



# Modification of a pencil graphite electrode with multiwalled carbon nanotubes capped gold nanoparticles for electrochemical determination of tramadol

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## ABSTRACT

A novel, low-cost and sensitive electrochemical detection method was proposed for quantitative measurement of tramadol (TRA) based on using a pencil graphite electrode (PGE) modified with multiwalled carbon nanotubes (MWCNTs) and gold nanoparticles (AuNPs). The electroanalysis of tramadol was carried out by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. Field emission scanning electron microscopy (FE-SEM) was used to investigate the surface morphology of the modified electrode and the electrochemical properties of f-MWCNTs/AuNPs/PGE surface were evaluated using electrochemical impedance spectroscopy (EIS) technique. The effect of several significant parameters such as pH, scan rate and immersion time of PGE in f-MWCNTs suspension was examined and they were optimized. The influence of interfering species including ascorbic acid, uric acid and citric acid was evaluated and the obtained results showed that the developed sensor has a high selectivity for tramadol molecules. A linear relationship was observed between differential pulse voltammetry response of the modified electrode and the concentration of TRA in the range of 12–100 and 100–3000 nM. The proposed electrode showed high sensitivity and selectivity for determination the concentration of tramadol in tablet and biological fluids.

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## 1. Introduction

Today, in all societies, the pharmaceutical compounds are part of the daily people life and in the meantime, the consumption of tranquilizers and opiate is increasing. Tramadol (TRA) is one of the painkillers and opioid analgesic which is used mostly by the young people. Tramadol, (1R,2R)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl) cyclohexanol, is an opioid atypical drug with the central effect which has an agonist effect on the  $\mu$ -opioid receptor. It attaches to the  $\mu$ -opioid receptor and then stimulates the function of the receptor and may imitate the regulatory action of the body [1,2]. The analgesic potency of tramadol is between weak opioid and morphine. This drug can be taken by injection, suppository and oral. Most of the reported side effects of tramadol have happened with intramuscular injection and in recent years, its use has increased in society [3]. The tramadol overdose can cause dizziness, vomiting, nausea, diarrhea, chills, hallucinations and respiratory problems since it is considered as a toxic material in nature [4]. Today, consumption of tramadol is one of the most predictable harmful behaviors in the world with over one million deaths annually. According to the World Health Organization, the use of this

deadly substance is released in many countries and it can cause great harm [4].

Quantitative determination of tramadol in biological samples has been investigated by some instrumental methods such as GC [5], GC-MS [6,7], HPLC [8], capillary electrophoresis [9,10], spectrophotometry [11,12], chemiluminescence [13] and electrochemical methods [14]. The electrochemical methods have attracted more attention in recent years due to their recognition properties, simplicity, low cost and fast response. However, for the purpose of direct determination of the analytes, electrochemical methods usually require the modification and application of materials such as carbon nanotubes, graphene and metal nanoparticles to amplify the electrochemical signals.

Multi-walled carbon nanotubes (MWCNTs) are the allotropes of carbon with a cylindrical nanostructure which are used extensively in the construction of electrochemical sensors because they are able to facilitate the electron transfer process in electrochemical reactions [15,16]. MWCNTs with high surface areas and unique physical properties, high stability and adjustable length, are a good option for biological and pharmaceutical applications [17].

The nanoscale materials such as gold metal nanoparticles (AuNPs), have a diagnostic capacity due to their joining up power with biological and pharmaceutical molecules [16]. The gold nanoparticles can be used as the catalyst for a large number of sensors owing to the stability and complete recovery of these nanoparticles in the redox processes.

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In this research work, a new electrochemical sensor was constructed for quantitative measurement of tramadol in solutions, using multiwalled carbon nanotubes (MWCNTs) and the gold nanoparticles (AuNPs). The pencil graphite with a wide potential window can be used as an electrode for electroanalytical applications. Pencil graphite electrode (PGE), with its graphite nature as an amplifier platform, also has a great effect in increasing the electrochemical signal and reducing the overvoltage. Therefore, PGE is an ideal substrate for designing and construction of electrochemical sensors.

The electrochemical sensor was fabricated via immersing of a pencil graphite electrode in multiwalled carbon nanotubes suspension and then deposition of the gold nanoparticles through the electrochemical method. The fabrication process of the sensor was characterized by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), field emission scanning electron microscopy (FE-SEM) and energy dispersive spectroscopy (EDS). The experimental results showed that the developed sensor has an excellent electrocatalytic activity for oxidation of tramadol molecules. The advantages of pencil graphite electrode are high electrochemical activity, good mechanical strength, low cost, easy method of preparation and ease modification, saving time and also good reproducibility. The constructed electrode showed good repeatability and reproducibility, high sensitivity and selectivity and also good recovery for determination of tramadol in tablet and biological fluids as real samples.

## 2. Experimental

### 2.1. Chemicals

Tramadol was obtained from Sajjad Daru Shargh Pharmaceuticals Company (Mashhad, Iran). MWCNTs (>95%, outer diameter 5–15 nm) was purchased from US research nanomaterials, Houston, USA. Hydrogen tetrachloroaurate trihydrate ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ ) was obtained from Sigma-Aldrich (USA). All the other chemicals and reagents used in this work were obtained from Merck (Darmstadt, Germany) and used without further purification. All solutions were prepared in deionized distilled water.

### 2.2. Instrumentation

All voltammetric measurements were performed using a  $\mu$ -Autolab III (Eco Chemie, The Netherlands) potentiostat/galvanostat equipped with NOVA software. A three electrode system was used with a modified pencil graphite electrode (PGE), an Ag/AgCl/KCl (3.5 M) and a platinum wire electrode as the working, reference and counter electrodes, respectively. The pencil graphite (type 2B, 0.7 mm diameter, KNOW Korea) was purchased from stationery store. The two other electrodes were purchased from Azar Electrode Company (Urmia, Iran). pH measurements were performed with a Metrohm pH meter (model 827 pH, Swiss made). The electrochemical impedance spectroscopy (EIS) measurements were carried out using Gill AC potentiostat (ACM). The morphology of fabricated electrodes was characterized by using a TESCAN BRNO-Mira3 LMU field emission scanning electron microscope (FE-SEM). Surface elemental analysis of the modified electrode was performed by energy dispersive X-ray (EDX) technique using Oxford-7353 EDX microanalyzer.

