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Original scientific paper

Electrochemical determination of tramadol using modified screen-printed electrode

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Abstract

The detection of tramadol using a screen-printed electrode (SPE) modified with La^{3+}/ZnO nano-flowers and multi-walled carbon nanotubes (La^{3+}/ZnO NFs-MWCNTs/SPE) is reported in this work. To examine electrochemical oxidation of tramadol, the modified electrode was implemented with the utilization of differential pulse voltammetry, chronoamperometry and cyclic voltammetry as diagnostic techniques. The proposed electrode displays favorable electrocatalytic behavior concerning tramadol oxidation with an approximately 330 mV potential shift to less positive potential. In the range of tramadol concentrations of 0.5 to 800.0 µM, differential pulse voltammetry displays the linear response. Tramadol detection limit of 0.08 µM was achieved within optimized testing conditions for this sensor of simple construction. Lastly, the fabricated sensor was utilized for the determination of tramadol in urine samples.

Keywords

La^{3+}/ZnO nano-flower; multi-walled carbon nanotubes; voltammetry.

Introduction

Drug analysis is vital for numerous uses, such as forensic science, quality control, and clinical applications. Tramadol is a synthetic analgesic applied in the cure of mild to severe chronic or acute pain. Together with other narcotics used to treat chronic, obstetric, and cancer pain control, as well as moderate surgical pain in adults and children, tramadol has been implemented since 1977. Tramadol has similar characteristics with codeine with a difference in having a methyl substitute on the morphine structure phenolic moiety. Tramadol restricts the reuptake of norepinephrine and serotonin while expediting the release of these neurotransmitters. It is categorized as an opioid and binds to the brain receptors (opioid receptors). It is also a synthetic codeine analog and not categorized as a controlled substance. Moreover, addicts may use it as an anti-addictive substance, although extreme tramadol dependence has been evident because of its impact on opioid receptors within the central nervous system [1,2]. There has been an increase in lethal tramadol poisoning in the past few years [3].

Numerous analytical methods have been implemented to determine drugs in clinical and pharmaceutical preparations. Such methods include high-performance liquid chromatography (HPLC) combined with quadrupole electrospray ionization mass spectroscopy (Q-TOF-MS), UV-Vis spectrophotometry coupled with chemometric analysis, ultrahigh performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) [4-8]. Compared to mentioned methods, electroanalytical methods are more advantageous in terms of higher sensitivity, lower equipment costs and simplicity. Numerous electrochemical methods are mentioned in the literature for analyte determination, with and without the use of modified electrodes [9-14].

In designing authentic and portable electrochemical sensing platforms with improved performance, screen-printed electrodes (SPEs) are the most promising components. These components are extremely versatile, user-friendly, and cost-efficient analytical tools and are of a field-based size. For example, SPEs provide the possibility for sample volume reduction to a few microliters and may easily be configured for multiple analytes detection while avoiding contamination or memory effects. These devices have displayed competency for *in situ* monitoring of pollutants and real-time analysis to prevent pollution.

In recent years, researchers have focused on designing and synthesizing nanomaterials for various applications due to their unique physical and chemical properties [15-19]. Lately, advances in nanoscience and nanotechnology have aided the progression of SPEs fabrication. Due to the specific characteristics of nanomaterials, SPE modification with nanostructured materials has paved the way for improved electroanalytical performances compared to bare SPEs. When doped with carbon nanotubes, metal nanoparticles *etc*., SPEs have exhibited improved sensitivity and detection limit [20,21].

In chemistry, a nano-flower is the name given to a compound of certain elements resulting in compositions identical to flowers from a microscopic point of view. In a few cases, these compositions are referred to as nano-bouquets or nano-trees. Within the scientific community, La^{3+}/ZnO nano-flowers ($La^{3+/}$ZnO NFs) are specific compounds that have attracted great attention because they can be implemented as catalysts and photocatalysts [22-24]. Multi-walled carbon nanotubes (MWCNTs) are widely used in electroanalytical chemistry owing to their unique one-dimensional (1D) structural, mechanical and electronic properties [25,26].

In this paper, La^{3+}/ZnO NFs and MWCNTs modified SPE is for the first time utilized as a sensitive, rapid, inexpensive and simple electrochemical sensor to determine tramadol.

