

Full Paper

Novel Sensor Based on 3-Mercaptopropyltrimethoxysilane Functionalized Carbon Nanotubes Modified Glassy Carbon Electrode for Electrochemical Determination of Cefixime

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Abstract- In this work, a highly dispersed Multi-walled carbon nanotube (MWCNT) was successfully functionalized with 3-mercaptopropyltrimethoxysilane (MPTS) molecule by silanization method. The MPTS functionalized MWCNT (MPTS-MWCNT) were structurally characterized by scanning electron microscope (SEM) and energy dispersive X-ray (EDAX) techniques. Moreover, glassy carbon electrode modified with MPTS-CNT (MPTS-MWCNT/GCE) was prepared by casting of the MPTS-MWCNT solution on GCE. The MPTS-MWCNT/GCE showed an excellent electrocatalytic activity towards cefixime (CEF). Bare GCE shows an ill-defined oxidation wave around 0.95 V for CEF, whereas MPTS-MWCNT/GCE shows a well-defined oxidation peak at 0.7 V vs. Ag|AgCl|KCl (3 M). Differential pulse voltammetry method was used to determine CEF in the concentration range of 0.6-20 μ M with a detection limit of 0.07 μ M (S/N=3). The present modified electrode shows good recovery results for CEF tablet and spiked CEF in human blood plasma sample. Possible interferences, such as: glycine, alanine, ascorbic acid, cephalexine, L-cysteine, glutathione and lysine for the detection of CEF at the MPTS-MWCNT/GCE were investigated. The results showed that L-cysteine and glutathione showed interference, whereas another mentioned compounds did not show interference.

Keywords- Cefixime, Functionalized carbon nanotube, 3-Mercaptopropyltrimethoxysilane, Modified glassy carbon electrode

1. INTRODUCTION

The discovery of carbon nanotube (CNT) in 1991 opened up a new era in material science and nanotechnology [1]. The subtle electronic behavior of CNTs reveals that they have the ability to promote electron-transfer reaction and have a high electrocatalytic effect when used as electrode materials [2,3]. Preparation, investigation, and application of nanomaterials are very important in chemical science [4-6]. During the past few years, considerable research efforts have been devoted to the synthesis, modifications, and applications of carbon nanotubes (CNTs), due to their appealing uniform and high specific surface area and unique physical and chemical properties [7-10]. In most cases, CNTs were studied in the electrochemical determination and exhibited good electrocatalytic performances. Recently, CNTs have been used as an ideal host for host-guest chemistry and functionalization. Modification of the surface of CNTs with different materials such as metals, metal oxides, complex metal oxides and polymers [9-13] can improve CNTs characteristics. The surface functionalization aids the carbon nanotube materials in becoming biocompatible, improving their solubility in physiological solutions and selective binding to biotargets. The functionalized CNTs were believed to be very promising in the fields, such as: preparation of functional and composite materials and biological technologies [14]. Also, nanotubes have large inner volumes, which can be filled with any desired chemical or biochemical species ranging in size from proteins to small molecules [15]. Thus, the development of simple and cost effective chemical methods for functionalization of carbon nanotube materials is becoming an area of growing fundamental and industrial importance [16].

Cefixime (CEF) is an oral third generation cephalosporin antibiotic useful for the treatment of susceptible infections, including gonorrhea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis and urinary-tract infections. CEF is available as the trihydrate [17-19]. Forty to fifty percent of the oral dose of cefixime is adsorbed from the gastrointestinal tract. There is no evidence of metabolism of cefixime in vivo [20,21]. Cefixime is a primary candidate for switch therapy owing to its very good efficacy and safety profile [22]. Since, multiple methods have been proposed for the analysis of cefixime using different analytical techniques [23-28]. However, although the selectivity and the detection limit are relatively satisfactory in these methods, the spectrophotometric, fluorimetric and chromatographic methods are rather time consuming methods and require large number of complicated steps to follow on for analysis. In comparison with other methods, voltammetric methods showed some advantages including the absence of influence of matrix effects, and higher selectivity and sensitivity because of the possibility of higher sample dilution. Consequently, the development of rapid, simple and accurate method with high sensitivity for the determination of CEF at trace levels in pharmaceutical and biological samples is of particular significance.

