Int. J. Electrochem. Sci., 6 **(2011)** 1355 - 1366

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Voltammetric Determination of Amoxicillin at the Electrochemical Sensor Ferrocenedicarboxylic Acid Multi Wall Carbon Nanotubes Paste Electrode

Masoud Fouladgar[1,*], *Mohammad Reza Hadjmohammadi* [2], *Mohammad Ali Khalilzadeh*[3], *Pourya Biparva*[2], *Nader Teymoori*[3], *Hadis Beitollah*[4],

[1] Department of Biochemistry, Falavarjan Branch, Islamic Azad University- Falavarjan-Iran
[2] Department of Chemistry, Mazandaran University, P.O. Box453, Babolsar, Iran
[3] Department of Chemistry, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran
[4] Department of Geology, Islamic Azad University-Zarand branch-Iran

[*]E-mail: fouladgar@iaufala.ac.ir

Received: 27 December 2010 / *Accepted:* 29 March 2011 / *Published:* 1 May 2011

A sensitive and selective electrochemical method for the determination of amoxicillin (AMX) was developed using a ferrocenedicarboxylic acid modified carbon natubes paste electrode (FDCCNTPE). The modified electrode exhibited good electrocatalytic activity for electrochemical oxidation of amoxicillin at the pH of 10.5 phosphate buffer solution. The diffusion coefficient ($D= 4.62 \times 10^{-5}$ cm^2 s^{-1}), and the kinetic parameter such as the electron transfer coefficient ($\alpha= 0.494$) of AMX at the surface of FDCCNTPE were determined using electrochemical approaches. Under the optimized conditions, the electrocatalytic oxidation peak current of amoxicillin showed two linear dynamic ranges with a detection limit of 8.7 nmol L^{-1} amoxicillin. The linear calibration ranges was in the range of 0.03-0.35 µmol L^{-1} and 0.50-32.70 µmol L^{-1} amoxicillin using square wave voltammetric method. Finally, this modified electrode was also examined for the determination of amoxicillin in real samples such as drug and urine.

Keywords: Amoxicillin, Ferrocenedicarboxylic acid, Carbon nanotube paste elecrode, Electrocatalysis

1. INTRODUCTION

Carbon nanotubes (CNTs), a new form of elementary carbon, are composed of graphitic sheets rolled into closed concentric cylinders with diameter of nanometers and length of micrometers. Since the discovery of CNTs in 1991 [1], they have attracted more and more attention in physical, chemical and material science fields [2] due to their unique characteristic such as high electrical conductivity, chemical stability and high mechanical strength and modulus [3]. CNTs consisting of cylindrical shells

of graphitic sheets with nanometer diameter were found in two distinct types of structures: the single-walled carbon nanotubes (SWNTs) and multi-walled carbon nanotubes (MWNTs). The subtle electronic properties of carbon nanotubes suggest that they have the ability to promote electron transfer reaction when used as the electrode material in electrochemical reaction, which provides a new way in the electrode surface modification for designing new electrochemical sensors [4] and novel electrocatalytic materials [6]. It has been reported that CNTs modified electrodes were successfully applied to study and determine many biological and organic molecules [7].

Amoxicillin, D-α-amino-*p*-hydroxybenzylpenicillin trihydrate, is one of the most frequently used β-lactam antibiotics in the world and it is employed to treat humans and animals [7, 8]. As others β-lactam antibiotics, AMX, presents a structure based on a β -lactam ring responsible for the antibacterial activity and variable side chains that account for the major differences in their chemical and pharmacological properties. After a single oral dose of 500mg, 60–86% of the drug excreted is unchanged in the urine during the first 6 hours. Despite a high level of clinical success, a serious mechanism of resistance had emerged demanding high dose regimen and new pharmacokinetic combination. AMX is one of the more important antibiotics used in the treatment of bacterial infections because of its fair safety and efficacy. As the clinical use of AMX became common, methods for its quantification in drugs and biological fluids have attracted the attention of investigators. Several methods were used for the determination of AMX, including in tablets or urine such as spectrophotometic [9-14], chromatography [15-17], LC/MS/MS [18] and electrochemical methods [19]. Most of the reported methods suffer from disadvantages such as complicated procedure, time consuming, requirement of expensive instruments and low detection sensitivity.

