Electrochemical Detection of Codeine in Pharmaceutical Tablets Using a Tungsten Oxide Nanoparticles and Carbon Nanotubes Modified Electrode

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An electrochemical sensor with a glassy carbon electrode (GCE) decorated with a composite of carbon nanotubes (CNTs) and tungsten oxide (WO_3) was prepared for the quantification of codeine. The crystal structure of the CNT-WO_3 composite was characterized by X-ray powder diffraction (XRD) and the morphology by scanning electron microscopy (SEM). In comparison with bare GCE, WO_3 modified GCE and CNT modified GCE, the CNT-WO_3 composite modified GCE revealed a well-defined oxidation of codeine. Besides, the current response exhibited a remarkable enhancement during the oxidation process. The current response of codeine oxidation peak is linearly related with the codeine concentration ranging from 0.005 to 20 μM with the detection limit of 0.02 μM. The improved voltametric behaviour and long-time stability of the electrochemical sensor make it successfully applied for codeine determination in the medicament and human urine.

1. INTRODUCTION

The detection of drugs in biological matrices, a field of great importance in analytical chemistry, have attracted intense interest in recent years as its widely used in drug therapy, drug intoxication and anti-drug control [1-3]. Accordingly, a wide variety of substances in the commercialized tablets are required to be detected accurately and precisely in the shortest time possible [4].

Codeine   ((5α,6α)-7,8-didehydro-4,5-epoxy-3-methoxy-17-methylmorphinan-6-ol)(structure shown in Fig. 1), generally explored as an infallible cure for pain and coughing, is a substance extracted from poppy. Morphine can be used for the preparation of codeine by methylation as well [5, 6]. Codeine, belongs to the same opium family as morphine and heroin, is used for the treatment of moderated pain. Although codeine induces less euphoria and sedation than morphine and heroin, it can also produce addiction, which makes the study of codeine a significant program in the area of toxicology [7]. The use of codeine inappropriately can result in the drug-dependent phenomenon, which is a famous problem. Therefore, it is forcible to control the concentration below 40 µg/L ranges (100 nM) when using codeine-containing drugs in some countries [8]. This drug has been detected in diverse matrixes using different methodologies such as gas chromatography [9, 10], liquid chromatography [10], chemiluminescence [11], electrophoresis [12] and electrochemistry [13-21]. Among all the methods mentioned above, some weaknesses exist in each technique (e.g., time consuming in sample pretreatment and the lack of sensitivity). Thus, to develop a rapid, sensitive, and simple (low cost) method for codeine determination is greatly demanded.
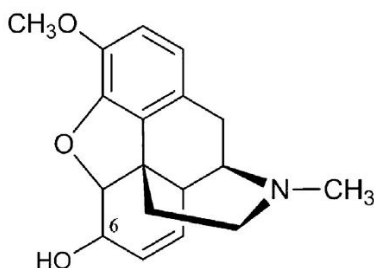

Figure 1. Chemical structure of codeine.

Comparing other detecting methods, electrochemical techniques have many advantages, such as simple, sensitive and economical in the analysis area. Moreover, it can give out some mechanism information. However, rather high potentials must be applied for the direct oxidation of the analyte, which leads to possible interferences. Electrode surface modification provides an opportunity to overcome this problem and enhance the determination performance. As shown in the experiments of Oliveira-Brett et al., electrochemical detection had been shown to be useful for codeine determination in the preparation process of drugs [22]. As reported by Shih et al., a screen-printed electrode modified with nontronite clay (NC/SPE) can be applied to the detection of codeine [23]. A novel electrochemical method with good determination performance was reported recently, of which the working electrode was an Au microelectrode and the detection technique was FFTCV [24]. The adopt of palladized aluminum electrode with prussian blue film on the surface (PB/Pd-Al) for codeine determination was studied as well [25]. Besides, using Pd-Al electrode by differential pulse voltammetry for the detection of paracetamol, ascorbic acid and codeine was also studied in their group [21, 26, 27].