In this contribution, we report a simple method to functionalize MWCNT with 3-mercaptopropyltrimethoxysilane (MPTS) molecule by silanization method. The MPTS–MWCNT was characterized by SEM and EDAX techniques. The electrocatalytic property of MPTS–MWCNT on the glassy carbon electrode was examined for the oxidation and the voltammetric determination of CEF in aqueous medium.

2. EXPERIMENTAL

2.1. Instrumentation

Electrochemical measurements were performed with potentiostat/galvanostat (Sama 500-c Electrochemical Analysis system, Sama, Iran). The three-electrode system consists of a MPTS–MWCNT/GCE as working electrode, Ag|AgCl|KCl (3 M) as the reference electrode and a platinum wire as an auxiliary electrode (Metrohm) were used in all voltammetric experiments. Hitachi S-3500N scanning electron microscope (SEM) with energy dispersive X-ray (EDAX) spectroscopy was used for surface image measurements and chemical characterization of a specimen.

2.2. Reagents and materials

Multi-walled carbon nanotubes (MWCNTs; outer diameter: 20-40 nm, length: 5-15 μm , purity >97) were purchased from Shenzhen Nano-Technologies Port Co., Ltd. (China). Cefixime and 3-mercaptopropyltrimethoxysilane (MPTS) were of analytical grade from Merck. The solvent used for the electrochemical studies was twice distilled water. Buffer solutions were prepared from orthophosphoric acid and its salts in the pH ranges 3.0–10.0. Potassium chloride from Fluka was used as the supporting electrolyte.

2.3. Functionalization of MWCNT

MWCNTs were purified using a mixture of sulfuric acid and nitric acid in a composition of 3:2 by volume. The functionalization of MWCNTs surface was carried out by reacting MWCNT with 3-mercaptopropyltrimethoxysilane molecule. About 10 mg of MWCNTs was added to 30 mL of ethanol and dispersed by ultra-sonication for 20 min. Then 5% solution of 3-mercaptopropyltrimethoxysilane added drop wise to the MWCNTs dispersed solution under vigorous stirring at 60 °C. The solution was kept in an oil bath at 60 °C for 12–14 h. The product (MPTS–MWCNT) was obtained by centrifugation and repeatedly washed with water, followed by drying at 80 °C for 10 h.

2.4. Preparation of modified electrode

Prior to modification, the bare GCE was polished on chamois leather with 0.05 μm alumina powder. Then it was thoroughly sonicated in deionized water and absolute ethanol, respectively. 1.0 mg MPTS–MWCNT was dispersed in 3 mL ethanol with the aid of ultrasonic agitation to give a highly dispersed solution. The cleaned GCE was coated by casting 5 μL of the MPTS–MWCNT dispersed solution and dried at 50 $^{\circ}\text{C}$ in an oven air to remove the solvent.

3. RESULTS AND DISCUSSION

3. 1. Morphology study of MPTS-MWCNT

The SEM images of MWCNT and MPTS-MWCNT are shown in Fig. 1. As shown, after functionalization of MWCNT with MPTS, the diameter of MPTS-MWCNT (Fig. 1b) is increased as compared to that of MWCNT (Fig. 1a) shows species of MPTS have been immobilized on the MWCNT.

