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ELECTROCHEMICAL CHARACTERISTICS OF TENOFOVIR AND ITS DETERMINATION IN DOSAGE FORM BY ELECTROANALYTICAL METHODS

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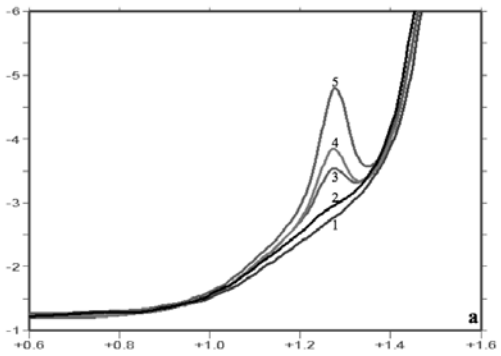
The fully validated, simple, reliable and rapid voltammetric techniques were developed for the determination of Tenofovir disoproxil fumarate from its dosage form, based on its electrochemical oxidation at a glassy carbon electrode. Tenofovir disoproxil fumarate has been studied by cyclic, differential pulse, square wave and adsorptive stripping voltammetric techniques. Different parameters were optimized for the sensitive assay. At the glassy carbon electrode, the effects of pH on the peak potentials and the peak current, buffer types, concentrations, scan rates were studied as details. Depending on pH, the electrooxidation was found irreversible and exhibited mixed diffusion under adsorption controlled process. Voltammetric assay is described for the determination of Tenofovir disoproxil fumarate by adsorptive stripping differential pulse voltammetry and adsorptive stripping square-wave voltammetry. Using adsorptive stripping differential pulse and adsorptive stripping square wave voltammetric methods, in pH 4.7 acetate buffer solution, a linear response was obtained within the range of $6.0x10^{-7}$– $6.0x10^{-5}$ M. The detection limits are estimated to be $1.02x10^{-7}$ M and $8.40x10^{-8}$ M with adsorptive stripping differential pulse and adsorptive stripping square wave voltammetry, respectively. These methods were successfully applied for the analysis of Tenofovir disoproxil fumarate from pharmaceutical dosage form. The repeatability, reproducibility, precision and accuracy of the methods in all working media were investigated. The standard addition method was used to obtain the accuracy results using recovery studies.



INTRODUCTION

The development and use of antiviral drugs for the treatment of viral infections such as acquired immune deficiency syndrome, hepatitis, avian and swine flu epidemics has become a very active area for the last few years. Substituted purine derivatives represent an important class of compounds actively studied as possible therapeutics against human immunodeficiency viruses (HIV).[1-3] Tenofovir disoproxil fumarate (TDF) is the prodrug of tenofovir, a nucleotide reverse transcriptase inhibitor active against HIV-1, HIV-2 and Hepatitis B virus. It blocks the HIV reverse transcriptase enzyme and combined with viral DNA.[4-5] The compound is broadly used worldwide for its efficacy and tolerability.[6]

The chemical structure of TDF (9-[(R)-2-[[bis[[(isopropoxy-carbonyl)oxy]methoxy] phosphinyl]methoxy]propyl]adenine fumarate (1:1)) is shown as below.[7]

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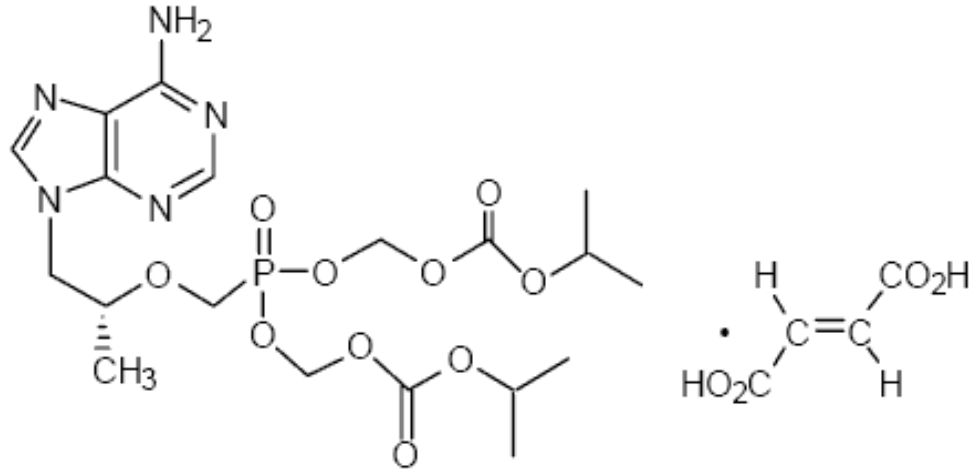

Scheme 1 – The chemical structure of TDF.