Among metal oxides, tungsten trioxide WO_3 is of great importance for current technology owing to its electro-optic, ferroelectric, electrochromic and semiconducting properties [28-32]. Moreover, it has been used as a sensing material due to its good electrical properties recently. To improve the performance of metal oxide based sensors such as ameliorating their sensitivity to target molecules and reducing their response time, combining other materials with metal oxide can be an effectively method [33, 34]. Except owing some properties in the same as other types of carbon, CNTs have featured advantages including rapid electrode kinetics, enhanced electronic properties, and a large edge plane/basal plane ratio, which makes them a promising candidate to be incorporated into electrochemical sensors. Therefore, the sensitivity, the kinetics of electron transfer and the limit of detection of CNT-based sensors are generally better than that of traditional carbon electrodes [35-38].

In this work, a WO_3/MWCNT composite was prepared by impregnation and precipitation method. Then a glassy carbon electrode (GCE) modified with the WO_3/MWCNT composite was constructed for quantification of codeine in pharmaceuticals. The proposed sensor demonstrated high electrocatalytic activity, excellent selectivity and good reproducibility for codeine determination.

2. EXPERIMENTS

2.1 Materials

Codeine, ethylic acid, ascorbic acid, citric acid, glucuronic acid, urea and tungsten (VI) chloride were purchased from Sigma Chem. Co. The electrolytes such as PBS (phosphate buffer) and ABS (acetate buffer) were obtained from Shanghai Pharma. BRBS (Britton-Robinson buffer solution) was synthesized by mixing boric acid, ethylic acid and orthophosphoric acid (each at same concentration of 0.04 M) in deionized water. The desired pH value of the mixture was adjusted with sodium hydroxide (0.2 M). CCVD technique was used for the preparation of MWCNTs. Acetylene was used as the carbon source and Fe, Co on the support $CaCO_3$ was used as the catalyst. The reaction occurred in a furnace with nitrogen flow at 700 ^{o}C. The mean diameter of the as-synthesized MWCNTs was 25-60 nm and the mean length ranged from hundred nanometers to few micrometers.

2.2 Preparation of WO₃/MWCNT composite

The MWCNTs suspension was prepared by adding 20 mg MWCNTs to 40 mL of the ethanol under sonication for 45 min. The precursor solution was prepared by adding 513.14 mg precursor in 20 mL of ethanol, then the mixture was kept stirring for 30 min to get completely dissolved. Then the precursor solution was added drop by drop to the MWCNTs suspension. After that 4 mL ammonium hydroxide was put into the mixed solution. For the sake of evaporating all existing solvent, the mixture was heated to a certain temperature which is around the boiling point of ethanol. After a period of time, gray powders were obtained with the complete removal of solvent. The obtained powders were dried in the oven at 120 ^{o}C for 24 h. The sample synthesized using the impregnation method was dealt with air at 460 ^{o}C.

2.3 Characterizations

X-ray powder diffraction (XRD) patterns were performed on Rigaku Miniflex-II diffractometer using Cu Ka radiation. Morphological images were obtained using Scanning Electron Microscopy (SEM, Hitachi S-4700).

2.4 Electrochemical termination of codeine

All electrochemical determinations were conducted at a CHI 660A Electrochemical Workstation which was purchased from Shanghai Chenhua Instrument Company. A three-electrode system (modified GCE as working electrode, 3 M Ag/AgCl electrode as reference electrode and platinum wire as counter electrode) was used throughout the entire experiments. Electrochemical impedance spectroscopy (EIS) with 5 mM $[Fe(CN)_6]^{3-/4-}$ as probe and 0.1 M KCl as electrolyte was studied for characterizing the electrode resistance performance. The specific parameters are set as follows: the frequency range from 10^1 to 10^5 Hz and the amplitude 5 mV. The CV tests were also performed with the voltage ranging from 0 to 1.0 V. The effect of pH condition was investigated by CV method.
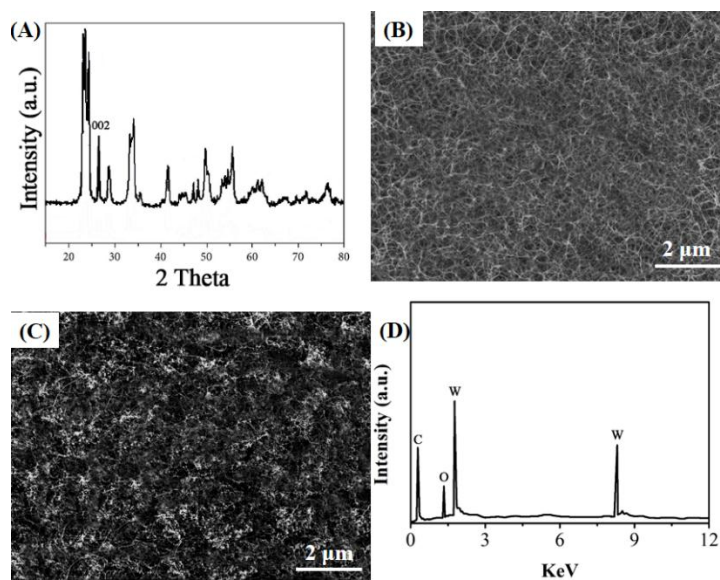
3. RESULTS AND DISCUSSION