In this study, we describe the use of ferrocenedicarboxylic acid as a mediator in the multi walled carbon nanotube paste electrode for the electrooxidation of AMX in aqueous media at pH 10.5. This electrode was used for the determination of AMX in pharmaceutical and urine samples. Results showed that, this electrochemical sensor were selective and sensitive for determination of AMX in real samples.

2. EXPERIMENTAL

2.1. Materials and apparatus

All of the solutions were freshly prepared using double-distilled water. Amoxicillin and ferrocendicarboxylic acid were analytical grade (Fluka, Buches, Switzerland). Graphite fine powder (Merck, Darmstadt, Germany) and paraffin oil (DC 350, Merck, density=0.88 g cm^{-3}) were used as binding agents for the graphite pastes. All flasks and containers before use were soaked in 6 M HNO3 at least for 24 hours and then rinsed with deionized water. The buffer solutions were prepared from ortho-phosphoric acid and its salts with pH range of 7.0-12.0. Voltammetric measurements were carried out using a computerized potentiostat/galvanostat (SAMA500 Electrochemical Analysis System, SAMA research center, Iran). A Pentium IV computer controlled all settings and data processing of the system. All the electrochemical studies were performed at 25 ± 1 °C. A three electrode assembly was employed for the experiments in a 50 mL glass cell containing an Ag/AgCl

electrode as reference electrode, a platinum wire counter electrode and multi wall carbon nanotubes paste electrode (MWCNTPE) as working electrode. All of the potentials were measured and reported *vs.* Ag/AgCl reference electrode. The pH of the solutions was controlled with a Metrohm pH meter (model 827 pH Lab).

2.2. Synthesis of multi-walled carbon nanotubes

The nanotubes were grown by chemical vapor deposition. Several transition metal catalysts have been shown to be active for generation of carbon nanotubes [20]. In this work MWCNTs were synthesized from acetylene on a Fe:Co: $CaCO_3$ catalyst at 720 ^oC [21]. For the production of carbon nanotubes, approximately 100 mg of catalyst containing 5 wt % Fe- Co with a mole ratio of 1:1 was weighed and spread into a thin layer onto a quartz boat positioned horizontally inside of a resistive tube furnace under nitrogen flow. The furnace temperature was then set at the reaction temperature, while accurately controlled. When temperature reached to 720 ^oC, acetylene was introduced at 3.0 ml/min, while the flow of nitrogen maintained at 200 ml/min. After rinsing the system with nitrogen, reaction product was collected from the quartz boat. For purification, raw MWCNT samples were sonicated (40 kHz) in diluted nitric acid (30% HNO_3) for 30 min, filtered, washed with distilled water to remove acid and finally dried at 120 ^0C overnight. The residue of as-prepared MWCNTs was placed inside a Pyrex tube and oxidized in a furnace at 350 ^oC in air for different time periods to remove carbon impurities. The diameter, length, purity and other specifications of synthesized MWCNTs are summarized in Table 1.

Table 1. Specification of synthesised multi-walled carbon nanotube by chemical vapor deposition method

Catalyst	Co: Fe
Color	Black
Purity	> 95%
Outside Diameter (OD)	8 – 15 nm
Inside Diameter (ID)	3 – 5 nm
Length	10 – 50 μm
Special Surface Area (SSA)	235 m^2/g
Bulk density	0.07 g/cm^3
True density	~2.1 g/cm^3

2.3. Preparation of the electrodes

A 0.01 g of ferrocenedicarboxylic acid was dissolved in diethyl ether and mixed with 89-times its weight of graphite powder and 10-times its weight of multi wall carbon nanotubes with a mortar and pestle. The solvent was evaporated and 5ml Paraffin were added to this mixture and mixed for 20 min until a uniformly-wetted paste was obtained. The paste was then packed into the end of a glass

tube. Electrical contact was made by pushing a copper wire down the glass tube into the back of the mixture. When necessary, a new surface was obtained by pushing an excess of paste out of the tube and polishing it on a weighing paper. Unmodified carbon paste was prepared in the same way without adding ferrocenedicarboxylic acid and carbon nanotubes to the mixture and was used for comparison purposes.

