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A Sensor for Determination of Tramadol in Pharmaceutical Preparations and Biological Fluids Based on Multi-Walled Carbon Nanotubes-Modified Glassy Carbon Electrode

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Summary: A chemically modified electrode is constructed based on multi-walled carbon nanotube modified glassy carbon electrode (MWCNTs/GCE). It is demonstrated that this sensor could be used for determination of pharmaceutical important compound tramadol (TRA). The measurements were carried out using differential pulse voltammetry (DPV), cyclic voltammetry (CV) and chronoamperometry (CA) methods. DPV experiments of various concentration of TRA showed two linear dynamic ranges. The first linear dynamic range was from 4 μM to 35 μM, and the second linear dynamic range was between 60 μM to 550 μM. A detection limit of 0.38 μM (S/N = 3) was obtained. The analytical performance of this sensor has been evaluated for the detection of TRA in human serum, human urine and some pharmaceutical preparations with satisfactory results.

Keywords: Electrode, Nanotube, MWCNTs/GCE, Tramadol, Differential Pulse Voltammetry.

Introduction

Tramadol (TRA) is a synthetic centrally acting analgesic agent, which was used for the relief of moderate to chronic pain and has no clinically relevant cardiovascular or respiratory depressant activity. TRA is generally said to be devoid of many serious adverse effects of traditional opioid receptor agonists, such as the risk for respiratory depression [1, 2] and drug dependence [1, 3]. Based on the latter, the abuse potential of TRA is considered to be low or absent [4-6], which is in contrast to other opioids. Its overall analgesic efficacy was comparable to that achieved using equianalgesic doses of morphine or alfentanil [7]. Several analytical methods for determination of TRA were proposed which are mostly based HPLC [8, 9], GC-MS [10, 11], spectrophotometry [12, 13], electrophoresis [14] and potentiometry [15, 16] methods. However, some of these methods suffer from some disadvantages such as high costs, long analysis times and requirement for sample pretreatment. Therefore, development of a simple, inexpensive, sensitive and accurate analytical method for determination of TRA is of great importance.

TRA is an electroactive compound which can be oxidized electrochemically. The development and application of electrochemical sensors for TRA analysis, with respect to its sensitivity, accuracy, and simplicity, has been of greater interest in recent years [17-19]. However those methods suffer high acidic solution requirement for analysis (pH of 2) which is far from biological pH [17], low linear dynamic range or high detection limit [18, 19]. Carbon nanotubes (CNT) represent an increasingly important group of nanomaterials with unique geometrical, mechanical, electronic and chemical properties [20, 21]. Such properties of CNT make them also extremely attractive for the task of electrochemical detection. CNTs are widely used to prepare modified electrodes and construct sensing films as they can enhance electron transfer rate and sensitivity [22, 23]. In this work we present the application of the multi-walled carbon nanotube modified glassy carbon electrode (MWCNTs/GCE) as a sensor for determination of TRA at biological pH with low detection limit and wide linear dynamic range.

Results and Discussion

Electroxidation Behavior of TRA on MWCNTs/GCE

The differential pulse voltammograms recorded for TRA at bare GCE, and MWCNTs/GCE are shown in Fig. 1. Curve **a** shows the voltammogram of 150 μM of TRA in PBS (pH 7.5) at GCE. Curve **b** displays voltammogram of TRA, at the same conditions as curve **a,** at MWCNTs/GCE. As can be seen, for the GCE the oxidation peak for TRA is very small. However the DPV of the TRA at the MWCNTs/GCE (voltammogram **b**) shows considerable increase in its oxidation peak current. In addition the corresponding peak potential of TRA

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shifts to less positive potential on MWCNTs/GCE. The presence of MWNTs could both increase the electrode surface area and facilitate the electron transfer between electrode and the analyte; therefore the enhancement in the corresponding electro-chemical oxidation peak current was observed.

