

Electrochemical behavior of tadalafil on TiO₂ nanoparticles–MWCNT composite paste electrode and its determination in pharmaceutical dosage forms and human serum samples using adsorptive stripping square wave voltammetry

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Abstract A sensitive and selective electroanalytical method for the determination of tadalafil (TAD) using adsorptive stripping square wave voltammetry at multiwalled carbon nanotube paste electrode (MWCNTPE) and modified TiO₂-multiwalled carbon nanotube paste electrode (TiO₂-MWCNTPE) was presented. The calibration curves were linear in the concentration range of 3.6–8.1 and 12.7–61.1 μM on MWCNTPE, 0.27–15.2 μM on TiO₂-MWCNTPE. The recommended method was successfully applied to the determination of the drug in tablets and human serum samples with good recoveries. The selectivity of the proposed method was considered in the presence of Ca²⁺, K⁺, Na⁺, 2-mercapto benzimidazole, thiourea, and dopamine by means of recovery tests. Interfering agents did not show considerable effect on TAD determination. No electroactive interferences from the tablet excipients and endogenous substances from biological material were detected. The possible electrooxidation pathway and the number of transferred electrons were also investigated.

Keywords Tadalafil · Voltammetry · TiO₂ nanoparticles · MWCNT composite paste electrode · Pharmaceutical dosage form

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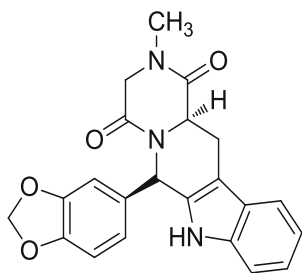
Introduction

Tadalafil (TAD) (Scheme 1), (6R,12aR)-6-(1,3-benzodioxol-5-yl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazino[1', 2':1,6]pyrido[3,4-b]indole-1,4-dione, is an oral drug that is used for treating impotence (the inability to attain or maintain a penile erection) and benign prostatic hyperplasia (BPH). It is in a class of drugs called phosphodiesterase inhibitors that also includes sildenafil and vardenafil. However, its chemical structure differs from that of sildenafil and vardenafil, reflecting differing pharmacological properties.

The mechanism whereby TAD improves the symptoms of BPH is not clear, but phosphodiesterase-5 is present in the muscles of the bladder and the prostate, and it has been suggested that the relaxation of these muscles may make the passage of urine less difficult (www.medicinenet.com/tadalafil/article.htm). Tadalafil is absorbed rapidly at mean C_{\max} (0.973 μM for 20 mg) observed at 2 h, thereafter, concentrations declined nearly mono-exponentially with the mean $t_{1/2}$ at about 17.5 h. Therefore, development of more effective analytical method is required for the routine analysis of TAD in biological fluids [1].

In the literature, there are several studies on the determination of TAD in pharmaceutical or biological samples. The methods in already published studies are liquid chromatography with UV detection [2–10], fluorescence detection [11], mass detection [12], gas chromatography with mass spectrometry (MS) detection [13], capillary chromatography [14], capillary electrophoresis [15], spectrophotometry [16, 17], and spectrofluorometry [18]. The reported methods have many shortcomings such as potential loss of drugs in the re-extraction procedure and the need of lengthy, tedious, and time-consuming plasma sample preparation and extraction process.

Among several analytical methods for detecting species in a particular solution, electroanalysis is one of the most



Scheme 1 Chemical structure of TAD

promising methods for its numerous advantages, such as low cost, sensitivity, reliability, accuracy, consistency, and its simplicity in use [19–21]. Since their discovery, many researchers in academics and industry have developed a widespread interest in using nanomaterial especially carbon nanotubes (CNT) as a biosensor. The reason for widespread utilization of CNTs in electrochemistry is their unique structural, mechanical, and electronic properties, which include the possession of hollow cores for storing guest molecules, high chemical and thermal stability, and high elasticity and conductivity [22]. CNTs show much better transfer characteristics with metal matrix composites. Efficient load transfer between a matrix and CNTs play a key role in the mechanical properties of composites and can lead to development of many super strong nanocomposites [23]. Nano-sized materials support constructively the catalytic sensitivity of CNT due to the combination of their electronic, adsorptive, mechanical, and thermal properties. Similarly, it has been demonstrated that titanium dioxide (TiO_2) nanoparticles is one of the most capable materials, which has good potential interest as a sensor electrode in electrochemistry [24, 25]. The combination of CNT and TiO_2 can provide significant effect for enhancing catalytic process [26]. With continued developments in the synthesis and production of CNTs, composite materials containing nanotubes are near-term applicable and will see innovations that take advantage of their special properties. In recent years, modified electrodes with nanoparticles have been used as a new type fabrication composite electrode, as they have many particular properties such as high chemical stability, excellent ionic conductivity, and wide electrochemical windows [27–30]. Moreover, high conductors that are multifunctional (electrical and structure) and highly anisotropic insulators and high-strength, porous ceramics are more examples of new materials that can come from nanotubes [31].

Even though a class of drugs called phosphodiesterase inhibitors such as sildenafil [32–34] and vardenafil [35] were studied by voltammetric methods, as yet, there has been no literature report for the electrooxidative behavior and redox properties of TAD, neither bare nor modified electrodes. In this paper, a composite paste electrode that has been made of multiwalled carbon nanotube (MWCNT) and incorporated into TiO_2 nanoparticles for determination of TAD is reported

for the first time. The aim of this work is to carry out a detailed investigation on the electrochemical behavior and possible oxidation mechanism of TAD with the proposed TiO_2 -MWCNTPE by using cyclic (CV) and adsorptive stripping square wave (AdSSWV) voltammetric techniques. This work was also aimed to develop a new, fully validated, rapid, and simple voltammetric method for the direct determination of TAD in bulk material, pharmaceutical dosage forms, and serum samples without any time-consuming extraction, evaporation, or separation steps prior to drug analysis.

Experimental

Apparatus

Voltammetric records were obtained by a Bioanalytical Systems-Epsilon potentiostat/galvanostat (BAS, Epsilon) analyzer connected with a BAS C-3 solid electrode cell stand. In all voltammetric measurements, BAS MF-2010 model carbon nanotube paste electrode ($\varnothing=3$ mm, $A=0.071$ cm²) was used as a working electrode, a platinum wire (BAS MW 1032) as the counter electrode, and an Ag/AgCl (3 mol L⁻¹ KCl) was used as a reference electrode. AdSSW voltammetric conditions were given as follows: pH 3.0 BR buffer solution as supporting electrolyte, $\Delta E_s=4$ mV, $f=200$ Hz, $\Delta E=40$ mV, $t_{acc}=40$ s, and $E_{acc}=0$ mV, due to the highest sensitivity, and selectivity pH measurements were performed by a Hanna pH meter with combined glass electrode. All experiments were conducted under room conditions.