2.4. Preparation of real samples

Ten tablets of amoxicillin were completely ground and homogenized. Then, 37 mg of the powders was accurately weighed and dissolved with ultrasonication in 100 mL of ethanol-water (1:1) solution. This solution was diluted 10-times, and then 1mL portion of solution was diluted in a voltammetric cell to 10 mL of 0.1 mol L^{-1} phosphate buffer (pH =10.5) for the analysis of the sample. Capsule samples were also prepared with the same procedure. The urine samples used for measurements were centrifuged and diluted two times with distilled water without any further pretreatment. The standard addition method was used for the determination of AMX in the real samples.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of FDCCNTPE

Recently the properties of ferrocenedicarboxylic acid carbon paste electrode (FDCPE) were studied in buffered aqueous solution by cyclic voltammetry [22-24]. Its cyclic voltammogram exhibited an anodic peak with E_{pa} =0.49 V and cathodic peak with E_{pc}= 0.38 V. Also, the obtained result showed that the redox process of Fc/Fc^+ in ferrocenedicarboxylic acid is independent from the pH of aqueous solution.

3.2. pH Effect on the electrochemical behavior of AMX

The electrochemical behavior of amoxicillin is dependent on pH value of the aqueous solution [19], whereas the electrochemical properties of Fc/Fc^+ redox couple are independent of pH.

Therefore, the electrochemical behavior of AMX in 0.1 M phosphate buffer solution with various pH (7.0<pH<12.0) were studied at the surface of FDCCNTPE by cyclic voltammetry. Figure 1 shows that the maximum electrocatalytic current for AMX was obtained at pH= 10.5. Therefore, pH 10.5 was chosen as the optimum pH for the determination of AMX at FDCCNTPE.

3.3. Electrocatalytic oxidation of AMX

AMX is an oxidisable compound and can be detected by electrochemical methods based on anodic oxidation. The use of chemically modified electrodes greatly increases the selectivity and

sensitivity toward this compound. As it is shown in Fig. 2 AMX produces a considerable anodic current at E_{pa}= 500 mV using FDCCNTPE, with a decrease at the reduction peak in reversal scan direction, which is a typical electrocatalytic process (Diagram 1).

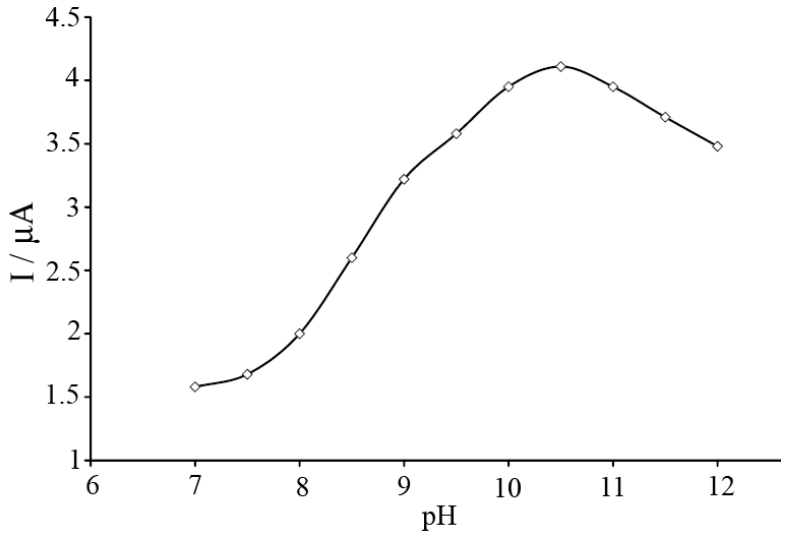

Figure 1. Current-pH curve for electro-oxidation of AMX in 0.1 M phosphate buffer solution with various pH values at the surface of FDCCNTPE with scan rate of 10 mV s^{-1}.