An extensive literature survey was carried out for the determination of TDF in pure, pharmaceutical formulations and human matrixes. In literature there exist studies related with HPLC,[8-19] spectrophotometry.[20-22] Also, the electrochemical reduction of TDF was investigated at hanging mercury drop electrode.[23] No electrochemical data about the oxidation behavior of TDF were found in the literature. The electrochemical and enzymatic oxidations pathways of drugs follow similar mechanistic pathways. Hence, the knowledge about electrochemical behavior of these compounds is important for biological interest.

In the last decade, electroanalytical techniques have been widely used for the assay of drugs in their pharmaceutical dosage forms and in biological samples.[23] The electroanalytical techniques have been used as an alternative method to the above mentioned analytical methods such as liquid chromatography (LC) and spectrophotometry. They have been shown to be perfect to determinate of drugs in various matrices without any extraction or evaporation steps.[24] Electroanalytical methods emerge with interplay between electricity and chemistry; in other words, they were used to measure current, potential, or charge and their relationship with the chemical parameters. Electrochemical studies provide evidence related to the mechanisms of biological electron-transfer processes. Electrochemistry has always supplied analytical methods characterized by instrumental simplicity, cost, and portability. For determination of trace amount of drugs which have an adsorptive character on working electrode surface, adsorptive stripping voltammetry has been shown as an effective analytical technique.

Glassy carbon electrode (GCE) is most commonly used carbon-based electrode. It has perfect mechanical and electrical properties, wide useful potential range, particularly in the anodic direction, and impermeability to gases. They allow many applications in many different areas, since their performances are relatively reproducible.[25] Additionally, carbon has a strong inertness and a rich surface chemistry.[26]

The goal of this study was to evaluate the electrochemical oxidation behavior of TDF at the glassy carbon electrode. Moreover, it is aimed to develop a new sensitive adsorptive stripping voltammetric procedure for quantification of TDF in the pharmaceutical formulation without any time-consuming extraction or evaporation steps.

RESULTS AND DISCUSSION

No previous electrochemical data were available regarding to the electrooxidative behavior of TDF in the literature. The electrochemical behavior of TDF was investigated on a bare GC electrode, which may offer some advantages for the use of such electrodes as sensors. The several measurements with different electrochemical methods including cyclic voltammetry (CV), differential pulse voltammetry (DPV), square wave voltammetry (SWV) and adsorptive stripping techniques such as adsorptive stripping differential pulse voltammetry (AdSDPV) and adsorptive stripping square wave voltammetry (AdSSWV) were performed with the purpose of obtaining detailed electrooxidative information on TDF.

Influence of the pH on the peak potentials and peak currents

The influence of the pH on the TDF peak current at a GCE was investigated. The voltammetric study was performed in a broad pH range between 1.0 and 9.0, 0.7 and 11.0 and 0.7 and 12.0 for CV, DPV, and SWV with different buffers using a GCE, respectively.

As seen in figure 1, TDF exhibit one distinct and well-defined anodic peak between pH 3.0 and 8.0 by SWV. After pH 8.0, the anodic peak or wave is hardly detected on the GCE surface. The sharp peak and better response is obtained in acetate buffer at pH 4.7 (Fig. 1 and Fig. 2). Hence, pH 4.7 acetate buffer was selected for further works, due to the fact that it gives the best response and it is suitable for analytical purposes.

By SWV, as seen in figure 2A, peak potential (E_p) shifted less positive potential values with the increasing of pH. E_p shifting is presented the linear response versus pH with a slope of -49.63 mV as seen below.

E_p (mV) = -49.63 pH + 1563.9; r: 0.996
(between pH 0.7 and 11.0) by SWV

Also using CV and DPV, the Ep-pH plots were obtained and related equations are given below:

E_p (mV) = -47.78 pH + 1517.8; r: 0.994
(between pH 1.0 and 9.0) by CV

E_p (mV) = -49.35 pH + 1530.6; r: 0.994
(between pH 0.7 and 12.0) by DPV

From the equations, the slope of E_p *vs.* pH values are close to 59 mV/pH, meaning that equal number of electrons and protons are transferred in the electrode reaction.

The detailed electrochemical study of 4×10^{-5} M TDF in pH 4.7 acetate buffer at GCE was performed using repetitive cyclic voltammetry. As seen in figure 3, over the swept potential range there is one oxidation peak appeared on GCE around at +1.33 V. No peaks were observed in the reverse scans that evidenced the irreversible nature of the oxidation process at GCE. At the second and higher cycles, the peak height of TDF was decreased. This result may be explained to the consumption of adsorbed TDF on the electrode surface.

