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Determination of trimebutine in pharmaceuticals by differential pulse voltammetry at a glassy carbon electrode

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Abstract

The differential pulse voltammetric (DPV) determination of trimebutine (TMB) was achieved at a glassy carbon electrode in acetonitrile/0.1 M $LiClO_4$. Trimebutine gave two irreversible, diffusion controlled peaks at 740 and 1318 mV versus Ag/AgCl reference electrode, respectively. The second oxidation peak was used to determine trimebutine concentrations in the range 1–50 $\mu g\,ml^{-1}$ with a detection limit ($3\sigma m$) of 0.3 $\mu g\,ml^{-1}$. Precision of the method (RSD, $n = 6$) within- and between-days obtained from six determinations at 5 $\mu g\,ml^{-1}$ was found to be 0.7 and 1.1%, respectively.

The method was successfully applied to the quantitation of TMB in granule dosage form (Debridat®) and recoveries between 98.4 and 101% were obtained. Excipients did not interfere with the assay and the results agreed well with those determined by previously established HPLC method.

Keywords: Differential pulse voltammetry; Glassy carbon electrode; Pharmaceuticals; Trimebutine

1. Introduction

Trimebutine [2-dimethylamino-2-phenylbutyl-3,4,5-trimethoxy benzoic acid] and its maleate are well-known compounds which act directly on smooth muscles and possesses motility-regulating function on the gastric-emptying kinetic. They are clinically used in the treatment of various digestive tract-disorders including dyspepsia, irritable bowel syndrome and post-operative ileus [1–3]. The inhibitory and excitatory actions of trimebutine on gastric muscles are due to inhibition of Ca^{2+} and depolarization of the membrane, respectively [4]. Trimebutine (TMB) exhibits weak opioid properties [5–8] and has been shown to influence the activity of visceral afferent nerves in the rat [9].

Many studies have dealt with the development of analytical methods for the determination of trimebutine and its metabolites in biological fluids, based on high-performance liquid chromatography using UV [10–12] mass spectrometry [13] and fluorescence detection [14]. The analysis of TMB and its metabolites in human plasma using GC–MS was described only in one paper [15]. Capillary zone electrophoresis was also used for the quantitation of trimebutine in plasma [16]. Comparatively, few methods have been developed for the assay of trimebutine in dosage forms [17,18].

An up-to-date literature survey revealed that no publications concerning the electrochemical behavior or the electroanalytical determination of trimebutine in pharmaceutical formulations or human fluids have been reported. This led us to study the voltammetric oxidation of TMB at a glassy carbon electrode in an attempt to develop a simple electrochemical method for its determination in pharmaceutical products.

Electrochemical methods have proved to be useful for sensitive and selective determination of many pharmaceutical compounds. These methods do not require tedious pre-treatment and involve limited pre-separation, and consequently reduce the cost of analysis [19,20].

This work is aimed to study the voltammetric oxidation behavior of trimebutine at a glassy carbon electrode using cyclic differential pulse and linear sweep voltammetry. The

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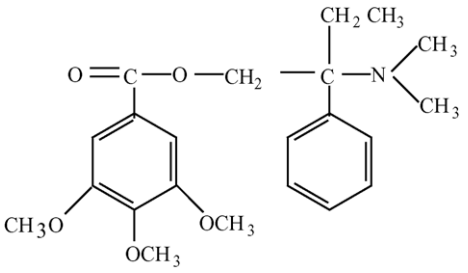

Scheme 1. Chemical structure of trimebutine.

observed electroactivity was applied for developing a differential pulse voltammetric method for the determination of trimebutine. The proposed method has been applied to the quantitation of TMB (Scheme 1) in pharmaceutical formulations.

2. Experimental

2.1. Chemicals and standards

Trimebutine was kindly supplied by PHARMAGREB (Tunisia). Lithium perchlorate (Normapur) and pyridine were purchased from Prolabo (France). Analytical-grade acetonitrile was obtained from Fluka (France).

Debridat® (product of Pfizer), labeled to contain 74.4 mg trimebutine per bag, was purchased from local pharmacy.