Experimental

Chemicals and apparatus

An Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands) controlled using standard electrochemical system software was used for electrochemical experiments. A standard three-electrode cell was implemented at 25 ± 1 °C. An Ag/AgCl/KCl (3.0 M) in addition to a platinum wire and La^{3+}/ZnO/SPE were used as a reference, auxiliary and working electrode, respectively. To conduct pH measurements, a 710 Metrohm pH meter was used.

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The remaining reagents were of analytical grade, including tramadol bought from Merck, Darmstadt, Germany. Phosphate buffer solutions (PBS) were prepared in the 2.0-2.9 pH range, using orthophosphoric acid and its salts.

La^{3+}-doped ZnO nanoflowers preparation

Each chemical applied to prepare nano-powder, *i.e.,* ammonia (25 % NH_3), zinc acetate ($Zn(CH_3COO)_2 \cdot 2H_2O$), thiourea ($(NH_2)_2CS$), and lanthanum nitrate ($La(NO_3)_3 \cdot 6H_2O$) were of analytical grades. All substances were dissolved in the deionized water. Ammonia was a complexing agent during the process of nano-powder procurement. ZnO nanostructures were procured *via* dissolution of 0.46 mol of zinc acetate in 80 mL of the deionized water, 0.18 mol of thiourea in 80 mL of deionized water, 0.0046 mol of lanthanum nitrate in 80 mL of deionized water as well as *via* addition of 19.76 mL of ammonia to 80 mL of the deionized water. The amounts of thiourea, zinc acetate, and ammonia solutions have been kept fixed at a 1:1:1 ratio. Afterward, zinc acetate solution was added to the reaction bath. Then, thiourea and lanthanum nitrate solutions were added, and the mix was shaken for a few seconds. Finally, ammonia was added slowly to the solution and the mixture was stirred for five minutes. Next, the bath temperature was increased to 80 °C. The formed precipitate was put aside overnight and filtrated. Afterward, ethanol was used to wash the precipitate. The resulting powder was dried at ambient temperature for a couple of days.

Electrode preparation

La^{3+}/ZnO NFs and MWCNTs were used as a coating material for the bare SPE. 1 mg of La^{3+}/ZnO NFs and 1 mg of MWCNTs were dispersed by ultrasonication to prepare a stock of La^{3+}/ZnO NFs-MWCNTs dispersion in 1 mL water. Then, 5 µl aliquot of the La^{3+}/ZnO NFs-MWCNTs/H_2O suspension was cast on the carbon working electrode and left to evaporate at room temperature.

The surface area of La^{3+}/ZnO NFs-MWCNTs/SPE and bare SPE were obtained by cyclic voltammetry (CV) using 1 mM $K_3Fe(CN)_6$ at different scan rates. Applying Randles-Ševčik formula [27] for La^{3+}/ZnO NFs-MWCNTs/SPE, the electrode surface was calculated to 0.097 cm^2 which was about 3.1 times greater than bare CPE.

Real samples preparation

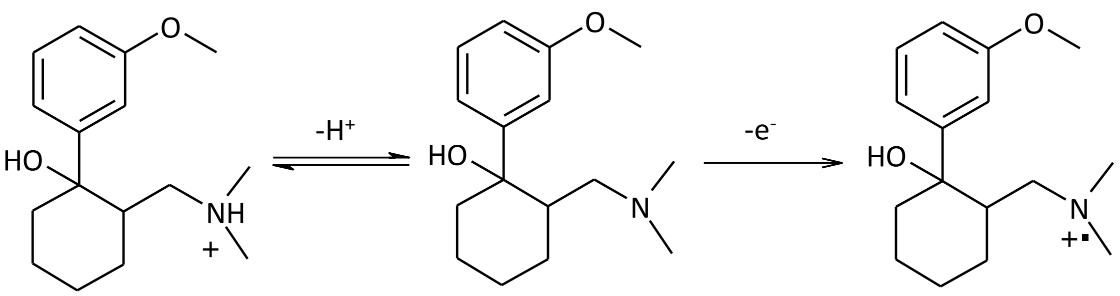
The preparation started by grinding five tramadol tablets (100 mg per tablet) obtained from Tehran Chemie Pharmaceutical Co, Iran. Then, 400 mg of the resulting powder was dissolved in 25 mL of water via ultrasonication. Various volumes of the diluted solution were then poured in a 25 mL volumetric flask and diluted with phosphate buffer solution (PBS) pH 7.0 to the mark. The proposed method was used to analyze the tramadol content using the standard addition method.