### 2.3. Preparation of modified electrode

A length of 1 cm of a pencil graphite rod was chosen as the electrode surface and the rest of its surface was covered by a Teflon bar (Fig. 1). Initially, a suspension containing 5 mg of functionalized multi-wall carbon nanotubes (MWCNT-COOH) in 2.5 ml of DMF was prepared and then the suspension was made uniform in an ultrasonic bath for 20 min. For modifying the surface of the pencil graphite electrode, it was immersed in the prepared suspension for a period of 105 min while the suspension was stirred by a magnetic stirrer with a medium speed. Then, the surface of the electrode was exposed to an IR light for drying out completely and creating a carbon nanotube film at the electrode surface. Then, the f-MWCNTs modified electrode was dipped into an electrochemical cell containing 0.5 mM  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  and 0.1 M  $\text{KNO}_3$  solution. The electrochemical deposition of gold nanoparticles was carried out by using chronoamperometry technique at a constant potential of  $-0.2$  V for 360 s. The modified electrode was washed with double-distilled water and was exposed to air (Fig. 1).

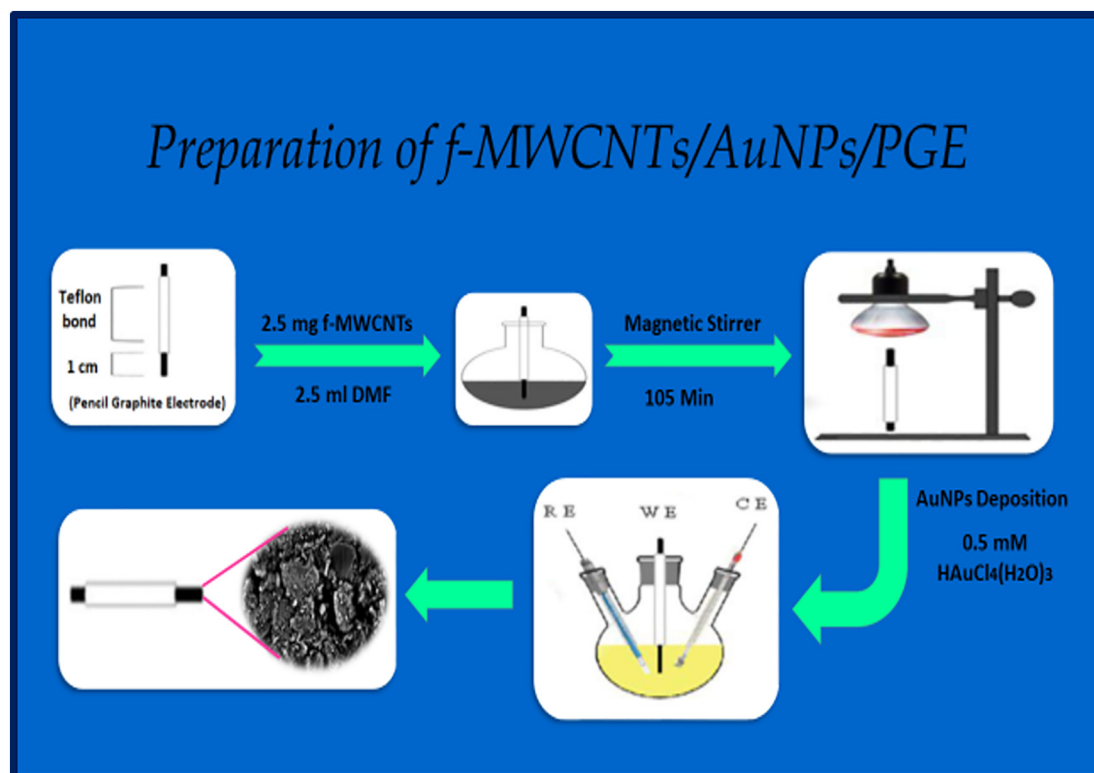


Fig. 1. Schematic illustration for preparation of f-MWCNTs/AuNPs/PGE.

