pharmaceutical samples.

4. Conclusions

The novel electroanalytical method employing an unmodified carbon nanotube paste electrode has shown to be effective alternative for AMT determination in pharmaceutical samples in presence of sulfuric acid as electrolyte. It was found that sulfuric acid improves the AMT electroxidation, probably due to the oxidation of alkylamine nitrogen atoms with one electron transfer and the formation of radical cations. The electrode can be prepared very easily and quickly. Besides that, it is highly stable for several measurements of AMT without the necessity of electrode surface regeneration or renewal. Finally, the procedure described herein can provide satisfactory detection limits and it presents the

Table 5
Comparison of different electroanalytical methods reported for the AMT determination.

Technique/sensor	Calibration curve (μmol L^{-1})	LOD (μmol L^{-1})	Application	Ref.
Voltammetry/polymer-modified carbon paste electrode	1.0–100	Not determined	Pharmaceutical formulations	[9]
Flow injection potentiometry/plastic membrane electrodes using amitriptylinum phosphotungstate (Am-PTA) and amitriptylinum phosphomolybdate (Am-PMA)	50.0–10,000	Not determined	Pharmaceutical formulations	[10]
Cyclic Voltammetry/carbon-polyurethane composite electrode	14.9–87.4	Not determined	Without application	[11]
MWCNT/SiO_2/Al_2O_3/Nb_2O_5/DNA (MWCNT/SN/DNA)	10.0–80.0	0.12	Pharmaceutical formulations	[12]
Potentiometry/Potentiometric amitriptyline-plastic membrane sensor using ion-pair complexes with triphenylstilbenylborate and tetra(2-chlorophenyl)borate	7.0–10,000 or 6.0–10,000	5.0 or 3.6 (response time of 1 min)	Pharmaceutical formulations	[35]
Unmodified carbon nanotube electrode for measurements in sulfuric acid medium	0.0–30.0	1.61	Pharmaceutical formulations	Present work

LOD = limit of detection.

Table 6
Application of the proposed method to pharmaceutical formulations and its validation by UV–vis spectrophotometry.

Sample	Amount labeled (mg/tablet)	Found value (mg/tablet)		Relative error (%)	
		Proposed method*	Reference method*	Re_1 (%)	Re_2(%)
I	75.0	73.9(±0.5)	70.6(±0.2)	4.6	−1.5
II	25.0	24.9(±0.2)	24.3(±0.4)	2.5	−0.5
III	25.0	25.8(±0.1)	23.4(±0.1)	10.2	3.2
IV	25.0	24.6(±0.3)	23.3(±0.1)	5.4	−1.7
V	25.0	25.3(±0.3)	26.2(±0.2)	−3.4	0.1
VI	25.0	25.3(±0.2)	23.6(±0.3)	7.2	1.2

Re_1 = method proposed vs. reference method; Re_2 = sensor vs. labeled value. Calculated test *t*-paired = 1.93, Critical *t*-test value = 2.57. Calculated *F*-test value (I) = 7.56, (II) = 2.56, (III) = 1.69, (IV) = 8.41, (V) = 1.69 and (VI) = 2.75, critical *F* value = 19.0, at 95% confidence level.

* Results taken as the mean of triplicate values ($n = 3$) ± standard deviation.

desired features for routine analyses in regard to good precision (intra- and inter-day) and accuracy.

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