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A sensitive electrochemical sensor for rapid determination of methadone in biological fluids using carbon paste electrode modified with gold nanofilm

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ABSTRACT

A novel and effective electrochemical sensor for the determination of methadone (MET) at pH 9.0 using gold nanoparticles, electrodeposited on a multi-walled carbon nanotube modified carbon paste electrode (GNPs/MWCPE), is introduced. The voltammetric behavior of MET at this modified electrode was studied using cyclic and square wave voltammetric techniques and the results were compared with those obtained at the multi-walled carbon nanotube modified carbon paste electrode (MWCPE). The oxidation of MET was irreversible and exhibited an adsorption controlled process at the GNPs/MWCPE and a diffusion controlled process at the MWCPE. The effect of various experimental parameters including pH, scan rate, and accumulation potential and time on the voltammetric response of MET was investigated. At the optimum conditions, the concentration of MET was determined using square wave voltammetry (SWV) in a linear range of 0.1–500.0 μmol L^{-1} with a correlation coefficient of 0.9901 at the GNPs/MWCPE, and 0.5–300.0 μmol L^{-1} with a correlation coefficient of 0.993 at the MWCPE and the detection limits were found to be 0.005 and 0.3 μmol L^{-1}, respectively. The proposed electrode was successfully applied to the determination of MET in a pharmaceutical dosage form, urine and saliva samples. The effects of common interferences, namely some of different cations and anions, on the current response of MET were investigated. This revealed that the GNPs/MWCPE shows excellent analytical performance for the determination of MET in terms of a very low detection limit, high sensitivity, very good repeatability and reproducibility.

1. Introduction

Methadone (6-dimethylamino-4,4-diphenyl-3-heptanone or Dolophine, MET, Scheme S1), which has been tested as an active agent in the treatment of patients addicted to heroin or morphine, is a powerful narcotic analgesic resembling morphine in its acting and use [1].

Because MET treatment replaces a short-acting opioid (heroin) with along acting opioid (methadone), it has been controversial since its inception particularly with regard to adequate dose levels [2,3]. It has also been observed that heroin addicts undergoing maintenance treatment with MET have significantly lower mortality than those who do not [4]. Besides, like other opioid medications, MET has the potential for abuse [5]. According to the Public Health Advisory of the United State Food and Drug Administration (USFDA) on November 27, 2006 the prescribing MET is complex. MET should only be prescribed for patients with moderate to severe pain, when their pain is not improved with other non-narcotic pain relievers. It may build up in the body to a toxic level if it is taken too often, if the amount taken is too high, or if it is taken with certain other medicines or supplements [6]. Therefore, many efforts have been made to develop a rapid and reliable sensor to determine the MET content of biological matrices for clinical and forensic purposes in the prevention of drug abuse and inadvertent overdose [1,6].

There are a variety of methods for the determination of MET, although few of them can be readily adapted to routine analysis, such as capillary electrophoresis [7], flow-injection analysis [8], liquid chromatography [9,10], HPLC [11–14], atomic absorption and atomic emission spectrometry [15] and gas chromatography [16]. Although these methods are sensitive and highly reliable, they are often time-consuming and require expensive apparatus and reagents.

Since the electrochemical procedures are simple and powerful, the cost is low and the miniaturization is possible, electroanalytical methods have proved to be useful for the development of very sensitive and selective methods for the determination of organic molecules, including drugs in dosage forms and in biological fluids [17,18].

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Additional application of electrochemistry includes the determination of electrode mechanisms. Carbon paste electrode (CPE), which is made up of carbon particles and an organic liquid, is widely applied in the electroanalytical community due to its low cost, ease of fabrication, high sensitivity for detection and renewable surface [19–25]. Lately, to improve the sensitivity, selectivity, detection limit and other features of CPE, chemically modified carbon paste electrodes (CMCPEs) have been used [26–30]. The operation mechanism of such CMCPEs depends on the properties of the modifier materials used to import selectivity and sensitivity towards the target species [23].

Gold nanoparticles (GNPs), with large surface area, good biocompatibility, high conductivity and electrocatalysis characteristics, have been used to improve the sensitivity and the detection limit in electrochemical studies [31–36]. They are also suitable for many surface immobilization mechanisms and can act as tiny conduction centers and can facilitate the transfer of electrons. Many works had been conducted to construct the immunosensors using CPE modified with gold nanoparticles [20,25,37–39].