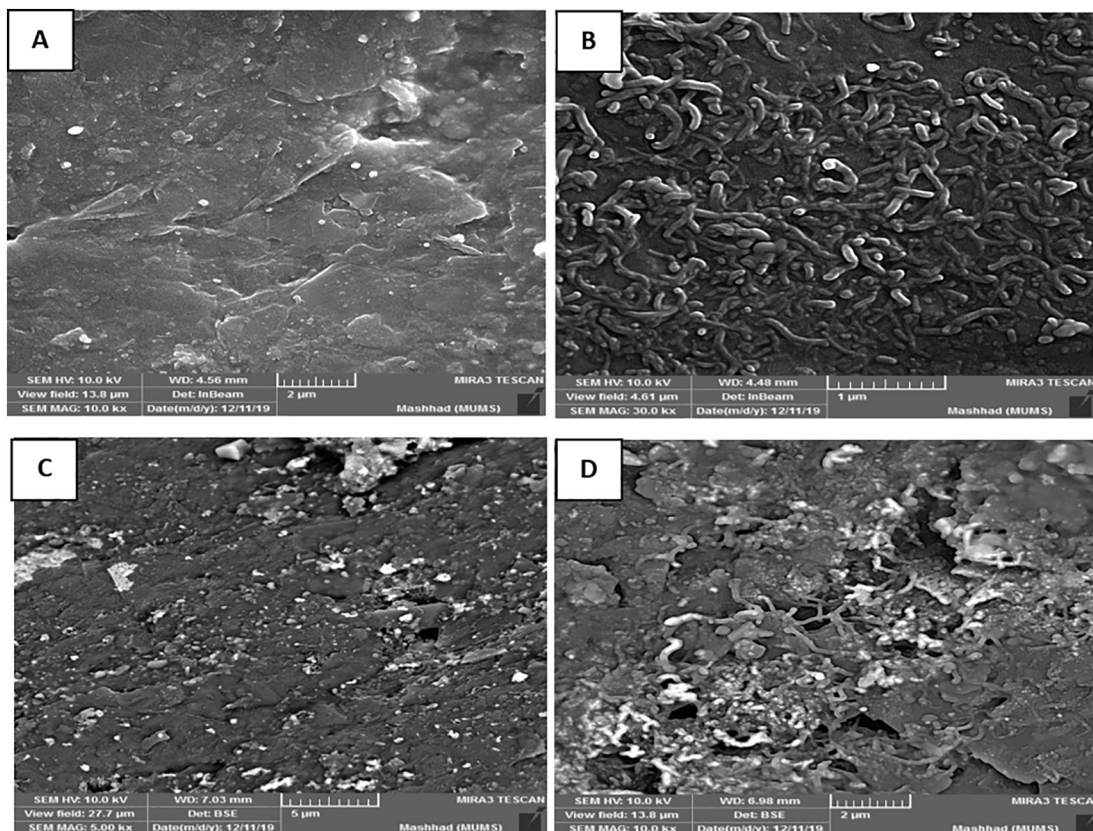


Fig. 2. Field emission scanning electron microscope (FE-SEM) images of (A) bare PGE, (B) f-MWCNTs/PGE, (C) and (D) f-MWCNTs/AuNPs/PGE.

#### 2.4. Preparation of real samples

The pharmaceutical sample (tramadol tablet from Sajjad Daru Shargh Pharmaceutical Co., Iran, 100 mg) was purchased from a local drug store. One tramadol tablet was weighted, crushed as a soft powder by moss and dissolved in 100 ml of deionized water. The solution was filtered by a filter paper and it was used as a real sample.

The urine sample was collected from a healthy person and diluted 50 times with 0.1 M phosphate buffer (PBS) to reduce the matrix effects.

Standard addition method was used for determination the concentration of TRA in the prepared solution.

### 3. Results and discussion

#### 3.1. Characterization of f-MWCNTs/AuNPs nanocomposite

##### 3.1.1. Field emission scanning electron microscope (FE-SEM)

The surface morphologies of the modified electrode were investigated by field emission scanning electron microscope (FE-SEM). Fig. 2, shows

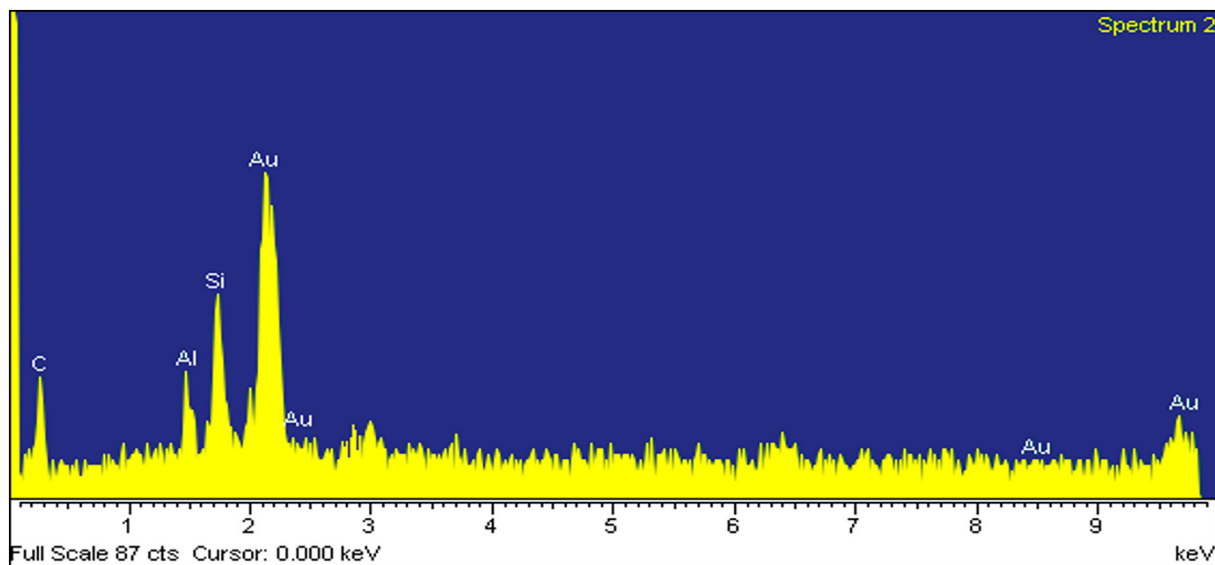


Fig. 3. EDS spectrum of f-MWCNTs/AuNPs/PGE.

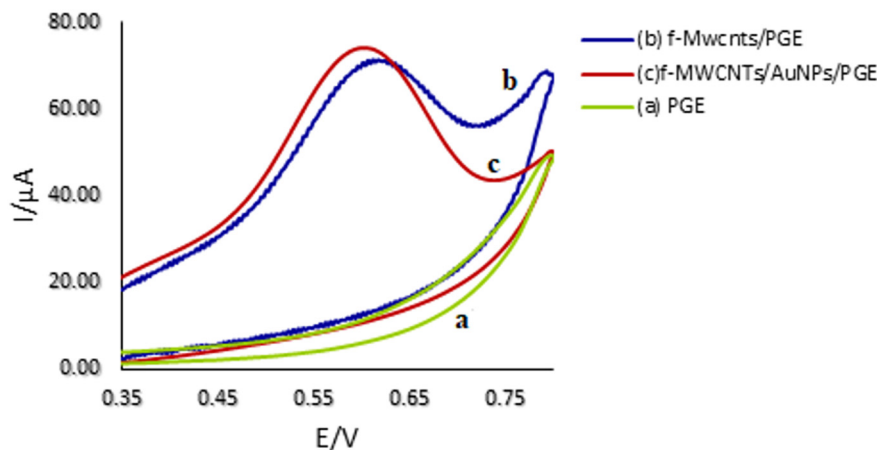


Fig. 4. Cyclic voltammograms of (a) bare PGE, (b) f-MWCNTs/PGE and (c) f-MWCNTs/AuNPs/PGE in 0.1 mM tramadol solution with the scan rate of 0.05 V/s.

the FE-SEM images of the bare PGE, f-MWCNTs/PGE and f-MWCNTs/AuNPs/PGE. At the bare PGE, the graphite layers can be seen obviously as cumulative and create porous and irregular sheet-like structure. After immersing of the pencil graphite electrode in f-MWCNTs suspension, the multiwalled carbon nanotubes are attached at the surface of the PGE (Fig. 2B). Figs. 2C & D, illustrate the FE-SEM images of the surface of f-MWCNTs/AuNPs/PGE after electrochemical deposition of gold nanoparticles.