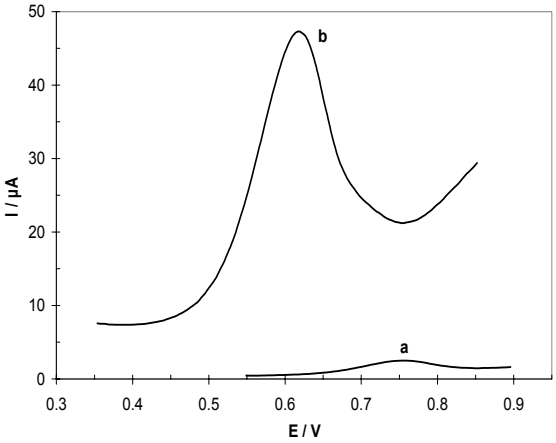

Fig. 1: Differential pulse voltammograms of 150 µM of TRA at (a) GC and (b) MWCNTs /GCE in 0.1M phosphate buffer solution (pH 7.5).

The effect of the scan rate on peak current of TRA was investigated in pH 7.5 of Phosphate buffer solution containing 100 µM TRA. Fig. 2 shows the cyclic voltammograms of the modified electrode at different scan rates from 10 to 500 mVs^{-1}. The anodic peaks current were proportional to the scan rate over the range 10–100 mVs^{-1} indicating adsorptive properties of the electrochemical process (Fig. 2, Inset A). At higher sweep rates, up to 350 mVs^{-1}, the plot of peak currents versus scan rate deviates from linearity and the peak current becomes proportional to the square root of the scan rate (Fig. 2, Inset B), indicating a diffusion controlled process.

Effect of Operational Parameters

The effect of pH of solutions on the electrochemical response of the MWCNTs/GCE towards the determination of 250 µM TRA was investigated using CV method in 0.1 M phosphate buffer solution scan rate of 60 mVs^{-1}. Variations of peak current with respect to pH of the electrolyte in the pH range from 4 to 10 are shown in Fig. 3. It can be seen that the anodic peak currents of TRA increases with raising the solution pH until it reaches 7.5 (Fig. 3, Inset A). However at higher pH the TRA oxidation peak current starts to decrease. Therefore the pH value of 7.5, which is close to biological pH value, was chosen as an optimum solution pH for further experiments. Variation of TRA oxidation peak potential with pH is in accordance with equation of Ep = -0.048 pH + 0.9874 (Fig. 3, Inset B). For a Nernstian process which number of transferred electrons is equal to number of transferred proton, the slope would be expected to be −59 mV pH^{-1} unit. The slope of -48 mV pH^{-1} suggests that the numbers of electrons and protons transferred in the oxidation reaction of TRA are equal.

The plot of the cyclic voltammogram anodic peak current versus accumulation time for 50 µM TRA solution was obtained. Initially, peak currents for this compound increase with accumulation time up to 45 s. However after 45 s of accumulation time, the peak currents reach a slight increasing and then plateau. As a consequence, the accumulation time of 45 s was chosen as an optimum time for further experiments.

Linear Dynamic Range and Detection Llimit of the Method

The electrochemical response of TRA in a 0.1M PBS pH 7.5 using MWCNTs/GCE is depicted in Fig. 4 and 5. Fig. 4 show differential pulse voltammograms and corresponding calibration curves obtained at MWCNTs/GCE in various concentrations of TRA. Application of DPV method two linear ranges was obtained. The first linear dynamic range was from 4 µM to 35 µM, with a calibration equation of Ip (µA) = 0.4629c (µM) + 0.4406 (R^2=0.9992) and the second linear dynamic range was between 60 µM to 550 µM with a calibration equation of Ip(µA) = 0.1019c (µM) + 16.831 (R^2=0.9987). A detection limit of 0.38 µM (S/N = 3) was obtained.

Fig. 5 displays chronoamperograms response of the rotated modified electrode (2500 rpm) with successive injection of TRA at an applied potential of 0.7 V in PBS (pH 7.5). Application of CA method showed that the linear dynamic range was from 10 µM to 700 µM, with a calibration equation of Ip(µA) = 0.2241c (µM) + 0.0117 (R^2=0.9998) and a detection limit of 0.96 µM (S/N = 3) was obtained.