Reagents

TAD was obtained from Abdi Ibrahim Pharm. Ind. Istanbul, Turkey. The commercial Lifta[®] tablets containing 20 mg of TAD were acquired from a local pharmacy. The following 0.04 M H_3BO_3 , 0.04 M H_3PO_4 , and 0.04 M CH_3COOH were used to prepare Britton–Robinson buffer (BR buffer) solutions extending from pH 2.0 to 10.0. All chemicals were provided from Merck, Darmstadt, Germany. MWCNT powder (mesh size, -270 , <53 μm) was obtained from Merck. TiO_2 used for modified electrode (1 % Mn doped; nanopowder, <100 nm (BET), ≥ 97) was provided from Sigma Aldrich. TAD stock solution was prepared daily by dissolving 50 mg TAD in 10.0 mL acetonitrile and kept from the light to avoid possible decomposition. Dilute solutions were prepared freshly by distilled water.

Preparation of the MWCNTPE and TiO_2 -MWCNTPE

MWCNTPE was prepared by mixing MWCNT powder and mineral oil with 70 % and 30 % mass percentages, respectively. The homogenized paste was inserted in a plastic syringe

needle using a 3-mm diameter copper wire (BAS MF 2010). Then, TiO₂-multiwalled carbon nanotube paste (MWCNTPE) electrodes were prepared with different mass percentages of TiO₂ such as 1, 5, 10, and 20 % to find the optimum blend. Modified-MWCNTPE electrode prepared as suitable composition by mixing 0.65 g of MWCNT powder with 0.05 g of TiO₂ nano-powder and 0.30 g mineral oil. Mineral oil was added as before and mixed again to get a homogenous modified TiO₂-MWCNTPE electrode (5 % TiO₂ by mass).

Tablet assay procedure

Five Lifta® tablets, containing 20 mg TAD, were accurately weighed and crushed to a homogeneous fine powder in a mortar. An accurate weight of this powder equivalent to one tablet content was weighed and transferred into a 100-mL calibrated flask, diluted with pH 3.0 BR buffer solution, and then sonicated for 10 min. The content of TAD was calculated from the corresponding regression equation.

Analysis of human serum samples

Human blood obtained from healthy volunteer was used for the TAD determination. Blood sample was centrifuged at 3,000 rpm at nearly 10 min at room temperature, and proteins in blood were separated from the serum. The obtained serum samples were stored at -4 °C until assay. For the analysis of serum, 3.6 mL of serum samples were spiked with 1.0 mL of TAD stock solution (5 mg mL⁻¹) and completed with 5.4 mL acetonitrile up to 10.0 mL, so that the final concentration of TAD was 500 µg mL⁻¹.

Recovery studies

Since other inactive ingredients of the pharmaceutical dosage forms may interfere with the analysis or affect its accurate quantification, potential effects from matrix components must be investigated. For this purpose, a known amount of the pure

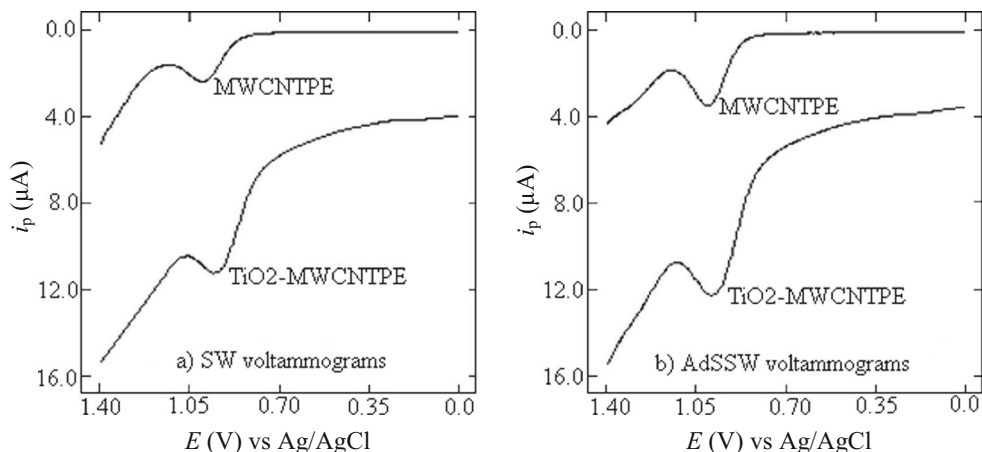
TAD was added to the pre-analyzed tablet formulation of TAD. The recovery results were obtained by using the related calibration equation for six repeated measurements.

Results and discussion

No previous electrochemical study about the sensitive, selective, and simple electroanalytical determination of TAD with neither MWCNTPE nor TiO₂-MWCNTPE has been performed. To demonstrate the usefulness of a MWCNTPE electrode for the determination of TAD which may offer advantages for the use of such electrodes as sensors, voltammetric behaviors of TAD on both MWCNTPE and TiO₂-MWCNTPE were investigated in detail by CV and AdSSWV.

AdSSWV methods have been applied to numerous drug active compounds, by which the low detection and determination limits could be achieved. Effect of “stripping” was proved by obtaining voltammograms using SWV on both electrodes. SW and AdSSW voltammograms of 5 µg mL⁻¹ TAD solutions with MWCNTPE and TiO₂-MWCNTPE were shown in Fig. 1a and b, respectively. When used adsorptive stripping voltammetric conditions, about 1.5 times increasing in the current response was obtained with TiO₂-MWCNTPE electrodes when compared with MWCNTPE. In addition, peak current of TAD at TiO₂-MWCNTPE using AdSSWV is 2.6-fold bigger than the peak current of TAD at MWCNTPE using SWV (Fig. 1) ($\Delta E_s=4$ mV, $f=200$ Hz, $\Delta E=40$ mV, $t_{acc}=40$ s and $E_{acc}=0$ mV pH 3.0 BR buffer solution). The resulting enhancement in kinetics of TAD oxidation, together with the considerable decrease in the background current probably makes TiO₂-MWCNTPE more sensitive using either SWV or AdSSWV for the determination of trace amount of TAD. The superiority of AdSSWV, and hence sensitivity, could be attributed to the contribution of adsorption phenomenon of TAD during electro-deposition process.