### 3.1.2. Energy dispersive spectroscopy (EDS)

The EDS spectrum in Fig. 3, provides direct elemental information of f-MWCNTs/AuNPs/PGE at the electrode surface. This spectrum clearly shows the presence of elements such as carbon, silicon, aluminum, and most importantly gold. The presence of Si and Al signals in the EDS spectrum is due to the presence of clay ( $\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2$ ) [18] at the electrode surface. The gold peak clearly shows the successful electrochemical deposition of the gold nanoparticles at the surface of the pencil graphite electrode.

## 3.2. Electrochemical characteristics

### 3.2.1. Electrochemical behavior of f-MWCNTs/AuNPs/PGE

In order to describe and compare the effect of presence and absence of the modifier layer on the surface of PGE, cyclic voltammetric technique (CV) was utilized [19]. The cyclic voltammograms of TRA at the bare PGE, f-MWCNTs/PGE and f-MWCNTs/AuNPs/PGE at a scan rate of 50 mV/s are shown in Fig. 4. As is evident in this Figure, the bare electrode does not show a significant peak for oxidation of TRA molecules (curve a). However, a relatively strong anodic peak of tramadol molecules is observed at about 0.61 V at the surface of f-MWCNTs/PGE (curve b). When the f-

MWCNTs/PGE is modified with the gold nanoparticles, the oxidation peak current of TRA molecules increases and the oxidation peak potential significantly shifts to the negative direction (curve c). This behavior may be attributed to the large specific surface area and high electrical conductivity of f-MWCNTs and also the good electrocatalytic activity of AuNPs which enhance the electron transfer process between the tramadol molecules and the electrode surface [20].

In order to obtain the electroactive area of the proposed sensor compared to the bare PEG and f-MWCNTs/PEG, cyclic voltammograms were recorded in a 5.0 mM  $\text{K}_4\text{Fe}(\text{CN})_6$  solution containing 0.1 M KCl at different scan rates. According to the Randles-Sevcik equation and the slope of the plot of  $I_p$  versus  $v^{1/2}$ , the electroactive area was calculated [21].

$$I_p (\text{A}) = 2.99 \times 10^5 n[(1 - \alpha) n_\alpha]^{1/2} A C D^{1/2} v^{1/2}$$

The electroactive areas were estimated to be  $3.09 \times 10^{-5}$ ,  $1.03 \times 10^{-4}$  and  $2.06 \times 10^{-4} \text{ cm}^2$  for PEG, f-MWCNTs/PEG and f-MWCNTs/AuNPs/PGE, respectively. Therefore, the surface modification can increase the specific surface area.

### 3.2.2. Electrochemical impedance spectroscopy (EIS)

The electrochemical impedance spectroscopy (EIS), can be used for investigation the interfacial electron transfer properties of electrodes at different modification steps. Fig. 5A and B display the Nyquist plots of the bare PGE, f-MWCNTs/PGE and f-MWCNTs/AuNPs/PGE in solutions 0.1 M KCl containing 5 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  and 1 mM TRA in PBS (pH 7.0), respectively. In the Nyquist plot, the diameter of the semicircle at high frequency equals to the charge transfer resistance and it can be used to describe the interface properties of the electrode [22,23]. The electrical equivalent circuit

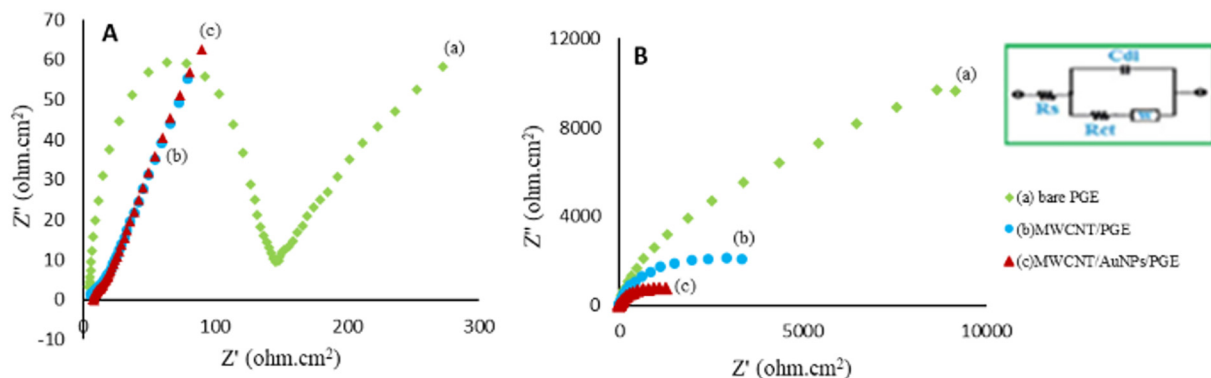


Fig. 5. Electrochemical impedance spectra of (a) bare PGE, (b) f-MWCNTs/PGE and (c) f-MWCNTs/AuNPs/PGE in (A) 5 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  (1:1) solution containing 0.1 M of KCl and (B) 1 mM TRA in PBS (pH 7.0).

which was used for the fitting of the experimental data is presented in the inset of Fig. 5. In this circuit,  $R_s$  and  $R_{ct}$  are the solution resistance and charge transfer resistance, respectively.  $W$  is the Warburg and  $C_{dl}$  represents the double layer capacitance. The values of the equivalent circuit elements obtained from the fitting of the experimental information are summarized in Table 1. The obtained results in Fig. 5A and Table 1, show that the bare PGE has the largest semicircle diameter in the high-frequency region which is due to the high resistance ( $140 \text{ k}\Omega\text{cm}^2$ ) toward the electron transfer. But the f-MWCNTs/PGE and f-MWCNTs/AuNPs/PGE, have the lower electron transfer resistances of  $20.84 \text{ k}\Omega\text{cm}^2$  and  $19.41 \text{ k}\Omega\text{cm}^2$ , respectively. As is evident in Fig. 5B, the impedance spectra in TRA solution are different in the presence and absence of AuNPs. The bare PGE shows the most electron transfer resistance ( $11.11 \text{ k}\Omega\text{cm}^2$ ). However, in the presence of f-MWCNTs, the electron transfer resistance is  $4.83 \text{ k}\Omega\text{cm}^2$ . When the surface of f-MWCNTs/PGE is modified with gold nanoparticle, the diameter of the semicircle decreases and the electron transfer increases ( $1.94 \text{ k}\Omega\text{cm}^2$ ) which demonstrates that the presence of f-MWCNTs and AuNPs at the surface of the fabricated electrode, can facilitate the electron transfer process between the TRA molecules and the electrode surface.

