

Development of a voltammetric procedure for assay of thebaine at a multi-walled carbon nanotubes electrode: quantification and electrochemical studies

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Abstract The study of electrochemical behavior and determination of thebaine (THEB), an opiate alkaloid, is described on a multi-walled carbon nanotube (MWCNT) modified glassy carbon electrode by adsorptive stripping voltammetry and electrochemical impedance spectroscopy. The results indicated that MWCNT electrodes remarkably enhance electrocatalytic activity toward the oxidation of THEB in a wide pH range of 2.0–10.0, and it shows two irreversible and diffusion-controlled anodic peaks. Then, a sensitive, simple, and time-saving cyclic voltammetric procedure was developed for the analysis of THEB in human urine samples. Under optimized conditions, the oxidation peak has two linear dynamic ranges of 1.0–80.0 and 100.0–600.0 μM , with detection limit of 0.23 μM and a precision of <4% (relative standard deviation for eight analysis).

Keywords Multi-walled carbon nanotube · Thebaine · Adsorptive stripping voltammetry · Electrochemical impedance spectroscopy

Introduction

With the rise in the use of illegal drugs, there is also an increased demand to identify their consumption. Therefore, forensic science laboratories must have specific and sensitive techniques to discriminate between the legal and illegal intake of opiates. Opiates were originally available from the opium poppy. The alkaloids that occur in the poppy include thebaine (THEB), morphine, noscapine, codeine, and papaverine.

Traditionally, only natural substances from opium and semisynthetic derivatives are termed opiates, whereas opioids are synthetic derivatives, e.g., methadone. However, in recent times, the term “opioid” has been understood to encompass opiates. Although they are commonly used as therapeutic agents, some of these compounds are also frequently abused as illicit drugs. These compounds may have severe side effects, including, but not limited to, drowsiness, dizziness, hypotension, unconsciousness, or mental clouding, which can lead to significant impairment [1–3]. THEB is a naturally occurring opiate that is introduced into the body, along with morphine and codeine, following the consumption of poppy seeds. Scientific literature has clearly demonstrated the distinct possibility of a morphine and/or codeine positive due to poppy seed consumption. The potential legal consequences of an opiate positive necessitates that laboratories, both drug testing and forensic, differentiate between an opiate positive due to poppy seed consumption. In a case where morphine and/or codeine has been detected, a subsequent THEB positive would indicate poppy seed consumption and could possibly rule out opiate use [4–8]. Presence of THEB in urine samples was investigated as a possible marker for poppy seed consumption [8, 9]. Therefore, a procedure that allows for the rapid and accurate determination of THEB is useful for the field of forensic toxicology.

Various analytical methods have been reported for the determination of THEB based on spectroscopy [10–12], capillary electrophoresis [13–18], enzyme-linked immunosorbent assay [19], chromatography [8, 9, 20–24], and differential pulse polarography [25]. Electrochemical techniques can be especially attractive for the determination of THEB because of their experimental simplicity and low cost. There are many papers dealing with the electrochemical behavior of opiates [3, 26–28]. While morphine is easily oxidized at relatively low potentials, most of the

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other opiates are not oxidizable except at high potentials. This is due to the fact that these opiates contain a phenol ether or ester instead of a hydroxyl group at their structures. Thus, compounds such as THEB, codeine, heroin, or papaverine are not readily detectable electrochemically. The modification of inert substrate electrodes with redox activated thin films or using ultrasensitive multi-walled carbon nanotube (MWCNT) electrodes offers significant advantages in design and development of new electrochemical sensors.

Carbon nanotubes continue to receive remarkable attention in electrochemistry. The modification of electrode substrates with MWCNTs for use in analytical sensing has been documented to result in low detection limits, high sensitivities, reduction of overpotentials, and resistance to surface fouling. MWCNTs have been introduced as electrocatalysts, and carbon nanotube-modified electrodes have been reported to give super performance in the study of a number of biological and pharmaceutical species [29–33]. Compton and Banks' group has demonstrated for most electroactive species, via the comparison of MWCNT-modified electrodes with edge plane pyrolytic graphite electrodes, that usually the electroactive sites of the MWCNTs are edge plane-like sites/defects that can occur at the ends of the nanotubes or along the nanotube where tube compartments terminate [34–36].

The objective of the current work is to develop a suitable and sensitive method for the determination of THEB, based on the unusual properties of carbon nanotubes, such as strong adsorptive ability, huge specific area, subtle electronic properties, and excellent electrocatalytic activity. The electrochemical behavior of THEB on the multi-wall carbon nanotube-coated glassy carbon electrode (GCE) was investigated by voltammetry and electrochemical impedance spectroscopy (EIS) techniques to better determine the interaction of THEB and the electrodes. The results showed that MWCNTs strongly enhanced the electron transfer rate of THEB oxidation, and the determination sensitivity of THEB was significantly improved. At the MWCNT-coated GCE, the remarkable peak current enhancement and negative shift of peak potential occurred for THEB oxidation, compared with that of a bare GCE. Consequently, an ultrasensitive adsorptive stripping voltammetric method based on the carbon nanotube-modified electrode was developed for the determination of THEB in urine samples. This newly proposed method provides some advantages such as high sensitivity, rapid response, low cost, and simplicity.