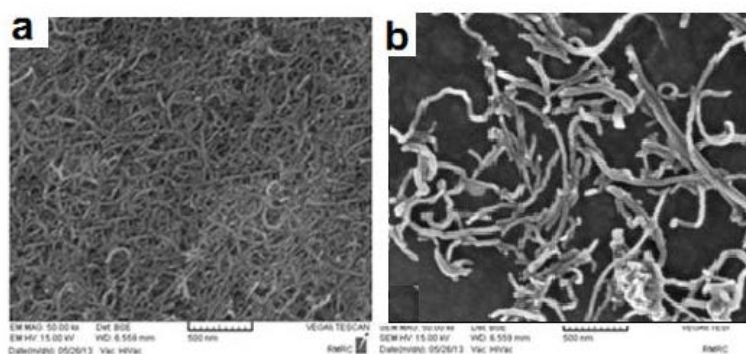


Fig. 1. SEM images of (a) MWCNT and (b) MPTS-MWCNT

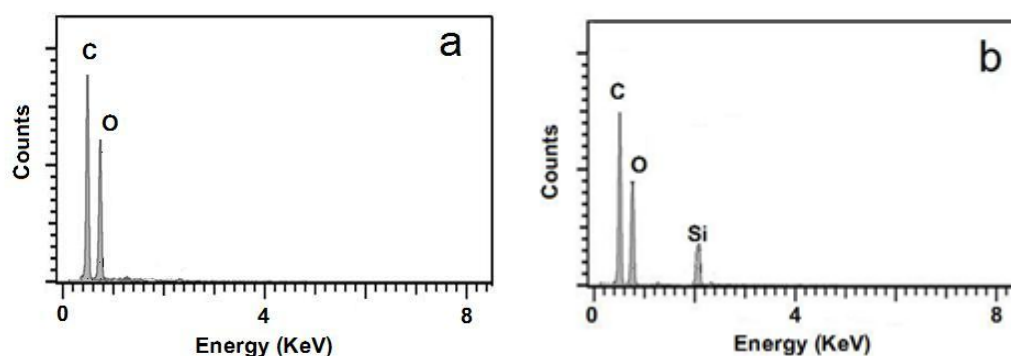


Fig. 2. EDAX patterns of (a) MWCNT and (b) MPTS-MWCNT

The EDAX spectrum shown in Fig. 2a indicates that the MWCNT contains only C and O elements, while the EDAX spectrum of MPTS-MWCNT (Fig. 2b) contains C, O and Si elements. This result confirms functionalization of MWCNT.

3.2. Electrochemical behavior of CEF at MPTS-MWCNT/GCE

We have examined the electrocatalytic activity of MPTS-MWCNT/GCE toward CEF electrooxidation in buffered solution (pH 7.00) using cyclic voltammetry (Fig. 3). We obtained higher oxidation peak current with less positive peak potential for CEF at the surface of MPTS-MWCNT/GCE in this condition. Bare GCE shows an (un-defined or broad) oxidation peak at 0.95 V vs. Ag|AgCl|KCl (3M) for CEF (curve c). On the other hand, the MPTS-MWCNT/GCE exhibits a well-defined oxidation peak for CEF at 0.70 V vs. Ag|AgCl|KCl (3 M) (curve d). Cefixime has NH groups that make cefixime as an oxidizable compound and can be detected by electrochemical methods based on anodic oxidation [25].

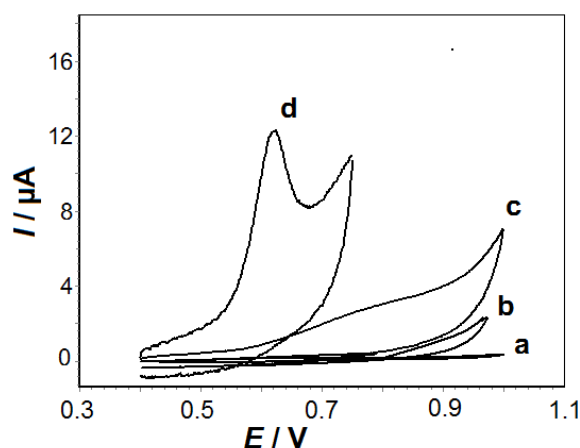


Fig. 3. Cyclic voltammograms of (a) GCE and (b) MPTS-MWCNT in 0.1 M PBS (pH 7.00) and 0.1 M KCl and (c) as (a) and (d) as (b) in the presence of 0.1 mM CEF at scan rate of 50 mV s^{-1}

The effect of the scan rates of potential on the cyclic voltammetric behavior of 0.1 mM CEF was studied at the surface of MPTS-MWCNT/GCE in 0.1 M PBS (pH 7.00). It was observed that the values of E_{pa} was shifted slightly to the positive direction with increasing scan rate of potential. The anodic peak current is linearly proportional to square of scan rate (Fig. 4), suggesting that the CEF electrochemical reaction at MPTS-MWCNT/GCE is a diffusion-controlled process.