In this work we report construction and application of a gold nanoparticles electrodeposited multi-walled carbon nanotube modified carbon paste electrode (GNPs/MWCPE) as an electrochemical sensor for the determination of MET in different samples. The performance of this sensor was compared with that of a multi-walled carbon nanotubes modified carbon paste electrode (MWCPE). Gold nanoparticles were deposited at the MWCPE surface by cyclic voltammetry. The electrochemical behavior of MET at the modified electrodes was investigated using CV and SWV techniques. According to the results, the GNPs/MWCPE displayed a low detection limit, and a high selectivity and sensitivity to MET determination in real samples. Since the limit of detection of the proposed sensor is better than that for some reported electrochemical methods [6], and also because of high selectivity and sensitivity of the method, the developed electrode was used to the square wave voltammetric (SWV) determination of trace amounts of MET in real samples, such as the saliva, urine and pharmaceutical dosage samples. To the best of our knowledge, a few reports have been published on the determination of MET in biological fluids and pharmaceutical samples using electrochemical sensors [6,1].

The gold nanoparticles/multi-walled carbon nanotubes modifier combined the unique electronic properties of MWCNTs and attributes of gold nanoparticles such as their high surface-to-volume ratio, high catalytic efficiency, good biocompatibility and chemical stability, thus, a synergistic effect occurred in GNP-MWCNT/CPE hybrids for exerting their enhanced electrocatalytic capabilities.

2. Experimental

2.1. Materials and apparatus

All the reagents and materials were of analytical grade from Merck or Sigma-Aldrich. All the solutions were prepared using double-distilled water. Britton–Robinson (B–R) buffer solutions ($CH_3COOH+H_3BO_3+H_3PO_4$) of pH 5–11(4.0×10^{-2} mol L^{-1}) were used as the supporting electrolyte. Stock solutions of MET (1×10^{-3} mol L^{-1}) were prepared in water and kept in the dark in refrigerator. Working solutions under voltammetric investigations were prepared by dilution of the stock solution with selected supporting electrolyte to give desired solutions containing MET.

A Metrohm Model 827 pH lab (Herisau, Switzerland) pH-meter with a combined glass electrode was used for pH measurements. A PAR-305 magnetic stirrer with a Teflon-coated magnet was used to provide the convective transport during the preconcentration step. The size and morphology of the nanoparticles were characterized by a scanning electron microscope (SEM-EDX, XL30 and Philips Netherland). Voltammetric systems were conducted using a potentiostat/galvanostat (Autolab PGSTAT302 N) and it was controlled by a computer using a Nova version 1.7 software. Three-electrode cell system was used to monitor the cyclic and square-wave voltammograms. A saturated Ag/AgCl electrode, a platinum wire and a modified carbon paste electrode were used as the reference, auxiliary and working electrodes, respectively.

2.2. Pretreatment of multi-walled carbon nanotube materials and modified carbon paste electrode

A pretreatment of the CNTs is usually necessary to eliminate graphitic nanoparticles, amorphous carbon, metallic impurities, and/or to improve the electron transfer properties and/or to allow further functionalization [40]. The preparation of multi-walled carbon nanotubes modified carbon paste electrode (MWCPE) with composition of 10.0% (w/w) MWCNTs, 65.0% (w/w) graphite powder and 25.0% (w/w) paraffin oil was performed as described in our previous work [24].

After fabrication of MWCPE, the electrode tips were smoothed manually with clean paper and then at a plane glass surface to produce a flat surface.

To construct the GNPs modified MWCPE (GNPs/MWCPE 2), MWCPE was immersed into a 6 mmol L^{-1} of hydrogen-tetrachloroaurate $HAuCl_4$ solution containing 0.1 mol L^{-1} KNO_3 (prepared in doubly distilled water, and deaerated by bubbling with nitrogen). A constant potential of −0.400 V vs. Ag/AgCl was applied for 100 s. Then, the modified electrode was washed with doubly distilled water and dried carefully [41].

2.3. Analytical procedure

The analysis of MET by ASWV was carried out in a 25.0 mL aliquot (pH 9.0 B–R buffer) using the following steps after purging with nitrogen for at least 5 min: (a) pre-conditioning or cleaning step was performed, by scanning the potential from 0.500 to 1.100 V vs. Ag/AgCl for 10 cycles before each measurement, to clean the surface of the modified electrode; (b) the preconcentration step was proceeded at 0.700 V vs. Ag/AgCl for 100 s; at the end of the preconcentration time, stirring was stopped and a 10 s rest period was allowed for the solution to become quiescent; (c) the adsorptive square wave voltammograms were recorded when the potential was swept from 0.500 to 1.25 V vs. Ag/AgCl. In the pre-conditioning and preconcentration processes, the detection solutions were stirred with a magnetic stirrer. The peak current at about 1.000 V vs. Ag/AgCl for MET was measured. All the measurements were carried out at room temperature (25.0 ± 0.1 °C). Calibration graph was prepared by plotting the net anodic peak current vs. MET concentration in the solution.

2.4. Analysis of real samples

The urine sample was taken from a healthy individual immediately before the experiments. Different amounts of MET was added to the urine sample. Acetonitrile removes proteins more effectively, the addition of 1–1.5 vol of acetonitrile in urine is sufficient to remove the proteins. After vortexing for 30 s, the mixture was centrifuged for 10 min at 3000 rpm to separate urine protein residues and the supernatant was taken carefully. Appropriate volumes of this supernatant were transferred into the 50.0 mL volumetric flasks and analyzed by the standard addition method.

