

PENCIL GRAPHITE ELECTRODE MODIFIED NANOSENSOR FOR DETECTION AND DETERMINATION OF TRAMADOL IN BLOOD SERUM

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Resumen: El tramadol (TD) es un fármaco pseudo-opioide que tiene un efecto de somnolencia. En esta investigación, el electrodo de grafito de lápiz (PGE) fue modificado por nano tubo de carbono (CNT) utilizado para la detección de TD en suero sanguíneo. Para la detección y determinación se utilizó el método de voltametría diferencial de pulso (DPV). Se investigó la influencia de las variables electroquímicas para el tipo de electrodo y el pH de la solución. El sensor fue diseñado para medir TD en el suero sanguíneo. A continuación se investigó el efecto de la interferencia del ácido ascórbico en la medición de TD con este sensor.

Bajo condiciones optimizadas, se observó una relación lineal para el pico anódico sobre la raíz cuadrada de la velocidad de exploración. Se observó una respuesta lineal sobre el intervalo de concentración de 5 - 25 M con un coeficiente de correlación (R^2) de 0,999 y un límite de detección (LOD) de 0,776 μM .

Se observó una distancia de 0,9 V entre el pico de oxidación del ácido ascórbico y el TD en el estudio de interferencia.

Palabras clave: CNT, sensor electroquímico, tramadol, electrodo lápiz grafito

Abstract: Tramadol (TD) is a pseudo-opioid drug that has a drowsiness effect. In this research, pencil graphite electrode (PGE) was modified by carbon nano tube (CNT) used for the detection of TD in blood serum. Differential pulse voltammetry (DPV) method was used for detection and determination. The influence of the electrochemical variables was investigated for type of electrode and pH of solution. The sensor was designed to measure TD in the blood serum. Then the effect of ascorbic acid interference on the measurement of TD was investigated with this sensor.

Under optimized conditions, a linear relation was observed for the anodic peak over the square root of scan rate. A linear response over the concentration range of 5- 25 M was observed with a correlation coefficient (R^2) of 0.999 and detection limit (LOD) of 0.776 μM .

Distance of 0.9V between was observed between oxidation peak of ascorbic acid and TD in interference study.

Keywords: CNT, electrochemical sensor, tramadol, pencil graphite electrode

1. INTRODUCTION

Tramadol is a drug used to relieve a lot of pain. TD is known to be an stimuli drug, and its administration without prescription gives rise to irreparable lesions in drug users. Hence, the sensors to detect this drug in biological samples even at very low levels are very significant.

In recent years, the need for methods with high sensitivity and selectivity for the determination of biological compounds is increasing. So far, various methods such as UV-Visible spectrometry, atomic absorption spectroscopy, high performance chromatography (HPLC), gas chromatography (GC), mass spectrometer (GC mass), and capillary electrophoresis have been used for this purpose.

But these methods require pre-condensation and expensive detectors, along with spoilage of the sample. Therefore, the use of a method such as an electrochemical analysis that is fast and relatively inexpensive and a non-destructive method would be cost effective and useful. [1-5].

Today, extensive applied research using electrochemical techniques has been used to analyze and identify various groups of substances in various environments, including pharmaceutical samples, biological contaminants and biological systems, from different stages of production to market. Because of the high voltage surplus of biological compounds, direct measurement of them at ordinary electrodes is difficult with electrochemical methods. This leads to a decrease in sensitivity and selectivity in the electrochemical analysis of these compounds. Accordingly, an attempt to improve the properties of the surface of the electrodes results in the use of electrodes modified by heterogeneous modulators, at the electrode surface or homogeneous modulators in solution [6].

In a study, a modified carbon paste electrode with titanium and zinc oxide nanopowders was used to determine TD in biological samples [7]. The production of commercial paste electrode for measuring TD in physiological samples without pre-treatment of the sample has also been reported. In this research, differential pulse voltametric method was used to identify TD. For this purpose, carbon nanotubes and molecularly imprinted polymer have been used to improve the conductive properties and increase selective properties, respectively [8]. In another research, magnetic nanoparticles coated with molecularly imprinted polymer were used to correct the carbon paste electrode. The sensor's ability to detect and determination of TD in human urine specimens and drug products (limit of detection) was obtained $0.004 \mu\text{M}$ [9].

