Turk J Pharm Sci 2019;16(2):184-190
DOI: 10.4274/tjps.galenos.2018.60783

ORIGINAL ARTICLE

Electroanalytical Determination of the Anti-inflammatory Drug Tenoxicam in Pharmaceutical Dosage Forms

Anti-enflamatuvar İlaç Tenoksikamın Farmasötik Dozaj Formlarından Elektroanalitik Miktar Tayini

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ABSTRACT

Objectives: The electro-oxidation behavior of the non-steroidal anti-inflammatory drug tenoxicam (TX) was studied on multiwalled carbon nanotube (MWCNT)-modified glassy carbon electrode (GCE) by cyclic voltammetry, differential pulse voltammetry (DPV), and square wave voltammetry (SWV).

Materials and Methods: The GCE was modified with MWCNT for sensitive determination of TX by voltammetric methods.

Results: The current peaks for TX occurred at around 0.520 V for DPV and 0.570 V for SWV when the potential was scanned in the positive direction. The oxidation process of TX showed irreversible and diffusion-controlled behavior. The linear responses were obtained in the range from 2×10^{-7} to 1×10^{-5} M with the limit of detection (LOD) 1.43×10^{-9} for DPV and from 8×10^{-9} to 8×10^{-6} with the LOD 9.97×10^{-10} for SWV in 1 M acetate buffer solution at pH 5.5.

Conclusion: Fully validated DPV and SWV were successfully applied for the determination of TX from pharmaceutical dosage form and yielded satisfying results.

Key words: Glassy carbon electrode, multiwalled carbon nanotubes, tenoxicam, voltammetry

ÖZ

Amaç: Non-steroidal antienflamatuvar ilaç etken maddesi tenoksikamın (TX) elektro-oksidasyon davranışı çok duvarlı karbon nanotüple (MWCNT) modifiye edilmiş camsı karbon elektrot (GCE) ile dönüşümlü voltametri, diferansiyel puls voltametri (DPV) ve kare dalga voltametri (SWV) ile çalışılmıştır.

Gereç ve Yöntemler: GCE, TX'in voltametrik metodlarla hassas tayini için MWCNT ile modifiye edilmiştir.

Bulgular: Potansiyel pozitif yönde tarandığında TX'in pik akımı 0.520 V civarında DPV ile, 0.570 V civarında SWV ile oluşmuştur. TX'in oksidasyon prosesi tersinmez ve diffüzyon kontrollü davranış göstermiştir. DPV ve SWV için doğrusal cevaplar sırasıyla 2×10^{-7}-1×10^{-5} M, 1.43×10^{-9} M yakalama alt sınırı (LOD) ile, 8×10^{-9}-8×10^{-6} M, 9.97×10^{-10} M LOD ile 1 M asetat tamponu pH 5.5 içinde elde edilmiştir.

Sonuç: Tamamen valide edilmiş DPV ve SWV başarılı bir şekilde TX'in farmasötik dozaj formundan miktar tayini için uygulanmış ve memnun edici sonuçlar elde edilmiştir.

Anahtar kelimeler: Camsı karbon elektrot, çok duvarlı karbon nanotüp, tenoksikam, voltametri

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Received: 24.01.2018, Accepted: 15.03.2018

INTRODUCTION

Tenoxicam (TX) (Figure 1) is a non-steroidal anti-inflammatory drug and shows analgesic, anti-inflammatory, and antirheumatic properties. TX, a member of the oxicams class, is widely used to relieve swelling, inflammation, stiffness, and pain associated with osteoarthritis, rheumatoid arthritis, arthrosis, ankylosing spondylitis, arthritic diseases such as tendinitis, bursitis, shoulder or hip periarthritis (shoulder-hand syndrome), sprains and injuries, and acute gout. TX inhibits prostaglandin biosynthesis both *in vitro* and *in vivo*. It shows a strong inhibitory effect *in vitro* on human metalloproteinase (stromelysin and collagenase) enzymes that stimulate cartilage destruction.[1]