A refrigerator was used to store the urine samples. 10 milliliters of the specimen were taken or centrifuged for 15 minutes at 2000 rpm to prepare the experiment samples. A 0.45 µm filter was used to filter the supernatant and various resulting volumes were diluted using 25 mL volumetric flasks *via* PBS pH 7.0. Various tramadol amounts were used to spike diluted urine samples.

Results and discussion

Electrochemical profile of the analyte on La^{3+}/ZnO NFs-MWCNTs/SPE

Since the electrochemical behaviour of tramadol is pH-dependent (*cf*. Scheme 1), the optimizing pH of the solution is necessary for obtaining the best results.



Scheme 1. *The probable electro-oxidation mechanism for tramadol at the surface of the modified electrode*

Oxidation peak current values taken from measured DPVs in 0.1 M PBS containing 200.0 µM of tramadol in a dependence on solution pH are presented in Figure 1, showing that the best results for tramadol electrooxidation at the modified electrode surface are obtained at pH 7.

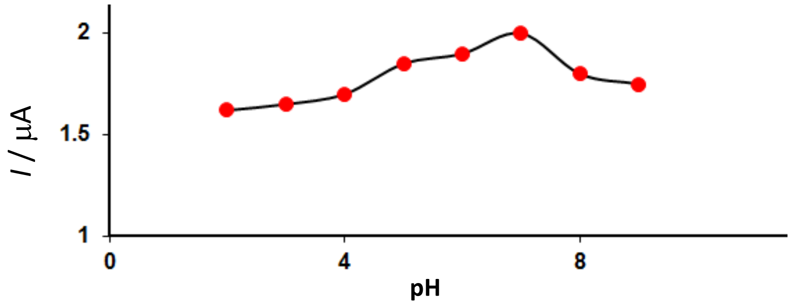


Figure 1. *Plot of Ip vs. pH taken from DPVs of* La^{3+}*/ZnO NFs-MWCNTs/SPE in 0.1 M PBS containing 200.0 µM of tramadol at different pH(2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0).*

The cyclic voltammograms for La^{3+}/ZnO NFs-MWCNTs/SPE (curve a) and unmodified SPE (curve b) were used to acquire 400.0 µM tramadol as presented in Figure 2. It is evident that at bare SPE, the maximum oxidation of tramadol takes place at 890 mV, while at La^{3+}/ZnO NFs-MWCNTs/SPE, it is attained at approximately 330 mV more negative value.

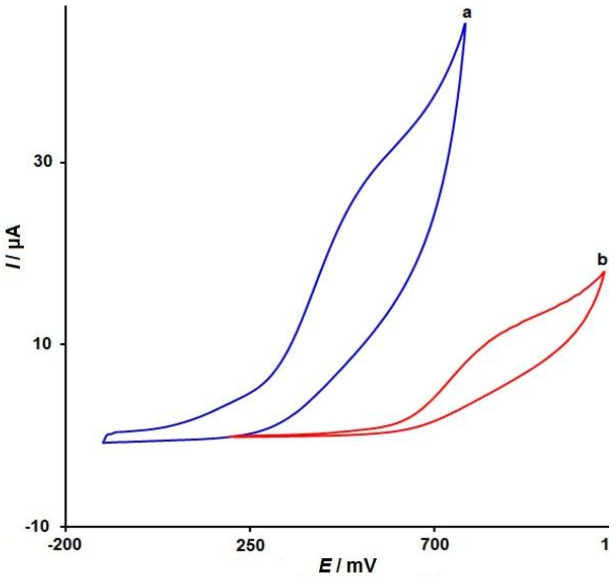


Figure 2. *Cyclic voltammograms of (a)* La^{3+}*/ZnO NFs-MWCNTs/SPE and (b) bare SPE in 0.1 M PBS (pH 7.0) in presence of 400.0 µM tramadol at the scan rate 50 mV* s^{-1}

Scan rate impact

The impact of potential scan rates on tramadol oxidation currents is presented in Figure 3. It is clear that by increasing the scan rate, peak currents were also increased. Moreover, it was proven that the oxidation process is diffusion-controlled according to the linear I_p plots against $v^{1/2}$, shown in the inset of Figure 3.