Fig. 1 Comparison of the characteristics of TAD peak currents at MWCNTPE and TiO₂-MWCNTPE electrodes in pH 3.0 BR buffer solution **a** SW voltammetric technique, **b** AdSSW voltammetric technique



Effect of potential modulation parameters

Peak currents for $5 \mu\text{g mL}^{-1}$ TAD in pH 3.0 BR buffer were obtained at MWCNTP electrode under different accumulation potentials within the ranges of 0–800 mV. As shown Fig. 2a, the peak currents depend on accumulation potentials, and 0 mV was chosen as an optimum potential due to its maximum sensitivity.

The influence of the accumulation time has been tested between 0 and 120 s in pH 3.0 BR buffer solution. As shown in Fig. 2b, the peak currents did not significantly change at the initial stages of accumulating time but noticeably decreased for further applications after 80 s, which could be attributed to the saturation of the target molecule on the electrode surface. The accumulation time was therefore selected as 40 s for subsequent analysis. The influence of amplitude has been tested in the potential range from 10 to 70 mV. The TAD peak signal reached up to the maximum current as the amplitude value approached 40 mV and remained nearly constant up to 70 mV (Fig. 2c). The influence of step potential on the TAD peak exhibited that the highest and best-regular response appeared with step potential 4 mV (Fig. 2d). The increments of frequency from 25 to 300 Hz caused both peak increments and potential shifts, respectively. On the other hand, frequency more than 200 Hz caused the peak shape of TAD to be broader and deformed. Thus, frequency was chosen as 200 Hz for subsequent analysis.

In order to compare their performances, the same optimum parameters were used for TiO_2 -MWCNTP

electrode. In summary; accumulation potential, accumulation time, potential step, frequency, and pulse amplitude were $E_{\text{acc}}=0$ mV, $t_{\text{acc}}=40$ s, $\Delta E_s=4$, $f=200$ Hz and $\Delta E=40$ mV, respectively.

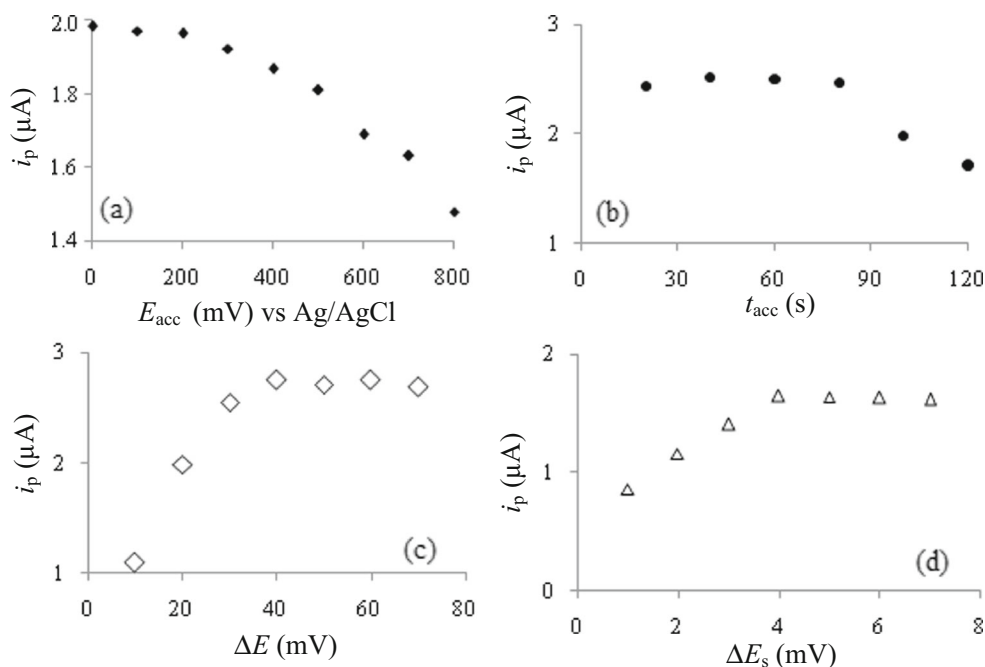
Influence of composition of TiO_2

In order to find the most effective composition of TiO_2 -MWCNT powders for the sensitive detection of TAD, various modified electrodes with different compositions were tested by SWV in the BR buffer at pH 3.0. The most regular peak responding to TAD had appeared using the electrode prepared with the 5 % (w/w) of TiO_2 nano particles. When mass percentage of TiO_2 in the modified electrode was increased with respect to MWCNT such as 10 % and 20 %, the peak current of TAD was dramatically decreased. This can be attributed to the strong adsorption behavior of TiO_2 nanoparticles. The maximum sensitivity for the oxidation peak was recorded for the composite electrode containing 5 % TiO_2 by mass. When the higher amounts of TiO_2 were introduced into the carbon nanotubes the electron transferring on the surface of the composite electrode had difficulties due to the higher than 5 % TiO_2 by mass.

Influence of scan rate

In order to understand whether the electrode process is diffusion- or adsorption-controlled, scan rate influence on

Fig. 2 The influence of experimental parameters on the AdSSWV determination of TAD in pH 3.0 BR buffer solution **a** accumulation potential (E_{acc}), **b** accumulation time (t_{acc}), **c** pulse amplitude and **d** step potential



the peak current was investigated within the ranges of 20 to 400 mV s^{-1} on both electrodes at pH 3.0 BR buffer solutions.