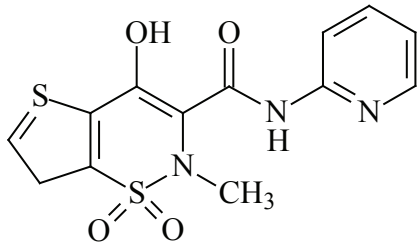

Figure 1. Chemical structure of TX

TX: Tenoxicam

In the literature, high performance liquid chromatography,[2-7] thin layer chromatography,[8] flow injection spectrophotometric analysis,[9-11] and spectrophotometric and spectrofluorimetric methods[12-15] are reported as methods for the determination of TX in pharmaceuticals and biological samples. These methods require mostly time-consuming sample preparation procedures such as extraction and the costly instrumentation makes their usage inconvenient. Electrochemical methods are user friendly, no pretreatment is required for them, and they use low-cost instrumentation and minimum amount of organic solvent compared to the reported analytical methods. Additionally, electrochemical methods supply high sensitivity, precision, accuracy, and wider linear dynamic range.[16,17]

TX was determined using a differential pulse polarographic method in pharmaceuticals and blood, with a static mercury drop electrode.[18] El-Maali et al.[19] investigated the electro-reduction behavior of TX and piroxicam at the static mercury drop electrode. The electro-reduction of TX was also investigated using a hanging mercury drop electrode.[20]

In recent years, working electrodes were modified with carbon nanotubes (CNTs) for electrochemical and bio-electrochemical studies.[21,22] CNTs can be used as electrode materials with useful properties; they show excellent high chemical stability, high mechanical strength, and a wide range of electrical conductivity. CNTs supply a modifier to promote electron transfer reactions between many biologically important species and the surface of the electrode. CNT-modified electrodes have been reported to have excellent electroanalytical properties such as low background current, wide potential window, high sensitivities, and low detection limits.[23] The excellent properties of CNTs make them extremely popular for obtaining chemical sensors and they are used for electrochemical detection.[24]

The aim of the present study was to develop a multiwalled (MW)CNT-modified glassy carbon electrode (GCE) for electroanalytical determination of TX and to investigate the electro-oxidative behavior of TX with voltammetric methods. The obtained MWCNT-modified GCE and fully validated voltammetric methods indicated a low detection limit, high selectivity and sensitivity, and good recovery results in the electroanalytical determination of TX.

EXPERIMENTAL

Instrumentation

All experiments were carried out using a three-electrode electrochemical cell with a GCE (Bioanalytical Systems, ϕ: 3 mm diameter) as the working electrode, a platinum wire as the counter electrode (Bioanalytical Systems), and a Ag/AgCl electrode (Bioanalytical Systems, 3.0 M KCl) as the reference electrode. All voltammetric measurements were performed using an Autolab Pgstat128n potentiostat/galvanostat with Nova 10.0 software (Metrohm-Autolab, The Netherlands). The pH measurements were carried out using a Hanna HI2211 pH meter (Romania) with an accuracy of ±0.05 pH at room temperature. All of the electrochemical measurements were performed at room temperature (25±1°C).

Reagents

TX was supplied by Deva (Turkey) and its pharmaceutical dosage form (Tilcotil® tablets, 20 mg of TX per tablet) was purchased from a pharmacy and was used without further purification. TX stock solutions (1×10^{-3} M) were prepared in methanol and stored at +4°C away from light. TX working solutions for the voltammetric investigation were prepared by direct dilution of the stock solution with the selected supporting electrolyte containing a constant amount of methanol (20% V/V). MWCNT were purchased from Nano-Lab (USA) with ~95% purity, 1-5 µm lengths and 30±10 nm diameter. *N,N*-Dimethylformamide (DMF) was from Fluka (Switzerland).

Britton–Robinson buffer solutions (0.04 M) were prepared at pH 3.0-8.0 from 0.04 M CH_3COOH (Merck, Germany), 0.04 M H_3BO_3 (Aldrich, USA), and 0.04 M H_3PO_4 (Merck, Germany). Acetate buffer solutions (1 M) at pH 3.5, 4.5, and 5.5 were prepared from 1 M CH_3COOH (Merck, Germany). Phosphate buffer solutions (0.1 M) were prepared from H_3PO_4 (Merck, Germany) for pH 2.0-4.0 and Na_2HPO_4 (Aldrich, USA) and NaH_2PO_4 (Merck, Germany) for pH 5.0-8.0. The pH values were adjusted with 5 M NaOH (Aldrich, USA) solution.

Sartorius Arium proUV nanopure water (resistivity ≥18 MΩ cm) and analytical reagents were used for the preparation of solutions.