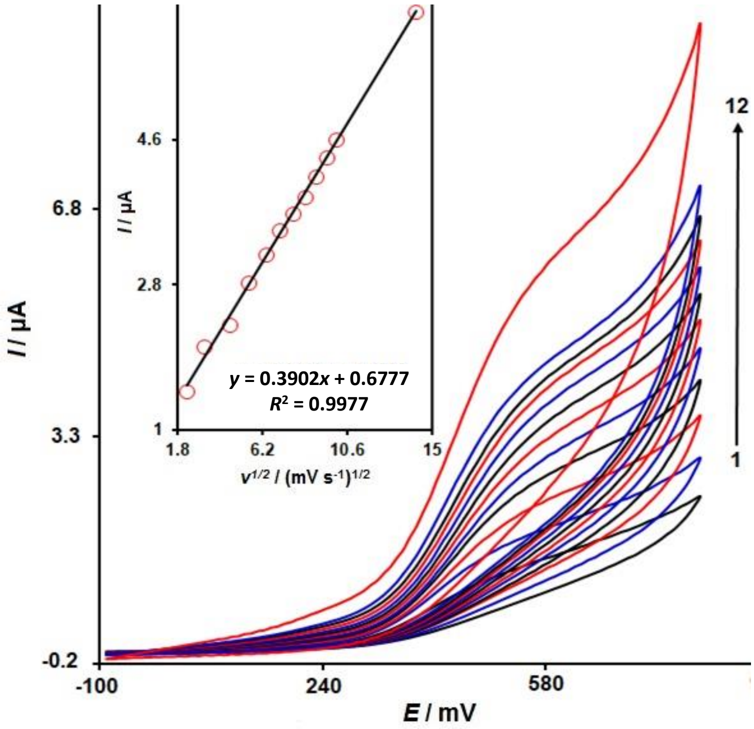


Figure 3. *Cyclic voltammograms of La^{3+}/ZnO NFs-MWCNTs/SPE in 0.1 M PBS (pH 7.0) containing 500.0 µM tramadol at various scan rates (numbers 1-12 correspond to 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 and 200 mV s^{-1}). Inset: variation of anodic peak current* vs. v$^{1/2}$

In this stage, Tafel plot has been drawn from data obtained from the ascending part of current voltage curve recorded at the scan rate equal of 5 mV s^{-1} for epinine (Figure 4). It is notable that this piece of voltammogram, which has been named the Tafel region has been influenced by the electron transfer kinetics between the substrate (tramadol) and La^{3+}/ZnO NFs-MWCNTs/SPE. The Tafel slope of 0.1948 V has been observed in complete agreement with contribution of 1 electrons in the rate determining phase of the electrode [22] supposing the charge transfer coefficient, α = 0.7 for tramadol.

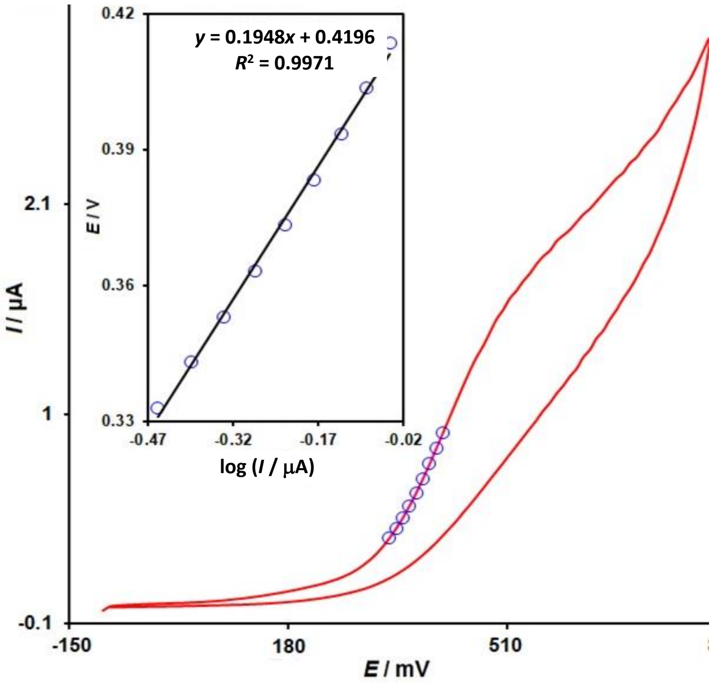


Figure 4. *Cyclic voltammogram (5 mV s^{-1}) of electrode in 0.1 M PBS (pH 7.0) containing 500.0 µM tramadol. The inset shows the Tafel plot derived from data denoted at raising part of CV*

Chronoamperometric analyses

Figure 5 presents chronoaperometric analysis of various concentrations of tramadol samples using La^{3+}/ZnO NFs-MWCNTs/SPE in PBS pH 7.0.