Following equations were obtained within the range 20 to 250 mV s^{-1} for both electrodes.

$$i_p(\mu\text{A}) = 0.44 v^{1/2} (\text{V s}^{-1}) - 0.595, r = 0.998 \text{ for the MWCNTPE}$$

$$i_p(\mu\text{A}) = 0.67 v^{1/2} (\text{V s}^{-1}) - 0.847, r = 0.999 \text{ for the TiO}_2\text{-MWCNTPE}$$

$$\log i_p(\mu\text{A}) = 0.43 \log v (\text{mV s}^{-1}) - 0.28, r = 0.991 \text{ for the MWCNTPE}$$

$$\log i_p(\mu\text{A}) = 0.63 \log v (\text{mV s}^{-1}) - 0.48, r = 0.994 \text{ for the TiO}_2\text{-MWCNTPE}$$

The logarithm of oxidation peak currents versus scan rates exhibited a linear relationship with a slope of 0.43 and 0.63, for the MWCNTPE and $\text{TiO}_2\text{-MWCNTPE}$ electrodes, respectively. The 0.43 value is close to the theoretical one of 0.5 for an ideal diffusion-controlled electrode process for MWCNTPE. For $\text{TiO}_2\text{-MWCNTPE}$, the experimental slope (0.63) than the theoretical one may be attributed to the partial involvement of the diffusive drug molecules in the electrode

reaction of the adsorbed ones. The overall electrode process is mixed diffusion-controlled with adsorption of the drug molecules at the electrode surface [36].

The oxidation peak potentials were simultaneously shifted (73 mV for MWCNTPE, 84 mV for $\text{TiO}_2\text{-MWCNTPE}$) to more positive potentials by the scan rates increments on the both electrodes. The linear relations between the peak potential and the logarithm of scan rate can be expressed as:

$$E_p(\text{V}) = 0.051 \log v (\text{V s}^{-1}) + 0.949, r = 0.990 \text{ for the MWCNTPE}$$

$$E_p(\text{V}) = 0.056 \log v (\text{V s}^{-1}) + 0.927, r = 0.986 \text{ for the TiO}_2\text{-MWCNTPE}$$

In order to determine the kinetic parameters of the electron-transfer process for the TAD oxidation on the MWCNTPE and $\text{TiO}_2\text{-MWCNTPE}$, cyclic voltammetric experiments were performed at different scan rates. The Laviron's theory for irreversible processes was then applied to calculate the number of electron transferred and the heterogeneous electron-transfer rate constant (k^0).

$$E_p = E^{0'} - \left(\frac{2.303RT}{\alpha nF} \right) \log \left(\frac{RTk^0}{\alpha nF} \right) + \left(\frac{2.303RT}{\alpha nF} \right) \log v$$

where $E^{0'}$ is the formal potential, T is the temperature in degrees Kelvin (298 K), α is the transfer coefficient, k^0 is the rate constant for the interfacial electron transfer process (per second), n is the number of electrons transferred in the rate determining step, v is the scan rate, F is the Faraday constant ($96,480 \text{ C mol}^{-1}$), and R is the universal gas constant ($8.314 \text{ K}^{-1} \text{ mol}^{-1}$).

Herein, for the MWCNTPE and $\text{TiO}_2\text{-MWCNTPE}$, slopes and αn values were 0.051, 0.056 and 1.16, 1.06, respectively. Generally, α is assumed to be 0.5 in totally irreversible electrochemical behavior. Therefore, the values of $n=2.32$ and 2.12 (~ 2) were obtained for the oxidation peak at MWCNTPE and $\text{TiO}_2\text{-MWCNTPE}$, respectively.

The value of $E^{0'}=0.969$ and 0.964 V were obtained from the intercepts of a plot of E_p versus v at MWCNTPE and $\text{TiO}_2\text{-MWCNTPE}$, respectively. From this, k^0 was calculated to be 96.07 s^{-1} for MWCNTPE and 178.29 s^{-1} for $\text{TiO}_2\text{-MWCNTPE}$. These indicates the efficiency of the MWCNTPE and $\text{TiO}_2\text{-MWCNTPE}$ electrodes in promoting the electron transfer between TAD and the electrode surfaces.

In this study, the forward peak was shifted at 53 and 54 mV per tenfold change in scan rate for MWCNTPE and $\text{TiO}_2\text{-MWCNTPE}$, respectively. According to Gossner [37], if both electrode kinetics and chemical kinetics are slow, the cyclic voltammograms are irreversible.

Influence of pH for MWCNTPE and $\text{TiO}_2\text{-MWCNTPE}$

In most analytical determination, it is well known that the pH of the supporting electrolyte has a major impact on the response of organic and inorganic drug compounds. This parameter was examined to detect involvement of the proton for the oxidation process on MWCNTPE and $\text{TiO}_2\text{-MWCNTPE}$.

For this aim, the effects of pH on both peak potential and current were studied within pH 2.0 to 10.0 using SWV for both electrodes. The peak potentials were shifted to less positive direction by increasing pH, indicating that the

oxidation of TAD was pH-dependent (Fig. 3a and b). The linear relationship between E_p and pH for both electrodes

exhibited a linear segment in the pH range of 2.0 to 10.0 as follows:

$$E_p(\text{mV}) = -29.13 \pm 0.03 \text{ pH} + 1107.9 \pm 0.8, r = 0.991 \text{ for the MWCNTPE}$$

$$E_p(\text{mV}) = -36.32 \pm 0.06 \text{ pH} + 1109 \pm 1, r = 0.995 \text{ for the TiO}_2\text{-MWCNTPE}$$

The E_p versus pH plot consists of three straight portions with slope values of 29.2 and 36.3 mV, MWCNTPE and TiO₂-MWCNTPE electrodes, respectively. The slope value = $(0.059 m/\alpha n)$, where α is the symmetry transfer coefficient and m and n are the number of protons and electrons involved in the electrode reaction, respectively. The αn values were calculated from Laviron equation. Hence, the m values were calculated as 0.48 and 0.61 (~1) for MWCNTPE and TiO₂-MWCNTPE electrodes, respectively [38, 39].

The influence of pH on the peak currents at both electrodes was also studied in detail, and the experimental results indicated that the proper curves and the maximum peak currents were obtained in pH 3.0 buffer solutions for both electrodes. Accordingly, due to the highest sensitivity and selectivity, the peak current obtained at pH 3.0 BR buffer solution was preferred for analytical purposes.