Experimental

Apparatus and reagent

Electrochemical measurements were carried out in a conventional three-electrode cell, powered by an electro-

chemical system comprising the Autolab system with PGSTAT 12 and FRA2 boards (Eco Chemie B. V., Utrecht, The Netherlands). The system was run on a PC using GPES and FRA 4.9 software. For impedance measurements, a frequency range of 100 kHz to 10 mHz was employed. The AC voltage amplitude used was 5 mV, and the equilibrium time was 20 min. The MWCNT-modified GCE, a graphite electrode, and a saturated Ag/AgCl reference electrode were employed as working, auxiliary, and reference electrodes, respectively. The MWCNTs were bought from Iran's Research Institute of Petroleum Industry and synthesized by chemical vapor deposition with a diameter of 8–15 nm, a length 50 μM , and a purity of 95%. The modified electrodes with carbon nanotube layers were characterized by scanning electron microscopy (SEM). THEB and other opiates were purchased from Temad (Tehran, Iran), and 0.002-M solutions of THEB were freshly prepared in methanol–water (1:1, v/v) solution and were used as the stock solution.

Universal buffer (boric acid, phosphoric acid, acetic acid, and sodium hydroxide, 0.1 M) solutions with different pH values were used for the study of the pH influence. All aqueous solutions were prepared with deionized water of resistivity not less than 18.0 M Ω at 25°C.

Preparation of MWCNT suspension and modified GCE

To eliminate metal oxide catalysts within the nanotubes, MWCNTs were refluxed in the 2.0 M HNO₃ for 15 h, and then washed with twice-distilled water and dried at room temperature. Of purified MWCNTs, 0.01 g was dispersed in 10 ml dimethylformamide (DMF) by using ultrasonic agitation to obtain a relative stable suspension. The GCE was carefully polished with 0.05 μm alumina slurry on a polishing cloth, and then washed ultrasonically in methanol and water, respectively. The cleaned GCE was coated by casting 300 μL of the black suspension of MWCNTs and dried in an oven at 60°C. The microscopic areas of the MWCNT-modified GCE and the bare GCE were obtained by CV using 1 mM K₃Fe(CN)₆ as a probe at different scan rates. For a reversible process, the Randles–Sevcik formula has been used:

$$I_{pa} = 2.69 \times 10^5 \left(A \text{ s mol}^{-1} \text{ V}^{-1/2} \right) n^{3/2} A C_O D_R^{1/2} \nu^{1/2}, \quad (1)$$

where I_{pa} (A) refers to the anodic peak current, n is the electron transfer number, A (cm²) is the surface area of the electrode, D_R (cm² s⁻¹) is the diffusion coefficient, C_O (mol cm⁻³) is the concentration of K₃Fe(CN)₆, and ν (V s⁻¹) is the scan rate. For 1 mM K₃Fe(CN)₆ in the 0.1 MKCl electrolyte, $n = 1$ and $D_R = 7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$; then, from the slope of the $I_{pa} - \nu^{1/2}$ relation, the microscopic areas can be calculated. On the bare GCE, the electrode surface area was found to be

0.0314 cm², and for the MWCNT-modified GCE, the microscopic area was nearly 2.9–3.0 times greater.

Analytical procedure

A 10-ml volume of 0.1 M universal buffer solution containing a suitable amount of THEB was added to the sample cell. For CV studies, the solution was stirred at about 50 rpm on the desired accumulation potential for a given period of time. Stirring was then stopped, the solution was allowed to rest for 15 s, and the electrode was scanned between 0.0 and 1.3 V. After each electrochemical measurement, the electrode was slightly rinsed with methanol and water to renew its surface.

Extraction and assay of THEB in human urine

Several organic solvents were tested for the extraction of THEB from human urine samples. Diethyl ether was found to be relatively efficient for this extraction. Figure 1 shows the influence of pH on the percentage of THEB extracted when the described procedure is followed. pH values of urine samples were adjusted by HCl and NaOH solutions. As can be seen, at pH9 or higher, the recovery results are near 100%.

For analytical measurements, the urine samples were centrifuged and diluted ten times at pH9, then, 2 ml of the sample was mixed with 8 ml of diethyl ether. The mixture was shaken and, after separation, the organic phase was evaporated to dryness at room temperature under an atmosphere of nitrogen. The residue was re-dissolved by adding 5 ml of the buffer (pH4.0) supporting electrolyte, and the cyclic voltammetric signal was recorded. The standard addition method was used to evaluate the content of THEB in the real samples.

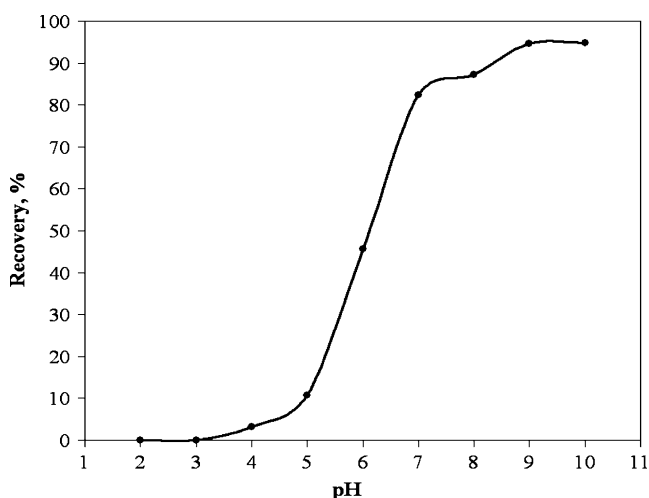


Fig. 1 Recovery of thebaine in the diethyl ether solvent as a function of pH

Results and discussion

Characterization of MWCNT-modified electrode

The dispersing state of the MWCNTs was examined by SEM. SEM micrographs showed that most of MWCNTs were well-dispersed in DMF and no longer entwined together.