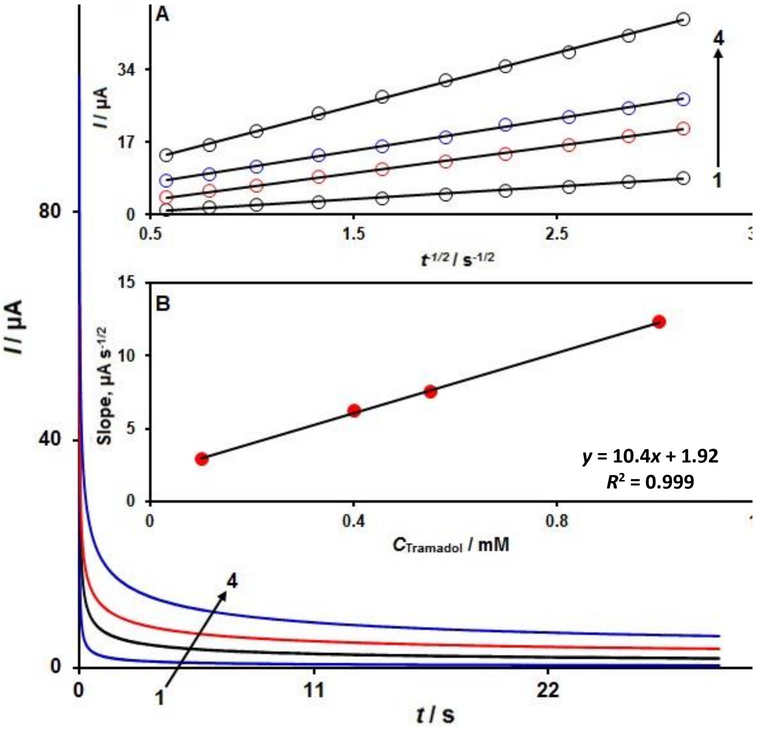


Figure 5. *Chronoamperograms obtained at La^{3+}/ZnO NFs-MWCNTs/SPE in 0.1 M PBS (pH 7.0) for different concentration of tramadol (1–4 correspond to 0.1, 0.4, 0.55, and 1.0 mM of tramadol). Insets: (A) plots of* I vs. t$^{-1/2}$ *obtained from chronoamperograms 1–4; (B) plot of slopes of straight lines against tramadol concentration*

Cottrell equation (1) for electroactive materials chronoamperometric analysis under mass transfer limited circumstances [27]:

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \qquad (1)$$

where C_b is the bulk concentration (mol cm^{-3}) of an analyte, D is diffusion coefficient (cm^2 s^{-1}), n is the number of transferred electrons, A is the electrode surface area (cm^2), and t is time (s). Best fitted linear plots of I *vs.* $t^{-1/2}$ for various tramadol concentrations are shown in Figure 5A. Figure 5B presents the resulting straight-line slopes *vs.* tramadol concentrations. The mean value of D was obtained as 9.2×10^{-6} cm^2 s^{-1} from the Cottrell equation and resulting slope for n = 1.

Calibration curve

Tramadol peak currents acquired using differential pulse voltammetry (DPV) a La^{3+}/ZnO NFs-MWCNTs/SPE were utilized for quantitative analysis of tramadol residing in PBS. DPV has an adventage over other electroanalytical techniques regarding superior characteristics and enhanced sensitivity for analytical application. DPV analysis (step potential of 0.01 V and pulse amplitude of 0.025 V) performed for a range of tramadol solutions in 0.1 M PBS at La^{3+}/ZnO NFs-MWCNTs/SPE is presented in Figure 6. A linear relationship between tramadol concentration and peak current is obtained within the concentration range of 0.5-800.0 µM with 0.9992 correlation coefficient and detection limit (3σ) estimated as 0.08 µM.