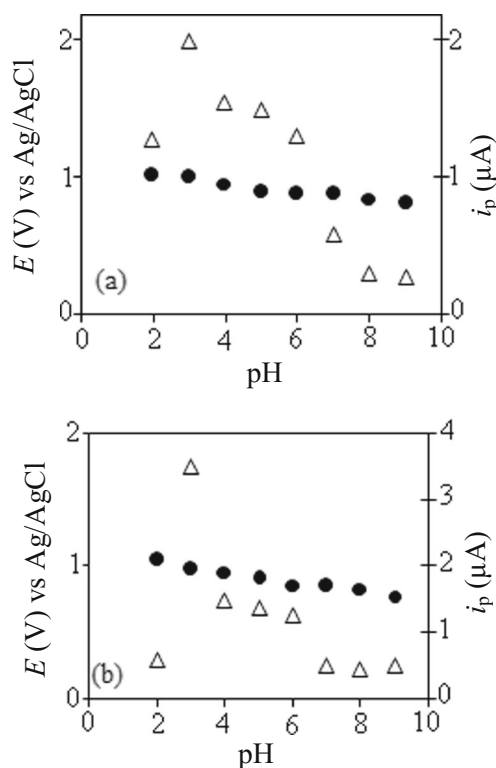


Fig. 3 The influence of pH on the determination of 5 $\mu\text{g mL}^{-1}$ TAD **a** MWCNTPE, **b** TiO₂-MWCNTPE (open triangle for the current, closed circle for the potential) ($\Delta E_s=5$ mV, $f=100$ Hz, $\Delta E=50$ mV, $t_{\text{acc}}=60$ s and $E_{\text{acc}}=0$ mV)

Electrochemical behavior of TAD

In order to characterize the electrochemical oxidation behavior of TAD, cyclic voltammetry (CV) was carried out in the potential range of 0 to 1.4 V. In this study, electrochemical oxidations behaviors of TAD on the MWCNTPE and the TiO₂-MWCNTPE were investigated.

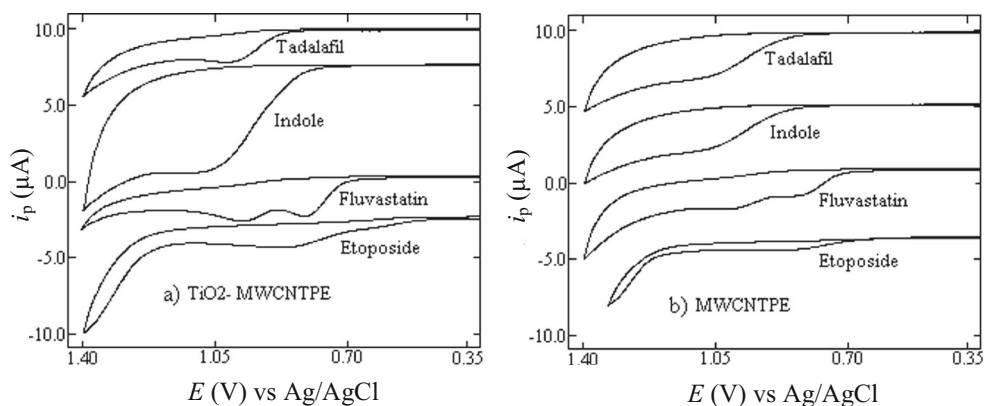
The cyclic voltammetric measurements of 300 $\mu\text{g mL}^{-1}$ TAD solution for both electrodes indicated irreversible behaviors (Fig. 4). Once the potential scanning was carried out from 0 to 1.4 V in the positive direction, single anodic response was detected on MWCNTPE and TiO₂-MWCNTPE at about +1.02 and +1.0 V, respectively. By reversing the potential scan from 1,400 to 0 mV, no reduction peak or wave corresponding to the anodic response of TAD was observed. After the second or further cycles, the TAD oxidation response was decreased and finally remained unchanged. This phenomenon may be due to the adsorption of TAD or its oxidative products at the electrode surface. After all, TAD exhibited an irreversible electrochemical behavior on both electrodes. Anodic peak current of TAD appeared at 1.0 V on TiO₂-MWCNTPE which was about 0.02 V less positive (not significant change) than observed at MWCNTPE. These results on TiO₂-MWCNTPE demonstrate that a strong enhancement in electron transfer rate of TAD is taking place. TiO₂ nanoparticles together with MWCNT provide an excellent electrochemical reactivity.

The SW voltammograms of 5 $\mu\text{g mL}^{-1}$ TAD was shown in Fig. 1 on MWCNTPE and TiO₂-MWCNTPE using both techniques. TAD exhibited an anodic peak which obtained peak current as 2.4 μA at MWCNTPE with an oxidation peak potential at +1.02 V. Using TiO₂-MWCNTPE, the peak current at +1.0 V was increased to 3.6 μA , and this indicates the presence of a synergy between TiO₂ nanoparticles and MWCNT (Fig. 1).

Oxidation mechanism of TAD

CV techniques are among the most suitable methods for the investigation of redox behavior of drug active compounds that can give insights into its metabolic fate [21, 40–45]. Electrochemical responses from the redox properties of drugs and biomolecules might have profound effects on the understanding of the redox mechanism related to the activity. In order to identify the possible functional group responsible from the

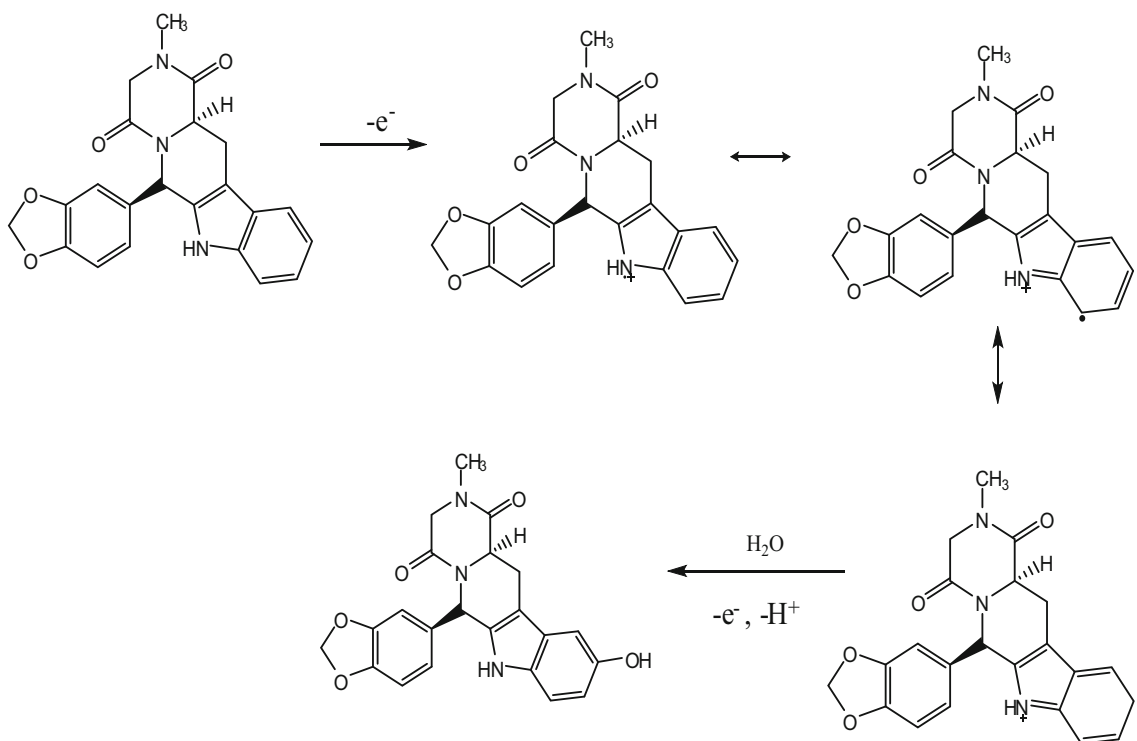
Fig. 4 Application of the model drugs for the illumination of the electrooxidation mechanism of TAD in pH 3.0 BR buffer solution **a** MWCNTPE, **b** TiO₂-MWCNTPE



