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Cephalexin Electrochemical Sensors based on Glassy Carbon Modified with 3-mercaptopropyltrimethoxysilane Functionalized Multi-walled Carbon Nanotubes

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Abstract- This research discusses the preparation of an electrochemical sensor for cephalexin (Ceph) based on multi-walled carbon nanotubes (MWCNT) functionalized with 3-mercaptopropyltrimethoxysilane (MPTS) molecule modified glassy carbon electrode (GCE). The MPTS functionalized MWCNT (MPTS-MWCNT) was structurally characterized by thermogravimetric analysis (TGA) and electrochemical impedance spectroscopy (EIS). In this area, glassy carbon electrode (GCE) modified with dispersed MPTS-MWCNT ethanol solution on the GCE, which MPTS-MWCNT was used as a new mediator for the voltammetric determination of cephalexin. Differential pulse voltammetry method was employed for determination of Ceph in phosphate buffer solution. Under the optimum conditions at pH 7.00, the oxidation of cephalexin at the surface of the modified electrode was occurred at 1.05 V *vs*. Ag|AgCl|KCl$_{3M}$ with higher oxidation current in compared to bare GCE. The MPTS-MWCNT/GCE showed an excellent electrocatalysis activity towards oxidation of Ceph. The oxidation peak current of cephalexin at the surface of MPTS-MWCNT/GCE in the differential pulse voltammetry are found to be linear over the range of concentration 0.5 to 50 μM and the lower detection limit are found to be 0.12 μM for this drug. The proposed method showed good recovery results for voltammetric determination of Ceph in the Ceph tablet solution and spiked Ceph in human serum sample.

Keywords– Cephalexin, Functionalized Multi-walled carbon nanotubes, Electrocatalysis, Electrochemical sensors, 3-mercaptopropyltrimethoxysilane

1. INTRODUCTION

Cephalexin (Ceph) is a semi-synthetic antibiotic of the cephalosporins group, which can treat a number of bacterial infections. It kills gram-positive and some gram-negative bacteria by disrupting the growth of the bacterial cell wall. It is an effective broad spectrum antibiotic, which have a structure similar to that of the penicillin and is used for patients allergic to penicillin. Cephalosporins are a series of antibiotics containing a β-lactam ring fused to a six-membered ring with a sulfur atom. Ceph has been used in the treatment of urinary tract infections, respiratory tract infections (including sinusitis, otitis media, pharyngitis, tonsillitis, pneumonia, and bronchitis), skin and soft tissue infections [1,2]. The widespread use of this compound and its clinical and pharmacological studies requires fast and sensitive analytical methods to determine the drug in pharmaceutical formulations and serum samples.

Carbon nanotubes (CNTs) are rolled up seamless cylinders of graphene sheets, exhibiting many unique physical, mechanical and chemical properties [3-7]. The unique properties of carbon nanotubes make them extremely attractive for the task of chemical sensors, in general, and electrochemical detection, in particular [8]. CNTs can be used as electrode materials with electrocatalytic properties and with enhanced current due to the larger surface area, for electrochemical and bioelectrochemical applications [9]. Modification of the surface of CNTs with different materials can improve CNTs characteristics [10,11]. The functionalized CNTs were believed to be very promising in the fields, such as: preparation of functional and composite materials and biological technologies [12]. Therefore, 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 [13].

Various method including chromatography [14-16], spectrophotometry [17], fluorescence method [18,19], immunoanalysis [20,21] and electroanalytical methods [22–24] for determination of cephalexin have been reported. 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 and simple method with high sensitivity for the determination of Ceph at trace levels in pharmaceutical and biological samples is of particular significance.

In this research, we characterize the synthesized MPTS–MWCNT by TGA and EIS techniques. Then, an electrochemical sensor was prepared based on the modified glassy carbon electrode with MPTS–MWCNT for voltammetric determination of Ceph in aqueous solution using differential pulse voltammetry.

2. EXPERIMENTAL

2.1. Instrumentation

The pH was measured using a pH meter model 250I on Analyzer using a combined electrode (glass electrode-reference electrode). The thermogravimetric analysis (TGA) was carried with thermogravimetric analyzer model Q50. Electrochemical studies were performed with potentiostat/galvanostat (Sama 500-c Electrochemical Analysis system, Sama, Iran). A conventional three-electrode system consisting of $Ag|AgCl|KCl_{3M}$ as the reference electrode, a MPTS–MWCNT/GCE as working electrode and a platinum wire as auxiliary electrode were used.