EIS can also provide some information about impedance changes of the electrode surface or its electron transfer ability during the modification process. Figure 2 shows the typical Nyquist plot of the imaginary impedance (Z'') vs the real impedance (Z') of the EIS obtained at the bare GC and MWCNTs electrodes. Curves include a semicircle part and a linear part; the semicircle part at higher frequencies corresponds to the electron transfer limited process, and the linear part at the lower frequencies corresponds to the diffusion process. Because impedance quantities depend on the microscopic areas of the electrodes and the surface area of the bare GC and modified electrodes were different, the impedance elements were normalized with respect to the electrode surface area during data analysis. Here, the electron transfer resistance, R_{ct} , for the bare GC and MWCNTs, equals 46.0 and 12.6 Ωcm^2 , respectively. Therefore, the charge transfer resistance of the MWCNT-modified electrode is significantly lower than that of the GC electrode, which means it has stronger ability in the redox reaction of hexacyanoferrate.

Electrochemical behavior of THEB on GCE and MWCNT-modified electrode

The voltammetric responses of 60 μM THEB on the bare and MWCNT-modified electrode were studied over the pH interval 2.0 to 10.0 using cyclic voltammetry. According to Fig. 3, it is observed that the anodic oxidation of THEB on the bare GCE shows an ill-defined peak attributed to the oxidation of a tertiary amine group and/or methoxy groups [26–28]. However, under similar conditions, THEB yields two well-defined and pH-dependent oxidation peaks on the MWCNT-modified electrode, and the oxidation peak current significantly increases as compared with that of bare GCE. This increase in the peak current and the lowering of oxidation potential are clear evidence of the catalytic effects of the MWCNTs toward the oxidation of THEB.

Considering and comparing the electrochemical behavior of similar compounds [3, 26–28], it is possible to draw some initial conclusions about the oxidation mechanism. The first anodic wave observed at pH4.0, $E_p = +1.03$ V, appears to be the result of tertiary amine group oxidation, and the second peak with $E_p = +1.31$ V can be due to the oxidation of methoxy groups. The details of this process are discussed in the following sections.

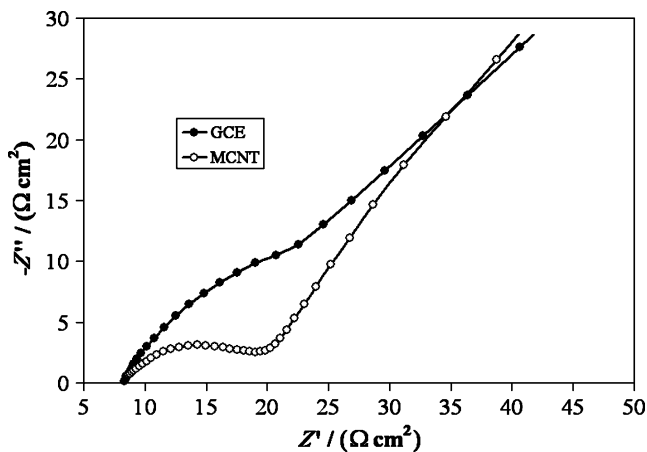


Fig. 2 The Nyquist diagrams of the impedance (Z'' vs. Z') of the (a) bare GC electrode and (b) multi-walled carbon nanotubes electrode. Other conditions; 0.1 mM $K_3[Fe(CN)_6]$ and 0.1 mM KCl solutions by applying a bias of 0.3 V and ac voltage with 5 mV amplitude in a frequency range from 0.01 Hz to 100 kHz

Effect of solution pH on the peak potentials and peak currents

The potential and current of mentioned peaks closely depend on the pH of the buffer solution. Experimental results of 60 μ M THEB in 0.1-M buffer solution at different pH values from 2.0 to 10.0 has been shown in Fig. 4. Direct oxidation of THEB presents a voltammetric signal on the entire pH range. When the pH is increased, it is observed that the first peak potential shifts to less positive values and shows one

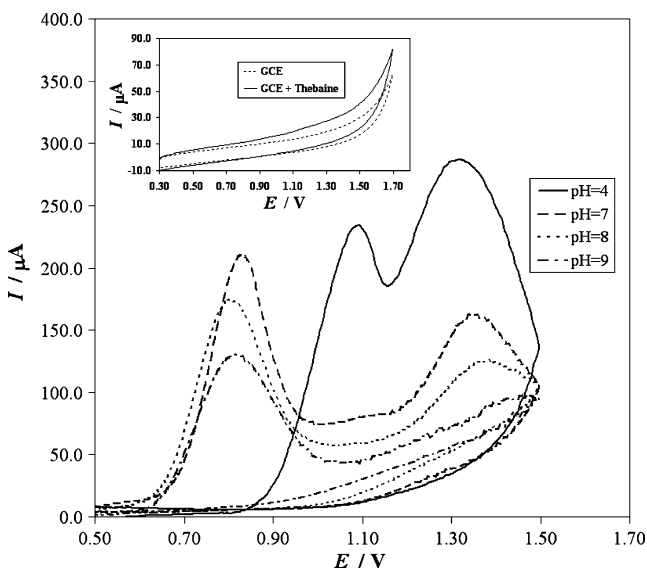


Fig. 3 Background-subtracted adsorptive stripping voltammograms of thebaine (THEB) at the multi-walled carbon nanotubes modified glassy carbon electrode (GCE) and in the various pHs. Other conditions; 60 μ M THEB, scan rate of 100 mV s^{-1} , accumulation potential of 0.0 V, and accumulation time of 6 min. *Inset* shows corresponding stripping voltammograms of THEB at the bare GCE electrode in pH4.0