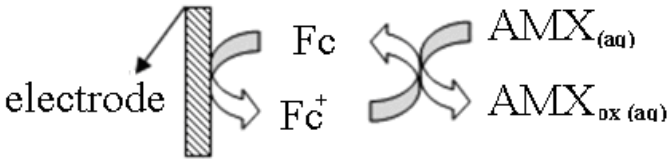

Diagram 1.

The utility of this modified electrode for oxidation AMX was evaluated by cyclic voltammetry.

The cyclic voltammetric responses of unmodified carbon nanotubes paste electrode in 0.1 M phosphate buffer (pH 10.5), without and with AMX in solution, are shown in Fig. 2 a and b respectively. If the electrode is modified with ferrocenedicarboxylic acid and then placed into the same AMX-containing electrochemical cell, a large anodic peak is observed without a cathodic counterpart (Fig. 2d). The current observed here is associated with AMX oxidation and not the oxidation of ferrocenedicarboxylic acid. Fig. 2c shows the cyclic voltammetric behavior of an electrode modified with ferrocenedicarboxylic acid in the absence of AMX in 0.1 M phosphate buffer with pH =10.5. It is apparent that the anodic current with the modified electrode in the absence of AMX is significantly less than that at solution containing it. At the surface of a bare electrode, AMX are not oxidized until 580 mV. As can be seen, electroactivity toward AMX on modified electrode was significant (Figs. 2 c,

d), with strongly defined peak potential, around 500 mV *vs.* Ag / AgCl electrode. Thus, a decrease in over-potential and enhancement in peak current for AMX oxidation were achieved with this modified electrode. Comparison of figures 2c and 2d shows that, the anodic peak current of mediator was greatly increased in the presence of AMX with respect to just Fc/Fc^{+} redox couple spiked in carbon nanotubes paste electrode. The corresponding cathodic peak was disappeared on the reverse scan of the potential.

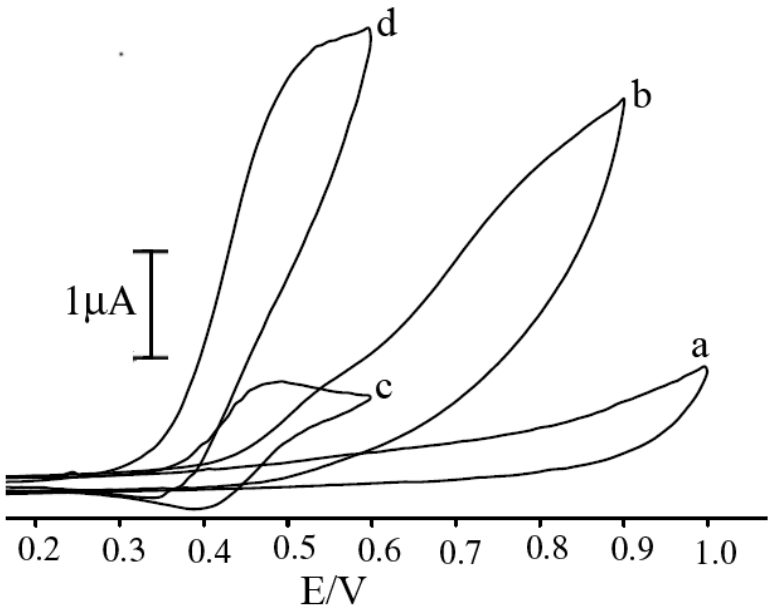

Figure 2. Cyclic voltammograms of FDCCNTPE with scan rate of 10 mV s^{-1} in the buffer solution (pH 10.5). (c) In the absence and (d) in the presence of 150 μmol L^{-1} AMX; (a) as (c) and (b) as (d) for an unmodified carbon nanotubes paste electrode, respectively.