Saliva sample was collected and centrifuged for 3 min with a rate of 3000 rpm. After appropriate dilution, the sample was analyzed according to the analytical procedure described above.

For pharmaceutical sample, 5 tablets were powdered and homogenized. Then about 10.0 mg of the homogenized powder was accurately weighed and dissolved in water and diluted to 50 mL. Finally, appropriate volumes of this solution were transferred into the electrochemical cell containing B–R buffer solution of pH 9 (the final volume of the solution in electrochemical cell was 25 mL) and then the electrochemical signal was determined.

3. Results and discussion

3.1. Morphologies of the different electrodes

The response of an electrochemical sensor is related to its physical morphology. In order to characterize the GNPs deposited on the electrode; the SEM analysis was carried out on the surface of modified electrodes. The SEM images from the surface of MWCPE and GNPs/MWCPE are shown in Fig. S1. Significant differences in the surface structure of MWCPE and GNPs/MWCPE were observed. By comparing the surface of two electrodes by SEM images, it was clear that, as expected, a high-coverage gold nanoparticles monolayer with well-defined order is formed and the metallic nanoparticles are located at different elevations over the substrate. The above results showed the successful attachment of gold nanoparticles on carbon paste electrode surface. Moreover, a random distribution and interstices among the nanoparticles were observed in the SEM image of GNPs/MWCPE exhibiting a large surface area.

3.2. Electrochemistry of MET at the GNPs/MWCPE

The electro oxidation of MET in B–R buffer solution of pH 9.0 was studied at the surface of bare CPE, MWCPE and GNPs/MWCPE using cyclic voltammetric technique in the potential range 0.500–1.250 V vs. Ag/AgCl. The CVs for 3.0×10^{-4} mol L^{-1} MET solution were recorded (Fig. 1I). The scanning was started at 0.500 V vs. Ag/AgCl in the positive direction in pH 9.0 B–R buffer solution as the supporting electrolyte. The CV for CPE showed a week peak in the positive-going scan (Fig. 1I curve A). Electrochemical oxidation of MET showed a very weak and irreversible peak with the potential of 1.010 V vs. Ag/AgCl at the MWCPE, while a well-defined oxidation peak was obtained at the potential of about 1.000 V vs. Ag/AgCl at the GNPs/MWCPE surface (Fig. 1I curves B and C). By reversing the potential at +1.250 V no reduction signal corresponding to the anodic response was observed on the cathodic branch. In the absence of MET no anodic peak current was observed for all three electrodes. Fig. 2II shows the anodic stripping square wave voltammograms (ASSWVs) for 3.0×10^{-5} mol L^{-1} of MET at the given conditions. As can be seen, the CPE exhibited one broad peak, and the MWCPE showed one broad peak with higher oxidation current in the potential range 0.500–1.250 V vs. Ag/AgCl for the electro-oxidation of MET. There is a well-defined stripping peak related to the oxidation of MET at the surface of GNPs/MWCPE. As the voltammograms show, multi-walled carbon nanotubes increased the surface of the electrode resulted in an enhancement in the electrochemical properties of the electrode [42], and the gold nanoparticles increased the surface of the electrode and also provided a good surface for electron transfer. As a result of synergistic effect from both the above factors, the response of gold nanoparticles/multi-walled carbon nanotubes modified CPE increased and the sensor exhibited excellent electrocatalytic activity and voltammetric performance to the oxidation of MET. As Fig. 1 shows, modification of the electrode surface with GNPs caused a dramatic increase in anodic signal and therefore, a significant increase in voltammetric sensitivity. The results illustrates that the GNPs/MWCPE could present a favorable activity towards the oxidation of MET, suggesting that this electrode will be an excellent sensor for MET determination.

3.3. Optimization of the variables

3.3.1. Influences of the pH

The influence of the pH on the oxidation peak current of 3.0×10^{-5} mol L^{-1} MET was studied by cyclic voltammetry using B–R buffer. It was observed that as the pH of the medium increased gradually, the peak potential kept on shifting towards less positive values, suggesting the involvement of proton in the oxidation reaction (Fig. 2).

The E_p for the oxidation of MET at the GNPs/MWCPE showed a linear relationship with the pH of the buffer solution regarding the following equation:

$$E_p(\mathrm{V}) = 1.525 - 0.058\ \mathrm{pH} \quad (r^2 = 0.9969)$$

The linearity observed in the plot can be explained by the changes in protonation of the acid–base functions in the molecule.