Preparation of the MWCNT-modified GCE

First 0.2% and 0.5% (mg mL^{-1}) MWCNT dispersions in DMF were sonicated for 4 h to obtain a homogeneous mixture. The GCE was polished with aqueous slurry of alumina powder (ϕ: 0.01

μM) on a polishing pad (Bioanalytical Systems polishing pad) and then rinsed with nanopure water before coating it. Four different suspensions of MWCNT in DMF 2.5 and 5 μL/0.2% and 1 and 5 μL/0.5% were dropped on the surface of the GCE to select suspension of MWCNT according to the optimum peak current obtained for TX. The selected dispersion of MWCNT in DMF for voltammetric determination of TX was dropped on the surface of the GCE. The resulting modified electrode was named an MWCNT-modified GCE. The MWCNT-modified GCE electrode dried overnight at room temperature. After each measurement, the electrode surface was cleaned using cyclic voltammetry (CV) in the potential range between -0.4 V and +1.0 V (3 cyclic) in buffer solution.

Pharmaceutical assay

Ten Tilcotil® tablets (each tablet includes 20 mg of TX) were first weighed and then powdered in a mortar. The needed amount of powder equivalent to 1×10^{-3} M of TX was diluted to 25 mL with methanol and sonicated for 10 min. The analyzed solutions were prepared by taking aliquots of the clear supernatant liquor and diluting with the selected supporting electrolyte. TX working solutions for voltammetric inquiries were prepared by direct dilution of the stock solution with 1 M acetate buffer solution at pH 5.5 containing a constant amount of methanol (20% V/V).

RESULTS AND DISCUSSION

The fabrication of the MWCNT-modified GCE was optimized to obtain the best MWCNT suspension for TX oxidation. The effect of the volume of MWCNT in DMF suspension on the peak current was investigated at four different loadings of MWCNT (2.5 and 5 μL/0.2%, 1 and 5 μL/0.5%) on the surface of the GCE. The coated electrodes with 2.5 μL and 5 μL for 0.2% and 1 μL and 5 μL for 0.5% of MWCNT suspension were used to determine 4×10^{-5} M TX by CV, differential pulse voltammetry (DPV), and square wave voltammetry (SWV). As shown in Figure 2, in DP voltammograms obtained from TX the peak current reaches its maximum value (2.47 μA) when the amount of MWCNT suspension (0.2%) is 2.5 μL. Thus, 2.5 μL for 0.2% MWCNT suspension was chosen to modify the GCE and this electrode was used for all electrochemical studies. Moreover, Figure 2 shows the response of TX obtained on a bare GCE (0.040 μA). The peak current of TX on the MWCNT-modified GCE (a) increased about 60-fold compared to the peak current of TX on the bare GCE (e).

Voltammetric behavior of TX at the MWCNT-modified GCE

Voltammetric responses of TX were checked out in detail by CV, DPV, and SWV using the MWCNT-modified GCE over the pH range of 2.0-8.0 in different buffer solutions. The cyclic voltammograms of 1×10^{-5} M TX solution exhibited an irreversible electrochemical oxidation process on the MWCNT-modified GCE in all working solutions (Figure 3). The CV scan was carried out from -0.40 V to 1.0 V in the positive direction and an anodic response of TX was observed at about +0.55 V at a scan rate of 100 mV s^{-1}.

The influence of pH on the peak current and potential was examined from pH 2.0 to 8.0 using CV, DPV, and SWV. The results acquired from CV, DPV and SWV showed similarity. Therefore, only DPV results for the main oxidation step are shown as E_p-pH and I_p-pH plots in Figure 4. The peak potentials of the responses were shifted to more negative potentials by increased pH. This is based on the oxidation of conjugate base at less positive potentials compared to the corresponding acid form. The TX oxidation peak that corresponds to the electroactive group in acid-base equilibrium with a pK_a of about 5.5[25] indicates pH dependence. Above pH 5.5, the peak potential is pH independent (Figure 4a). The linear relationship between