Repeatability and Long-Term Stability of the Electrode

The repeatability of the analytical method for determination of TRA has been studied. Indeed, the relative standard deviation (RSD) of 1.67 and 1.15 % for 50.0 and 100.0 µM TRA respectively in ten consecutive determinations has been obtained.

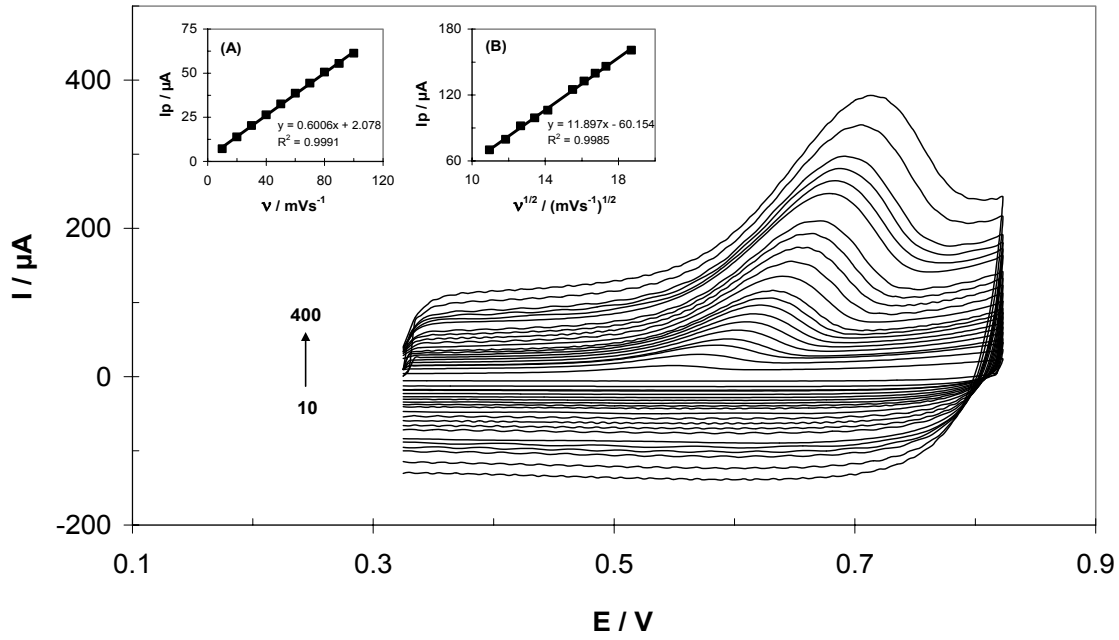

Fig. 2: Effect of scan rates on CVs of 100 µM TRA at (a) 10, (b) 20, (c) 30, (d) 40, (e) 50, (f) 60, (g) 70, (h) 80, (i) 90, (j) 100, (k) 120, (l) 140, (m) 160, (n) 180, (o) 200, (p) 240, (q) 260, (r) 280, (s) 300, (t) 350 and (u) 400 mVS^{-1}. Insets: (A) Plot of peak currents as a function of scan rate of potential. (B) Plot of peak currents as a function of square root of the scan rate of potential.