electro oxidation of TAD, the CV experimental results and their curves were compared with those of some selected model compounds containing indole and benzodioxol groups.

Although the exact oxidation mechanism was not determined, some conclusions about the potentially electroactive centers under working conditions could be reached. Some model compounds were used for understanding the electrooxidation mechanism of TAD. For this, indole, fluvastatin sodium, which contains indole ring in their structures and etoposide, which contains benzodioxol ring in its structure of TAD, has been studied. Several measurements with different electrochemical techniques were performed using various pH values (pH 3.0 and 7.0 BR-buffer solution) for all these model compounds and TAD in order to obtain information about the oxidation behavior. CV measurements

on the anodic direction showed the irreversible nature of the oxidation process with model compounds (Fig. 4). TAD and model compounds (except etoposide which contains benzodioxol ring and methoxy group) exhibit anodic responses nearly at the same potentials. This indicates that the oxidation of TAD is due to the indole moiety. From the CV curves, the voltammetric behavior of indole derivatives, which are structurally related to TAD, may be postulated by the oxidation of this group [21, 40–51]. Our results on model compounds show that the electro active center responsible from the anodic peak was the nitrogen atom on the indole ring. In all studied pH values, indole response was obtained clearly and separately. The anodic oxidative behavior of TAD was also comparable to the indole oxidation of the model



Scheme 2 Possible oxidation pathways of TAD

Table 1 Analytical performance data for the determination of TAD by AdSSWV using MWCNTPE and TiO₂-MWCNTPE at pH 3.0 BR buffer solution

Parameters	Performance data (MWCNTPE)		Performance data (TiO ₂ -MWCNTPE)
	Low conc.	High conc.	
Peak potential (V)	+1.02	+1.02	+1.0
Linearity range (μM)	3.60 to 8.05	12.70 to 61.10	0.27–15.20
Slope ($\mu\text{A}/\mu\text{M}$)	0.082 \pm 0.003	0.163 \pm 0.007	0.072 \pm 0.002
Intercept (μA)	0.12 \pm 0.02	1.41 \pm 0.28	0.56 \pm 0.02
Correlation coefficient	0.995	0.997	0.997
Limit of determination, LOD (μM)	0.11	3.70	0.08
Limit of quantification, LOQ (μM)	0.36	12.30	0.27
Repeatability of peak potential (RSD%) ^a	0.237	0.158	0.447
Repeatability of peak current (RSD%) ^a	0.551	0.238	0.322
Reproducibility of peak potential (RSD%) ^b	0.27	0.25	0.50
Reproducibility of peak current (RSD%) ^b	1.54	0.76	0.90

Relative standard deviation, $\text{RSD} = \left(\frac{s}{\bar{x}}\right)100$

^a $n=7$

^b $n=9$

compounds as reported in the literature [52]. Fluvastatin sodium is C-3 indole derivatives. Two oxidation peaks were observed like other C-3 indole derivatives [52]. Electrochemical behavior of fluvastatin sodium investigated as details in our previous paper. It gave two oxidation peaks at +0.8 and at +0.9 V in pH 3 phosphate buffer. Electrochemical reaction at boron-doped diamond electrodes involves two electrons and two protons [53]. Chemical structure of TAD is different than fluvastatin sodium molecule. For this reason, its oxidation

mechanism is a little bit different. Hence, we may suggest that TAD is irreversibly oxidized with two electrons and one proton at electrodes. Comparative studies on indole, fluvastatin sodium, were realized by CV as a function of pH in order to identify the oxidation process of TAD. Considering the aforementioned comparison for the oxidation process of TAD, and bearing the oxidative process of nitrogen atom on the indole ring in mind, leads finally to hydroxylation of the benzene ring [54] (Scheme 2).

Fig. 5 AdSSW voltammograms for the calibration graph of TAD **a** TiO₂-MWCNTPE, **b** MWCNTPE; **a** pH 3.0 BR buffer solution, **b** 0.6 μM , **c** 2.6 μM , **d** 5.12 μM , **e** 7.7 μM , **f** 8.9 μM , **g** 10.2 μM , **h** 11.5 μM , **k** 12.7 μM , **l** 15.2 μM ($\Delta E_s=4$ mV, $f=200$ Hz, $\Delta E=40$ mV, $t_{\text{acc}}=40$ s and $E_{\text{acc}}=0$ mV)