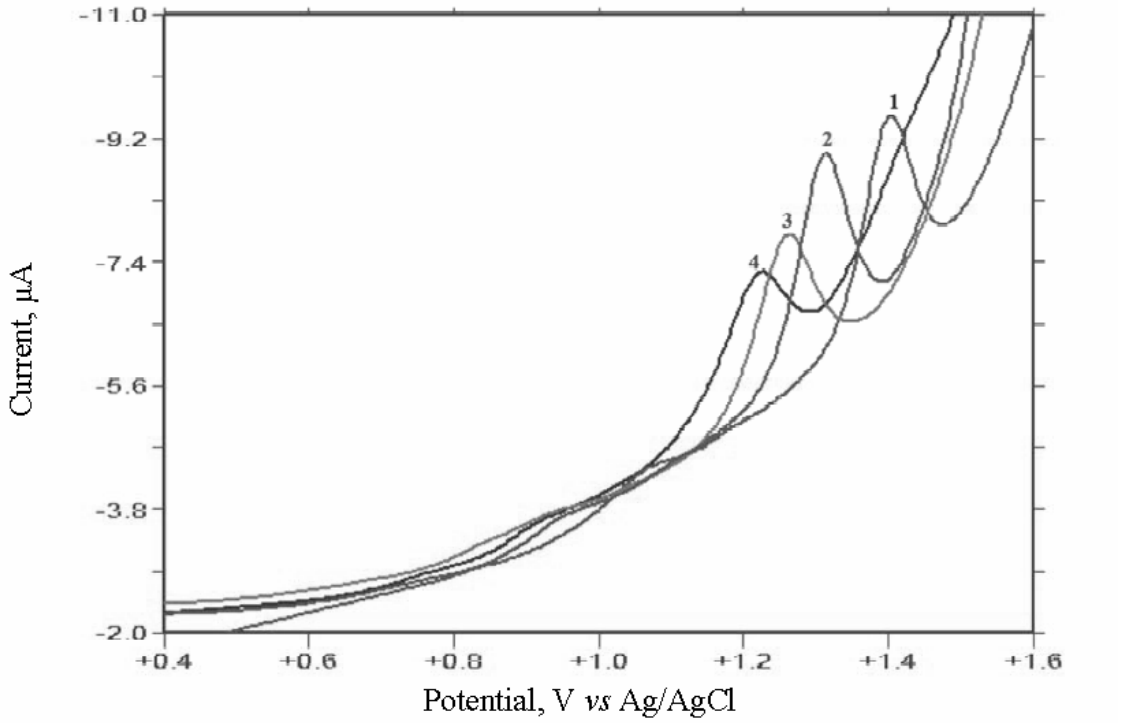

Fig. 1 – SW voltammograms of $4x10^{-5}$M TDF solution on different pH values as pH 2 phosphate buffer (1); pH 4.7 acetate buffer (2); pH 6 phosphate buffer (3); pH 7.0 BR buffer (4).