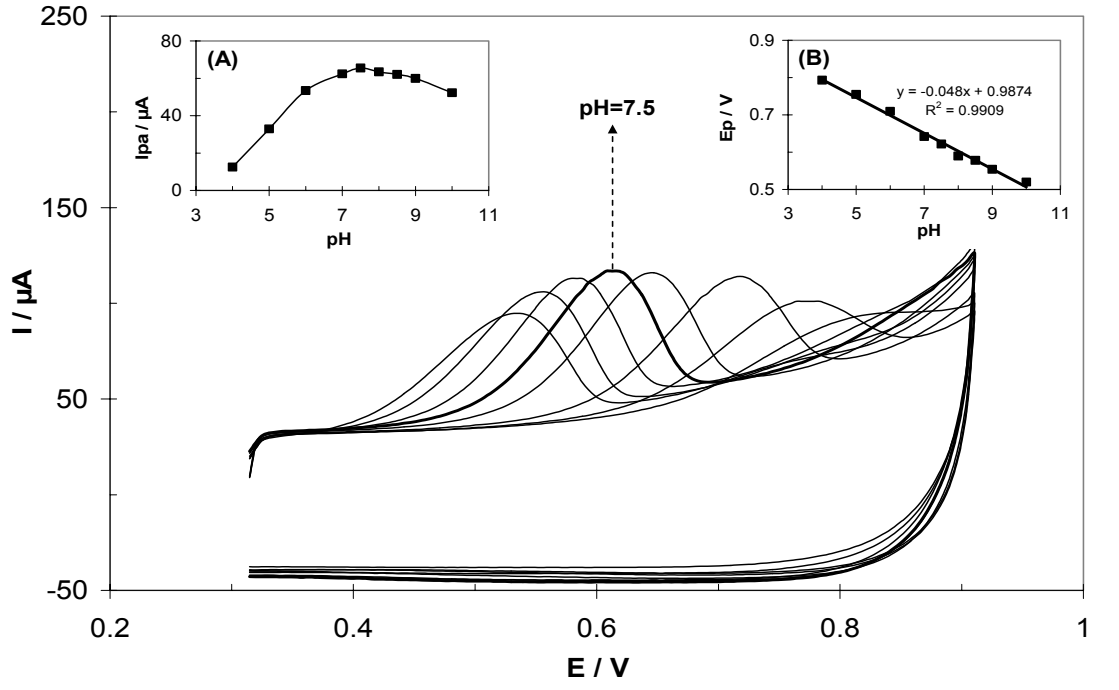

Fig. 3: Effect of pH on the CVs of oxidation of 250 µM TRA compound at MWCNTs/GCE. Insets: (A) Plot of peak currents as a function of pH buffer; (B) Plot of potential of peaks (Ep) as a function of pH buffer.