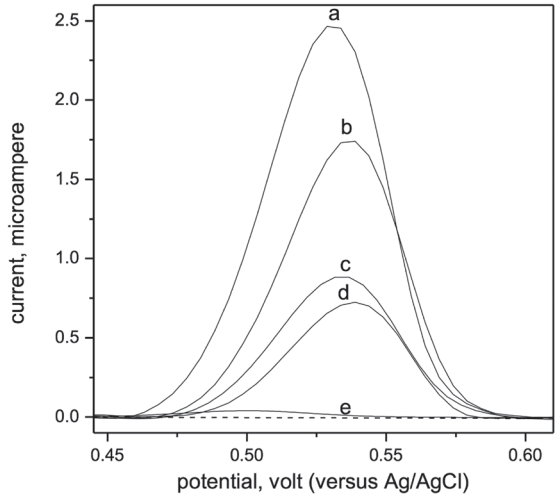

Figure 2. Differential pulse voltammograms 4×10^{-5} M of TX in 0.04 M Britton–Robinson buffer at pH 5.0 a) 0.2% 2.5 μL, b) 0.2% 5 μL, c) 0.5% 1 μL, d) 0.5% 2.5 μL of MWCNT-modified GCE, e) bare GCE. Dash line; 0.04 M Britton–Robinson buffer solution on 0.2% 2.5 μL of MWCNT-modified GCE

TX: Tenoxicam, MWCNT: Multiwalled carbon nanotube, GCE: Glassy carbon electrode

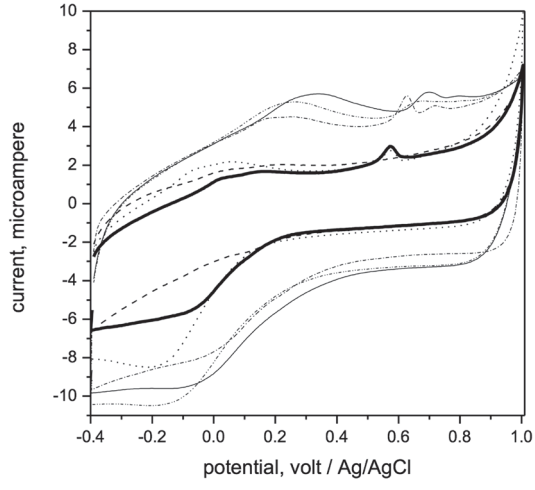

Figure 3. Cyclic voltammograms of 1.0×10^{-5} M TX in 1 M acetate buffer at pH 3.5 (–.–.), pH 5.5 (_), 0.04 M Britton–Robinson buffer pH 3.0 (_), pH 4.0 (–..–), 0.1 M phosphate buffer at pH 7.0 (....) with MWCNT-modified GCE. 1 M acetate buffer at pH 5.5 (---); scan rate 100 mV s^{-1}

TX: Tenoxicam, MWCNT: Multiwalled carbon nanotube, GCE: Glassy carbon electrode

E_p and pH can be clarified according to the following equation between 2.0 and 5.5 in all supporting electrolytes: E_p(mV)= -24.7pH+654.2 (r=0.9987). The slope value (-24.7) was about half of -59.0 mV/pH, and so it was inferred that the number of protons is half of the number of electrons transferred in the TX reaction. This can be attributed to the oxidation of the amide group in the structure of TX.

The impact of pH on the TX peak current on the MWCNT-modified GCE indicated that the peak current of TX was maximum in 1 M acetate buffer at pH 5.5 (Figure 4b). Thus, 1 M acetate buffer was selected as the supporting electrolyte for the quantitative determination of TX from pharmaceutical dosage forms.

Scan rate studies were performed to understand the electrochemical process for TX at the surface of the MWCNT-modified GCE. The electrochemical behavior of 8×10^{-5} M TX in 1 M acetate buffer at pH 5.5 was investigated at different scan rates ranging from 5 to 200 mV s^{-1} by CV. The peak potential of TX solution was shifted in the anodic direction when the scan rate was increased (Figure 5). A plot of peak current versus the scan rate showed a straight line with a slope of 0.0118 (equation 1). This indicated that the electrochemical reaction is checked by the diffusion of the electroactive species to the MWCNT-modified GCE surface.[26,27] Related equations are noted below:

$I_p = 0.0118\ \upsilon + 0.15$; r=0.997 (n=8) (Equation 1)

It was also observed that the anodic peak current of TX shifted to a higher positive value when the scan rate was increased. This shows the irreversibility of the oxidation reaction of TX on the MWCNT-modified GCE.[28]

Calibration curve and method validation

Quantitative analysis of TX for validation studies was performed using DPV and SWV. The calibration curves for DPV and SWV were drawn by plotting the peak current versus the TX concentration. TX responses were linear between the ranges of 2×10^{-7} and 1×10^{-5} M for DPV and 8×10^{-9} and 8×10^{-6} M for SWV. Equations obtained from the calibration data were as follows:

I_p(μA) = 52349 μM - 0.0209; r=0.997 (n=10) for DPV (Equation 2)

I_p(μA) = 25472 μM + 0.0039; r=0.997 (n=14) for SWV (Equation 3)

DP and SW voltammograms for various concentrations of TX are shown in Figures 6a and 6b, respectively.