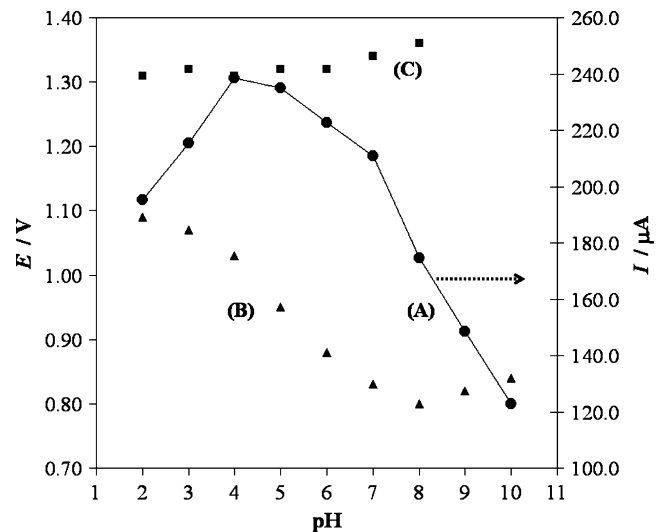


Fig. 4 Effect of pH on the peak current and potential at the modified electrode. *A* Peak current of the first reaction, *B* peak potential of the first reaction, and *C* peak potential of the second reaction. Other conditions, scan rate of 100 mV s^{-1} , 60 μ M thebaine, accumulation potential of 0.0 V, and accumulation time of 6 min

break in the slope at pH8.0. This value is near to the pK_a value of THEB, which equals 8.2 at 20°C [37]. In acidic medium, the tertiary amine group of THEB is protonated to give a cationic species. When the pH is increased, $\text{pH} > 8.0$, the deprotonation of the tertiary amine group is performed. Also, the potential of a second peak does not change significantly in the pH range of 2.0–8.0, indicating that no hydrogen ions participate in this oxidation reaction.

By treatment of MWCNTs in nitric acid, carboxylic groups can be created on the surface of nanotubes.

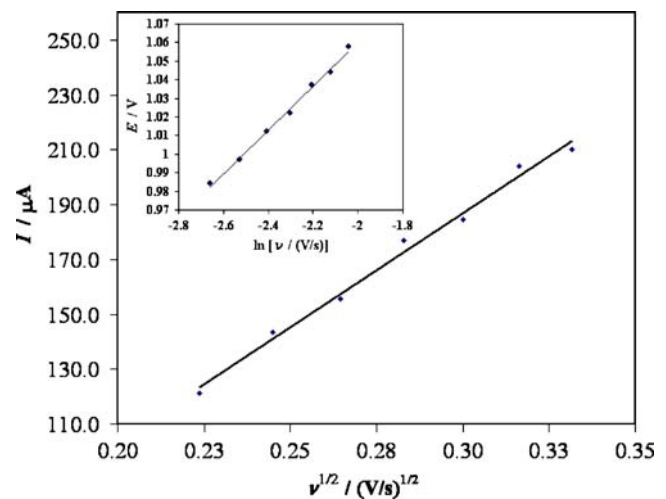
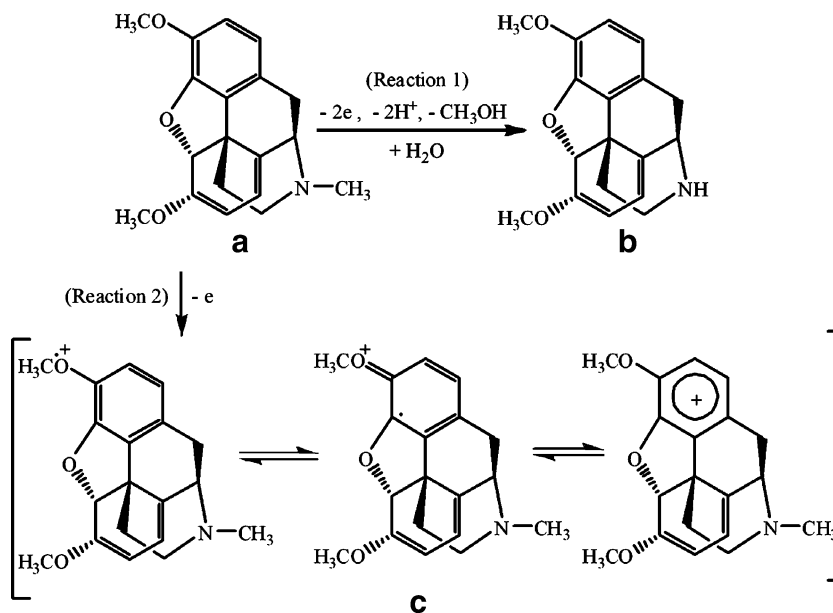


Fig. 5 Variation of the peak current with the square root of scan rate on the modified electrode. *Inset* shows a linear relationship between the peak potential and the logarithmic scan rate. Other conditions, 60 μ M thebaine, pH4, accumulation potential of 0.0 V, and accumulation time of 6 min