Standard stock solution of (1000 $\mu g\,ml^{-1}$) trimebutine was prepared by dissolving 25 mg TMB standard powder in 25 ml acetonitrile and kept in the freezer at − 18 °C. Working standards of TMB were freshly prepared just before assay, by adding appropriate amounts of stock solution directly to the voltammetric cell. The supporting electrolyte (0.1 M $LiClO_4$) was prepared by dissolving the appropriate amount in acetonitrile.

2.2. Apparatus

All experiments of cyclic and differential pulse voltammetry were performed with a Radiometer-potentiostat model POL 150 associated to an MDE 150 stand which is equipped with a three-electrodes system consisting of a glassy carbon disk working electrode ($\phi = 5$ mm), an Ag/AgCl (3 M KCl) reference electrode and a platinum wire as auxiliary electrode. The system was monitored with a personal computer using TraceMaster 5 software (Radiometer, France) for data acquisition and subsequent analysis.

The glassy carbon working electrode was polished at the beginning of the study, using aqueous slurry of 0.3 μm aluminum oxide on a smooth polishing cloth for 2 min. The electrode was then thoroughly washed with purified water and sonicated in acetone for 2 min. Before its use in voltammetric measurements, the electrode was immersed in supporting electrolyte and then was cleaned electrochemically by cycling potential scan for three cycles between 0 and 1.8 V at scan rate of 100 $mV\,s^{-1}$.

2.3. Procedure

In a typical run, a 5 ml of the supporting electrolyte was transferred into a clean, dry cell and the required volume of the standard stock solution of trimebutine was added by micropipette (Gilson, France). After a stirring period of 30 s, a cyclic or differential pulse voltammogram was recorded. Differential pulse voltammograms were recorded using a scan rate of 5 $mV\,s^{-1}$ with pulse amplitude of 50 mV and a pulse width of 20 ms.

The TMB standard curve was constructed by plotting the intensity of the anodic (DPV) current peak against the corresponding trimebutine concentration in order to cover the range of 1–50 $\mu g\,ml^{-1}$.

2.4. Analysis of pharmaceutical dosage form

For the determination of trimebutine in pharmaceuticals, an accurately weighed portion of the mixed contents of 10 bags, equivalent to 74.4 mg of TMB was transferred to a 50 ml volumetric flask and dissolved in acetonitrile. The mixture was sonicated for 5 min in an ultrasonic bath and then completed to the mark with acetonitrile.

The amount of TMB in pharmaceutical formulation was determined by adding a suitable volume (35 μl) of the clear supernatant liquor to a voltammetric cell containing 5 ml of supporting electrolyte. The analysis was done using anodic differential pulse voltammetry and referring to the calibration curve.

3. Results and discussion

3.1. Cyclic voltammetry

The cyclic voltammogram of trimebutine, typically recorded at a glassy carbon electrode (Fig. 1) exhibited two well-defined anodic peaks (O_1 and O_2) at about 0.79 and 1.39 V versus Ag/AgCl. The two observed processes are irreversible, since no cathodic peak was recorded in the reverse scan even when the sweep is reversed at 1 V, just before the beginning of the second oxidation peak.

The linearity ($R^2 > 0.99$) of the peak currents versus $\nu^{1/2}$ plots of O_1 and O_2 for 50 $\mu g\,ml^{-1}$ TMB solution indicates that the processes are diffusion-controlled in the whole scan rate range studied (5–1000 $mV\,s^{-1}$).