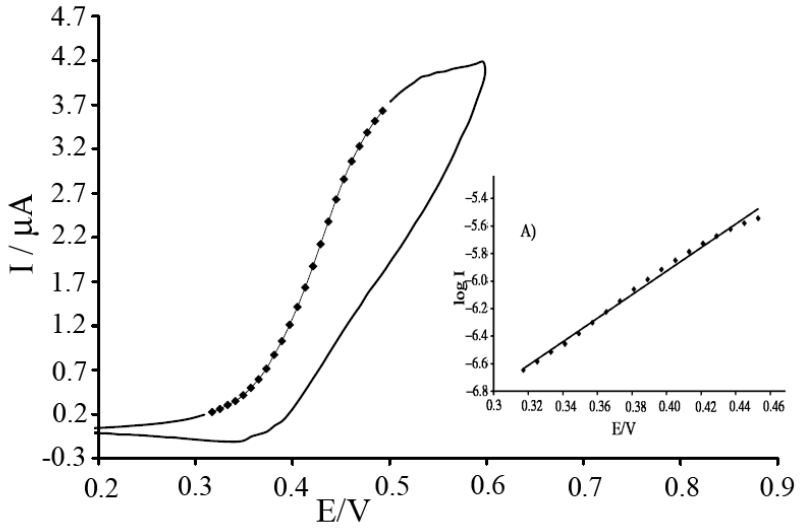

Figure 3. Tafel plot for FDCCNTPE in the presence of 120 μmol L^{-1} AMX in 0.1M phosphate buffer solution (pH =10.5) at scan rate $10mVs^{-1}$.

Results showed that AMX oxidation potential at an unmodified carbon nanotubes paste electrode was irreversible with two peak potential at 580 and 780 mV and that at the FDCCNTPE appeared at 500 mV *vs.* Ag/AgCl at pH=10.5. Therefore, the oxidation of AMX at the surface of the FDCCNTPE occurs at a potential about 80mV less positive than that of an unmodified carbon nanotubes paste electrode.

In order to get the information about the rate determining step, a Tafel plot was developed for FDCCNTPE using the data derived from the raising part of the current–voltage curve (Fig. 3 insert). The slope of the Tafel plot is equal to $n(1-\alpha)F/2.3RT$ or to 8.5458 V decade^{-1}. Using this data gives $n\alpha$ = 0.494. If assuming n = 1, then α = 0.494.

The following equation was used to calculate transfer coefficient [25, 26]:

$$E_P = (b/2) \log v + \text{constant} \quad (1)$$

Based on this equation, the slope of E_p *vs.* log v is b/2. The slope of E_p *vs.* log v was found to be 0.0591 V. Thus, b = 2 × 0.0591 or 0.118 V. According to one electron transfer in Fc/Fc^+ process, the transfer coefficient (α) is equal to 0.5. In addition, the value of αn_α (n_α is the number of electrons involved in the rate determining step) was calculated for the oxidation of AMX at pH 10.5 with the both modified and unmodified carbon nanotubes paste electrodes according to the following equation [27-29]:

$$\alpha n_\alpha = 0.048/(E_P - E_{P/2}) \quad (2)$$

Where $E_{P/2}$ is the potential corresponding to $I_{P/2}$. The values for αn_α were found to be 0.50 and 0.20 at the FDCCNTPE and unmodified carbon paste electrode, respectively. These values show that the overpotential of AMX oxidation is reduced at the surface of FDCCNTPE with a great enhances in the rate of the electron transfer process. This phenomenon is thus confirmed by the larger I_{pa} values recorded during cyclic voltammetry at FDCCNTPE.

3.4. Chronoamperometric measurements of AMX

Double step potential chronoamperometry was also used to investigate the electrochemical behavior of aqueous buffered solution (pH 10.5) containing various concentration of AMX at FDCCNTPE by setting the working electrode potential at 0.25 V (at the first potential step) and 0.70 V (at the second potential step) *vs.* $Ag|AgCl|KCl_{sat}$ (Fig. 4).

As can be seen, there is not any net cathodic current corresponding to the reduction of mediator in the presence of AMX, when the potential is stepped from 0.25 V to 0.70 V *vs.* $Ag|AgCl|KCl_{sat}$. However, in the presence of AMX the charge value associated with forward chronoamperometry is significantly greater than that observed for backward (Fig. 4 insert A (b′, d′)). The linearity of electrocatalytic current *vs.* $\upsilon^{1/2}$ shown that, the current is controlled by diffusion of AMX from bulk solution toward the surface of electrode which shows near-Cottrellian behavior. Therefore, the slope of linear region of Cottrell's plot can be used to estimate the diffusion coefficient of AMX. The slopes of

these lines were used to estimate the diffusion coefficient (D) of AMX in the concentration ranges of 200 – 400 μmol L^{-1}. The mean value of the D found to be 4.62 $\times 10^{-5}$ cm^2 s^{-1}. Therefore, results show that mediator at the surface of FDCCNTPE could catalyze the oxidation of AMX.