2.2. Reagents and materials

Cephalexine and 3-mercaptopropyltrimethoxysilane (MPTS) (Scheme 1) were of analytical grade from Merck. Buffer solutions were prepared from orthophosphoric acid and its salts in the pH ranges 3.00 – 8.00. Potassium chloride from Fluka was used as the supporting electrolyte. The solvent used for the experimental studies was twice distilled water. Multi-walled carbon nanotubes (MWCNTs) with outer diameter: 20-40 nm, length: 5-15 µm and purity>97 were purchased from Shenzhen Nano-Technologies Port Co., Ltd. (China).
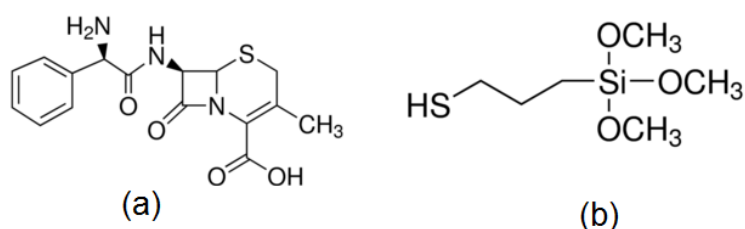
Scheme 1. The structures of: (a) cephalexin and (b) 3-mercaptopropyltrimethoxysilane

2.3. Preparation of modified electrode

First, the bare GCE was polished on polishing micro-cloth with 0.05 µm alumina powder for 2 min. Then, it was cleaned in an absolute ethanol solution in the ultrasonic bath and rinsed thoroughly with deionized water. 1.0 mg of synthesized MPTS–MWCNT [25] was dispersed in 3 mL ethanol with the aid of ultrasonic agitation to give a highly dispersed solution. Finally, 5 µL of the MPTS– MWCNT dispersed solution was dropped onto the surface of the glassy carbon electrode and it was dried in an oven air at 50 °C to remove the solvent.

3. RESULTS AND DISCUSSION

3.1. Thermal analysis of MPTS- MWCNT

Thermal analysis is a simple analytical technique that measures the weight loss of a material as a function of temperature. The Thermal analysis was conducted for MWCNT and MPTS-MWCNT powders (Fig. 1) to examine its phase formation during calcinations. It can be seen from Fig. 1, that the MPTS-MWCNT curve loses its mass in two distinct steps; the first weight loss is in the range of 50-150 °C, suggesting the evaporation of surface adsorbed water. The second weight loss appeared in 155-255 °C, signifying the decomposition of MPTS molecule. As compared to MPTS-MWCNT, the MWCNT loses its weight in three steps (Inset of Fig. 1). The first step is in the range of 40-130 °C, revealing the evaporation of surface adsorbed water molecules. Second step of weight loss appeared in 145-315 °C, suggesting the decomposition of labile oxygen (CO, CO_2, COOH and H_2O vapors) containing functional groups of MWCNT [26,27]. The third step of mass loss is in the range of 640-870 °C, signifying the partial decomposition of MWCNT itself. Also, the mass loss rate with temperature of MPTS-MWCNT was significantly lower and more residues were formed, which indicates the significant improvement in thermal stability of MPTS-MWCNT compared to MWCNT.
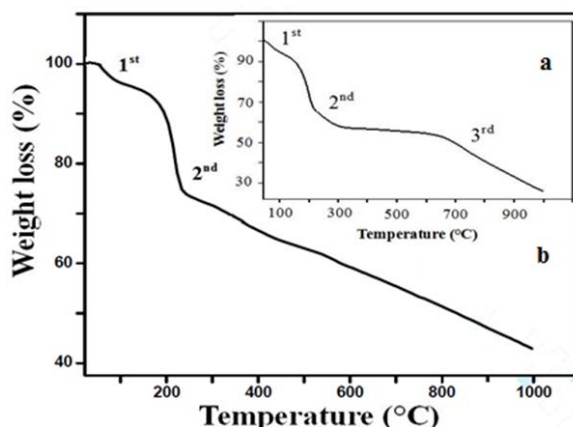