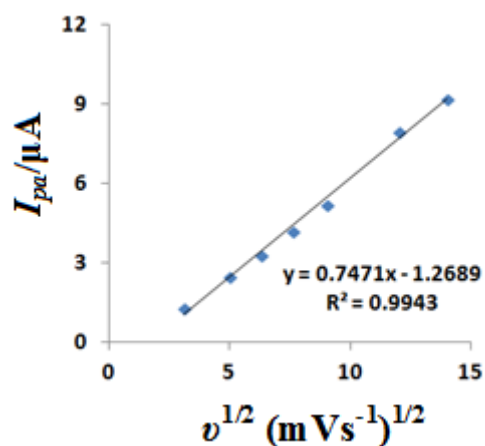


Fig. 4. Plot of anodic peak currents of 0.1 mM CEF in 0.1 M PBS (pH 7.00) and 0.1 M KCl supporting electrolyte at the surface of MPTS-MWCNT/GCE at various scan rates of potential (10, 25, 40, 50, 80, 140 and 200 mV s^{-1}) vs. $v^{1/2}$

3.3. Effect of pH on the CEF electrooxidation

It well known, the electrochemical behavior of CEF is dependent on the pH value of the aqueous solution [26].

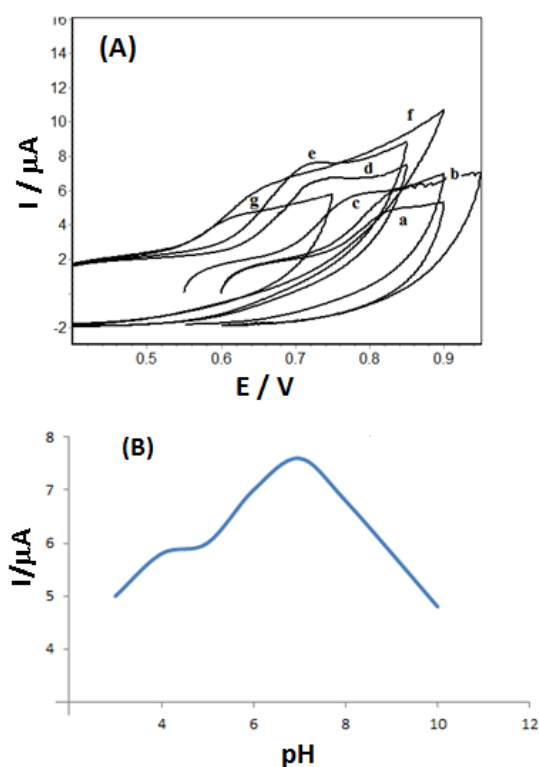


Fig. 5. (A) Cyclic voltammograms of MPTS-MWCNT/GCE in presence of 0.1 mM CEF in 0.1 M PBS and 0.1 M KCl as supporting electrolyte at various pHs: (a) 3, (b) 4, (c) 5, (d) 6, (e) 7, (f) 8 and (g) 10 at scan rate 50 mV s^{-1} ; (B) Plot of oxidation peak current vs. pH values

The effect of pH value on the electrooxidation of CEF at the surface of MPTS-MWCNT/GCE was investigated through the use of different 0.1 M phosphate buffer solutions (pH 3.00–10.00) (Fig. 5). The current response of CEF at MPTS-MWCNT/GCE increases from pH 3.00 to 7.00 and then a decrease is obtained at pH values higher than 7.00. Therefore, pH 7.00 was chosen as optimum pH for electrocatalytic oxidation of CEF at MPTS-MWCNT/GCE and further studies were performed at pH=7.00.

3.4. Voltammetric determination of CEF

Differential pulse voltammetry (DPV) method was used to examine the sensitivity of MPTS-MWCNT/GCE towards the detection of CEF. Fig. 6A shows the DPV curves obtained for CEF at MPTS-MWCNT/GCE in 0.1 M phosphate buffer solution, PBS (pH 7.00). The dependence of the oxidation peak currents with respect to concentration of CEF was linear 0.6–20 μM (Fig. 6B) with a detection limit of 0.07 μM DPV at MPTS-MWCNT/GCE.