Figure 2. (A) XRD of WO_3/MWCNT composite. SEM images of (B) MWCNT and (C) WO_3/MWCNT composite on electrode surface. (D) EDX of WO_3/MWCNT composite.

The crystal structure of the WO_3/MWCNT composite was characterized by XRD and the result was shown in Fig. 1A. Both the diffraction peaks related to WO_3 and MWCNT phase can be observed. The diffraction peaks of WO_3 indicate that the crystallization was in a single phase, assigned to

monoclinic WO_3 (JCPDS 72-0677). Besides, the main peak at $2\theta = 26.02°$ and some weak peaks around corresponded to (002) plane of graphitized CNTs.

The morphology of WO_3/MWCNT composite was measured by SEM. Fig. 1B indicated that a layer of CNTs was dispersed homogeneously on the electrode surface with no aggregation. Fig. 1C showed that WO_3 nanoparticles were distributed on the surface of CNTs. W, O and C were the major elements as revealed by EDX results (Fig. 1D), which indicated the successful formation of WO_3/MWCNT composite.

Electrochemical impedance spectroscopy (EIS) was explored to study the influence of surface modification on the electrochemical performance of GCE. Fig. 3 shows all testing EIS curves of bare GCE, MWCNT/GCE and WO_3/MWCNT/GCE with the solution of 5 mM $[Fe(CN)_6]^{3-/4-}$. The EIS curves are composed of two parts: a semicircular as an indication of the electron transfer limited process status of the working electrode and a straight line as an indication of the diffusion process status of the electrode. The electron transfer resistance value can be calculated by the diameter of the semicircular. Fig. 3 demonstrated that the bare GCE showed a relatively bigger semicircular than the other two electrodes. After modified with MWCNT, the diameter of semicircular obtained on MWCNT/GCE was decreased in comparison with that obtained on bare GCE, suggesting that the charge transfer was greatly enhanced by the immobilization of MWCNT on GCE. The results here were resulted from the excellent conductivity of the MWCNT surface. A similar semicircular was observed on the WO_3/MWCNT/GCE electrode, demonstrating that a low-electron-transfer resistance to the redox-probe can be achieved by modification of the GCE electrode.
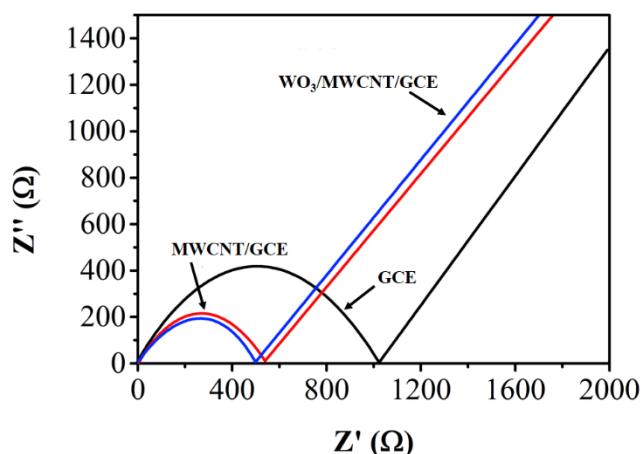

Figure 3. Nyquist diagrams of bare GCE, MWCNT/GCE and WO_3/MWCNT/GCE in 5 mM $K_4[Fe(CN)_6]$ + 0.1 M KCl.