Fig. 1. TGA curve of MPTS-MWCNT; Inset: TGA curve of MWCNT

3.2. Cyclic voltammetric behavior of Ceph at MPTS-MWCNT/GCE

The cyclic voltammetric behavior of 1.0 mM Ceph was studied at the surface of MPTS-MWCNT/GCE in 0.1 M phosphate buffer solution, PBS (pH 7.00) (Fig. 2). The results confirm that the oxidation of Ceph at the surface of the bare GCE has a low peak current at 1.2 V *vs*. Ag|AgCl|KCl_{3M}, whereas an increase in the oxidation peak current and decrease in the anodic peak potential of Ceph could be observed at the surface of MPTS-MWCNTs and MWCNTs modified electrodes (Fig. 2, curves d and e). Therefore, MPTS-MWCNT/GCE showed a higher oxidation peak current for Ceph that demonstrate high electrocatalytic effect

of functionalized carbon nanotubes toward Ceph oxidation. The proposed mechanism for the oxidation of Ceph is illustrated in Scheme 2.
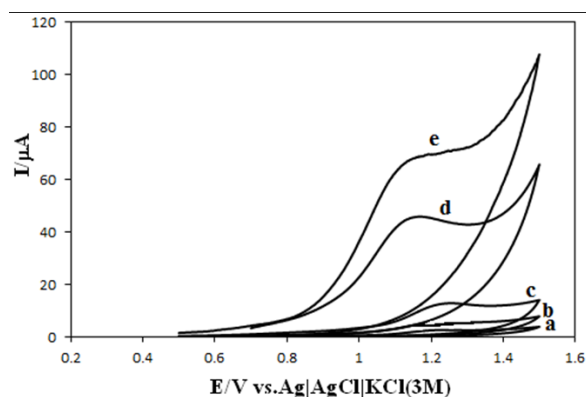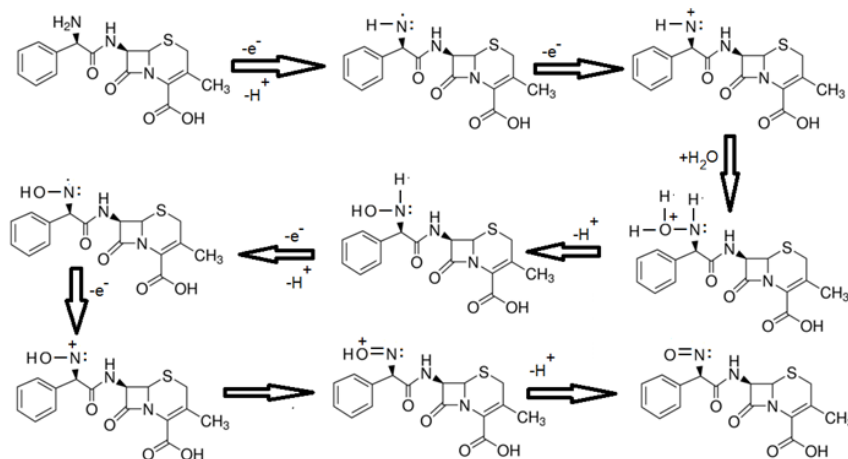

Fig. 2. Cyclic voltammograms of (a) GCE and (b) MPTS-MWCNT in 0.1 M PBS (pH 7.00) and 0.1 M KCl and (c) as (d), MWCNT/GCE and (e) as (b) in presence of 1 mM Ceph at scan rate of 50 mV s^{-1}