In a study of the peak potentials shift as a function of scan rate, the peak potential (E_{p1}) of the first wave (O_1) was observed to move anodically with increasing sweep rates. The plot of $E_{p1} = f(\log \nu)$ resulted in a straight line ($R^2 = 0.99$) with a slope (0.058/2 αn at 20 °C) of 58.4 mV/decade, and the αn value obtained (= 0.5) pointed to an irreversible oxidation process. Controlled potential coulometric experiments

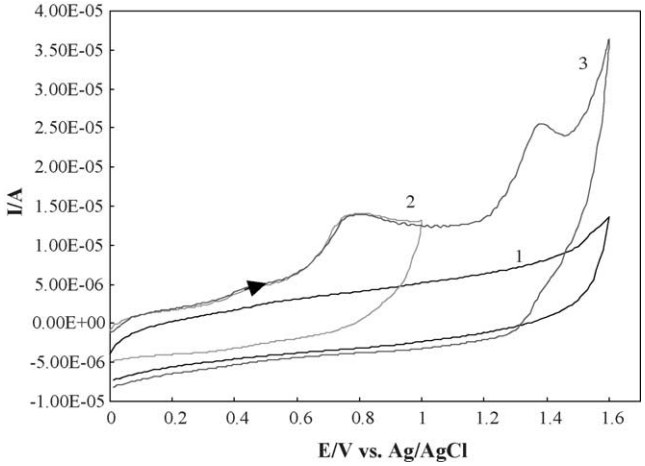

Fig. 1. Cyclic voltammograms of (50 μg ml^{-1}) trimebutine in ACN-$LiClO_4$ (0.1 M): (1) Residual current; (2) scan reversed at 1 V vs. ref.; (3) scan reversed at 1.6 V vs. ref. Electrode, glassy carbon; scan rate, 100 mV s^{-1}.

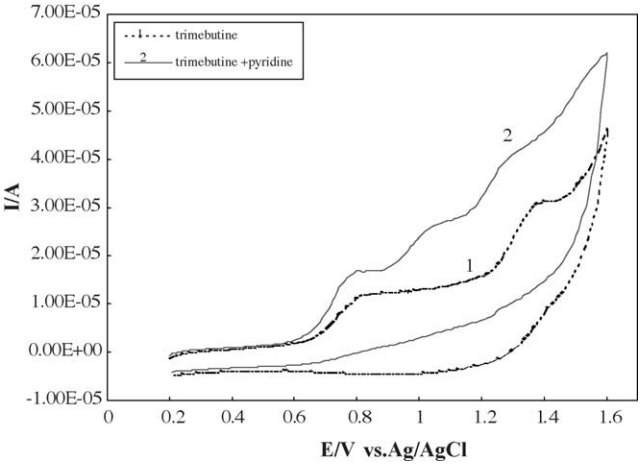

Fig. 3. Cyclic voltammograms of (50 μg ml^{-1}) trimebutine in (1) ACN-$LiClO_4$ (0.1 M) and (2) in the presence of pyridine. Electrode, glassy carbon; scan rate, 100 mV s^{-1}.

carried out at 1 V showed that a one-electron stoichiometry of $n = 1.06 \pm 0.03$ is involved in the first step. When the second peak was considered, an anodic shift of 18.5 mV per 10-fold increase in scan rate was observed. A shift of this magnitude corresponds theoretically to a fast electron transfer followed by a second-order reaction [21,22]. To confirm this behavior, the system was further investigated using linear sweep voltammetry on a rotating disk electrode. Current–potential curves for the oxidation of trimebutine were recorded at different rotation speeds and the obtained voltammograms are shown in Fig. 2. The limiting currents of the observed two waves were proportional ($R^2 > 0.99$) to the square root of the rotation speed (between 200 and 1600 rpm), which indicates that the electrode processes are controlled by diffusion. In addition, logarithmic analysis of the recorded waves, made in the form E versus $\log(I_L - I/I)$ plot, exhibited good linearity with slopes close to − 121 mV and − 63 mV per decade ($E_1(\text{mV}) = -121 \log(I_{L1} - I/I) + 748$, $R^2 = 0.994$; $E_2(\text{mV}) = -63.2 \log(I_{L2} - (I - I_{L1})/I - I_{L1}) + 1359$, $R^2 = 0.997$). This behavior is in good accordance with the previously discussed cyclic voltammetric results.