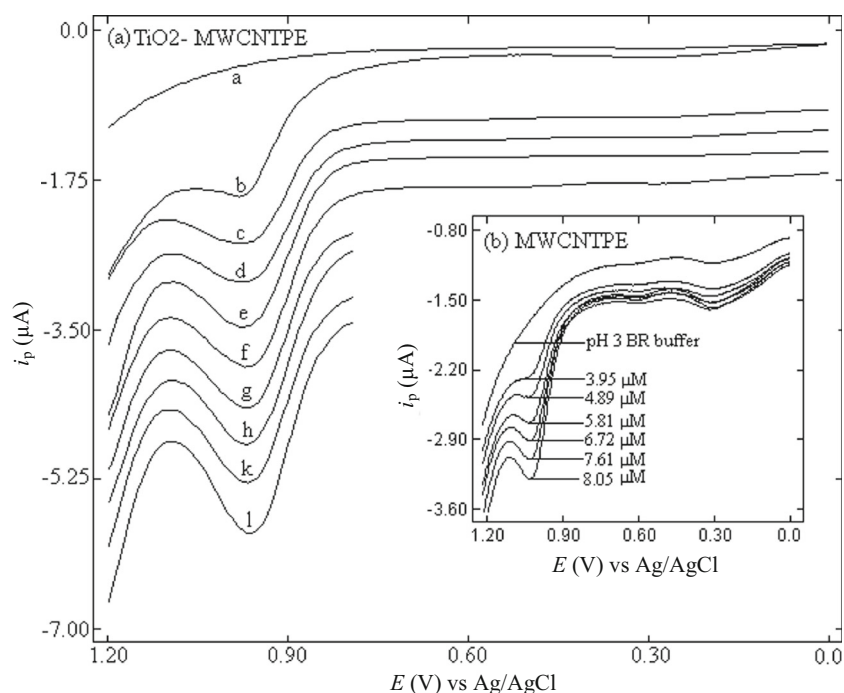


Table 2 Effect of interfering species on the determination of TAD

Interfering species	Recoveries (%)			
	Ratio of analyte to interference 1:1 (m/m)	Ratio of analyte to interference 1:2 (m/m)	Ratio of analyte to interference 1:5 (m/m)	Ratio of analyte to interference 1:10 (m/m)
2-mercapto benzimidazole	97.1	95.5	94.1	93.6
Ca ²⁺	93.4	86.9	84.3	80.3
K ⁺	100.2	95.3	95.4	88.7
Na ⁺	98.4	92.4	89.3	86.7
Thiourea	99.5	99.4	87.5	82.7
Dopamine	93.4	82.7	78.6	63.3
Uric acid	96.2	83.6	80.3	76.5

Calibration curve using MWCNTPE and TiO₂-MWCNTPE electrodes

The results of pre-trial showed that it was possible to apply SWSV techniques to the quantitative analysis of 3.6 μM TAD using MWCNTPE and TiO₂-MWCNTPE electrodes. In the calibration curve, there were two linear sections for MWCNTPE extending from 3.6 to 8.05 μM and

12.7 to 61.1 μM (Table 1). To our advantage, we had focused to the first section because these drugs generally are found at a low concentration in human body. On the other hand, there was a single linear part on TiO₂-MWCNTPE electrodes with a much lower detection limit compared with the bare electrode (Fig. 5). The linear equations for both electrodes could be displayed as follows:

$$I_p(\mu A) = 0.082 \pm 0.003 C(\mu M) + 0.12 \pm 0.02, r = 0.995 \text{ (between 3.6 and 8.05 } \mu M) \text{ using MWCNTPE}$$

$$I_p(\mu A) = 0.163 \pm 0.007 C(\mu M) + 1.41 \pm 0.028, r = 0.997 \text{ (between 12.7 and 61.1 } \mu M) \text{ using MWCNTPE}$$

$$I_p(\mu A) = 0.072 \pm 0.002 C(\mu M) + 0.56 \pm 0.02, r = 0.997 \text{ (between 0.27 and 15.2 } \mu M) \text{ for the TiO}_2\text{-MWCNTPE}$$

Effect of some interference substances at TiO₂-MWCNTPE

The selectivity of the proposed method was considered in the presence of some cations and bio-molecules by means of recovery tests. The interfering effects were estimated by comparing

the peak currents in the presence of the interfering species to that in their absence. The recoveries for a 10.0 μg mL⁻¹ TAD in the presence of 2-mercapto benzimidazole, thiourea, dopamine, uric acid, or Ca²⁺, K⁺, and Na⁺ are presented in Table 2. Dopamine is a member of the catecholamine family that plays active roles in the brain as a neurotransmitter. As shown in Fig. 6, dopamine exhibited an oxidation peak at about 0.76 V in pH 3.0 BR buffer solution and did not show serious interfering effect on the

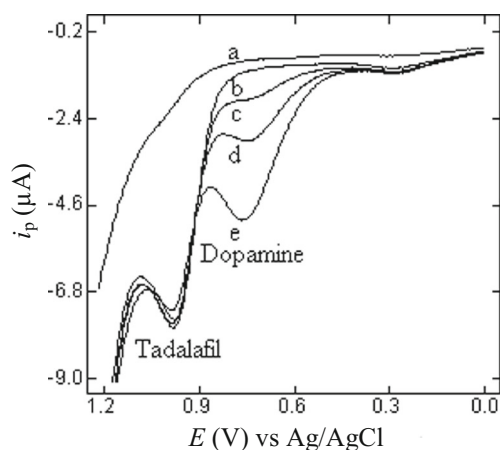


Fig. 6 AdSSWV determination of TAD in the presence of dopamine **a** 10.0 mL pH 3.0 BR buffer solution, **b** 10.0 μg mL⁻¹ TAD, **c** 10.0 μg mL⁻¹ TAD+10.0 μg mL⁻¹ dopamine, **d** 10.0 μg mL⁻¹ TAD+20.0 μg mL⁻¹ dopamine, **e** 10.0 μg mL⁻¹ TAD+50.0 μg mL⁻¹ dopamine, (ΔE_s=4 mV, f=200 Hz, ΔE=40 mV, t_{acc}=40 s and E_{acc}=0 mV)

Table 3 Analysis of TAD in commercial pharmaceutical dosage forms Lifta®

	MWCNTPE	TiO ₂ -MWCNTPE
Labeled claim (mg)	20.0	20.0
Found (mg±mg) ^a	19.98±0.3	19.99±0.4
RSD (%)	1.4	1.8
Relative error (%)	-0.1	-0.05
Added (mg)	20.0	20.0
Found (mg±mg) ^a	19.95±0.23	19.96±0.21
RSD (%)	1.00	0.91
Relative error (%)	-0.25	-0.20

^a n=6; $\bar{X} \pm \frac{t_s}{\sqrt{n}}$ at 95 % confidence level

Table 4 Comparison of proposed voltammetric method for the determination of tadalafil with the reported data