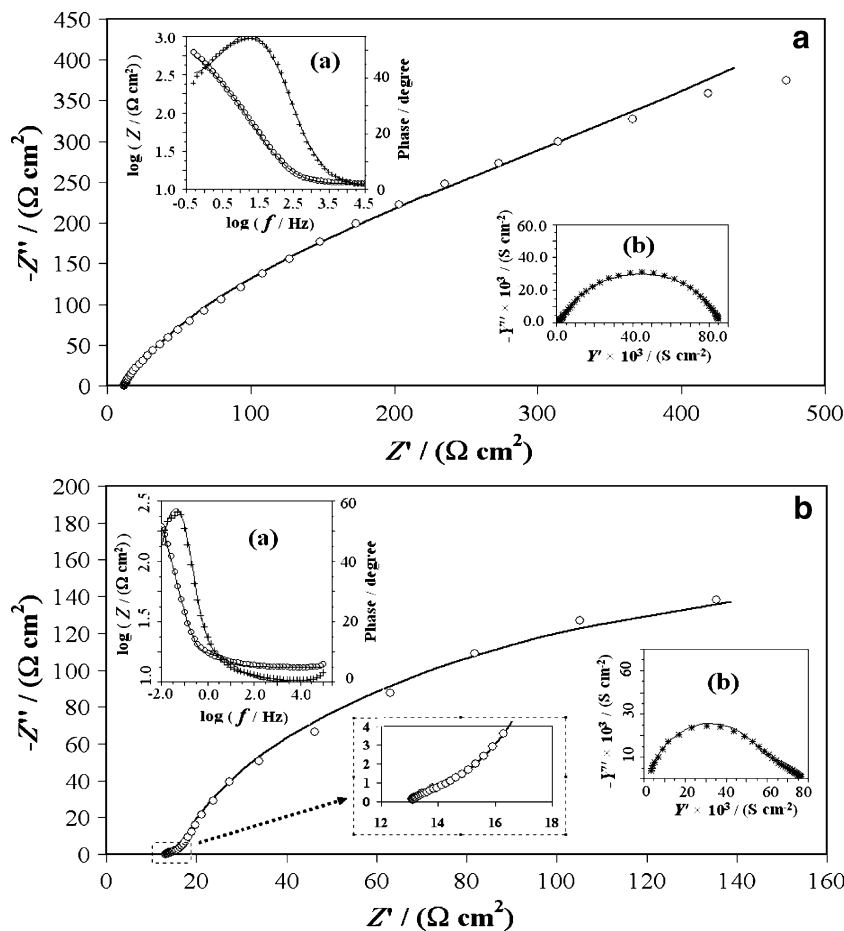
Scheme 1 Proposed oxidation mechanism of thebaine on the multi-walled carbon nanotubes modified electrode (a–c)

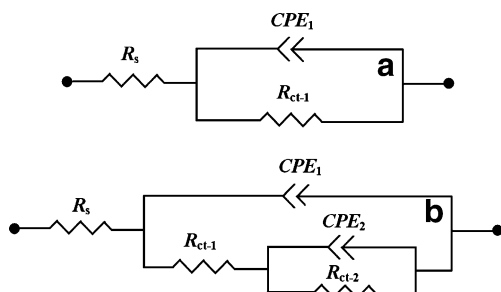


Therefore, in the highly acidic solutions, protonated THEB cannot properly be interacted with a working electrode surface, and its oxidation current amount is relatively low. In the conditions that THEB molecules have positive

charge and carboxylic groups of MWCNTs are deprotonated (pHs < 7) and obtain negative charge, there is better interaction between analyte and nanotube film. This point is observed at pH4 (see Fig. 4), and electro-oxidation current

Fig. 6 The Nyquist plots of the impedance (Z'' vs. Z') acquired for 60 μ M thebaine in pH4.0 on the GC electrode (a) and multi-walled carbon nanotubes electrode (b). Bias in both diagrams was 0.9 V with 5 mV ac voltage amplitude and frequency range of 0.01 Hz to 100 kHz. Insets show their related bode plots (a) and admittance (Y'' vs. Y') plots (b). Points show the experimental data and the full line is calculated from the optimized parameters





Scheme 2 The equivalent circuits compatible with the Nyquist diagrams represented in Fig. 6 (a, b)

is the highest. Therefore, pH4.0 was selected as the optimized value for the determination of THEB.

The calibration curve of E vs pH for the first oxidation peak, in the pH range of 3.0–7.0, has a slope of -57.7 mV pH^{-1} . This slope approximately suggests that the

$$E(V) = E'_0(V) + \frac{RT}{(1-\alpha)nF} (V) \left[0.780 + \ln \left[\left(\frac{D^{1/2}}{k_0} \right) / (s^{1/2}) \right] + \ln \left[\left(\frac{(1-\alpha)nFv}{RT} \right)^{1/2} / (s^{-1/2}) \right] \right] \quad (2)$$

Here, E'_0 is the formal potential, α is the transfer coefficient, n is the number of electrons involved in an electrode reaction, F is the Faraday constant, D_R is the diffusion coefficient, k_0 is the standard heterogeneous rate constant, R is the gas constant, T is the absolute temperature (300 K), and in the parentheses are the units of the arguments. The relationship between the peak potential and the logarithmic scan rate ($40\text{--}110 \text{ mV s}^{-1}$) is shown in the inset of Fig. 5. Based on the slope of the fitted line in this figure, with the equation $E(V) = 0.118 \times \ln[v(\text{Vs}^{-1})] + 1.296$, $R^2 = 0.9982$, $RT/2(1-\alpha)nF = 0.118 \text{ V}$, and the value of $n(1-\alpha)$ was calculated to be 0.11. For $n=2$, $\alpha=0.95$, which indicates that the activation free energy curve is not symmetrical in such an irreversible oxidation process.