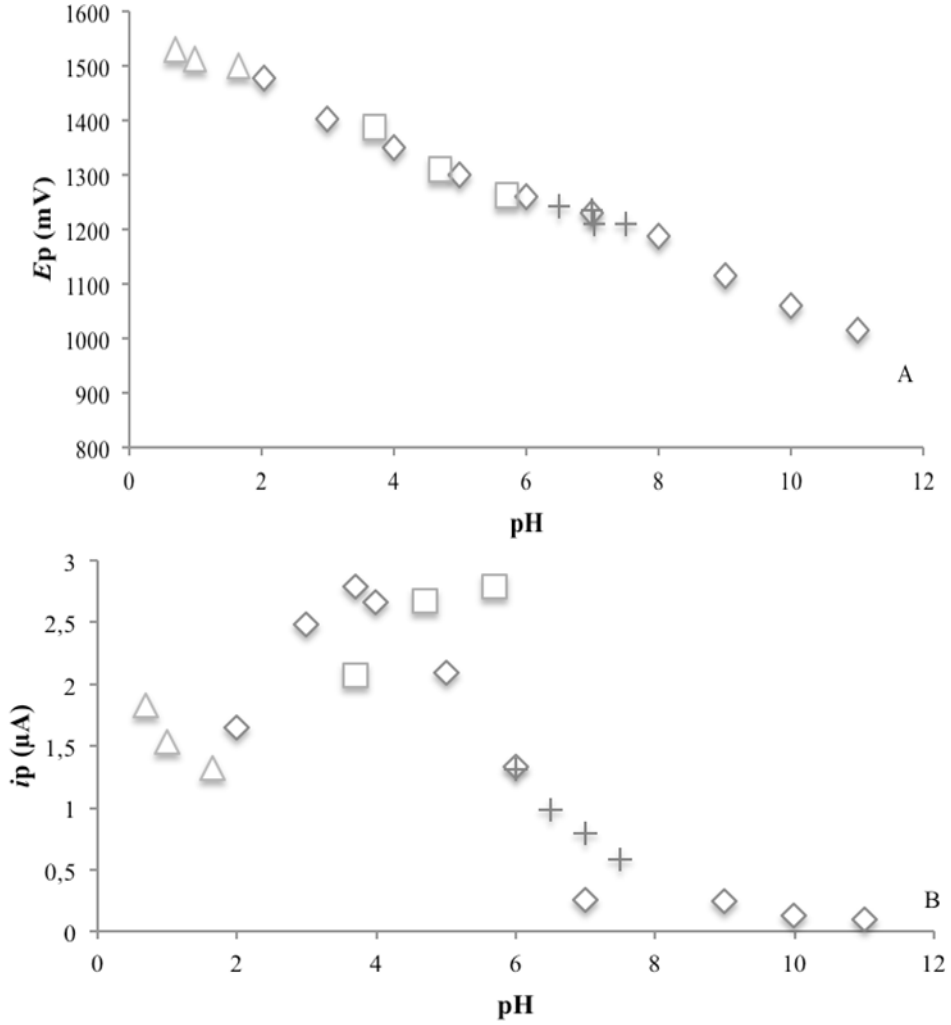

Fig. 2 – Effect of pH on TDF peak potential (A) and peak current (B); TDF concentration 4 x 10^{-5} M by using SWV. (△) 0.1 M H_2SO_4; (◊) Britton–Robinson; (□) acetate; (+) phosphate buffers.

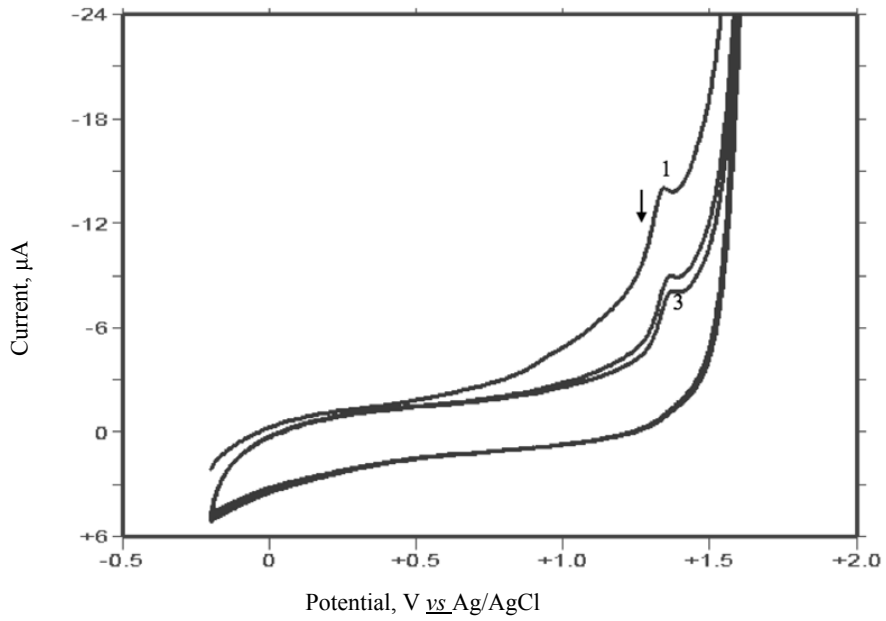

Fig. 3 – Repetitive cyclic voltammogram of 4x10^{-5} M TDF solutions in acetate buffer pH 4.7; Scan rate, 100 mV/s.