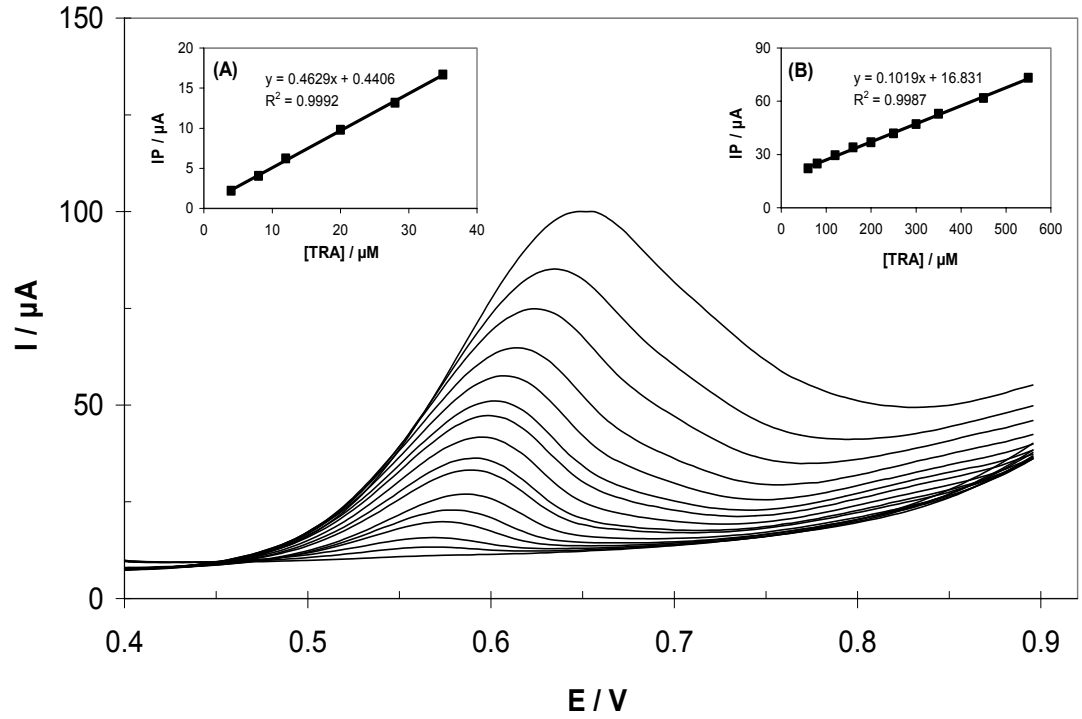

Fig. 4: DPVs of different concentrations of TRA as (a) 4, (b) 8, (c) 12, (d) 20, (e) 28, (f) 35, (g) 60, (h) 80, (i) 120, (j) 160, (k) 200, (l) 250, (m) 300, (n) 350, (o) 450 and (o) 550. Insets: (A) The first linear dynamic range of TRA. (B) The second linear dynamic range of TRA.

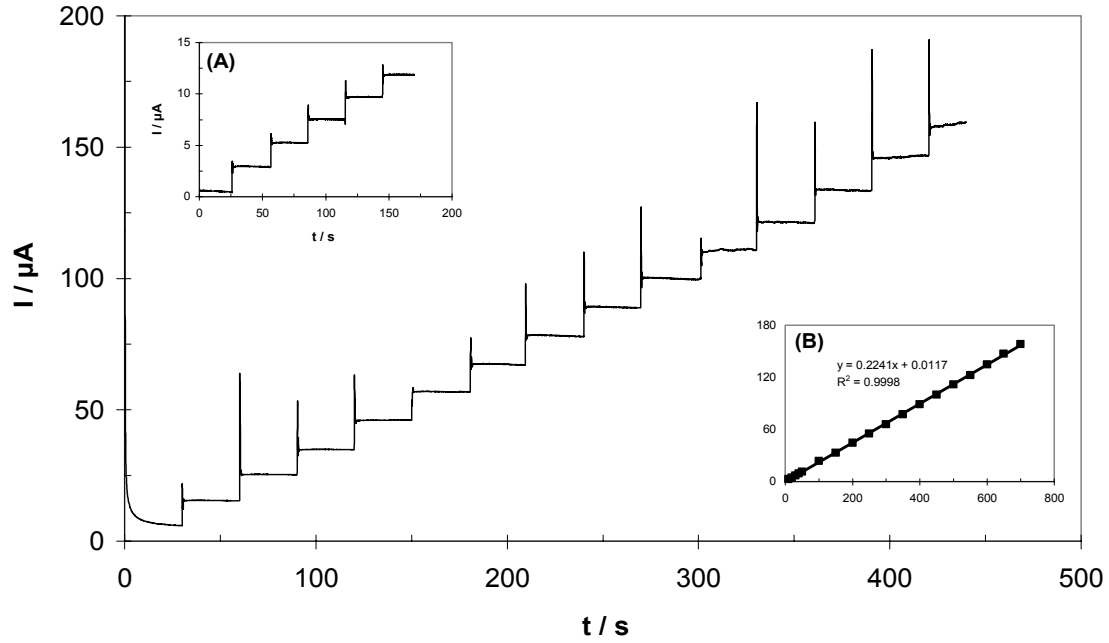

Fig. 5: Amperometric response at rotating MWCNTs/GCE for determination of TRA by successive additions of 50 µM TRA. Insets: (A) successive additions of 10 µM TRA. (B) Corresponding calibration curve.

Another attraction of the proposed modified electrode is that the resulting modified electrode is of a good long-term stability. Stability of the proposed electrode was tested by measuring the decrease in voltammetric current during repetitive DPV measurements of TRA after storing the electrode in solution or air for certain period of time. For example, determination of 40 μM TRA in 0.1 M PBS (pH 7.5), when the modified electrode was subjected to an experiment every 30 min, gave less than 8.1 % decrease in the voltammetric currents after 24 h. When the electrode was stored in the atmosphere for 10 days, the current response reduced less than 9.8 % when the electrode subjected to the solution containing 100 μM TRA.

Interference Studies

The effects of common interfering species in solution of 50 μM TRA under the optimum conditions were investigated. The results are summarized in Table-1 and show that they do not significantly affect the height of the peak currents for TRA. The tolerance limit was defined as the concentrations which give an error of ≤ 10 % in the determination of TRA compound. The data confirm that the proposed method is free from interferences of the most common interferants.