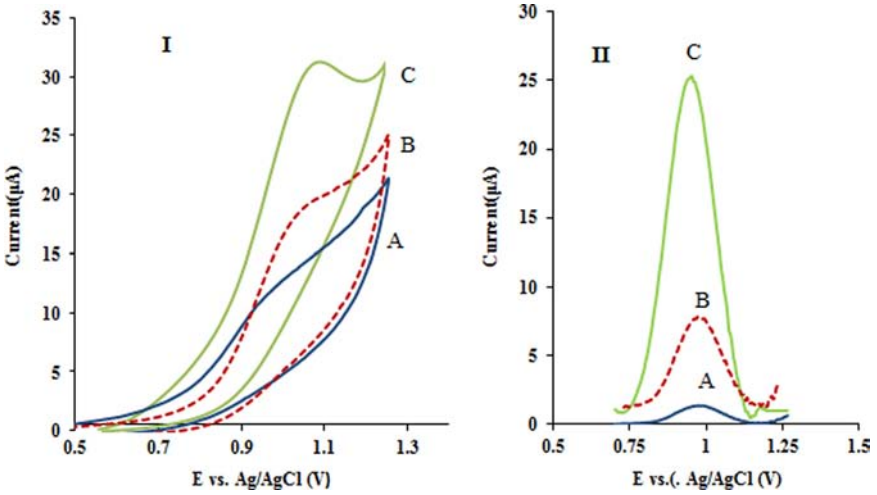

Fig. 1. (I) Cyclic voltammograms for 3.0×10^{-4} mol L^{-1} MET in B–R buffer solution of pH 9.0 obtained at (A) CPE, (B) MWCPE and (C) GNPs/MWCPE. (II) Square wave voltammograms for (A) CPE, (B) MWCPE and (C) GNPs/MWCPE in 3.0×10^{-5} mol L^{-1} of MET in pH 9.0 B–R buffer solution. Conditions: deposition potential, 0.700 V vs. Ag/AgCl; deposition time, 100 s; resting time, 10 s; SW frequency, 25 Hz; pulse amplitude, 0.100 V.

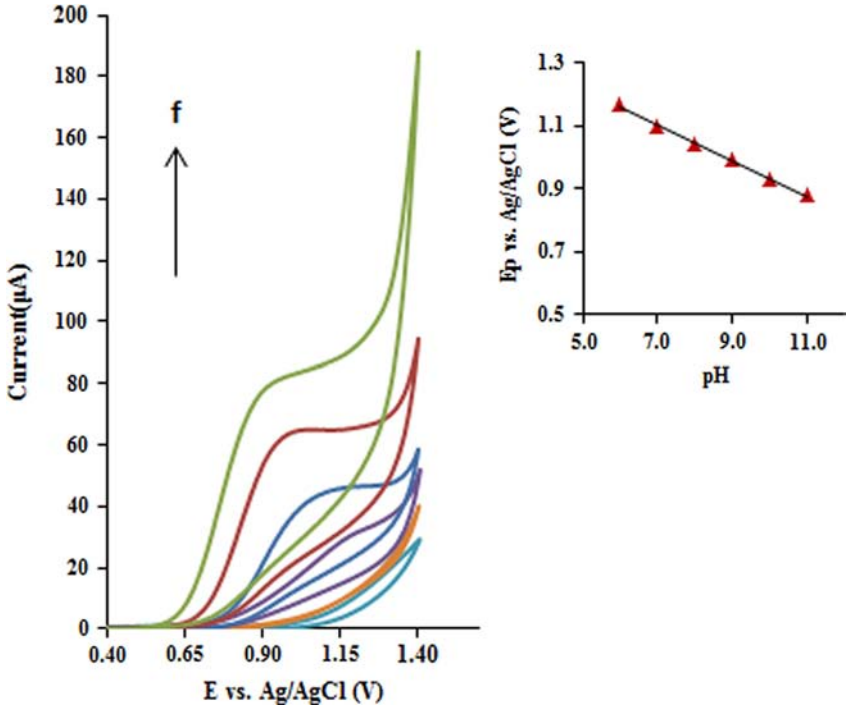

Fig. 2. Effect of pH in the range 6.0–11.0 (a–f), using B–R buffer, on the voltammetric response for 3.0×10^{-4} mol L^{-1} MET at GNPs/MWCPE. Inset: anodic peak potential as a function of pH.

Concerning the observed slopes of 58 mV/pH for MET, it can be concluded that equal numbers of electrons and protons are involved in the electrode reaction. The study of the influence of pH on SW anodic peak current was also carried out to determine the pH value for the maximum signal (Fig. S2). The height of the peak reached a maximum and the shape of the curves was better in B–R buffer of pH 9.0. This supporting electrolyte was chosen with respect to sharp response and better peak shape for the construction of calibration curve and for the determination of MET in standard solution and biological samples.