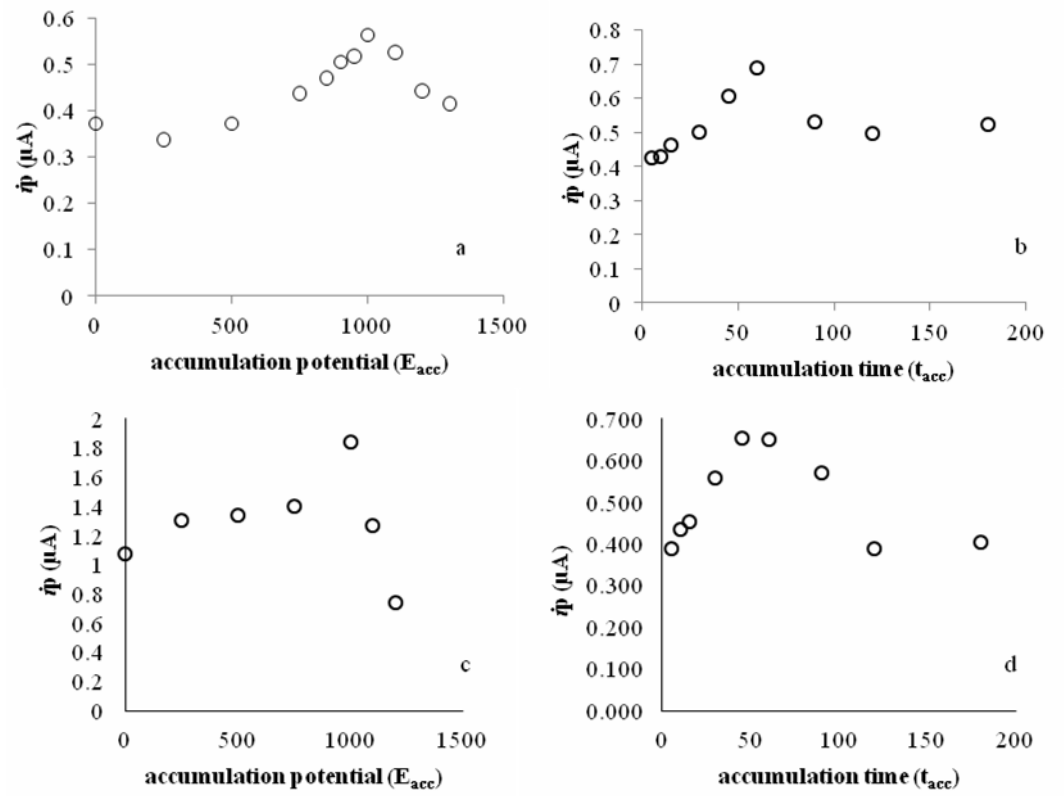

Fig. 4 – Effect of accumulation potential on the peak current (a); and effect of accumulation time on the peak current (b) using AdSDPV; Effect of accumulation potential on the peak current (c); and effect of accumulation time on the peak current (d) using AdSSWV.

Influence of the scan rate

The scan rate studies were realized between 5 and 1000 mVs^{-1} range on the peak current and peak potential of TDF. The results were assessed whether the processes on GCE were under diffusion or adsorption control or mix diffusion under adsorption control. By increasing the scan rate from 5 to 1000 mV s^{-1}, the peak potential also shifted to more positive values (about 69.4 mV) confirming the irreversibility of the oxidation process.

The irreversibility was also studied with a Tafel treatment of voltammetric curves.[24] The Tafel plots (*log i vs E*) were obtained with a scan rate of 5 mVs^{-1} beginning from a steady-state potential in acetate buffer at pH 4.70 for GCE. The αn value of anodic reaction from the slope of the linear part of the Tafel plot was found to be 0.240. The exchange current densities (i_o) were calculated as $3.56x10^{-11}A.cm^{-2}$. These values with the other results as absence of cathodic peak and peak potential positive shifting by increasing scan rate remarked that the anodic reaction is irreversible. 4×10^{-5} M solution of TDF, a linear dependence of the peak currents (i_p) upon the scan rate v (mVs^{-1}) was found. This equation is given below;

$$i_p\ (\mu A) = 0.013v\ (mVs^{-1}) + 1.09\ (r: 0.993;\ n:10)$$

The peak current was linearly proportional to the square root of the scan rate according to the following equation:

$$i_p\ (\mu A) = 0.45v^{1/2}\ (mVs^{-1}) - 1.24\ (r: 0.989\ ;\ n: 10)$$

The logarithm of oxidation peak currents (*log* i_p) versus logarithm of scan rates (*log* v) exhibited a linear relationship with a slope of 0.66 which can be expressed mix diffusion and adsorption controlled process.[27] The obtained equation is noted below:

$$log\ i_p\ (\mu A) = 0.66\ log\ v\ (mVs^{-1}) - 0.84$$
$$(r: 0.999\ ;\ n: 10).$$

Optimization of the method parameters

The quantitative electroanalytical methods are developed for the determination of TDF content in drug dosage form. To detect the trace amounts of electroactive compound in pharmaceuticals, DPV

and SWV have been extremely useful. SWV gives the best ratio of peak-to-background current and provide sharper and better defined peaks, leading to an enhanced resolution.

For voltammetric analysis, the influence of the SWV parameters on the peak current of TDF was studied. These parameters were frequency, pulse amplitude, and step potential. The dependence of the step potential was investigated in the range between 2 and 10 mV. The maximum peak current of TDF was obtained when the step potential was 8 mV. The pulse amplitude was studied in the range from 10 to 50 mV, and optimum value of 35 mV was chosen. Between the ranges of 10 and 50 Hz on the TDF peaks, the peak current was increased up to 30 Hz, hence 30 Hz was chosen as the optimum parameter.

Previously, the adsorptive character of TDF onto the GCE surface was identified by CV. With this result, the analytical methods are continued using adsorptive stripping techniques for the more sensitive assay.