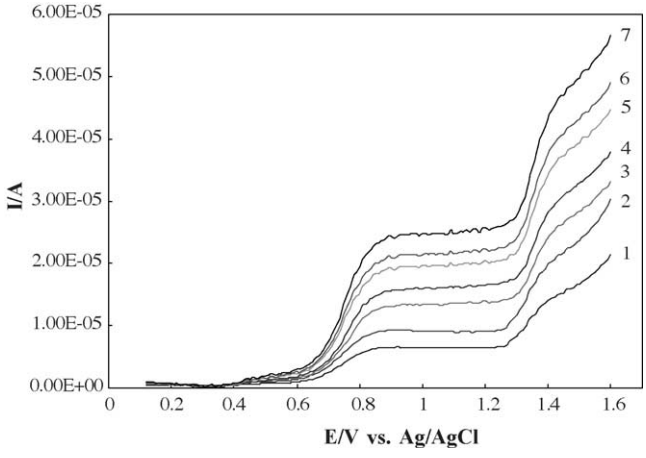

Fig. 2. Rotating disk voltammograms for the oxidation of (50 μg ml^{-1}) trimebutine at (1) 200, (2) 400, (3) 600, (4) 800, (5) 1000, (6) 1200, and (7) 1600 rpm. Electrode, glassy carbon; scan rate, 10 mV s^{-1}.

The addition of small amounts of a base such as pyridine-induced dramatic changes in the voltammograms (Fig. 3). Indeed, the addition of pyridine produced an increase of the first peak, which undergoes a shift towards less positive potentials with the appearance of a third wave located at 1.05 V versus Ag/AgCl. This indicates that the radical cation emerging from the first step is readily deprotonated by pyridine, generating neutral radical species, characterized by an oxidation potential which is clearly lower than that of the parent radical cation.

Taking into account all these results together with recently published work on the electrochemical-oxidation of trimethoxytoluene [23], an hypothetic mechanism may be tentatively invoked. The first oxidation wave corresponds to an irreversible monoelectronic oxidation of TMB to give a radical cation which undergoes a subsequent electron transfer at the second wave. The variation of the second oxidation-peak potential with the scan rate proves unambiguously [22] that the rate-determining step of the chemical evolution of the obtained product is a second-order process, probably involving dimerization.

In basic medium, we suggest that the initial oxidation is followed by a deprotonation step leading to a neutral radical which is easier to oxidize than the parent radical cation.

3.2. Analytical application

Using differential pulse voltammetry, two consecutive peaks were obtained. Only the second anodic peak (O_2) was chosen for analytical determination purposes, since it was sharper, better defined and more reproducible than the first peak.

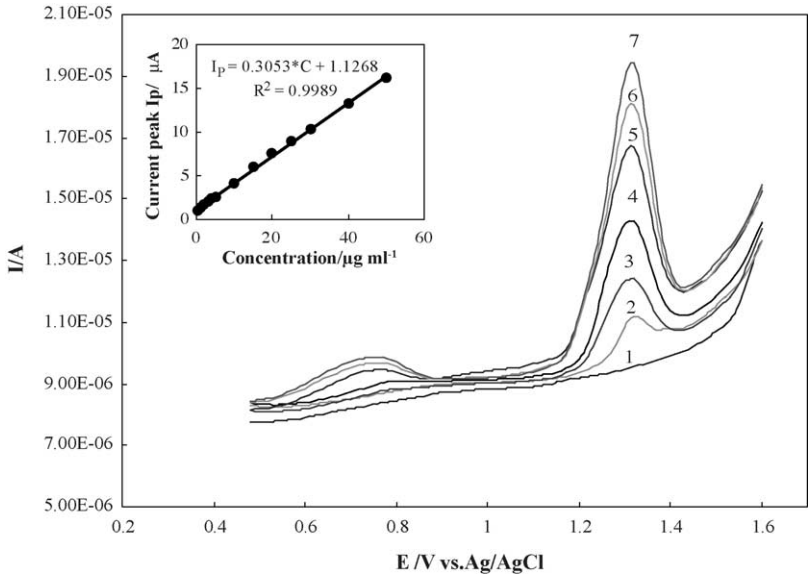

Fig. 4. Differential pulse voltammograms of different concentrations of trimebutine in ACN-$LiClO_4$ (0.1 M). Electrode, glassy carbon; scan rate, 5 mV s^{-1}; pulse amplitude, 50 mV; pulse width, 20 ms. Trimebutine concentration: (1) 0 μg ml^{-1}, (2) 2 μg ml^{-1}, (3) 5 μg ml^{-1}, (4) 10 μg ml^{-1}, (5) 15 μg ml^{-1}, (6) 20 μg ml^{-1}, (7) 25 μg ml^{-1}.