3.3.2. *Effect of potential scan rate*

The influence of potential scan rate on the oxidation reaction of MET at the MWCPE and GNPs/MWCPE was studied by cyclic voltammetry. CVs for a 5.0×10^{-4} mol L^{-1} of MET in B–R buffer solution of pH 9.0 at the gold nanoparticles and multi-walled carbon nanotubes modified carbon paste electrodes with scan rates ranging from 5 to 200 mV s^{-1} were investigated (Fig. 3a). Scan rate studies were carried out to assess whether the processes on GNPs/MWCPE were under diffusion or adsorption control. The linear relationship between the oxidation peak current (I_p) and the scan rate (ν) indicates that the electrode process was controlled predominantly by adsorption process rather than diffusion process (Fig. 3b). The regression equation, in B–R buffer solution of pH 9.0, was

$$I_p(\mu A) = 0.8755\nu(\text{mV s}^{-1}) + 18.22 \quad (r^2 = 0.998)$$

The effect of scan rate on the cyclic voltammetric response of MET at MWCPE, in B–R buffer of pH 9, was also investigated. The results showed a linear relationship between I_p and square root of scan rate ($\nu^{1/2}$). This indicated that, unlike GNPs/MWCPE, for MWCPE the electrode process was predominantly controlled by diffusion process rather than adsorption process. The equation was as follows.

$$I_p(\mu A) = 8.2308\nu^{1/2}(\text{mV}^{1/2}\ \text{s}^{-1/2}) + 52.454 \quad (r^2 = 0.9934)$$

As can be seen in Fig. 3a, the E_p was dependent on the scan rate. The plot of E_p vs. log υ was linear (Fig. 3c); having a correlation coefficient of 0.994 and the relation between peak potential and log υ can be communicated by the equation

$$E_p(\text{V}) = 0.0825 \log\ \nu(\text{V s}^{-1}) + 1.1096$$

For an adsorption-controlled and irreversible electrode process, according to Laviron [43], E_p is defined by the following equation:

$$E_p = E^{\circ\prime} + (2.303RT/\alpha nF)\log(RTk_o/\alpha nF) + (2.303RT/\alpha nF)\log\ \upsilon$$

where α is the transfer coefficient, k_0 is the standard heterogeneous rate constant of the reaction, n is the number of electrons transferred, υ is the scan rate and $E^{o\prime}$ is the formal redox potential. Other symbols have their common meanings. Thus, the value of αn can be easily calculated from the slope of E_p vs. log υ. In this system, the slope was found to be 0.096, taking $T=298$ K and substituting the values of R and F, αn was calculated to be 0.72. The value of α can be calculated as [43,44]

$$E_{p/2} - E_p = 1.857(RT/\alpha F)$$

where $E_{p/2}$ is the potential when the current is at the half peak value. From this, we obtained the value of α to be 0.3628 for the GNPs/MWCPE. Therefore, the number of electrons (n) transferred in the electro-oxidation of MET was calculated to be 2. Also this investigation was done at the surface of MWCPE and based on the results a value of electron coefficient of 0.29 was obtained and the number of electrons transferred was also found to be 2.

The CVs for the oxidation reaction of MET at both the electrodes did not show cathodic counterpart of the observed anodic peak, at any of the investigated pHs and scan rates, in the investigated potential range. The results confirmed that MET undergoes a two electron and two proton irreversible oxidation process. The proposed electrooxidation mechanism is shown in Scheme S2 [6,45].

At the surface of MWCPE the diffusion coefficient was calculated according to the chronoamperometric studies. Chronoamperometry was employed to investigate the electrochemical behavior of the aqueous buffered solution of pH 9 containing