For analytical measurements, accumulation potential (E_{acc}) and accumulation time (t_{acc}) values for AdSDPV and AdSSWV were also optimized. The effect of the E_{acc} on the peak current was studied when the t_{acc} was 60 s for the $4x10^{-6}$ M TDF. With the E_{acc} between the ranges of 0 and 1300 mV, E_{acc} value was selected as 1000 mV for AdSDPV (Fig. 4a) and AdSSWV (Fig. 4c). The effect of the t_{acc} on the peak current was studied when E_{acc} was 1000 mV; t_{acc} value was selected as 60 s for AdSDPV (Fig. 4b) and AdSSWV (Fig. 4d).

Analytical Applications

To develop a voltammetric assay of TDF, adsorptive stripping pulse voltammetric techniques were selected. The DPV and SWV are effective and rapid voltammetric techniques with advantages comprising low detection limits and good discrimination against background currents.[28-29] And, adsorptive stripping voltammetry of pulse techniques were studied for TDF which have interfacial adsorptive character onto the GCE. These techniques are greatly efficient techniques to assay of trace amount of a wide range of species.[30]

Hence, for the sensitive quantitative analysis of the TDF, AdSDPV and AdSSWV techniques were developed. The advantages of AdSDPV and AdSSWV over the other techniques are selectivity, greater sensitivity, the concomitant ease of measuring larger currents better defined at lower concentration.

The data obtained for the TDF oxidation using GCE demonstrates a good possibility for developing an electroanalytical methodology for TDF assay. The plot of peak current versus the frequent respective concentration of TDF was found to be linear in the concentration range between $6x10^{-7}$ and $6x10^{-5}$ M using both AdSDPV and AdSSWV techniques. Above the concentration of $6x10^{-5}$ M, loss of linearity was presumably owing to the adsorption of TDF on the GCE surface. Also, using AdSDPV and AdSSWV, selected calibration voltammograms were shown in figure 5.

Validation data from the analytical procedures for the quantitative assay of the TDF was examined via evaluation of the limit of detection (LOD), limit of quantification (LOQ), repeatability, reproducibility, recovery, precision and accuracy (Table 1). LOD and LOQ values confirmed the sensitivity of the proposed method, were illustrated in Table 1. The LOD and LOQ were calculated using the following equations:

$$LOD= 3s/m, LOQ=10s/m$$

where s, is the standard deviation of the peak currents (n: 5), m is the slope of the calibration line.[31] LOD values were calculated as 1.02×10^{-7} and 8.39×10^{-8} for AdSDPV and AdSSWV, respectively. LOQ values were also calculated as $3.39x10^{-7}$ and $2.80x10^{-7}$ for AdSDPV and AdSSWV, respectively.

The repeatability and reproducibility results were presented as RSD% in Table 1. The low values of standard error of the slope, intercept and also greater correlation coefficient than 0.999 confirm the precision of the proposed voltammetric methods. As seen in Table 1, the validation results have good precision, accuracy and reproducibility.

To assessment the stability of the prepared solution, the solution was exposed different conditions such as room temperature, in the oven, refrigerator, and water bath for 24 hours. As a result of the analyzing samples in the selected time interval, it was observed that the sample were highly affected from the water bath and oven. After 24 hours, this effect can be obtained as higher than 10% degradation and also this effect on the response is less than %10 in room temperature. There is no different response or degradation in the sample when keeping it in the refrigerator after 24 hours.