One of the main purposes of this research is to invent a new, quick and inexpensive method for identifying TD. For this purpose, modified graphite electrode, which is inexpensive, available and has the ability to be repeatable, and glassy carbon electrode (GCE), comparatively used to identify TD. Then, the detection and performance limits of this electrode were investigated in blood samples by differential pulse method. Then, the limit of detection and performance of this electrode were investigated in blood serum by differential pulse voltammetry method. In completing these studies, TD interactions with ascorbic acid was investigated.

2. EXPERIMENTAL

All reagents were of analytical grade: TD and methanol were obtained from Sigma Aldrich

USA. The GF of 10 nm in diameter and purity $\geq 95\%$ was purchased from Sigma (St. Louis, MO, USA). A Britton-Robinson Buffer (BRB) solution of 0.5 M. (pH 11) ($0.04 \text{ M H}_3\text{PO}_4$, $0.04 \text{ M H}_3\text{BO}_3$, and $0.04 \text{ M CH}_3\text{COOH}$) was prepared prior to use. All the solutions were prepared with double distilled water (DDW). The pencil graphite (Rotring Co, Germany, and Type H) was available as pencil lead with diameter of 2.0 mm and used as-received. Methanol was used as solvent for TD solution preparation. To prepare blood sample 0.6 ml of blood sample was added to 3.6 ml of Britton-Robinson Buffer (0.04 M , $\text{pH}=11$) and KCl (0.2 M) under continues stirring.

An Autolab PGSTAT 30 with the GPES 4.9 software package (Eco Chemie, The Netherlands) was used for electrochemical experiments. Three-electrode cell consists of a PGE (with a diameter of 2.0 mm. Azarelectrode) and glassy carbon electrode as working electrode, a platinum wire as auxiliary electrode, and a saturated calomel electrode (SCE) as reference electrode were used for electrochemical measurements.

Electrochemical characterization was conducted using DPV at scan rate of 50 mV s^{-1} in the range of 0.1-1 V performed in 0.04 M Britton-Robinson buffer solution ($\text{pH } 11.0$) containing 2 M KCl in the presence or absence of analyte. PGE electrode was polished to soft polish after completing each test and before starting another test. In surface modification of the PGE electrode, certain values of CNT was poured in dimethylformamide and then placed in an ultrasonic bath to be well dispersed. Then, three μL of it, were dumped onto the electrode with the sampler and used after evaporation of dimethylformamide. Before recording voltamogram, solution was stirred for several seconds in order to homogenization.

3. RESULT AND DISCUSSION

3.1. Electrode investigation

Considering the complexity of the preparation of the GCE electrode and its economical feasibility, in the first step of the experiments, a comparison was made between the behavior of the GCE and the PGE. For this purpose, the surfaces of both electrodes were modified by pyrrole, carbon nanotube and graphen. The Modified electrodes were used to measure tramadol by differential pulse voltammetry method. The results of this study are presented in figures 1 and 2. The results show that the electrodes modification with nanotubes was better than graphene and better than pyrrole. Also, the comparison of the obtained voltammograms for the GCE and PGE shows that the results obtained from these two electrodes are very close to each other. Therefore, it is possible to use the PGE in

continues, considering the convenience of preparation and cost-effectiveness.

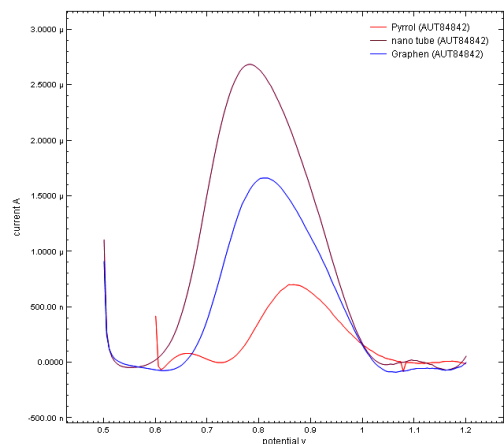


Fig.1. DPV responses of 0.04 M Britton–Robinson (pH 11) solution using GCE modified by CNT, graphene and pyrrole in the presence of 40 μ M tramadol.

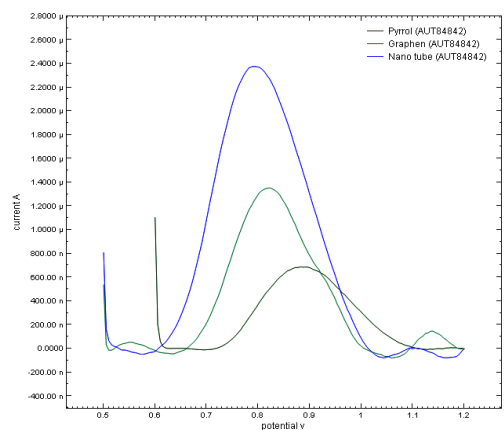


Fig.2. DPV responses of 0.04 M Britton–Robinson (pH 11) solution using PGE modified by CNT, graphene and pyrrole in the presence of 40 μ M tramadol.