The select of the electrolyte is of great importance in the electrochemical analyst. The electrolyte will affect the condition of the electrode-solution interface, which indirectly affect the thermodynamics and kinetics of the charge transfer [39]. Therefore, the influence of various electrolytes (e.g., PBS, ABS and BRBS) on COD oxidation using BDDF electrode was investigated. Although the results of cyclic voltammograms revealed that PBS, ABS and BRBS provided a similar

current response, the current peak obtained by using BRBS was best-defined. Therefore, BRBS was proven to be the most fittable electrolyte and selected to be used for all the following measurements.

Cyclic voltammograms of the WO_3/MWCNT/GCE electrode with or without 5 μM codeine were recorded and the results were shown in Fig. 4A. In the condition without codeine, there was no redox peak even if the potential ranging from 0.6 to 1.3 V, showing that WO_3 and MWCNT weren't electroactive in the tested potential range. When 5 μM codeine was added in blank solution, a anodic current peak with 72.4 μA appeared. Fig. 4B compared the electronic performance of bare GCE, MWCNT/GCE and WO_3/MWCNT/GCE electrodes at the codeine concentration of 5 μM. The very small oxidation of codeine observed at the bare GCE electrode suggested no electroactivity of codeine at bare GCE. In case that the GCE surface was modified with MWCNT, an anodic current emerged with the peak current being 16.6 μA. Surprisingly, an observably anodic peak appeared with large current of 72.4 μA by using the WO_3/MWCNT/GCE electrode. We might speculate that the synergistic effect of WO_3 and MWCNT greatly increased the current. In addition, the observed large background current when using WO_3/MWCNT/GCE electrode revealed the synthesized composite with large surface area [33]. As a whole, the synergetic effect of WO_3 and MWCNT leads to a remarkable enhancement of the current of codeine.
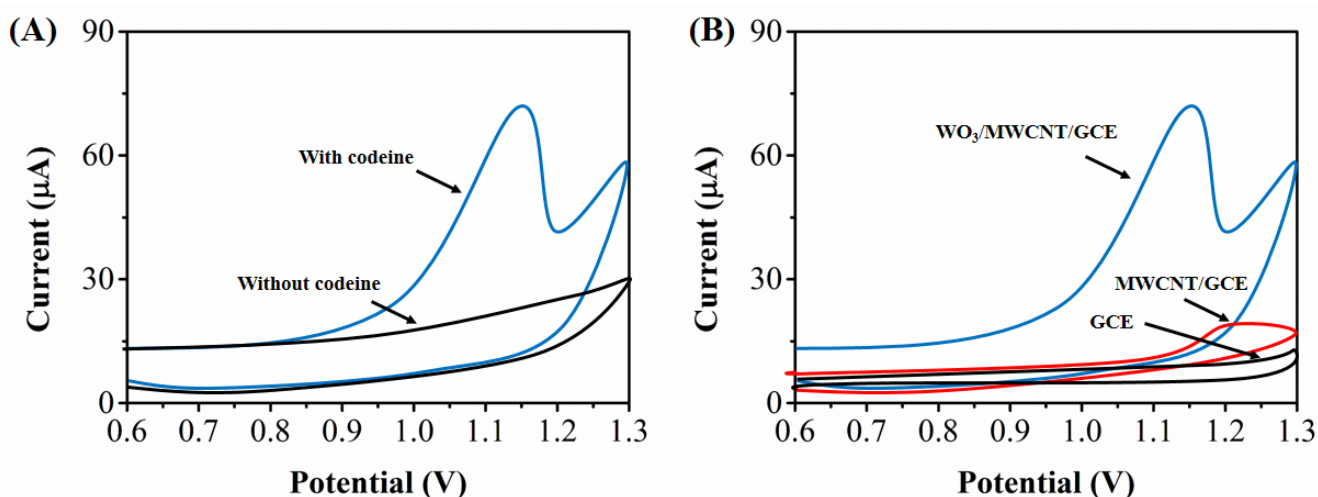

Figure 4. (A) Cyclic voltammograms of WO_3/MWCNT/GCE in the absent and present of 5 μM codeine in 0.1 M BRBS. (B) Cyclic voltammograms of bare GCE, MWCNT/GCE and WO_3/MWCNT/GCE in 0.1 M BRBS.