Limit of detection (LOD) and limit of quantification values were calculated according to 3s/m and 10s/m, respectively (s is the standard deviation of the peak currents obtained from three sequential measurements and m is the slope of the related calibration graph).[29-32] The characteristics of the calibration curve results for DPV and SWV are shown in Table 1.

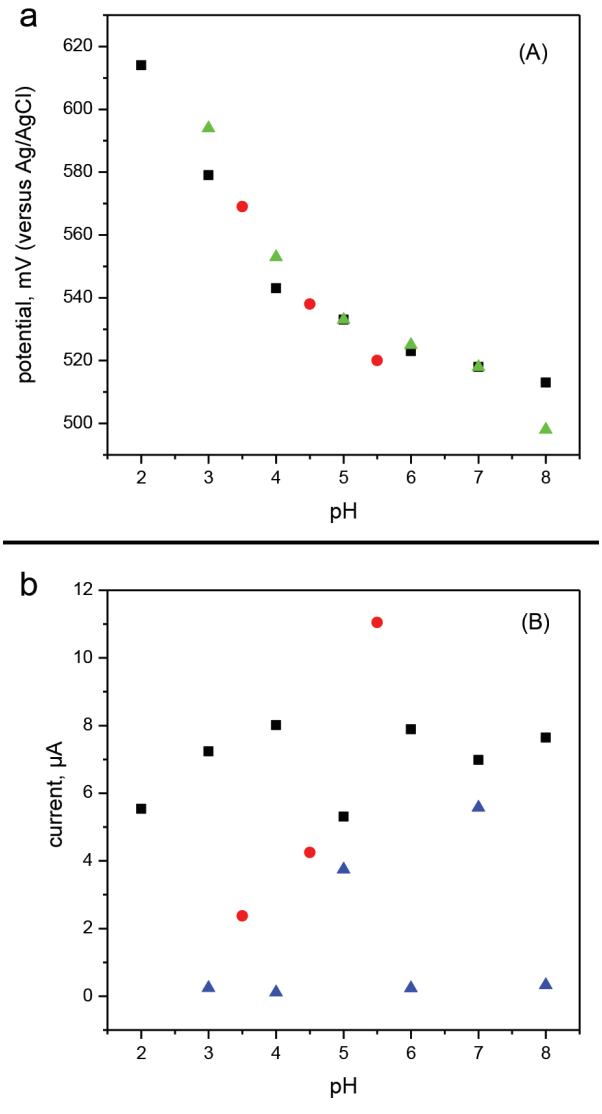

Figure 4. Plots of peak potential (E_p), versus pH a) and peak current (I_p), versus pH b) from differential pulse voltammograms of 1.0×10^{-5} M TX with MWCNT-modified GCE. Squares indicate 0.1 M phosphate buffer solution, triangles 0.04 M Britton–Robinson buffer solution, and circles 1 M acetate buffer solution

TX: Tenoxicam, MWCNT: Multiwalled carbon nanotube, GCE: Glassy carbon electrode

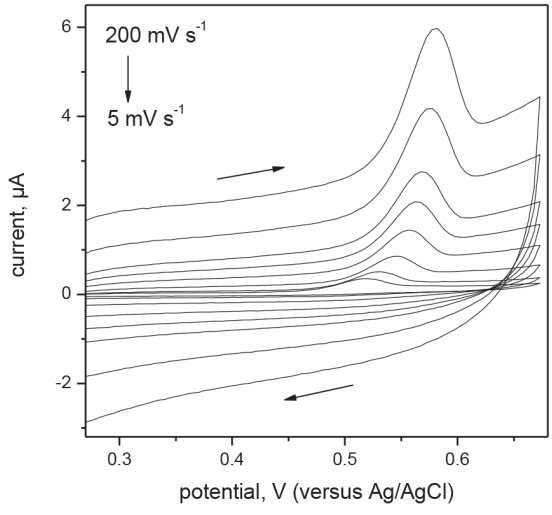

Figure 5. Cyclic voltammograms of 8.0×10^{-5} M of TX in 1 M acetate buffer solution at pH 5.5 at scan rates of 5, 10, 25, 50, 75, 100, 150, and 200 mV s^{-1} with MWCNT-modified GCE