Scheme 2. The proposed mechanism of Ceph electrooxidation

Fig. 3A shows the cyclic voltammograms of the MPTS-MWCNT/GCE in 0.1 M PBS (pH 7.00) in the presence of 1.0 mM Ceph in 0.1 M PBS (pH 7.00) and 0.1 M KCl as supporting electrolyte at various scan rates of potential. It was observed that the values of oxidation peak potential (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. 3B), suggesting that the oxidation of Ceph at MPTS-MWCNT/GCE is not reversible, although, the peak currents are controlled through diffusion process.
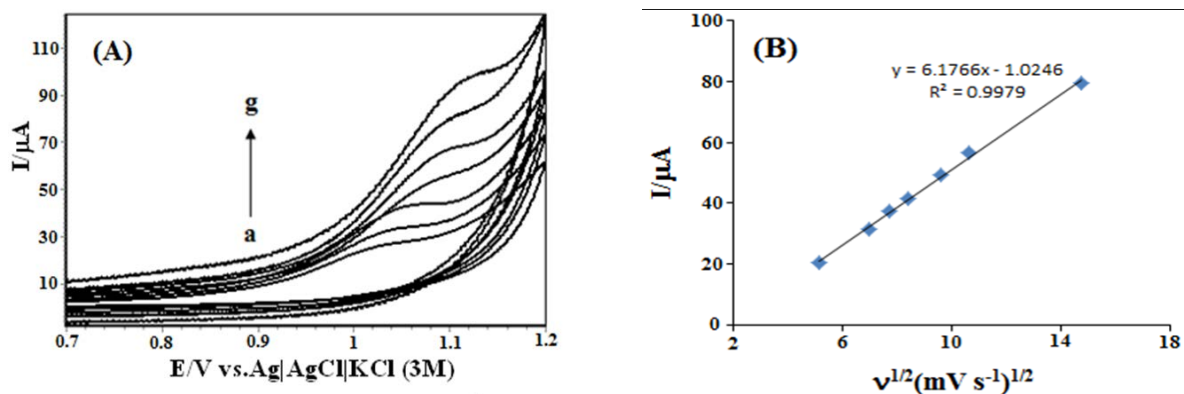
Fig. 3. Cyclic voltammograms of 1 mM Ceph in 0.1 M PBS (pH 7.00) and 0.1 M KCl as supporting electrolyte at the surface of MPTS-MWCNT/GCE at various scan rates: (a) 20, (b) 40, (c) 50, (d) 60, (e) 80, (f) 100 and (g) 200 mV s^{-1}; (B) Plot of peak currents *vs.* square of scan rates

3.3. The Ceph electrooxidation in various pH values

The electrochemical behavior of Ceph is dependent on the pH value of the aqueous solution [28]. Therefore, the cyclic voltammetric behavior of Ceph at the surface of MPTS-MWCNT/GCE was investigated in 0.1 M phosphate buffer solutions with different pH values (pH 3.00–8.00) (Fig. 4A). The oxidation peak current of Ceph at MPTS-MWCNT/GCE increases from pH 3.00 to 7.00 and reached to its maximum value at pH 7.00 (Fig. 4B), which pH 7.00 was chosen as optimum pH for electrocatalytic oxidation of Ceph at MPTS-MWCNT/GCE and further studies were performed at pH=7.00.
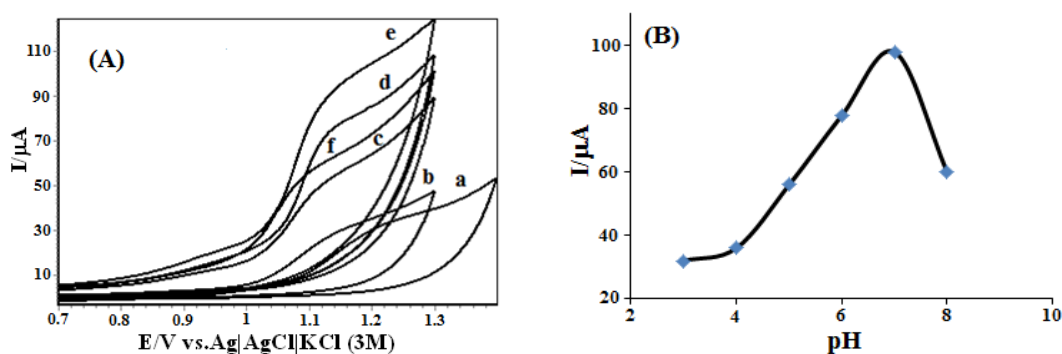

Fig. 4. (A) Cyclic voltammograms of 1 mM Ceph in 0.1 M PBS (pH 7.00) and 0.1 M KCl as supporting electrolyte at the surface of MPTS-MWCNT/GCE at various pH values: (a) 3.0, (b) 4.0, (c) 5.0, (d) 6.0, (e) 7.0 and (f) 8.0 at scan rate 50 mV s^{-1}; (B) Plot of oxidation peak current *vs.* pH values