3.2. Effect of pH on electrochemical behavior of TD

One of the factors influencing the voltammetric properties of TD is the pH of the solution being measured. The effect of pH on the electrochemical behavior of TD on modified PGE in 0.04 M Britton–Robinson buffer solution was investigated by running DPV scan in the solution pHs of 3 to 12. According to Fig. 4, with the reduction of pH, the oxidation potential of TD is transferred to positive potentials. In this figure, the values of the oxidation peak potential of TD are shown. It is found that the peak intensity in alkaline medium is better than acidic medium, and in the pH of 9 the best intensity was observed for oxidation peak of TD. This is used as optimum pH for subsequent

experiments. According the proposed mechanism for oxidation of TD (Fig. 3) and the production of positive-charge particles, it is clear that reaction products in alkaline solution will be more stable. In other words, the positive charged TD react with hydroxyl ions and the oxidation reaction is facilitated and catalyzed due to continuous consumption of the products.

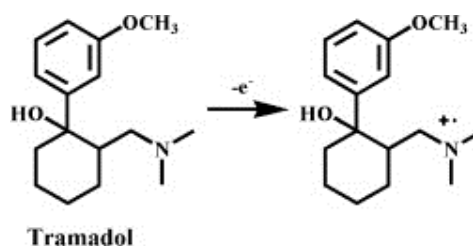


Fig. 3. Schematic illustration of tramadol oxidation

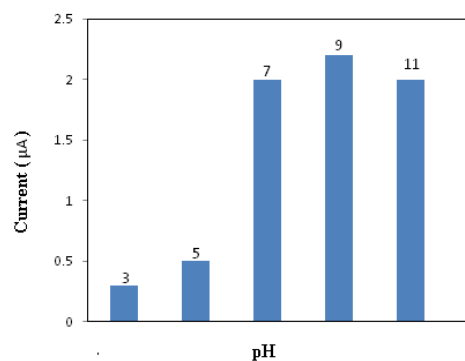


Fig.4. Effect of solution pH on the current peak of 40 μ M of TD in 0.1 M Britton–Robinson solution containing 0.1 M KCl

3.3. Calibration curve and detection limit

In order to plot the calibration curve of TD solution, modified PGE in optimized condition was placed in the cell containing 2 ml of Britton–Robinson solution (0.1M) with pH of 9. Then, certain concentrations of TD were added to the cell and the current changes were recorded. Figure 5 shows the voltammogram of TD solutions with different concentrations in a linear range at concentrations between 2 and 25 μ M. It is observed that the intensity of the oxidation peak of TD is increased by increasing the concentration of this material.

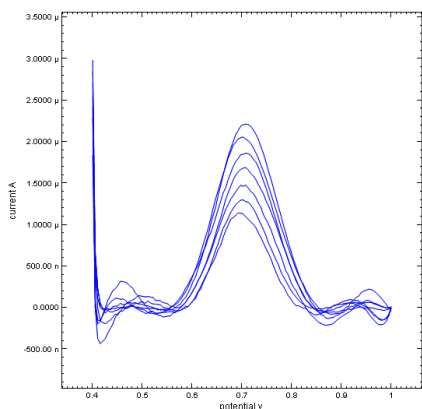


Fig.5. DPV of TD in 0.1 M Britton–Robinson solution containing 0.1 M KCl at different concentrations.

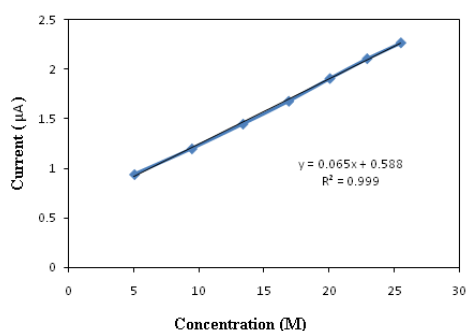


Fig.6. Calibration curve of TD at different concentrations

A calibration curve was plotted using the data obtained from the current-concentration curve. In order to verify the accuracy of the calibration voltammogram and to find the interpolation of points anodic peak position changes in different concentration of TD are presented in Fig. 6. Linear regression (0.999) obtained from calibration curve indicates the correlation between the data obtained from the calibration graph of TD.