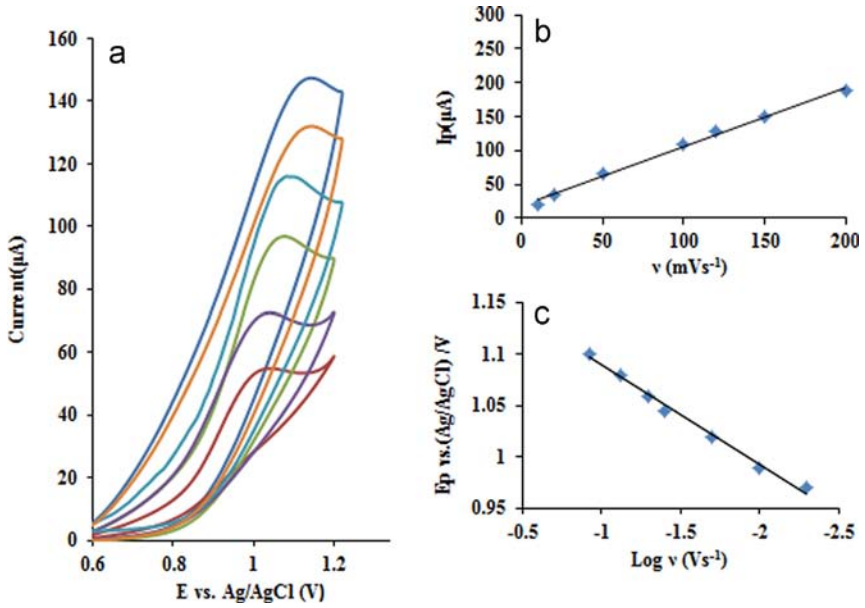

Fig. 3. (a) Dependence of the cyclic voltammetric response at GNPs/MWCPE on sweep rate in 0.1 mol L^{-1} B–R buffer of pH 9.0 containing 5.0×10^{-4} mol L^{-1} MET. Scan rate from bottom to top: 10, 20, 50, 75, 100, 120, 140, 200 mV s^{-1}. (b) Variation on the peak current with the scan rate. (c) Plot of peak current vs. logarithm of scan rate.

various concentrations of MET at the MWCPE by setting the working electrode potential at 1.000 V vs. Ag/AgCl (Fig. S3). The linearity of the current vs. $t^{-1/2}$ showed that the current is controlled by diffusion of MET from the bulk of the solution towards the surface of the electrode that causes a Cottrellian behavior. Therefore, the slope of the linear region of the Cottrell's plot can be applied to the estimation and determination of the diffusion coefficient of MET. A plot of I vs. $t^{-1/2}$ for MWCPE in the presence of MET gives a straight line, the slope of which can be used to the determination of the diffusion coefficient of MET (D) (Fig. S3, inset). The value of D was found to be 1.41×10^{-4} cm^2 s^{-1}.

3.3.3. *Effect of accumulation time and potential*

It was significant to fix the accumulation potential and accumulation time when adsorption studies were intended. Both parameters could affect the amount of adsorption of MET at the electrode. Bearing this in mind, the effect of accumulation potential and time on ASWVs signals were studied (Fig. S4). The effect of the accumulation potential on the anodic stripping peak current was examined in the potential range 0.300–0.900 V vs. Ag/AgCl under the above optimum conditions. By changing the accumulation potential from 0.300 to 0.700 V vs. Ag/AgCl, the peak current increased because the applied potential is near the oxidation potential of MET and therefore the MET molecules tend to acumulate at the electrode surface. But at the potentials higher than 0.700 V vs. Ag/AgCl the peak current decrased dramatically, because by applying the potentials that are higher or about the oxidation potential of MET, the drug molecules are oxidized as they reach the electrode surface and therefore their accumulation at the surface does do not take place. Therefore, 0.700 V vs. Ag/AgCl was selected as the accumulation potential in the procedure. The dependence of the maximum stripping peak current on the accumulation time was also examined (Fig.S5). Under the other optimum conditions, there was a linear relationship between the stripping peak current and accumulation time in the range 20–100 s. However, with further increase in accumulation time beyond 100 s, the peak current tended to be almost stable. Therefore, the optimal accumulation time of 100 s was chosen in further investigates.

Table 1
Optimum values of the studied parameters on the MWCPE.

Parameter	Range studied	Optimum value
Accumulation potential (V)	0.3–0.9	0.5
Accumulation time (s)	20–300	100
Pulse amplitude (V)	0.01–0.18	0.09
Frequency (Hz)	10–100	25

Other optimum conditions for the SWV response were recognized by measuring the current dependence on instrumental parameters counting pulse amplitude, resting time, pulse duration, and frequency. These parameters were optimized for obtaining maximum signal-to-noise ratio. Optimum values for the studied parameters were chosen as 0.100 V, 10 s, 5 s and 25 Hz for pulse amplitude, resting time, pulse duration and frequency, respectively.

The influence of optimization parameters on the oxidation reaction of MET at the MWCPE was also studied by SWAV. These parameters were optimized for obtaining maximum signal-to-noise ratio. Optimum values for the studied parameters are given in Table 1.

3.4. *Analytical characterization and method validation*

Calibration graphs were constructed under the optimum conditions described above using GNPs/MWCPE and MWCPE. Fig. 4a and b shows the net square wave voltammograms for different concentrations of MET obtained under the optimum conditions at the GNPs/MWCPE and MWCPE, respectively. As seen in Fig. 4a, for the GNPs/MWCPE the calibration graph was linear in the MET concentration range 0.01–500 μmol $L^{-1}$with a regression line equation of I_p (μA)=0.3896C_{MET} (μmol L^{-1})+15.19, (R^2=0.9912). For the MWCPE, the calibration graph was linear in the MET concentration range 0.5–300 μmol L^{-1} with regression equation of I_p (μA)=0.184C_{MET} (μmol L^{-1})+5.0797, (R^2=0.9932). The limit of detection, defined as LOD=3S_b/m, where LOD, S_b and m are the limit of detection, standard deviation of the blank and the slope of the calibration graph, respectively, was found to be 0.005 μmol L^{-1} and 0.3 μmol L^{-1} for the GNPs/MWCPE and