From the mentioned results, the following mechanism (Scheme 1) can be presented for the oxidation of THEB at

number of electron transfer is equal to that of hydrogen ions taking part in the electrode reaction.

Effect of scan rate on the peak currents and peak potentials

The effects of scan rate on the peak current and peak potential at the modified GCE in a 0.1-M universal buffer were investigated by cyclic voltammetry in the presence of $60 \mu\text{M}$ THEB at pH4.0. According to Fig. 5, the anodic peak current linearly increases with the square root of the scan rate, and the following equation is obtained: $I(\mu\text{A}) = 830.43 \times v^{1/2}(\text{Vs}^{-1})^{1/2} - 62.15$, $R^2 = 0.9961$, indicating a diffusion-controlled oxidation process occurring at the modified GCE. The scan rate of 100 mV s^{-1} was selected as the optimum for the determination of THEB.

The linear relationship of peak potential (E) and logarithmic scan rate (v) of an irreversible process obeys the following equation [38]:

the surface of MWCNT-GCE. The first oxidative reaction process can be attributed to the contribution of two electrons and two protons for the oxidation of the tertiary amine group to generate the secondary amine, and the second peak reaction belongs to oxidation of 3-methoxy groups (Fig. 3 and Scheme 1).

From the slope of the linear dependency of the anodic peak current on the square root of the potential sweep rate and using the Randles–Sevcik equation for totally irreversible electron transfer processes [38],

$$I_p = (2.69 \times 10^5) n^{3/2} \alpha^{1/2} A D^{1/2} C^* v^{1/2}, \quad (3)$$

where α is the electron transfer coefficient, n is the number of exchanged electrons, A is the surface area of the working electrode, C^* (mol cm^{-3}) and D ($\text{cm}^2 \text{ s}^{-1}$) are the bulk

Table 1 The values of the elements in equivalent circuit and the corresponding relative errors for the oxidation of thebaine on the bare glassy carbon and multi-walled carbon nanotubes electrodes

Electrode	$C_{\text{THEB}} (\mu\text{M})$	CPE ₁		$R_{\text{ct-1}} (\Omega \text{ cm}^2)$	CPE ₂		$R_{\text{ct-2}} (\Omega \text{ cm}^2)$
		$Y_0 \times 10^4$ ($\text{S cm}^{-2} \text{ s}^n$)	n		$Y_0 \times 10^4$ ($\text{S cm}^{-2} \text{ s}^n$)	n	
GCE	60.0	0.029 (3.78%)	0.7866 (0.78%)	430.00 (4.35%)	–	–	–
MWCNT	60.0	16.01 (6.42%)	0.7476 (2.12%)	7.02 (3.25%)	28.84 (3.46%)	0.8743 (3.26%)	367.00 (4.61%)
MWCNT	100.0	15.89 (4.23%)	0.8893 (1.45%)	6.45 (3.56%)	28.75 (1.59%)	0.9124 (4.23%)	295.62 (3.87%)
MWCNT	500.0	15.67 (3.24%)	0.9143 (2.23%)	6.20 (2.76%)	28.89 (1.67%)	0.9326 (2.46%)	198.94 (2.78%)

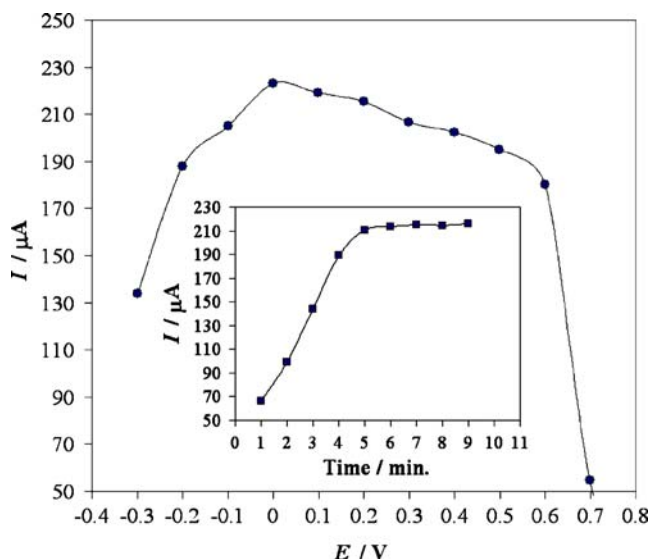


Fig. 7 Effects of accumulation potential on the oxidation peak current of 60 μM thebaine at scan rate of 100 mV s⁻¹ and pH7.5. Inset shows effect of accumulation time on the signal

concentration and diffusion coefficients of the electroreactant species, respectively, and ν is the potential sweep rate; the apparent diffusion coefficient for THEB species has been obtained $3.2 \times 10^{-2} \text{cm}^2 \text{s}^{-1}$, using $\alpha = 0.95$ and $n = 2$. By using adsorptive stripping voltammetry technique, a relatively high amount of THEB species is adsorbed on the electrode of nanotube films, having high specific area. This event, along with electrocatalytic behavior of nanotubes, leads to significantly enhancement of the oxidation current signal and the slope of Eq. 3, therefore increasing the apparent diffusion coefficient of THEB at this new condition.

EIS studies

EIS was also employed to investigate the oxidation of THEB on both GC and MWCNTs electrodes. Figure 6

shows the bode plots and the Nyquist plots of the impedance ($\Omega \text{ cm}^2$) and admittance (S cm^2), on the GC (a) and MWCNT (b) electrodes recorded at 0.9 V as dc-offset for 60 μM THEB in pH4.0.