References	Methods	Dynamic concentration range ($\mu\text{g/mL}$)	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)	Recovery (%)
[2]	LC-UV	0.005–2	0.001	0.0033	–
[3]	LC	10–100	–	–	–
[4]	LC	50.7–152.10	–	–	100.26
[5]	LC-UV	50–150	0.01	0.025	98.84
[6]	LC	10–200	0.021	0.066	100.95
[7]	Stability-indicating LC	10–100	–	–	–
[8]	LC-UV	0.01–0.8	0.0015	0.010	–
[9]	LC-UV	0.01–2	–	0.01	>77
[10]	LC-UV	0.1–5	0.1	–	99.5
[11]	LC-fluorescence	0.1–2	0.04	0.1	>98.0
[12]	LC-MS/MS	0.0005–1	1×10^{-8}	0.0005	91.07
[13]	GC/MS	0.002–0.5	0.0007	0.002	92.1–98.9
[14]	Micellar electrokinetic capillary chromatography	1–20	0.61	2.0	98.0
[15]	Capillary electrophoresis	200–5000	200	350	99.5
[16]	UV spectrophotometric	2–20	–	–	99.5–100.2
[17]	UV-visible spectrophotometric	0.4–2	–	–	99.89
[18]	Spektrofluorophotometric	4–20	2.08×10^{-4}	6.94×10^{-4}	97.18–100.02
This work	SWSV	0.11–5.92	0.031	0.10	100.8

determination of TAD. The influence of some cationic species found commonly in living organisms, such as Ca^{2+} , K^+ , and Na^+ was also studied. They displayed no interfering effects, since they could not be further oxidized near the peak potential of TAD at +1.0 V. The recovery of $10.0 \mu\text{g mL}^{-1}$ TAD in the presence of Ca^{2+} , K^+ , and Na^+ , and biomolecules with the mass ratios of 1:1, 1:2, 1:5, and 1:10 was extended from 80.3 % to 100.2 %. The interfering species such as urea, uric acid, and their derivatives have also vital importance as physiological compounds for life. These interfering agents did not show considerable effect on the TAD quantization. The degrees of the recoveries in the presence of latter species were consequently between 93.4 % and 99.5 % in their 1:1 mass ratios.

Application of analyte

Determination of TAD from pharmaceutical dosage forms and spiked human serum samples were studied in details using both electrodes. Also, studies on tablets and spiked serum samples were applied to investigate the reliability of the developed voltammetric methods.

Determination of TAD in pharmaceutical dosage forms and recovery studies

In order to investigate the applicability of the proposed methods in pharmaceutical dosage form, a commercial Lifta[®] tablets

were used. To determine whether recipients in the pharmaceutical dosage form interfere with the analysis, the accuracy of the proposed methods were evaluated by recovery tests after addition of known amounts of pure drug to the pre-analyzed formulations of TAD (Table 3). A tablet, “commercial pharmaceutical dosage forms Lifta[®]”, claims to contain 20 mg TAD was dissolved in 40.0 mL of acetonitrile and was used daily and prepared by dilution of the stock solution to $10 \mu\text{g mL}^{-1}$ with supporting electrolyte. The obtained results showed (Table 3) that the validity of the proposed methods was applied successfully to the quantitative for determination of TAD on both electrodes. According to the literature survey, voltammetric determination of TAD has not yet been reported, and therefore, the present method was compared with the other techniques. To evaluate the performance of the present method on the basis of its linearity range, detection limit, and recoveries, it was

Table 5 Determination of spiked TAD in serum samples

Medium	MWCNTPE Serum	TiO ₂ -MWCNTPE Serum
Add of TAD (mg)	0.5	0.5
Amount found (mg \pm mg)	0.502 ± 0.004	0.504 ± 0.012
Average recovery (%)	100.4	100.8
RSD (%)	0.32	0.96
Relative error (%)	+0.4	+0.8

$$n=3; t: 95 \% \text{ confidence level} = \left\{ \bar{X} \pm \frac{t_s}{\sqrt{n}} \right\}$$

compared with some other methods reported in the literature for TAD determination, and the results were summarized in Table 4. These results make it evident that the developed method is superior to previously reported ones in terms of dynamic concentration range [3–7, 10, 11, 14–18], detection limit [10, 11, 14, 15], and recoveries [5, 6, 9, 11–14, 18].

Determination of TAD in spiked human serum samples

The optimized AdSSWV method was also successfully applied to determination of TAD spiked to human samples in pH 3.0 BR buffer solution. Only obtained serum results were shown in Table 5. The mean percentage recoveries of TAD based on an average of five replicate measurements was found to be equal to 100.3 and 100.9, MWCNTPE and TiO₂-MWCNTPE, respectively (Table 5). The results are obviously accurate and precise.

Conclusions

In the presented work, the electrochemical behavior of TAD is investigated by CV and AdSSWV using MWCNTPE and TiO₂-MWCNTPE. The analytical performances of TiO₂-MWCNTPE were better than the bare MWCNTPE electrode. The peak current of TAD at TiO₂-MWCNTPE using AdSSWV was 2.6-fold higher than that of TAD at MWCNTPE using SWV. TiO₂-MWCNTPE composites with good oxidation catalysis for TAD would promote the ability of the electrode to eliminate the interferences from some cations and bio-molecules. The possible electrooxidation pathway and the number of transferred electrons were investigated as details. From the CV curves, the voltammetric behaviors of indole derivatives, which are structurally related to TAD, were determined, and it was postulated that the anodic peak was due to the oxidation of this group. This method provides a new way to construct a modified electrode for sensitive and selective determination of TAD from tablet dosage forms and human serum samples compared with MWCNTPE. A simple and convenient precipitation procedure makes this proposed method more feasible for the determination of TAD in human serum. The applicability of this method has been demonstrated by successfully analyzing the serum samples of the clinical study. The developed methods provide a selective, fast, experimentally convenient, cost-effective, high-throughput, and simple approach to the determination of TAD in tablet dosage forms and human serum, without the necessity of sample pretreatment or any time-consuming extraction and evaporation steps prior to the analysis. When comparing with the already published methods (Table 4), the proposed method shows the similar sensitivity, determination limit, and linearity ranges. But some of the published methods, especially liquid chromatography (LC)-MS and fluorimetric

methods, are more sensitive than our proposed method. However, the proposed electroanalytical assay has many advantages such as simplicity, selectivity, less solvent consumption, and the lack of extraction processes. The proposed assay might be an alternative to the LC techniques in therapeutic drug monitoring, or the experimental data might be used for the development high-performance liquid chromatography-electrochemical detection method. Furthermore, the presented method could possibly be adopted for pharmacokinetic studies, as well as clinical and quality control laboratories.

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