3.4. Electrochemical impedance spectroscopy

The electrochemical ability of the immobilized MPTS-MWCNTs onto GCE was investigated using electrochemical impedance spectroscopy (EIS). Fig. 5 shows Nyquist diagrams of the imaginary impedance (Z_{im}) *vs.* the real impedance (Z_{re}) of the EIS obtained at unmodified GCE (a), and MPTS-MWCNTs modified electrode (b) in a solution containing 10 mmol L^{-1} of $Fe(CN)_6^{3-/4-}$ redox couple. It has been found that the diameter of the Nyquist semicircle (charge transfer resistance, R_{ct}) to be decreased with the immobilization of MPTS-MWCNTs onto the surface of GCE. Hence, the interfacial electron transfer occurs rapidly, resulting a decrease in the electron transfer resistance.
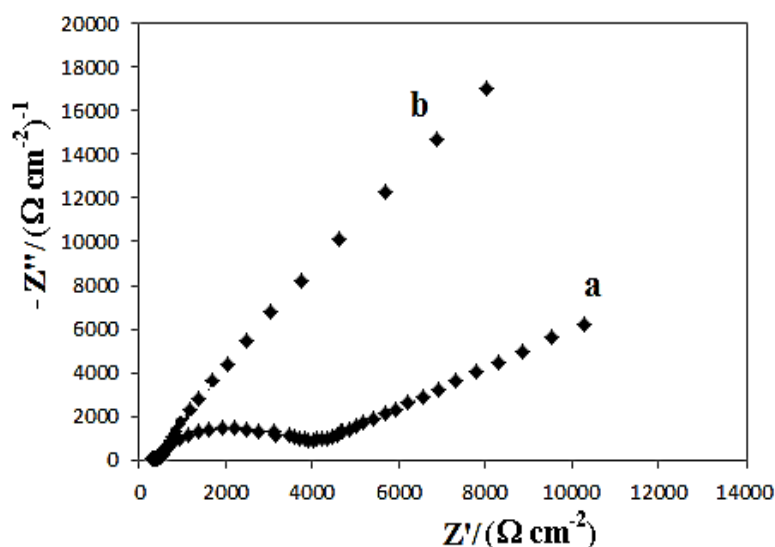

Fig. 5. Nyquist plot of 10 mmol L^{-1} $Fe(CN)_6^{3/4-}$ at: (a) an unmodified GCE; (b) MPTS-MWCNTs modified electrode

3.5. Voltammetric determination of Ceph at MPTS-MWCNT/GCE

Differential pulse voltammetry (DPV) method was used for voltammetric determination of Ceph at the surface of MPTS-MWCNT/GCE (Fig. 6A). Under the optimum experimental conditions, when the concentration of Ceph changed from 5.0×10^{-7} to 5.0×10^{-5} M, the anodic peak current of Ceph and its concentration declared linear relationship with a detection limit of 0.1 μM at MPTS-MWCNT/GCE (Fig. 6B).
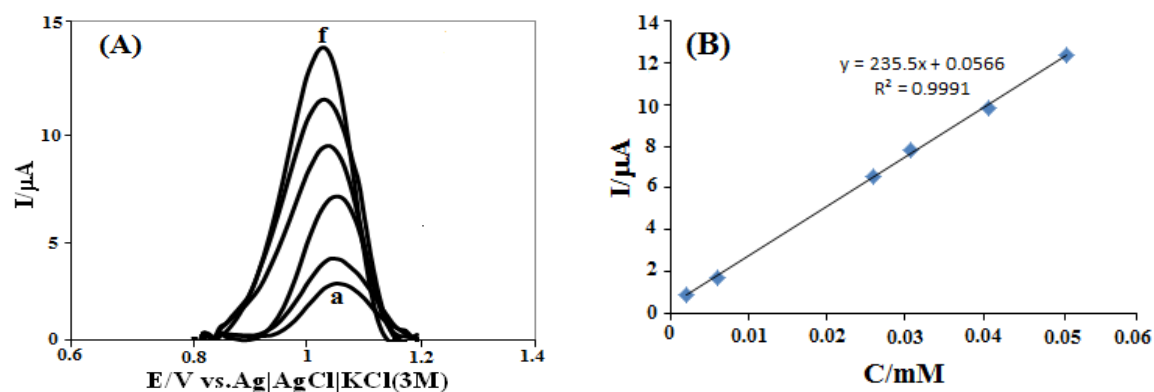
Fig. 6. (A) Differential pulse voltammograms of MPTS-MWCNT/GCE in 0.1 M PBS (pH 7.00) in the presence of (a) 0.5, (b) 5, (c) 25, (d) 30, (e) 40 and (f) 50 µM of Ceph in 0.1 M PBS (pH 7.00); (B) Plot of oxidation peak current *vs.* Ceph concentration