### 3.3. Effective parameters

#### 3.3.1. Effect of pH

One of the parameters which effects, the analytical performance, electrochemical characteristic and also the oxidation mechanism of TRA molecules is the pH of the solution [24]. In order to optimize this parameter, cyclic voltammetric measurements were carried out for TRA solutions containing  $0.1 \text{ M}$  PBS in a wide pH range (4–9). The corresponding cyclic voltammograms are shown in Fig. 6A. As is evident in Fig. 6A, the oxidation peak current of TRA molecules increases from pH 4 to pH 7 and then decreases. Therefore, pH 7 was selected as an optimum value in the subsequent experiments. Since the oxidation of tramadol molecules involves the removal of protons, therefore, this oxidation process occurs extremely hard in acidic environments [25]. According to the slope of the anodic peak potential versus pH plot in Fig. 6B in which  $dE_p/dpH = -2.303 \text{ mRT}/nF$  [26,27], the  $m/n$  ratio is obtained 1 which indicates that the equal number of protons and electrons are involved in the oxidation process of TRA molecules in solutions.

#### 3.3.2. Effect of scan rate

Cyclic voltammetric measurements of the tramadol solution were carried out using f-MWCNTs/AuNPs/PGE at pH 7 at different scan rates (Fig. 7A). The cyclic voltammograms show that as the scan rates increases, the peak current increases and the peak potential moves to more positive values which indicates that the electrochemical oxidation of tramadol molecules is irreversible. The plot of the anodic peak current ( $I_p$ ) versus the square root of the scan rates ( $v^{1/2}$ ) is linear which shows that the electrooxidation of tramadol molecules at the electrode surface is diffusion controlled (Fig 7B). The relationship between the intensity of the peak current and the square root of the scan rate for an irreversible electrode reaction is shown by Randles-Sevcik Eq. [28]:

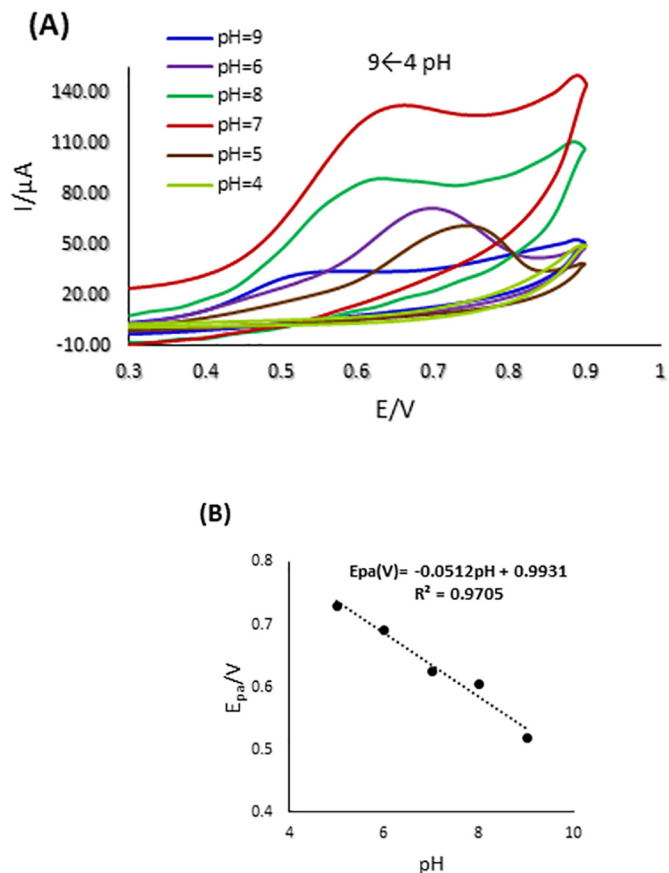
$$I_p(A) = 2.99 \times 10^5 n[(1-\alpha)n_\alpha]^{1/2} A C D^{1/2} v^{1/2}.$$

In this case, the calculated electrode surface area ( $A$ ) was  $2.06 \times 10^{-4} \text{ cm}^2$ , the concentration of tramadol ( $C$ ) was  $10^{-4} \text{ mol/cm}^3$

**Table 1**

Electrochemical impedance spectroscopy response at the different electrodes in (A)  $5 \text{ mM}$   $[\text{Fe}(\text{CN})_6]^{-3/-4}$  ( $1:1$ ) solution containing  $0.1 \text{ M}$  of KCl and (B)  $1 \text{ mM}$  TRA in PBS (pH 7.0).

Electrode	(A)					(B)				
	$R_s$ ( $\Omega \text{ cm}^2$ )	$R_{ct}$ ( $\text{k}\Omega \text{ cm}^2$ )	$n$	$C_{dl}$ ( $\mu\text{F cm}^{-2}$ )	$W$ ( $\text{k}\Omega \text{ cm}^2$ )	$R_s$ ( $\Omega \text{ cm}^2$ )	$R_{ct}$ ( $\text{k}\Omega \text{ cm}^2$ )	$n$	$C_{dl}$ ( $\mu\text{F cm}^{-2}$ )	$W$ ( $\text{k}\Omega \text{ cm}^2$ )
PGE	3.00	140.00	0.896	10.77	0.06	3.96	11.11	0.907	14.33	1.95
MWCNT/PGE	4.00	20.84	0.367	3088.10	0.08	4.70	4.83	0.915	305.03	0.17
MWCNT/AuNPs/PGE	6.94	19.41	0.426	8804.03	0.08	4.90	1.94	0.844	176.33	0.03



**Fig. 6.** (A) Cyclic voltammograms of TRA at the surface of the f-MWCNTs/AuNPs/PGE at various pHs (B) the relationship of the oxidation peak potential ( $E_p$ ) with pH.

and the diffusion coefficient ( $D$ ) was  $9.17 \times 10^{-5} \text{ cm}^2/\text{s}$  [29]. The  $(1-\alpha)n_\alpha$  was calculated by [30]:

$$E_p - E_{p/2} = 47.7/(1-\alpha)n_\alpha \text{ mV at } 25^\circ\text{C}$$

where  $E_p$  is the peak potential and  $E_{p/2}$  is the half-height potential for tramadol oxidation.