TX: Tenoxicam, MWCNT: Multiwalled carbon nanotube, GCE: Glassy carbon electrode

We determined the precision of the improved methods by repeatability and reproducibility studies. For the experiments 6×10^{-6} M TX solution in 1 M acetate buffer at pH 5.5 was used. To calculate relative standard deviation (RSD %) values for DPV and SWV, five measurements were taken from different solutions with the same TX concentrations in a day for repeatability and on different days of a week for reproducibility. These results (Table 1) demonstrated that the developed methods with the MWCNT-modified GCE were good in terms of precision, accuracy, repeatability, and reproducibility.

Stability studies of the MWCNT-modified GCE were performed as a function of time. For the purpose of the peak current 4×10^{-5} M TX was examined with DPV for 1 M acetate buffer solution at pH 5.5 on the same MWCNT-modified GCE stored at room temperature 2 months. After 4 and 8 weeks, the modified electrode kept 99.65% and 98.41% of the peak current of TX, respectively. After 2 weeks the peak current value kept only

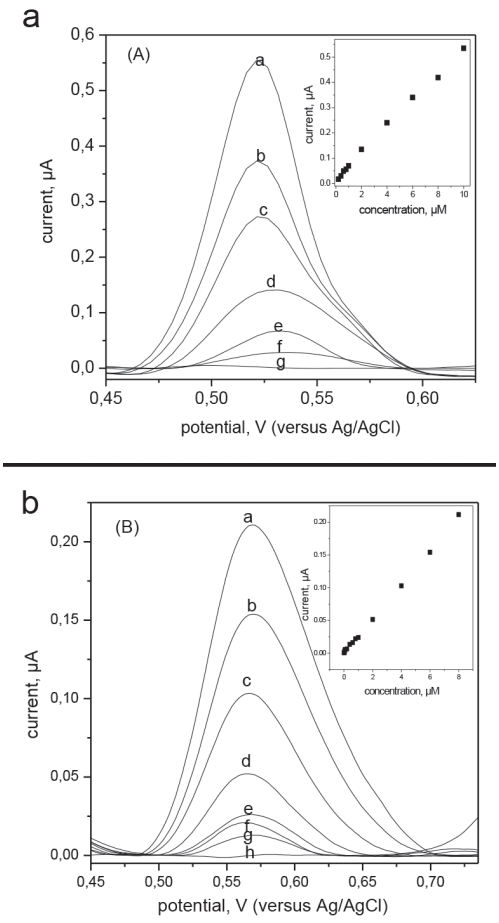

Figure 6. (a) Differential pulse voltammograms a) 1×10^{-5} M, b) 6×10^{-6} M, c) 4×10^{-6} M, d) 2×10^{-6} M, e) 1×10^{-6} M, f) 4×10^{-7} M TX in 1 M acetate buffer solution at pH 5.5, g) 1 M acetate buffer solution at pH 5.5 with MWCNT-modified GCE; (b) Square wave voltammograms a) 8×10^{-6} M, b) 6×10^{-6} M, c) 4×10^{-6} M, d) 2×10^{-6} M, e) 1×10^{-6} M, f) 6×10^{-7} M, g) 4×10^{-7} M TX in 1 M acetate buffer solution at pH 5.5, h) 1 M acetate buffer solution at pH 5.5 with MWCNT-modified GCE

TX: Tenoxicam, MWCNT: Multiwalled carbon nanotube, GCE: Glassy carbon electrode

Table 1. Validation data of calibration lines for the quantitative determination of TX by DPV and SWV on MWCNT-modified GCE in 1 M acetate buffer at pH 5.5

	MWCNT-modified GCE	
	DPV	SWV
Peak potential (V)	0.520	0.570
Linearity range (M)	2.0×10^{-7}-1.0×10^{-5}	8.0×10^{-9}-8.0×10^{-6}
Slope (µA µM^{-1})	52349	25472
Intercept (µA)	-0.0209	+0.0039
Correlation coefficient	0.997	0.997
Limit of detection (M)	1.43×10^{-9}	9.97×10^{-10}
Limit of quantification (M)	4.33×10^{-9}	3.02×10^{-9}
Repeatability of peak current (Relative standard deviation %)*	0.675	0.411
Repeatability of peak potential (Relative standard deviation %)*	0.044	0.319
Reproducibility of peak current (Relative standard deviation %)*	0.704	0.896
Reproducibility of peak potential (Relative standard deviation %)*	0.961	0.538