Table-1: Maximum tolerable concentration of interfering species

Interfering species	$C_{int}/\mu M$
L-dopa	**300**
Dopamine	**250**
L-alanin	**150**
uric acid	**120**
ascorbic acid	**120**
Aspartic acid	**400**
xanthine(XA)	**100**
caffeine	**180**
Vitamin E	**80**
***Vitamin B*$_1$**	**75**

C_{int} refers to interfering compound concentration

Analytical Applications

The applicability of the MWCNTs/GCE was examined for the determination of TRA in human serum (Table-2) and human urine (Table-3). The differential pulse voltammograms were obtained by spiking known amounts of TRA in the human serum and human urine samples using MWCNTs/GCE at optimum conditions as described earlier. Applicability of the MWCNTs/GCE was examined for the determination of TRA in its three pharmaceutical preparations as tablet, capsule and ampoule (Table-4).The concentrations were obtained by using the calibration plots. The results are summarized in Table-2 and 4. The recoveries were acceptable and they confirm that the proposed methods could be efficiently used for the determination of trace amounts of TRA in biological systems and various pharmaceutical preparations.

Table-2: Determination of TRA in human serum with MWCNTs/GCE (n=5)

Sample	Added (μM)	Found (μM)	R.S.D. (%)	Recovery (%)
1	**0.00**	**0.00**	**-**	**-**
2	**10.00**	**9.85**	**2.9**	**98.5**
3	**20.00**	**19.92**	**2.2**	**99.6**
4	**40.00**	**40.85**	**1.9**	**102.1**

Table-3: Determination of TRA in urine sample with MWCNTs/GCE (n=5).

Sample	Added (μM)	Found (μM)	R.S.D. (%)	Recovery (%)
1	**0.00**	**0.00**	**-**	**-**
2	**10.00**	**9.93**	**2.8**	**99.3**
3	**25.00**	**26.07**	**2.3**	**104.3**
4	**50.00**	**49.11**	**1.6**	**98.2**

Table-4: Determination of TRA in pharmaceutical Samples with MWCNTs/GCE (n=5).

Sample	*Declared content (mg)*	*Found* [a] *(mg)*	*R.S.D. (%)*	*Recovery (%)*
Tablet	**50**	**49.01**	**2.9**	**98.0**
Capsule	**50**	**48.27**	**3.3**	**96.5**
Ampoule	**50**	**51.62**	**2.5**	**103.2**

Experimental

Reagents and Solutions

All chemicals and solvents were of analytical grade and used without further purification. Multi-walled carbon nanotubes (MWCNTs) (>95 wt%, 5-20 nm) was purchased from PlasmaChem GmbH company. Tramadol (TRA) is obtained from Fluka chemical company and ampoules of TRALGIDOL® (labeled to contain 50 mg tramadol hydrochloride per ampoule) were obtained from Osvah pharmaceutical company (Tehran, Iran). Capsules and tablets of RUZ-TRAMADOL® (labeled to contain 50 mg TRA per capsule or Tablet) were purchased from Ruz-Daru pharmaceutical company (Tehran-Iran).

Stock standard solutions of 10 mM TRA were freshly prepared in 0.1 M phosphate buffers of pH 7.5. All TRA solutions were prepared by diluting the stock standard solutions using 0.1 M phosphate buffer (pH 7.5). The 0.1 M Phosphate buffer solutions (PBS) were prepared by dissolving appropriate amounts of sodium hydrogen phosphate and sodium dihydrogen phosphate in 250 mL volumetric flask. Electrochemical experiments on TRA were carried out in 0.1 M PBS at pH 7.5.