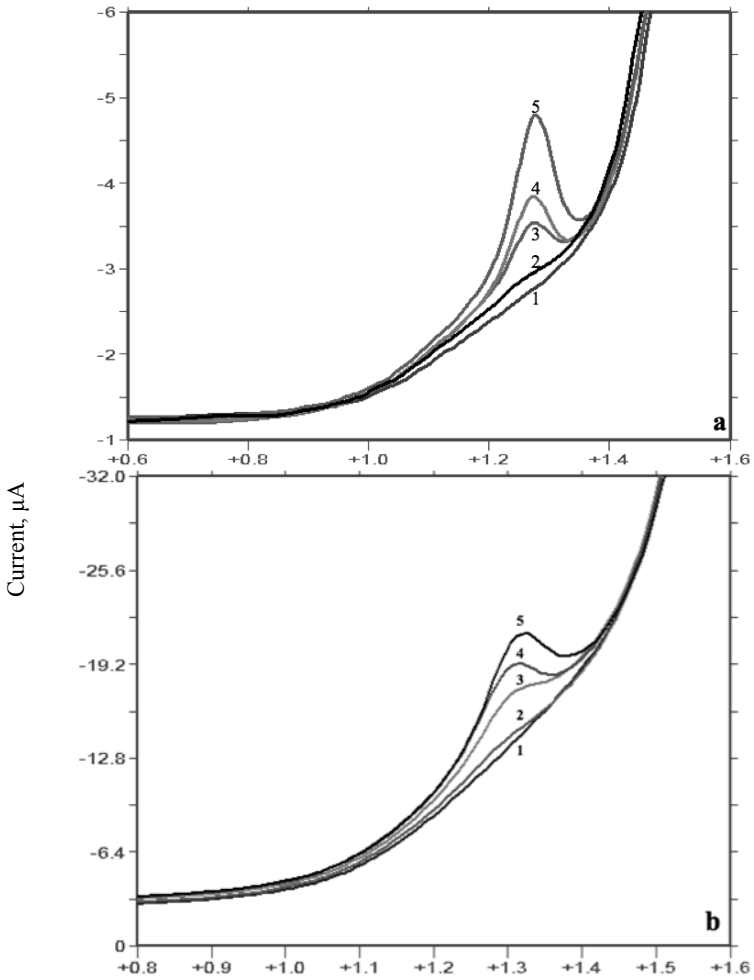

Fig. 5 – AdSDPV (a) and AdSSWV (b) obtained for the determination of TDF in acetate buffer. 1) Blank; 2) 8.0×10^{-7} M; 3) 6.0×10^{-6} M; 4) 1.0×10^{-5} M; 5) 2.0×10^{-5} M.

Table 1

Regression data of the calibration lines for quantitative determination of TDF in acetate buffer at pH 4.7 using AdSDPV and AdSSWV

	AdSDPV	**AdSSWV**
Measured Potential (mV)	1336.4	1328.5
Linearity range (M)	$6x10^{-7}$-$6x10^{-5}$	$6x10^{-7}$-$6x10^{-5}$
Slope (µA/M)	$7.01x10^{4}$	$2.05x10^{5}$
Standard Error of slope	$1.04x10^{3}$	$2.13x10^{3}$
Intercept(µA)	$7.34x10^{-2}$	$1.82x10^{-1}$
Standard Error of intercept	$2.40x10^{-2}$	$4.91x10^{-2}$
Correlation coefficient	0.999	0.999
LOD (M)	$1.02x10^{-7}$	$8.39x10^{-8}$

Table 1 (continued)

LOQ (M)	3.39×10^{-7}	2.80×10^{-7}
Repeatability of peak current* (RSD%)	0.197	0.290
Repeatability of peak potential* (RSD%)	0.156	0.136
Reproducibility of peak current* (RSD%)	0.242	0.330
Reproducibility of peak potential* (RSD%)	0.206	0.232

* Each value is the mean of 5 experiments.

Table 2

Results of the assay from TDF tablets and the recovery assay

	AdSDPV	**AdSSWV**
Labeled claim (mg)	245.00	245.00
Amount Found (mg)*	245.01	246.40
RSD%	1.12	1.77
Bias %	-0.040	-0.571
Added(mg)	20.00	20.00
Found (mg)*	20.07	20.10
Recovery%	100.35	100.50
RSD% of recovery	1.69	1.50
Bias%	-0.35	-0.5

* Each value is the mean of 5 experiments.

Table 3

Effect of interfering species on the determination of TDF

	TDF:AA (1:1)	**TDF:UA (1:1)**	**TDF: Glucose (1:1)**	**TDF: NaCl (1:1)**	**TDF: KCl (1:1)**	**TDF: Dopamine (1:1)**
Average Recovery* %	98.37	99.48	98.64	101.42	100.14	100.029
RSD %	0.940	0.676	1.662	1.052	1.177	1.761
Bias %	1.620	0.513	1.350	-1.422	0.146	0.029
	TDF:AA (1:10)	**TDF:UA (1:10)**	**TDF: Glucose (1:10)**	**TDF: NaCl (1:10)**	**TDF: KCl (1:10)**	**TDF: Dopamine (1:10)**
Average Recovery* %	80.15	88.39	121.21	106.43	106.34	112.33
RSD%	1.296	2.160	1.961	1.531	0.739	1.960
Bias %	19.85	11.600	-21.218	-6.431	-6.343	-12.330

* Each value is the mean of 5 experiments.

Assay of TDF in Pharmaceutical Dosage Form

The proposed AdSDPV and AdSSWV techniques were successfully applied to the direct determination of TDF in tablet dosage form. At the start of analysis, time-consuming preparation steps and pre-treatment was not required for samples with voltammetric techniques. The results, which

summarized in Table 2, show that AdSDPV and AdSSWV methods were successfully applied for the determination of TDF in VIREAD® tablets (245 mg/tablet).

Recovery Studies of TDF

For obtaining the accuracy results, recovery studies were realized using standard addition method. After addition of known amounts of the pure drug to the various pre-analyzed formulation of TDF, the recovery studies were carried out. It was verified that the excipients present in the tablets do not interfere with the analysis. The results demonstrate the validity of the proposed method for an accurate determination of TDF in tablets (Table 2). These results ascertain that AdSDPV and AdSSWV methods had adequate accuracy and precision. These methods could be applied to detect of TDF in pharmaceutical dosage form without any interference from the excipients.