Generally, both the potential and current of the peak will be influenced by the pH of the electrolyte during detecting biologically compounds by electroanalysis. In this study, the effect was studied using BRBS as the electrolyte with the pH ranging from 2 to 12. As depicted in Fig. 5, the peak potential (Ep) of codeine changed in the direction of less positive potentials with the increasing pH of electrolyte, which strongly confirmed that protons played a significant role in the codeine oxidation process. The Ep-PH dependence is linearly related during the entire studied pH range, indicating that the numbers of electrons and protons involved in the oxidation procedure were exactly the same. It can

be clearly observed from Fig. 5, the peak current (Ip) was also related with pH. The peak current of codeine increased rapidly with the ascending pH until the value of PH being 7.0. Then the current began to descending moderately. Based on the obtained results shown above, BRBS with PH of 7.0 was chosen as the optimized electrolyte.
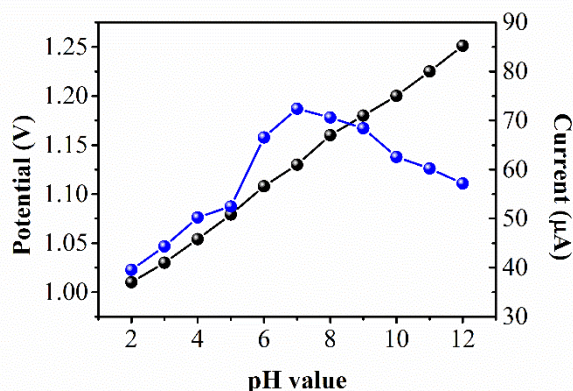

Figure 5. Effect of pH on the peak potential and peak current of 5 μM codeine in 0.1 M BRBS on WO₃/MWCNT/GCE.

As depicted in Fig. 6, the curve plotted by peak current against codeine concentration ranging from 0.005 to 20 μM was used for evaluate the applicability of DPV in codeine detection. A linear relationship was obtained between the current responses and the codeine concentrations. Namely, the constructed WO₃/MWCNT/GCE sensor could provide linear responses during codeine determination of which the concentration ranges from 0.005 to 20 μM. The detection limit was 0.02 nM. The relative standard deviation (RSD) of 0.9% was obtained by conducting measurements ten times under exactly the same conditions at the codeine concentration of 10 μM. Table 1 shows the comparison of our proposed codeine sensor with several existing reported electrochemical sensors. Results showed that the detection linear range and the detection limit of codeine using WO₃/MWCNT/GCE is comparable with other electrochemical sensors. The WO₃/MWCNT/GCE showed an excellent performance in the codeine detection in trace range. Specifically, the WO₃/MWCNT/GCE had a lower detection compared with that of the Graphene–CoFe₂O₄/CPE (0.03 to 12μM) [40]. On the other hand, the detection limit of the WO₃/MWCNT/GCE can be down to nano-molar level, which is much lower than that of the porous silicon/palladium nanostructure fabricated electrochemical sensor [41] and chemically modified-palladized aluminum electrode [42]. The extremely low RSD value can be an indication of the excellent repeatability. The low limit of detection and first-rate repeatability make the method a fittable technique for accurate determination of codeine. Therefore, the WO₃/MWCNT/GCE can be potentially used for determination of the codeine in real environmental samples.