Fresh human serum samples were prepared from Razi Institute of Vaccine and Serum Company

(Tehran, Iran). The serum and urine samples were filtered and diluted 20 times with 0.1 M PBS of pH 7.5 and checked for the determination of the recovery by spiking with TRA. The different pharmaceutical preparations of TRA as capsules, tablets (each labeled as 50 mg TRA content) and injections (labeled as 50 mg TRA per mL content), were selected for the analysis of TRA content using our proposed procedure. Ten capsules (or tablets) were accurately weighed and powdered in a mortar. An amount of the sample equivalent to one in tablet or capsule content was dissolved in 70 mL of 0.1M PBS (pH 7.5). After sonication for 10 minutes, the solutions were filtered by Whatmann No. 42 filter paper (Whatmann, Middlesex, UK), the residues were washed three times with 10 mL appropriate solvent, and the filtrate volumes were adjusted to 100 mL also using the same solvent. Ampoule content (1 mL) was consecutively diluted to reach appropriate concentration of TRA with 0.1 M PBS of pH 7.5.

Instrumentation

All the voltammetric measurements were carried out using nanotube modified glassy carbon electrode (MWCNTs/GCE) as a working electrode, Ag/AgCl/3M KCl as a reference electrode and platinum wire as an auxiliary electrode. DPV, CV and CA experiments were carried out using an Autolab PGSTAT 30 Potentiostat Galvanostat (EcoChemie, The Netherlands) coupled with a 663 VA stand (Metrohm Switzerland). All potentials given are with respect to the potential of the reference electrode. pH measurements were performed with a Metrohm 744 pH meter using a combination glass electrode.

Modification of the Electrodes

A glassy carbon electrode (GCE, 2-mm diameter, Metrohm) was polished with 0.3 and 0.05 µm aluminum slurry and rinsed thoroughly with triply distilled water. The GC electrode was cleaned by ultrasonic agitation for 5 min in ethanol and then distilled water, individually. The electrode was dried under nitrogen gas flow. A solution of 1 mg mL^{-1} MWCNTs–DMF was prepared by dispersing 1mg of MWNTs in 1 mL DMF. Then the solution was sonicated by ultrasonic agitation for 30 minute. 20 µl of MWCNTs–DMF solution was placed on the GC electrode surface. The electrode was then dried at room temperature to obtain MWCNTs/GCE. The fabricated MWCNTs/GCE was placed in the electrochemical cell containing 0.1M PBS and several cycles in the potential windows of 0.4 to 1 V were applied using CV method to obtain stable responses. The electrochemical surface area of the modified MWCNTs/GCE and bare GCE were determined by cyclic voltammogram measured between -0.1 to 0.6 V in 4mM ferricyanide solution (0.1 M phosphate buffer, pH 7.0) at different scan rates (not shown). The modified MWCNTs/GCE showed surface area of 9.8 times of GCE.

General Procedure

10 mL solution containing appropriate amount of TRA in 0.1 M PBS at pH 7.5 was transferred into the voltammetric cell. The voltammograms were recorded by applying positive-going potential from 0.4 to 0.8 V. The differential voltammogram showed anodic peak around 0.62 V corresponding to TRA compound which its height was proportional to its concentration in solution. The calibration curves were obtained by plotting anodic peak currents of TRA versus the corresponding concentrations. All experiments were carried out under open circuit condition. After each measurement, the MWCNTs/GCE rinsed carefully with distilled water to remove all adsorbate from electrode surface and to provide fresh surface for next experiments.

Conclusion

In this paper we introduced a sensor based on multi-walled carbon nanotube modified glassy carbon electrode. MWCNTs can increase anodic peak currents by enhancement of electron transfers of TRA compound on the electrode surface. The results indicated that MWCNTs/GCE facilitates the determination of TRA with good sensitivity and selectivity. The electrode showed high stability in repetitive experiments due to high water stability and high mechanical strength of MWCNTs. The effects of potential interfering compounds were studied, and it was found that the proposed procedure is free from interferences of most common interfering compounds. The proposed sensor was used in determination of TRA in some real samples like human serum, urine and some drugs, without the necessity of sample pretreatments or time-consuming extraction, with satisfactory results. The simple fabrication procedure, high speed, reproducibility, high stability, wide linear dynamic range, low detection limit, high sensitivity and its applicability in biological pH, suggest that the proposed sensor is an attractive candidate for practical applications.

Acknowledgments

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