Interference studies

The effect of probable interferences on the electroanalytical determination of TDF was studied. The AdSSWV technique was preferred for interference studies. The study was realized by adding some metal ions and possible biological molecules to a solution containing 4.0×10^{-6} M TDF in pH 4.7 acetate buffer. For interfering species, the tolerance limit was considered as the maximum concentration that gave a relative error less than ± 10.0%. Na^+, Cl^-, K^+, ascorbic acid, dopamine and uric acid and glucose solutions were added to the working media, separately.[32] According to the obtained results; Na^+, Cl^-, K^+, ascorbic acid, dopamine and uric acid and glucose solutions do not interfere with the TDF determination with the selectivity ratio of about 1:1. When TDF: interference ratios were 1:10, TDF signals were changed more than 10% except Na^+, Cl^- and K^+ (Table 3).

EXPERIMENTAL

Apparatus

Electrochemical measurements were performed using BAS 100W Electrochemical Analyzer, associated with one-compartment glass electrochemical cell and a three-electrode system. GCE was polished with aqueous slurry of alumina powder on a micro cloth pad (BAS polishing pad) manually and rinsed with water before use. All measurements were studied at room temperature.

The pH measurements were carried out with a pH meter Model 538 (WTW, Austria) using a combined electrode (glass electrode-references electrode) with an accuracy of ± 0.05 pH.

Operating conditions for SWV were: pulse amplitude, 35 mV; frequency, 30 Hz; potential step 8 mV and; for DPV were: pulse amplitude, 50 mV; pulse width, 50 ms; scan rate, 20 mV s^{-1}. Voltammetric analysis were carried out in pH 4.7 acetate buffer the accumulation potential at + 1.00 V was applied for a selected deposit time (60s) with stirring. After the stirring finished, waited 10s rest period. TDF was measured using AdSDPV and AdSSWV. All measurements were obtained at ambient temperature of the laboratory.

Reagents

TDF and VIREAD® tablets (245 mg/tablet) were kindly supplied by Gilead Pharmaceutical, USA. TDF standard stock solutions were prepared daily by direct dissolution in doubly distilled water and then stored in the refrigerator. All chemicals used were of reagent-grade quality (Merck or Sigma) and doubly distilled water was used throughout the experimental work. Working solutions for voltammetric investigations were prepared by dilution of the stock solution with the selected supporting electrolytes. 0.1 M H_2SO_4, Britton-Robinson (pH 2.0-9.0), phosphate (pH 6.0–7.0) and acetate (pH 3.7–5.7) buffers were used as supporting electrolytes.

All solutions were protected from light, and they were used within several hours in order to avoid hydrolysis. Ascorbic acid and dopamine were purchased from Sigma.

Tablet assay procedure

Five VIREAD® tablets were weighed and ground in a mortar to obtain the homogeneous fine powder. An accurately weighed portion of this powder equivalent to a stock solution of a concentration about 1.0×10^{-3} M was transferred into a 100 mL calibrated flask and completed to the volume with deionized water. The mixture was sonicated for 1h to effect the complete solution. Hence, the working solutions of the tablet dosage form were prepared exactly as the standard solutions. To obtain a final solution, taking appropriate aliquots of the clear supernatant liquor and diluting with the selected supporting electrolyte were realized. Voltammograms were recorded according to the AdSDPV and AdSSWV parameters as in pure TDF. The amount of TDF per solution was calculated using the linear regression equation obtained from calibration curve of pure TDF.

Recovery Studies

The recovery experiments were realized by the standard addition method, to investigate the accuracy, precision and reproducibility of the proposed voltammetric techniques. To investigate whether the excipients show any interference with the analysis, known amounts of the pure drug was added into the tablet formulation. The recovery results were determined based on at least five data.

CONCLUSION

The electrooxidative behavior of TDF was studied on GCE for the first time. The obtained

results revealed that the oxidation of TDF is an irreversible pH-dependent process in a mixed diffusion under adsorption controlled mechanism. AdSDPV and AdSSWV techniques were developed in this study for the reliable analysis of TDF in pharmaceutical dosage form in pH 4.7 acetate buffer at GCE. The analytical procedures were fully validated. Concentration dependency was linear within the studied range. Analytical performance of the developed method showed a low detection limit and reproducible result. The prepared sample solution are stable at least 24 h in the refrigerator. However, it was observed that the solutions were highly affected from the water bath and oven resulting that TDF can be affected to the heating conditions (*eg.* oven, water bath, room temperature). The proposed electrochemical techniques suggests sensitive, fast, cost-effective and simple approach for the determination of TDF in tablet dosage form without any interference from tablet excipients.

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