Table 1. *Comparison of analytical results for determination of tramadol by DPV method at La^{3+}/ZnO NFs-MWCNTs/SPE and some modified electrodes reported in the literature*

Electrochemical sensor	Linear range	Limit of detection	Ref.
Carbon nanoparticles/glassy carbon electrode	10- 1000 µM	1 µM	[28]
Graphitic carbon nitride/Fe_3O_4 nanocomposite/carbon paste electrode	0.2–14.0 and 14.0–120.0 µM	0.1 µM	[29]
poly(Nile blue)/glassy carbon electrode	1.0×10^{-6}- 3.1×10^{-4} M	5.0×10^{-7} M	[30]
La^{3+}/ZnO NFs-MWCNTs/SPE	0.5-800.0 µM	0.08 µM	This work

Table 1 presents a comparison of La^{3+}/ZnO NFs-MWCNTs/SPE analytical performance created in this work with other electrochemical sensors involved in tramadol analysis [28-30].

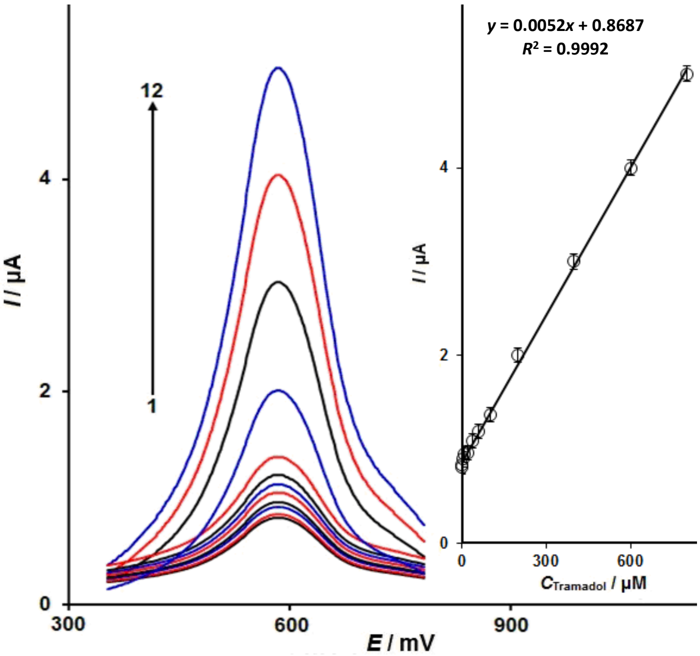


Figure 6. *DPVs of La^{3+}/ZnO NFs-MWCNTs/SPE in 0.1 M (pH 7.0) containing different concentrations of tramadol (1–12 correspond to 0.5, 1.0, 10.0, 20.0, 30.0, 40.0, 60.0, 100.0, 200.0, 400.0, 600.0 and 800.0 μM of tramadol). Inset: plot of the peak current vs. tramadol concentration in the range of 0.5-800.0 μM*

Real samples analysis

The proposed method was implemented to determine tramadol in urine samples and tramadol tablets to examine the relevance of modified electrode application in tramadol determination in real samples. The standard addition method was implemented for analysis, and the results are presented in Table 2. The ascertained tramadol recovery was favorable and reproducibility results were displayed according to the mean relative standard deviation (RSD).

Table 2. *Application of La^{3+}/ZnO NFs-MWCNTs/SPE for determination of tramadol in tramadol tablet and urine samples (n=5)*

Sample	Concentration of tramadol, μM		Recovery, %	RSD, %
	Spiked	Found		
Tramadol tablet	0	6.0	-	3.5
	2.0	7.9	98.7	2.7
	3.0	8.2	102.5	2.4
	4.0	9.9	99.0	1.9
	5.0	11.1	100.9	2.3
Urine	0	-	-	-
	5.0	5.1	102.0	2.1
	7.5	7.4	98.7	3.2
	10.0	10.3	103.0	2.7
	12.5	12.4	99.2	2.8

Conclusion

In summary, a rapid and simple method based on La^{3+}/ZnO NFs-MWCNTs/SPE was designed for the electrochemical determination of tramadol. The proposed sensor displayed excellent electrocatalytic activity and high recognition capability toward the electrochemical detection of tramadol. This sensor demonstrated wide detection range (0.5 - 800.0 μM) and low limit of detection (80.0

nM) towards tramadol. The sensor was also applied to detect tramadol in real samples, and satisfactory results were obtained.

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