For the sake of evaluating the selectivity of our electrochemical method, the influence of interfering agents on the detection of codeine should be considered. The experiments were conducted under the conditions as follows: BRBS with PH of 7.0 being the electrolyte and the concentration of codeine being 10 μM. Table 2 showed the experimental results by some possible interfering agents of

ions and molecules which were typically the components of tablets and biological samples. Whether the agent interfered seriously or not, the standard is that the codeine signal changes more than 5%.
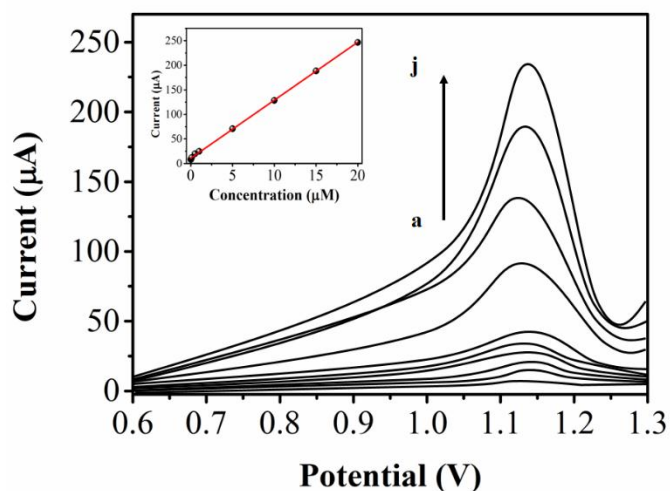

Figure 6. DPV of various codeine concentrations in 0.1M BRBS at pH 7.0 on WO_3/MWCNT/GCE.

Table 1. Comparison of codeine detection using our proposed method with other literatures.

Electrode	LDR[a] (μM)	LOD[b] (μM)	Reference
Acetylcholinesterase/ screen-printed electrode	-	20	[43]
Porous silicon/palladium nanostructure	1-700	0.4	[41]
Chemically modified-palladized aluminum electrode	2-50	0.8	[42]
Graphene–$CoFe_2O_4$/CPE	0.03-12	0.011	[40]
Boron-doped diamond film electrode	0.1-60	0.08	[44]
Flow injection analysis system with electrochemical detection	7-50	3	[22]
WO_3/MWCNT/GCE	0.005-20	0.002	This work

[a] Linear detection range
[b] Limit of detection

Table 2. Influence of potential interfering agents on the voltammetric response of 10 μM codeine.

Interfering species	Concentration (μM)	Current change (%)
Glucose	100 μM	1.51
Uric acid	100 μM	3.44
Folic acid	100 μM	4.21
Ascorbic acid	100 μM	3.54
Hydrogen peroxide	200 μM	4.01
Cl^- and CO_3^-	200 μM	1.20 and 0.91
K^+, Na^+ and Ca^{2+}	200 μM	0.98, 0.91 and 1.32

As can be seen from the values of codeine signal changes in Table 1, the interference by those interfering ions and molecules on the peak current of codeine was not significant. The only exception is adding 100-fold excess of glucose, and the oxidation signal change of codeine is more than 5%. Therefore, the proposed method can selectively determine codeine in the solutions concluding interfering agents as well.

Table 3. Determination of codeine in commercial tablets.

Diluted concentration (μM)	Detection (μM)	Added (μM)	Detection (μM)	Recovery (%)
5	4.966	5	9.957	99.57
5	4.959	10	14.873	99.15
10	10.002	10	19.866	99.33
10	9.982	20	30.212	100.71

The method of standard additions was explored for the determination of codeine concentration in tablets. To evaluate the accuracy of the electrochemical method, the aliquots amount of codeine standard solution was added. Table 3 presented the average results of standard deviations and half width of the confidence interval by six replicate measurements. The recovery values obtained were between 99.15 and 100.71%. The satisfactory recovery values demonstrated that no remarkably interferences existed. Therefore, the electrochemical method can becomingly determine the concentration of codeine in drugs with desired accuracy.

4. CONCLUSIONS

In conclusion, a WO$_3$/MWCNT composite modified GCE electrode was constructed for quantification of codeine. A linear relationship was found between the current responses and the codeine concentrations ranging from 0.01 to 60 μM. The limit of detection of the proposed sensor was 0.08 nM. The electrochemical codeine sensor owns low detection limit and excellent repeatability, which make it successfully applied for codeine detection in pharmaceutical tablets.

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