3.2.1. Calibration and validation

Initially, the optimal instrumental parameters for the quantitation of trimebutine were studied and the effect of scan rate, pulse height and width were investigated. With regard to peak current intensity and symmetry, the following parameters: 5 mV s^{-1}, 50 mV and 20 ms were chosen as the most convenient values.

Differential pulse voltammetry was applied to the quantitation of trimebutine and yielded a well-defined anodic peak at 1318 mV versus Ag/AgCl that increased linearly with increasing amounts of trimebutine (Fig. 4). The plot of peak current versus the respective concentration of trimebutine was found to be linear in the concentration range (1–50 μg ml^{-1}) and was represented by the linear equation: I_p (μA) = 0.305 C(μg ml^{-1}) + 1.126, R^2 = 0.999.

The method precision was investigated by repeatedly (n = 6) measuring a standard solution containing 5 μg ml^{-1} TMB within a day and over three consecutive days. The average relative standard deviations (RSD) for intra- and inter-day measurements were found to be 0.7 and 1.1%, respectively. The limit of detection (LOD) and quantification (LOQ) were calculated as (3 σ/m) and (10 σ/m), respectively [16], where σ is the standard deviation of the intercept and m is the slope. The calculated LOD and LOQ were found to be 0.3 μg ml^{-1} and 1 μg ml^{-1}, respectively.

3.2.2. Determination of trimebutine in pharmaceutical product

In order to evaluate the applicability of the proposed method, six commercial samples of Debridat® containing trimebutine were studied. Furthermore, to test the reliability of this method, Debridat® granular was also determined with a previously published HPLC method [10]. Results summarized in Table 1 show that DPV method yield an average concentration in good agreement (98.7% recovery) with the label claimed (74.4 mg/bag) with a standard deviation of 1.3%. Moreover, values obtained by DPV fitted reasonably well with those obtained by the chromatographic method. Indeed, statistical analysis based on Student's t-test showed no significant difference between the two methods (confidence limit > 95%).

Table 1
Determination of trimebutine in phamaceutical formulations (n = 6) using the proposed DPV method and reported HPLC method [10]

	Debridat®*	
	DPV	HPLC
Mean (mg/bag)	73.4	73.8
RSD%	1.3	1.8
t-Test (significance level 0.05, n = 6))	Calculated value 0.312 < theoretical value 2.571	

* Claimed concentration is 74.4 mg/bag.

The effect of additives (*polysorbate 80, orange flavor, aspartame and saccharose*) present as excipients in the pharmaceutical formulation on TMB determination, was studied by spiking the drug solutions with two additional levels at 2 and 4 μg ml^{-1} TMB. The recoveries were ranged between 98.4 and 101%, with an average of 99.5%. This indicates that excipients do not interfere with the determination of trimebutine.

All these results demonstrate that sensitivity and selectivity of DPV method are sufficient for the determination of TMB in pharmaceutical preparation.

4. Conclusion

The electrochemical oxidation of trimebutine at a glassy carbon electrode was studied and the obtained results were put to analytical advantage in the design of a new DPV method for rapid determination of the active drug in pharmaceutical formulations.

The proposed method has proved to be selective and sensitive without any statistically significant difference when compared to established HPLC method. Moreover, the method showed high precision (0.7–1.1%), good accuracy (recovery = 99.5%) and a reasonably low limit of detection ($0.3\,\mu g\,ml^{-1}$).

The main advantages of the proposed method are related to its rapidity, simplicity and low cost. It can be applied to the determination of trimebutine in pharmaceutical preparations without any separation or complex sample preparation, since there was no significant interference from the excipients.

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