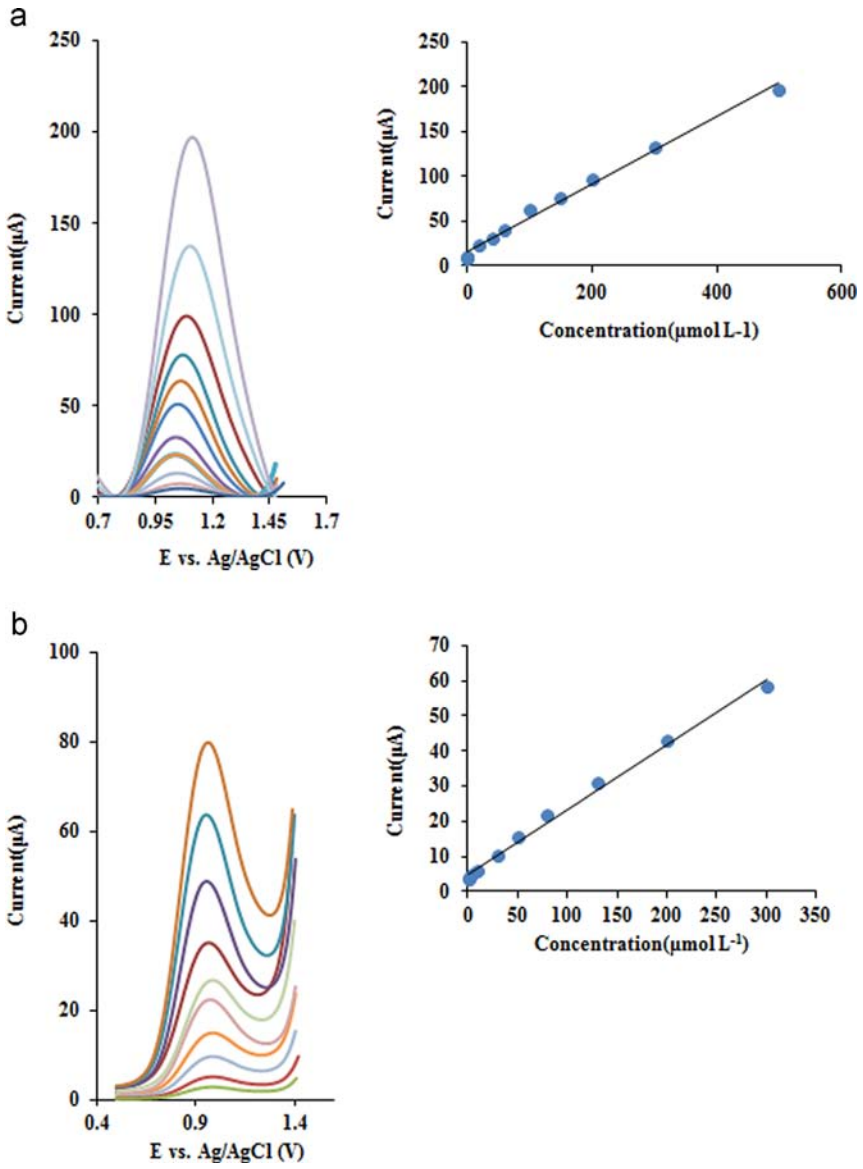

Fig. 4. Net square wave voltammograms for (a) GNPs/MWCPE (concentration from bottom to top: 0.01, 0.5, 1, 20, 40, 60, 100, 150, 200, 300, 500 μmol L^{-1} of MET) and (b) MWCPE (concentration from bottom to top: 0.5, 1, 5, 10, 30, 50, 80, 130, 200, 300 μmol L^{-1} of MET) in the solutions with different concentrations of MET in optimum conditions.

MWCPE, respectively. S_b was estimated by five replicate determinations of the blank signal.

In order to study the repeatability of the electrode preparation procedure, solutions of 30 μmol L^{-1} MET were analyzed. The repeatability of the electrodes on the determination of MET was evaluated by performing five determinations with the same standard solutions of MET using the same electrodes. The relative standard deviation (RSD) for the response was 2.39% and 2.55% for GNPs/MWCPE and MWCPE, respectively. The reproducibility of the response of the electrodes was also studied. Five electrodes were prepared from the same batch and were evaluated by performing the determination of 30 μmol $L^{-1}$of MET solution. The RSD for the responses between electrodes was 2.98% and 3.22% for the GNPs/MWCPE and MWCPE, respectively. The results showed that the repeatability and reproducibility of the sensors for the determination of MET are acceptable. The storage stability of the designed sensors was also investigated. For detection of 30 μmol L^{-1} MET, there was no significant decrease in current responses in 8 weeks. Therefore, the GNPs/MWCPE and MWCPE sensors were stable for 8 weeks. After that the response of the electrodes decreased and the noise in the responses increased. The high reproducibility and stability indicated that the modified electrodes were suitable for the analysis of real samples.