As observed in Fig. 6, a linear response observed for TD over a concentrations range of 5-25 M with a correlation coefficient (R^2) of 0.999. The detection limit of TD in the solution was calculated using the data of calibration curve and standard deviation equation as our previous work [مقاله سیفی][10]. The detection limit (LOD) of 0.776 μM was calculated for TD using this method.

3.4. Analysis in blood serum

In view of the capabilities for determination of TD, the modified electrode was employed for realistic sample containing this compound. The blood sample was diluted by a factor of ten by adding a two-to-one ratio of the Briton-Robinson 0.1M buffer with $\text{pH} = 9$ and KCl, 0.1M to reduce the interference caused by various complex compounds in blood.

Typical DPVs for determination of TD in blood serum are shown in Fig. 7 B. Linear equation of regression, $I_p \text{ (nA)} = 0.075 \text{ CTD } (\mu\text{M}) + 0.493$, $R^2 = 0.999$, in Fig. 7B (inset) was used for determination of TD in a blood serum sample.

The recovery for the spiked samples ranged between 99.64% and 103.40%, indicating that the detection procedures were free from interference by the blood serum sample matrix.

The results obtained for detection limit and linearity range of TD detection are comparable with similar studies by Afkhani et al. which was used a modified carbon nanotube nanoparticle-modified carbon paste electrode and a detection limit of 0.004 micromoles [9].

The results obtained with respect to the application of graphite electrode and carbon nanotubes are comparable to that of the Afshar et al., which was used modified glassy carbon electrode with polymer molecules and multi-wall carbon nano tube. In this way, they report a detection limit of about 0.5 μM for TD [7].

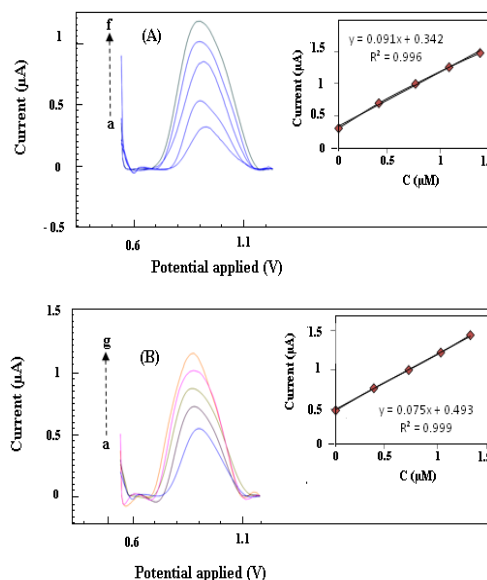


Fig.7. (A) DPVs of different concentrations of TD at the PPGE: (a) missing sample, (b) 3.64 μM , (c) 6.96 μM , (d) 10 μM and (e) 12.8 μM . Inset: related calibration graph. (B) DPVs of TD with concentrations (a) missing sample, (b) 3.53 μM , (c) 6.76 μM , (d) 9.73 μM and (e) 12.47 μM added to a blood sample. Inset: related calibration graph.

3.5. Interference with ascorbic acid

A DPV method was conducted to investigate the interference of ascorbic acid with TD in the sample containing 5 μM of TD and 15 mmol/L ascorbic acid and plotted the corresponding voltammogram in figure 8. The result demonstrated that the ascorbic acid peaks is at -0.1V and the TD peak is at 0.8V with spaced at about 0.9 volts. Therefore no

interference effect was observed for ascorbic acid to TD.

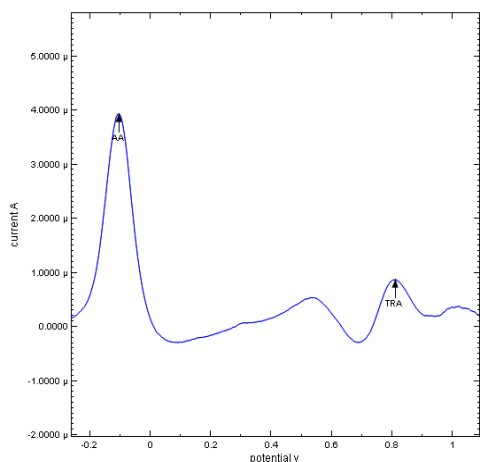


Fig.8. DPV of 5µM TD and 15 mmol/L ascorbic acid in 0.1 M Britton–Robinson solution containing 0.1 M KCl

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