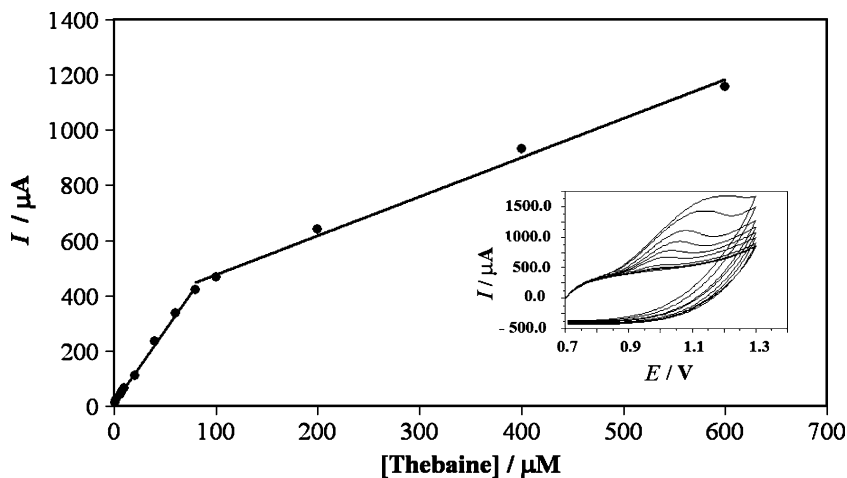
The Nyquist diagram of MWCNTs electrode and their related bode and admittance plots on this figure represent two overlapped, slightly depressed capacitive semicircles. The small and depressed semicircle has appeared at high frequencies near the origin, related to the charge transfer resistance of the first step of THEB oxidation (first peak in Fig. 3), with the combination of double-layer capacitance. The low-frequencies semicircle can be related to the second step of the oxidation process, and it has a relatively large diameter (charge transfer resistance) with respect to the high-frequencies semicircle. This result stems from the fact that the dc-offset potential supplies low over-potential for the second oxidation process. The diffusion process observed during the oxidation process using the MWCNT electrode most probably appeared at very low frequencies with a high time constant; it did not appear in the sweeping frequency range in the Nyquist plot. However, the GC electrode represents a high time constant semicircle that shows that the charge transfer resistance of the electrochemical oxidation process on this electrode is very high.

The equivalent circuits compatible with the Nyquist diagrams recorded for the GC and MWCNT electrodes are depicted in Scheme 2a and b, respectively. The model equivalent circuits contain the solution resistance (R_s), the charge transfer resistance of the first and second oxidation processes (R_{ct-1} and R_{ct-2}), and their related constant phase elements corresponding to the double-layer capacitances (CPE_1 and CPE_2). Impedance of CPE element can be expressed as [39, 40]:

$$Z_{CPE} = (Y_0 j \omega)^{-n}, \tag{4}$$

where Y_0 (the admittance parameter, $\text{S cm}^{-2} \text{s}^{-n}$) and n (dimensionless exponent) are two parameters independent

Fig. 8 Calibration curves for the determination of thebaine at the optimum conditions. Inset show the some of the raw voltammograms



of frequency; $j=(-1)^{1/2}$ and ω =angular frequency= $2\pi f$. Z_{CPE} corresponds to the constant phase angle element (CPE) impedance. $Y_0=C_{dl}$ only when $n = 1$, and n is related to α (phase angle) by $\alpha=(1-n) 90^\circ$. So, $n = 1$ and $\alpha = 0$ stand for a perfect capacitor, and lower n values directly reflect the roughness of the electrode surface. When $n = 0.5$, it is equal to a Warburg impedance. When $n = 0$, CPE is reduced to a resistor.

In the mentioned circuits, the charge transfer resistance of the electrode reaction is the only circuit element that has a simple physical meaning describing how fast the rate of charge transfer during electro-oxidation of THEB changes with the electrode potential or analyte concentration. The most widely accepted explanation for the presence of constant phase elements and the appearance of depressed semicircles in the Nyquist plots is the microscopic roughness, causing an inhomogeneous distribution in the solution resistance, as well as in the double-layer capacitance [41].

Table 1 shows the values of the equivalent circuit elements obtained by fitting the experimental results. The goodness of the fit can be judged by the estimated relative errors presented in the parentheses. According to the values of the electrical equivalent elements reported in this table, upon increasing the concentration of THEB on the MWCNTs electrode, the charge transfer resistances (R_{ct-1} and R_{ct-2}) decreased due to the facile occurrence of the faradic process related to the electro-oxidation process. Moreover, the concentration of THEB shows relatively little effect on CPE_1 and CPE_2 , related to the double-layer capacitances. The electron transfer resistance, R_{ct-1} , for the bare GC and MWCNT electrode, equals 430.0 and $7.02 \Omega cm^2$, respectively, indicating the faster charge transfer rate for oxidation of THEB on the MWCNT electrode surface, due to the electrocatalytic effect of nanotubes on the electro-oxidation process.