3.5. Interferences

In order to evaluate the selectivity of the GNPs/MWCPE and MWCPE sensors for the determination of MET, the influence of some common species on the determination of MET under the optimum conditions were investigated. The tolerance limit for the interfering species was considered as the maximum concentration that gave a relative error less than $\pm$5.0% at a concentration of 30.0 μmol $L^{-1}$of MET. The influence of morphine and the main metabolite of methadone, 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), was investigated. The results showed that they have no interfering effect on the determination of MET. Ascorbic acid, citric acid, uric acid, oxalic acid, glucose, acetaminophene, caffeine, K^+, Ca^{2+}, NO_3^-, NH_4^+ and Mg^{2+} have no effect on the I_p of MET even when presented in 150 fold excess over it. This suggested that the determination of MET in the

Table 2
Determination of MET in human urin and saliva samples by the proposed method ($N=5$).

Sample	Methadone concentration			
	Added (μmol L^{-1})	Found (μmol L^{-1})	Recovery (%)	Inter-day percision (%)
Urine	–	0.00	–	–
	1	1.03 ± 0.02	103.0 ± 1.87	2.10
	5	5.10 ± 0.05	102.0 ± 0.98	2.34
	20	19.68 ± 0.17	98.4 ± 0.85	3.29
	50	49.10 ± 0.19	98.2 ± 0.38	2.76
	150	151.00 ± 0.23	100 ± 0.01	1.68
Saliva	–	0.00	–	–
	1	0.98 ± 0.01	98 ± 1.00	1.65
	5	4.87 ± 0.11	97.4 ± 1.20	2.07
	20	20.50 ± 0.08	102.5 ± 0.39	2.85
	50	51.03 ± 0.21	102.0 ± 0.41	3.21
	150	149.01 ± 0.36	99.0 ± 0.02	2.73

pharmaceutical and biological samples at GNPs/MWCPE is not affected significantly by the common interfering species present along with the moleculesof interest.

3.6. Analytical applications

The GNPs/MWCPE sensor was successfully applied to the determination of MET in human urin, saliva and tablet samples. The tablet, saliva and urine samples were prepared as discussed in Section 2.4. MET was determined after addition to the human urine and saliva samples and the recovery values were calculated. The results are given in Table 2. According to Table 2 the recovery values were between 97.4% and 103.5% that are acceptable. MET in methadone hydrochloride tablets (inactive ingredients: lactose monohydrate, magnesium stearate, microcrystalline cellulose and silicon dioxide) was determined using both the proposed and standard spectrophotometric [46] methods and the values of 40.15 ± 0.39 mg MET $tablet^{-1}$ ($n=5$) and 39.7 ± 0.35 mg $tablet^{-1}$ ($n=3$) were obtained, respectively. For comparing the result of the suggested method with that of the spectrophotometry, the student t-test was applied. The calculated value of t (2.3) for $P=0.05$ was smaller than the critical value ($t_{4,\ 0.05}=2.78$). Therefore, it can be concluded that there is no significant difference between the results obtained by the two methods for $P=0.05$. Thus the sensor provides a good alternative for the determination of MET in real samples.

4. Conclusions

In this work, we constructed an electrochemical sensor for rapid and sensitive determination of MET using electrodeposition of gold nanoparticles at the MWCPE and comprised the performance of this sensor with the MWCPE towards the electrooxidation and determination of MET. The gold nanoparticles modified electrode showed to be efficient for the electrocatalytic oxidation of MET. Furthermore it was very stable and efficient for the immobilization of MET at the surface of the modified electrode. GNPs/MWCPE, by combining the benefits of GNPs/MWCNTs and MWCPE, exhibited excellent electrocatalytic activity and voltametric performance for the oxidation and determination of MET. According to the obtained results, the sensitivity for the determination of MET by GNPs/MWCPE was more than two times higher than that for MWCPE; and the detection limit for the determination of MET using the GNPs/MWCPE was about 50 times better than that obtained using the MWCPE. The electroxidation behavior of MET on two new modified carbon paste electrodes was studied and established. The proposed voltammetric methods can be applied to the direct analysis of pharmaceutical dosage form and biological samples without the need for separation or complex sample preparation, since there was no interference from the excipients and endogenous substances. In comparision with the other methods reported for the determination of MET, such as chromatographic methods, the proposed method is more cost effective and faster, uses simpler instruments and avoides application of organic solvents. The method provides a low detection limit and a wide dynamic range. Therefore, the proposed method can be used as an alternative to the chromatographic techniques in the therapeutic drug monitoring. In comparison with other electrochemical sensors, the proposed electrochemical sensor shows excellent analytical performance for the determination of MET in terms of small detection limit, wide linear dynamic range, high sensitivity, very good repeatability and reproducibility [1,6].

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.03.003.

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