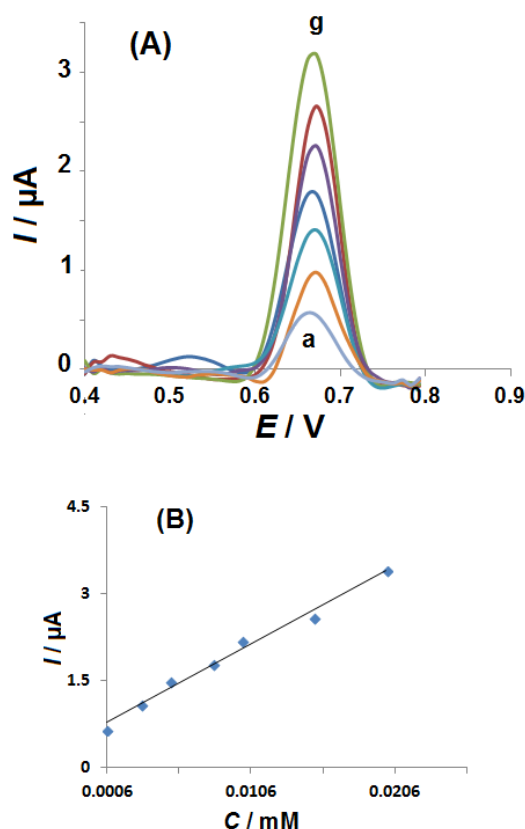


Fig. 6. (A) Differential pulse voltammograms of MPTS-MWCNT/GCE in 0.1 M PBS (pH 7.00) in the presence of (a) 0.6, (b) 3, (c) 5, (d) 8, (e) 10, (f) 15 and (g) 20 μM of CEF. (B) Plot of oxidation peak current vs. CEF concentration

The observed linear dynamic range, detection limit and the oxidation potential of CEF were compared with that reported analytical data by other research groups in the literature and are given in Table 1 [25-28]. As can be seen from Table 1, analytical parameters for CEF detection of the sensor prepared in this work are comparable with other results. Further, the present modified electrode was highly stable and no tedious procedure was involved in electrode modification.

Table 1. Analytical parameters for voltammetric determination of CEF at different modified electrodes

Electrode	LDR (μM)	LOD (μM)	Method	$E_{\text{CEF}}^{\text{a}}$ (mV)	Ref.
NiFe ₂ O ₄ -MWCNTs-GCE ^b	0.1-600	0.09	CV	900	[26]
HMDE ^c	0.06-200	0.06	SWV ^d	900	[28]
GNPs/MWCPE ^e	0.01-200	0.003	SWV	950	[27]
HMDE	0.06-0.11	0.008	SWV	900	[25]
MPTS-CNT/GCE	0.6-1000	0.07	DPV	670	This work

^aOxidation potential of CEF

^bNiFe₂O₄-Multi wall carbon nanotubes modified glassy carbon electrode

^cHanging mercury drop electrode

^dSquare wave voltammetry

^eGold nanoparticles electrodeposited on a multi walled modified carbon paste electrode

^fDifferential pulse polarography

3.5. Sample analysis

In order to verify the reliability of the proposed method, the MPTS-MWCNT/GCE was applied to analysis of CEF in tablet (tablet of CEF containing 200 mg, from Farabi Co., Iran) and blood plasma samples. For tablet analysis, the tablet of CEF containing 200 mg was homogenized. Then, an enough weight of the homogenized powder was accurately weighed and transferred into the electrochemical cell containing 20 mL 0.1 M PBS (pH 7.00). The analysis was carried out using the standard addition method. For plasma analysis, 2.0 mL of the sample solution plus 18.0 mL of 0.1 M PBS (pH 7.00) were transferred into the cell to measure the CEF contents using standard addition method. The analytical results are summarized in Table 2. The data obtained for the analysis CEF showed that there is no

significant difference between the spiked contents and those obtained by the proposed method. Therefore, the satisfactory results obtained with this method confirm the strong applicability of the MPTS-MWCNT/GCE in practical analysis. The data obtained for the analysis CEF was compared favorably with that obtained by UV- spectrophotometric method [28]. There is no significant difference between the obtained data by UV-spectrophotometric method and those obtained by the proposed method with the satisfactory recovery.