According to the slope of the line in Figure 7B which is equal to  $7 \times 10^{-5}$ , the total number of electrons involved in the electrochemical oxidation of tramadol molecules was calculated to be 2 electrons. The results obtained from the study of pH and scan rate effects indicate that the electrooxidation of tramadol should be a two-electron and two-proton process. This result is consistent with the oxidation mechanism of TRA molecules which is presented in Fig. 8. According to this mechanism, in the first step due to the removal of one electron from N atom of amin group, an aminium radical cation (1) is generated. In the second step, a proton is removed from the  $\text{CH}_3$  group and a free radical is formed (2). Afterward, removing of one electron from the radical, results in the formation of an iminium cation (3). In the last step, the iminium cation hydrolyzes through formation of hemiaminal which in turns decomposes to the final product

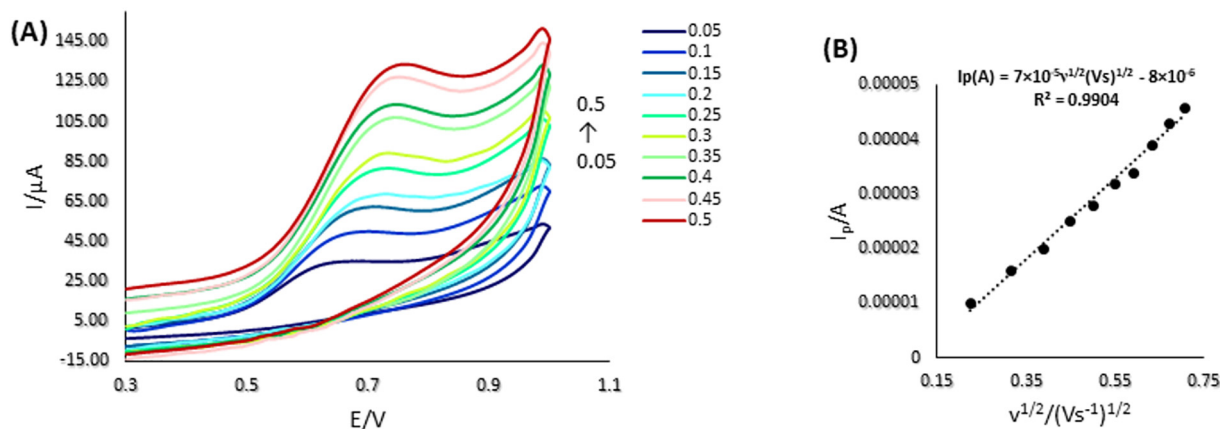


Fig. 7. (A) Cyclic voltammograms of TRA at the surface of f-MWCNTs/AuNPs/PGE in 0.1 M PBS (pH 7.0) at various scan rates from 50 to 500 mVs<sup>-1</sup>. (B) The plot of  $I_{pa}$  vs.  $v^{1/2}$ .

(4). This oxidation mechanism for TRA molecules is also reported in reference [31].

### 3.3.3. Effect of immersion time of PGE in f-MWCNTs suspension

The effect of the immersion time of PGE in f-MWCNTs suspension on the electrochemical response of the fabricated electrode toward the TRA molecules was investigated by using differential pulse voltammetry (DPV) measurements (Fig. 9). For this purpose, a suspension containing 5 mg of f-MWCNTs in 2.5 ml of DMF was prepared and the surface of graphite pencil electrodes was modified by immersing the electrodes in the f-MWCNTs suspension at different times from 15 to 120 min. As is evident in Fig. 9, the oxidation peak current increases from 15 to 105 min and then decreases. Therefore, 105 min was chosen for the modification of the electrode surface. It seems that at higher immersion times, the amount of carbon nanotubes increases and reaches to the saturation level which makes the electron transfer process between the TRA molecules and the electrode surface difficult.

### 3.4. Calibration plot

Under optimum experimental conditions, the quantitative measurements of TRA in solutions were performed by differential pulse voltammetry (DPV) using f-MWCNTs/AuNPs/PGE at pH 7. As can be seen from Fig. 10, the calibration curve is linear in the ranges of 12–100 nM and 100–3000 nM with respect to the concentration of tramadol. The correlation coefficients were found to be 0.9875 and 0.9919 in these concentration ranges, respectively. The detection limits of the method which were obtained by means of  $LOD = 3S_b/m$  were  $5 \times 10^{-9}$  M and  $1.3 \times 10^{-7}$  M and the limit of quantitations based on using  $LOQ = 10S_b/m$  were found to be  $20 \times 10^{-9}$  M and  $4.3 \times 10^{-7}$  M for the first and second linear ranges, respectively.

In order to compare the performance of the proposed electrochemical sensor with some of the modified electrodes for measurement the concentration of tramadol, the obtained analytical parameters are presented in Table 2. As can be seen in this Table, the proposed sensor has a wider linear range and a lower detection limit than the other reported electrodes.