TX: Tenoxicam, DPV: Differential pulse voltammetry, SWV: Square wave voltammetry, MWCNT: Multiwalled carbon nanotube, GCE: Glassy carbon electrode, *Obtained from five experiments

Table 2. Compared parameters obtained using different electrodes for the determination of TX

Electrode	Method	Linear range (M)	Limit of detection (M)	References
Static mercury drop electrode	Differential pulse polarography	7.41×10^{-8}-5.90×10^{-5}	7.41×10^{-8}	18
Static mercury drop electrode	Square wave adsorptive stripping voltammetry	8.0×10^{-10}-1.0×10^{-5}	1×10^{-10}	19
Hanging mercury drop electrode	Differential pulse polarography	1.24×10^{-6}-9.79×10^{-6}	-	20
MWCNT-modified	Differential pulse voltammetry	2.0×10^{-7}-1.0×10^{-5}	1.43×10^{-9}	This work
GCE	Square wave voltammetry	8.0×10^{-9}-8.0×10^{-6}	9.97×10^{-10}	

TX: Tenoxicam, MWCNT: Multiwalled carbon nanotube, GCE: Glassy carbon electrode

95.12%. Consequently, the MWCNT-modified GCE demonstrated long-term stability.

In the literature, electroanalytical determination of TX has been achieved with various electrodes. In Table 2, the results obtained in the present study and from other voltammetric studies in the literature were compared in terms of electrode, linearity range, and LOD. El-Maali et al.'s[19] study demonstrated a wider linearity range and a lower LOD value. However, the use of a mercury electrode is a disadvantage because of the highly toxic nature of the mercury. In the present study, the MWCNT-modified GCE provided a good linear range and detection limit with SWV and the MWCNT-modified GCE. Additionally, it has some advantages such as easy preparation, user friendliness, and long-term stability. As a result, the MWCNT-modified GCE can be used more safely and sensitively in the electroanalytical determination of TX.

Tablet analysis

DPV and SWV methods developed using the MWCNT-modified GCE were applied for the determination of TX in pharmaceutical dosage forms (Tilcotil® tablets). Each tablet in pharmaceutical dosage form contains 20 mg of TX. The DPV and SWV methods were applied in direct determination of TX in pharmaceutical dosage form without pretreatment such as extraction or evaporation steps. Furthermore, recovery studies with the proposed methods and modified electrode were also carried out via adding known amounts of pure TX to pharmaceutical form. Five repetitive experiments were done using the related calibration curve, which is a straight line, and the obtained results are demonstrated in Table 3. As shown in Table 3, the results were satisfactory and indicated the validity of the methods and modified electrode for the determination of TX in pharmaceutical form.

Table 3. The results for the determination of TX from tablet dosage forms and recovery experiments in 1 M acetate buffer at pH 5.5 by DPV and SWV on MWCNT-modified GCE

	Tablet (mg)	
	Differential pulse voltammetry	Square wave voltammetry
Labeled claim (mg)	20	20
Amount found (mg)*	19.871	20.260
Relative standard deviation %	0.714	0.638
Bias %	0.645	-1.3
Added (mg)	20.00	20.00
Found (mg)*	20.035	20.018
Average recovered (%)	100.865	100.307
Relative standard deviation % of recovery	0.799	0.704
Bias %	-0.865	-0.307

TX: Tenoxicam, DPV: Differential pulse voltammetry, SWV: Square wave voltammetry, MWCNT: Multiwalled carbon nanotube, GCE: Glassy carbon electrode

CONCLUSIONS

In the present study, a MWCNT-modified GCE was prepared for sensitive determination of TX. The fully validated DPV and SWV results demonstrated high sensitivity and reproducibility and repetitively via the developed sensor. The developed sensor was used for the determination of TX in pharmaceutical form by DPV and SWV without any pretreatment. The results were recovered in high percentages. In addition, the prepared electrode in this study is very useful in voltammetric studies of TX due to its high accuracy, sensitivity, stability, and repeatability, as well as its practical preparation. The sensor and method for determining accurate TX concentrations can be used in biological samples for pharmacokinetic studies and quality control laboratories.

Conflicts of Interest: No conflict of interest was declared by the authors.

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