Effects of accumulation potential and time

The oxidation peak currents of 60 μM THEB were measured by cyclic voltammetry after 6 min of accumulation at different potential from -0.3 to $+0.7$ V in pH4.0. According to Fig. 7, it is observed that the adsorptive stripping response of THEB increases rapidly upon increasing the accumulation potential (Fig. 7), then it rapidly diminishes at

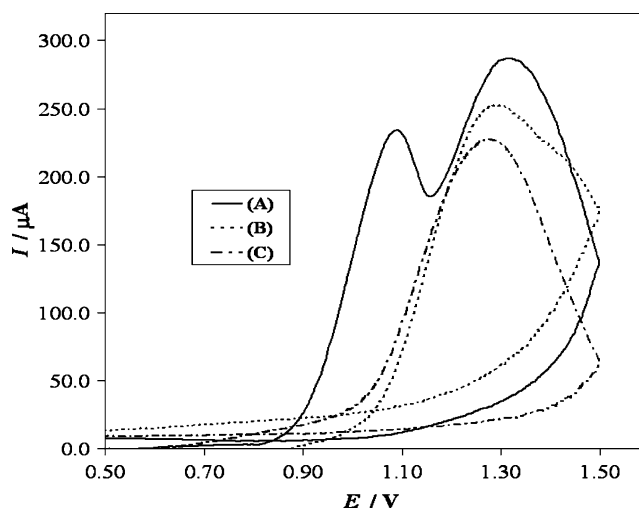


Fig. 9 Background-subtracted stripping voltammogram of 60 μM *A* thebaine, *B* oxycodone, *C* codeine on the multi-walled carbon nanotubes modified glassy carbon electrode. Other conditions; scan rate of 100 mV s^{-1} , pH4.0, accumulation potential of 0.0 V, and accumulation time of 6 min

the points that are near to the formal oxidation potential of THEB. Here, the accumulation potential of 0.0 V was selected as an optimum condition, and the following experiments were thus employed at this point.

Inset on Fig. 7 shows the influences of accumulation time on the oxidation peak current of 60 μM THEB. The oxidation peak current increases greatly at first, and then reaches a steady amount after 6 min due to adsorption saturation.

Performance of the system for THEB measurements

The calibration plot of THEB, based on the first oxidation peak, shows two linear curves in the concentration region of 1.0–80.0 and 100.0–600.0 μM with a detection limit of 0.23 μM (see Fig. 8). Using adsorptive stripping cyclic voltammetry, the optimum conditions were selected as pH4 (universal buffer) with a scan rate of 100 mV s^{-1} , and the accumulation time of 6 min at a potential of 0.0 V. Equations of linear least square calibration curves over this range are $I(\mu A) = 5.26 \times C_{THEB} + 12.21 (R^2 = 0.9974)$ and $I(\mu A) = 1.42 \times C_{THEB} + 333.29 (R^2 = 0.9964)$. Relative standard deviation (RSD) of <4% for 60 μM THEB (for eight analyses) showed excellent reproducibility. No obvious changes in the anodic peak current were found for

Table 2 Determination of thebaine in the urine samples at pH 4.0

Sample number	Added (μM)	Found ^a (μM)	Recovery (%)	RSD (%)
1	10.0	9.18 ± 1.73	91.8	4.34
2	40.0	39.37 ± 2.56	98.4	2.32
3	60.0	59.23 ± 0.74	98.7	0.47

^a Average of five replicate measurements

the same sample concentration when the modified electrode was kept under ambient conditions with the room temperature of about 30°C for 1 month or longer, and the modified electrode is stable.

The analytical characteristics of this method, such as detection limit, linear dynamic range, sensitivity, and precision for determination of THEB, are comparable or better than other sensitive methods such as capillary electrophoresis with chemiluminescence detection [14], spectroscopy [10–12], and polarography [25]. In comparison to most of the published chromatographic methods, which require lengthy and tedious pretreatment procedures, the proposed method is rapid, low cost, and very simple. However, the chromatographic and electrophoresis procedures have the advantages of simultaneous quantitation of the opiates when their conditions are properly selected.

Interference studies

Under optimized experimental conditions described above, the effects of some foreign species on the determination of THEB at 60 μM level were evaluated in detail. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than 3% for determination of THEB. Seven-hundred-fold of Na^+ , K^+ , NO_3^- , Ca^{2+} , Mg^{2+} , SO_4^{2-} , F^- , and urea, 200-fold of NH_4^+ , Cl^- , Br^- , CO_3^{2-} , glucose, sucrose, lactose, and fructose, and 80-fold of codeine, oxycodone, and papaverine solutions have almost no influence on the current response of THEB. All these indicated the peak current of THEB is not affected by all conventional cations, anions, and organic substances, but morphine and noscapine can be interfered.

Application

To evaluate the applicability of the proposed method, the recovery of THEB was determined in the urine samples by adding the standard value of THEB to them. Samples were pretreated in accordance with the section “[Extraction and assay of THEB in human urine](#),” and the standard addition method was used for the analysis of prepared samples. The data given in Table 2 show the satisfactory results for analytical determination of THEB in these real samples.

In this research, cyclic voltammetry was used to examine the determination of THEB in the presence of oxycodone and codeine. Figure 9 shows the background-subtracted cyclic voltammograms of these compounds on the modified electrode. Its observed peak potential of THEB is about 0.2 V far from that of oxycodone and codeine. This behavior provides relatively suitable conditions for analytical measurement.

Conclusion

The results discussed above demonstrate that the electrochemical response of THEB by adsorption stripping voltammetry on MWCNTs film can remarkably be enhanced in the high pH range of 2.0–10.0. Electrochemical studies for identifying the oxidation mechanism confirm the contribution of two electrons and two protons for the oxidation of the tertiary amine group to generate the secondary amine in the first reaction, and in the next step oxidation of 3-methoxy groups with exchanging one electron. Analysis by this method is comparable to other sensitive techniques, and the simplicity, sensitivity, selectivity, and short time of process are the main advantages of this procedure, making it useful for routine analysis. This modified electrode can be properly used for determination of THEB in human urine samples with satisfactory results.

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