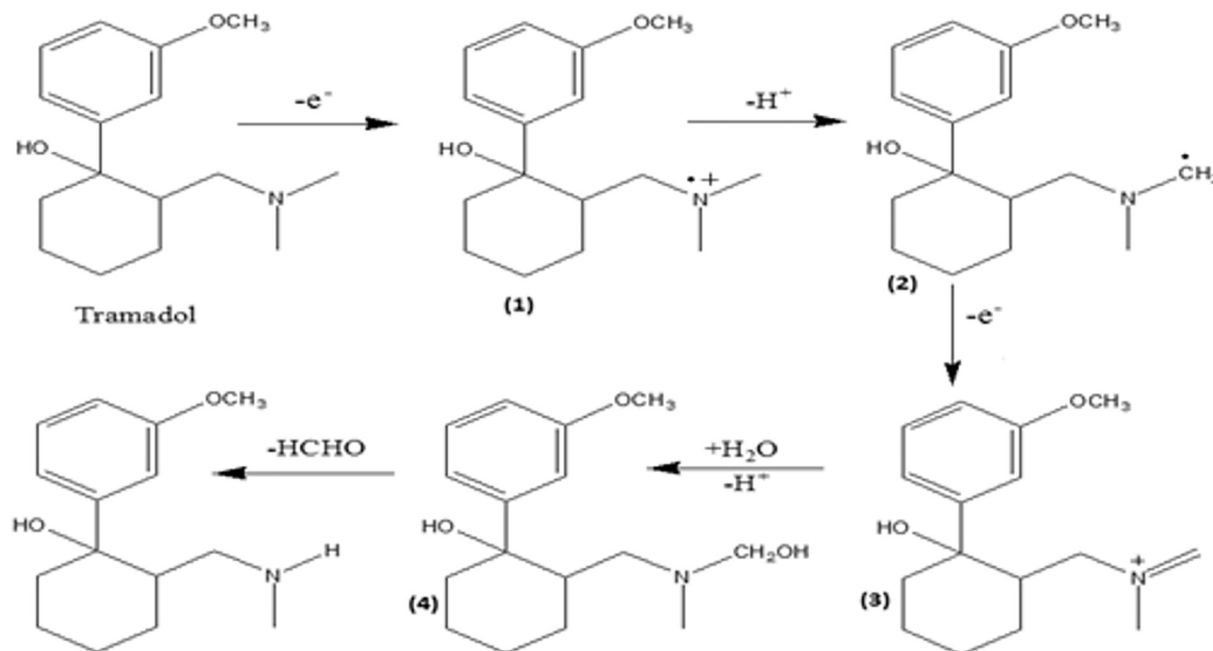


Fig. 8. Suggested oxidation mechanism for TRA molecule.

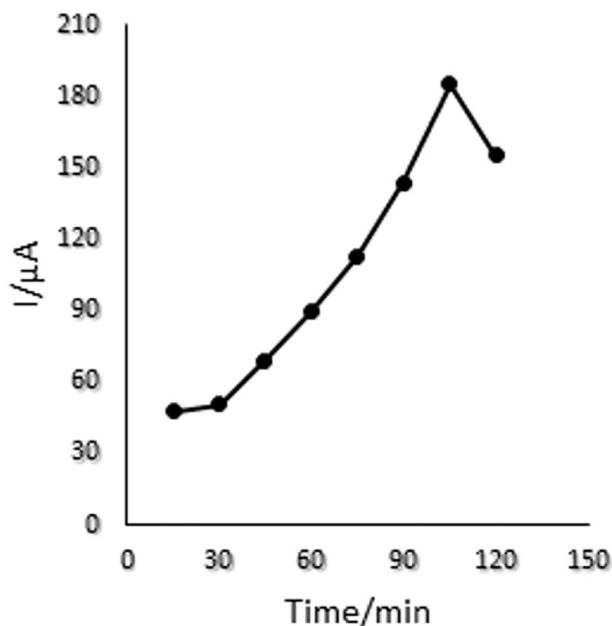


Fig. 9. Effect of immersion time of PGE in f-MWCNTs suspension.

### 3.5. Reproducibility, repeatability and stability of f-MWCNTs/AuNPs/PGE

In order to evaluate the reproducibility of the modified electrode, 5 modified electrodes were prepared under the same experimental conditions and the concentration of tramadol in solution (0.1 mM) was measured by differential pulse voltammetry (DPV) and the relative standard deviation was found to be: 2.4%. The repeatability of the modified electrode was investigated by sequential measurements ( $n = 5$ ) of 0.1 mM tramadol solution and the relative standard deviation (RSD) was calculated 2.2% which indicates the good repeatability of the developed electrode. According to these experimental results, the reproducibility and repeatability of the proposed sensor are acceptable.

Table 2

Comparison of detection limits and dynamic ranges of some electrochemical sensors for the detection of TRA.

Modified electrode	Linear range ( $\mu\text{M}$ )	LOD ( $\mu\text{M}$ )	References
CNP-GCE <sup>a</sup>	10–100, 100–1000	5	[32]
D50wx2/GNP/GCPE <sup>b</sup>	0.03–42	0.01	[33]
MWCNTs/GCE <sup>c</sup>	2–300	0.36	[34]
AuNPs/CNTs/GCE <sup>d</sup>	10–450, 600–1000	0.068	[29]
Glassy carbon electrode	15–75	2.2	[35]
LPME-BE/HPLC <sup>e</sup>	0.3–130	0.12	[36]
GPE <sup>f</sup>	0.1–1.5	0.004	[21]
Graphene/NiFe <sub>2</sub> O <sub>4</sub> nanocomposite	0.01–9	0.003	[37]
PAMDAN/Pt <sup>g</sup>	5.0–30	0.327	[38]
f-MWCNTs/AuNPs/PGE	0.012–0.1, 0.1–3	0.005	This paper

<sup>a</sup> Carbon nanoparticles modified glassy carbon electrode.

<sup>b</sup> Glassy carbon paste electrode (GCPE) modified with a cation exchanger resin, Dowex50wx2 and gold nanoparticles.

<sup>c</sup> Multiwalled carbon nanotube modified glassy carbon electrode.

<sup>d</sup> Au nanoparticles and multiwalled carbon nanotube modified glassy carbon electrode.

<sup>e</sup> Back extraction liquid phase micro extraction-high performance liquid chromatography.

<sup>f</sup> Graphite pencil electrode.

<sup>g</sup> Platinum electrode modified with poly 8-(3-acetylrimino-6-methyl-2,4-dioxopyran)-1-aminonaphthalene.