A comparison of the reported works by other research groups, with the present work is tabulated in Table 1 [22, 28-30]. Compared to boron-doped diamond thin-film electrode [22] and heated glassy carbon electrode [30], MPTS-MWCNT/GCE shows improved LOD. The ease and one step procedure of making MPTS-MWCNT, shown in this work, might be however an advantage when it comes to sensing in biological fluids with Ceph concentrations in the low µM region. 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 Ceph at different modified electrodes

Electrode	LDR (µM)	LOD (µM)	Method	Ref.
BDD[a]	800-2500	500	CV	[22]
HMDE[b]	0.1-250	0.05	Polarography	[29]
HGCE[c]	0.6-50	0.15	SWV	[30]
AuNP/GCE	1-1000	0.013	SWV	[28]
MPTS-MWCNT/GCE	0.5-50	0.12	DPV	This work

[a] Boron-Doped Diamond Thin-Film Electrode
[b] Hanging mercury drop electrode
[c] Heated Glassy Carbon Electrode

3.6. Sample analysis of Ceph

For demonstrate the ability of this proposed method for voltammetric determination of Ceph, the MPTS-MWCNT/GCE were applied to analysis of Ceph in Ceph capsule (cephalexin capsule containing 250 mg, from Farabi Co., Iran) and blood serum (from Dr. Safiri laboratory, Iran, Babolsar) samples. In this direction, the cephalexin capsule containing 250 mg, whose shell were removed, were ground to homogeneous exiguous powder in a mortar. Then, an enough weight of the homogenized powder was accurately weighed and transferred into the electrochemical cell containing 10 mL 0.1 M PBS (pH 7.00). The analysis was carried out using the standard addition method. For serum analysis, 1.0 mL of the sample solution plus 9.0 mL of 0.1 M PBS (pH 7.00) were transferred into the experimental cell to measure the Ceph contents using standard addition method. The analytical results are summarized in Table 2. The data obtained for the analysis Ceph 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 Ceph was compared favorably with that obtained by spectrophotometric method [29]. There is no significant difference between the obtained data by spectrophotometric method and those obtained by the proposed method with the satisfactory recovery.

Table 2. Determination of Ceph in tablet and blood serum samples at surface of MPTS-CNT/GCE in 0.1 M PBS (pH 7.00), n=3

Sample	Spiked (µM)	Found (µM)	Labled (mg)	Found (mg)	(Mean recovery ± SD) (%)		t_{exp}	F_{exp}
					Proposed method	**Comparative method**		
Tablet	–	–	250	241.5	96.6±1.2	100.2±0.6	4.1	4.34
Serum	6	5.67	–	–	93.45±1.4			
Serum	8	7.73	–	–	94.5±2.96			

Theoretical values for t=4.6 and F=6.39 (p=0.05)

3.7. Interference study

In order to voltammetric determination of cephalexin in samples at the MPTS-MWCNT/GCE, possible interferences were investigated by the addition of various compounds such as: histidine, aspartic acid, ascorbic acid, cefixime, L-cysteine, glutathione and lysine with concentration of 2.0 mM into pH 7.00 PBS in the presence of 1.0 mM Ceph by cyclic voltammetry. The results indicated that lysine and aspartic acid showed

interference, whereas another mentioned compounds did not affect to oxidation peak of this drug.

4. CONCLUSION

In this paper, we used the synthesized MPTS-MWCNT as a modifier for preparation of modified GCE as an electrochemical sensor for determination of Ceph in aqueous medium. The obtained results demonstrate the excellent electrocatalytic activity towards Ceph oxidation at the surface of MPTS-MWCNT modified GCE and the oxidation peak current of Ceph at the MPTS-MWCNT-GCE can be used for determination of Ceph in aqueous solution, so an acceptable linear dynamic range and detection limit can be obtained. The electrode can be prepared simply and can be used for simple, selective and precise voltammetric determination of Ceph in the real samples.

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