Table 2. Determination of CEF in tablet and blood plasma samples at surface of MPTS-MWCNT/GCE in 0.1 M PBS solution (pH 7.00)

Sample	Spiked(mg)	Found(mg)	Mean recovery (%)	t_{exp}	F_{exp}
	Proposed method	UV method			
Tablet	1.00	0.9494±1.1	100.4±0.5	1.92	4.84
Plasma	1.00	0.92	92.0±3.8		
Plasma	2.00	1.90	95.0±4.2		

Theoretical values for $t=2.31$ and $F=6.39$ ($p=0.05$)

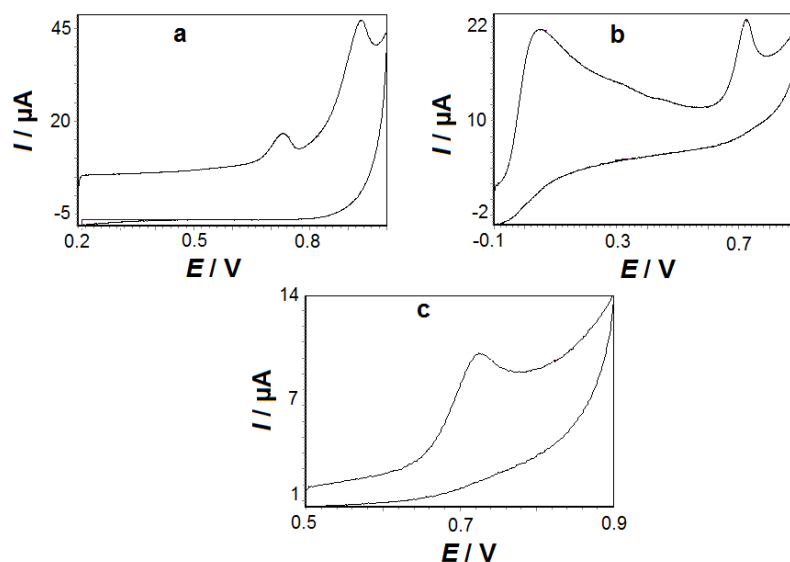


Fig. 7. Cyclic voltammograms of MPTS-MWCNT/GCE in the presence of (a) 1.0 mM CEF +10.0 mM cephalaxine, (b) 1.0 mM CEF +1.0 mM ascorbic acid and (c) 1.0 mM CEF +1.0 mM L-cysteine in 0.1 M PBS (pH 7.00) and 0.1 M KCl as supporting electrolyte at scan rate 50 mV s^{-1} .

3.6. Interference study

Possible interferences for the detection of CEF at the MPTS-MWCNT/GCE was investigated by the addition of various compounds such as glycine, alanine, ascorbic acid, cephalexine, L-cysteine, glutathione and lysine into pH 7.00 PBS in the presence of 1.0 mM CEF by cyclic voltammetry. The results showed that L-cysteine and glutathione showed interference, whereas another mentioned compounds did not show interference (Fig. 7).

4. CONCLUSION

In summary, we demonstrated that MWCNT was successfully functionalized with 3-mercaptopropyltrimethoxysilane (MPTS) molecule by silanization method. The MPTS-MWCNT is highly suspensible in water. The present study demonstrates the excellent electrocatalytic activity towards CEF oxidation at the surface of MPTS-MWCNT modified GCE. The bare GCE showed a broad or un-defined oxidation peak for CEF, whereas the MPTS-MWCNT/GCE not only showed a well-defined stable oxidation peak, but also enhanced its oxidation current at less positive oxidation potential. The MPTS-MWCNT on the surface of GCE showed excellent electrocatalytic activity toward CEF oxidation, indicating that the present method may be used for practical applications.

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