The stability of the proposed sensor was also investigated. For this purpose, the f-MWCNTs/AuNPs/PGE was utilized for determination the same concentration of TRA for 10 days. After each measurement, the electrode was placed in a refrigerator at 4 °C. The modified electrode showed 95.63% of the initial electrochemical response after 10 days which indicates the good stability of the modifier layer on the surface of PGE.

### 3.6. Interferences study

The effect of the presence of some interfering compounds such as ascorbic acid, uric acid and citric acid on the electrochemical measurement of tramadol was also investigated using the fabricated electrode. For this purpose, solutions of TRA (0.1 mM) and the above-organic compounds with concentrations of 10 and 100 times higher than TRA were prepared at

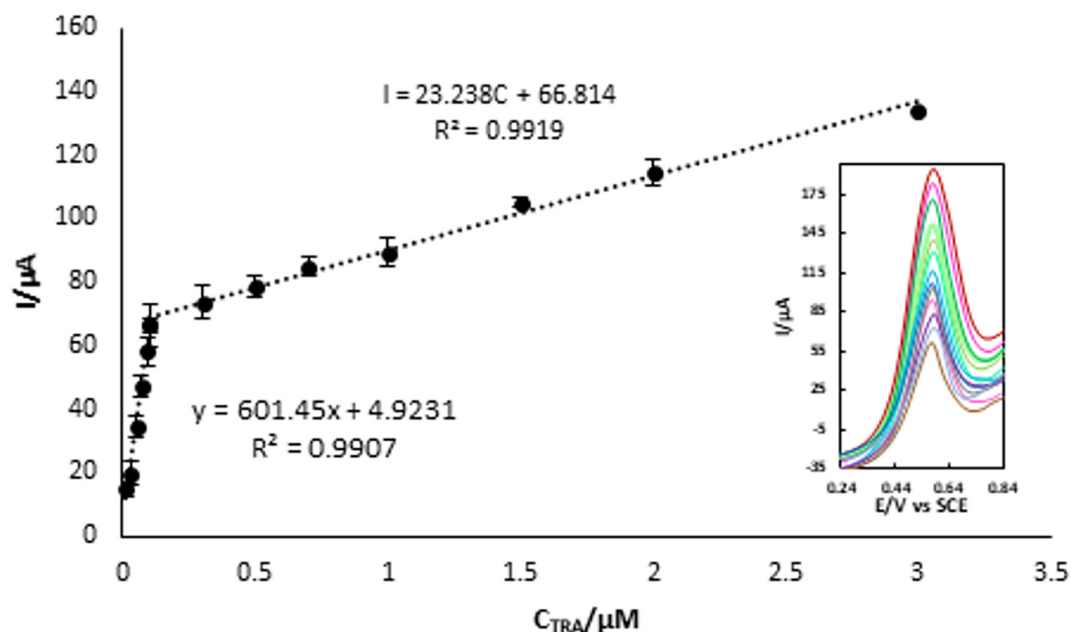


Fig. 10. Calibration curve of f-MWCNTs/AuNPs/PGE in the concentration range of 0.012 to 3  $\mu\text{M}$  of tramadol. The error bars represent the standard deviation for three duplicate measurements. Inset is the differential pulse voltammograms of modified electrode in different concentrations.

**Table 3**  
Effect of some interfering compounds on the determination of tramadol.

Interferent	% Recovery (10 times)	% Recovery (100 times)
Ascorbic acid	102	97
Uric acid	99	101
Citric acid	98	103

pH 7 and they were measured by differential pulse voltammetry. The values of recoveries are given in Table 3. As is evident in this Table, the presence of these three organic acids does not interfere with the quantitative measurement of tramadol in solutions. Since the  $pK_a$  values of ascorbic acid, uric acid and citric acid are 4.2, 5.4 and 3.13, respectively [31], therefore, they do not adsorb at the surface of the modified electrode with a negative charge at pH 7 and hence these organic acids do not interfere in the determination of tramadol in solutions.

### 3.7. Analytical application of modified electrochemical sensor

The applicability of the f-MWCNTs/AuNPs modified electrode for quantitative measurement of tramadol in a pharmaceutical sample (100 mg tablet) and a human urine sample was studied using differential pulse voltammetry technique, and the experimental results are shown in Tables 4 and 5, respectively. The samples were prepared by the procedures which are described in the real samples' preparation section. The standard addition method was used for determination the concentration of TRA in the prepared samples ( $n = 3$ ). The tramadol content of the tablets which was determined by the proposed sensor, was found to be: 101.2 mg per tablet, which is very close to its actual value (100 mg per tablet). The obtained recoveries for all of the samples, were in the range of 99.70%–101.83% with RSDs of 0.16–1.53%, indicating that the response of the constructed electrochemical sensor is independent of the matrix of real samples and it can be used for determination TRA in real sample solutions.

## 4. Conclusion

In the present study, the multiwalled carbon nanotubes and gold nanoparticles were used as the modifier materials on a pencil graphite electrode for construction of a new electrochemical sensor for voltammetric determination of tramadol in solutions. The combination of carbon nanotubes and gold nanoparticles improved the electrocatalytic activity of the sensor toward the oxidation of tramadol molecules in solutions. The modified electrode showed a good selectivity, low detection limit and wide linear range. Also, the proposed sensor provided good repeatability, reproducibility and stability. The constructed electrochemical sensor is a promising tool for determination of tramadol in both pharmaceutical and biological samples.

### CRediT authorship contribution statement

**Simin Kolahi-Ahari:** Investigation, Writing - original draft, Formal analysis. **Behjat Deiminiat:** Validation, Writing - review & editing, Supervision, Project administration. **Gholam Hossein Rounaghi:** Validation, Writing - review & editing, Supervision, Funding acquisition.

**Table 4**  
The results for determination of tramadol in a tablet ( $n = 3$ ).

Real sample	Initial concentration (nM)	Added (nM)	Founded (nM)	Recovery (%)	RSD (%)
Tramadol	50	6	56.11	101.83	1.04
tablet	300	6	306.08	101.33	0.16

**Table 5**  
The Results for determination of tramadol in human urine samples ( $n = 3$ ).

Real sample	Added (nM)	Founded (nM)	Recovery (%)	RSD%
Urine	40	39.94	99.85	1.53
	80	79.76	99.70	0.73

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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