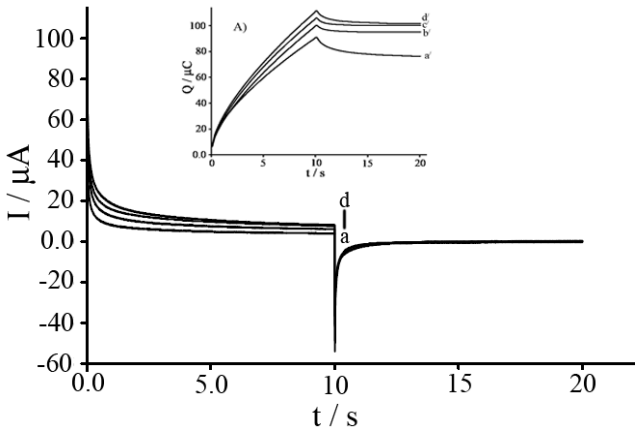

Figure 4. Chronoamperograms obtained at the FDCCNTPE in the absence a) and presence of b) 200, c) 300 and d) 400 μmol L^{-1} of AMX in the buffer solution (pH =10.5). Insert A) Shows the charge-time curves: a′) for curve a), b′) for curve b), c′) for curve c) and d′ for curve d).

Chronoamperometry can also be employed to evaluate the catalytic rate constant, *k*, for the reaction between AMX and the FDCCNTPE according to the method of Galus [30]:

$$I_C / I_L = \gamma^{1/2}[\pi^{1/2} \text{erf}(\gamma^{1/2}) + \text{pxp}(-\gamma)/\gamma^{1/2}] \quad (3)$$

where I_C is the catalytic current of AMX at the FDCCNTPE, I_L is the limiting current in the absence of AMX and $\gamma = kC_bt$ (C_b is the bulk concentration of AMX) is the argument of the error function. In the cases where γ exceeds 2, the error function is almost equal to 1 and therefore the above equation can be reduced to:

$$I_C / I_L = \pi^{1/2} \gamma^{1/2} = \pi^{1/2} (kC_bt)^{1/2} \quad (4)$$

where *t* is the time elapsed (sec). The above equation can be used to calculate the rate constant of the catalytic process *k*. Based on the slope of the I_C / I_L *vs.* $t^{1/2}$ plot; *k* can be obtained for a given AMX concentration. The calculated value of *k* is1.3 $\times 10^3$ M^{-1} s^{-1} using the slope of I_C/I_L *vs.* $t^{1/2}$ plot. This value of *k* represents the sharp feature of the catalytic peak observed for catalytic oxidation of AMX at the surface of FDCCNTPE.

3.5. Electrocatalytic determination of AMX

Since square wave voltammetry (SWV) is more sensitivity than cyclic voltammetry [31], so it was used for determination of AMX (Fig. 5). Results show two linear segments with different slopes

for AMX concentrations: for the first segment (0.03-0.35 µmol L^{-1} AMX), the regression equation was Ip(µA) = 13034 C_{AMX} + 3.4409(r^2 = 0.9879, n =7) and for the second segment (0.50-32.70 µmol L^{-1} AMX) , the regression equation was Ip = 306.37C_{AMX} + 9.7912 (r^2 = 0.9895, n= 6), where C_{AMX} is mM concentration of AMX. The detection limit obtained for the first segment was 8.7 nmol L^{-1} AMX according to the definition of $Y_{LOD} = Y_B + 3\sigma$ [32]. Detection limit, linear dynamic range and the sensitivity for AMX with FDCCNTPE in this case is comparable and even better than those obtained by several other modified electrodes (Table 2).

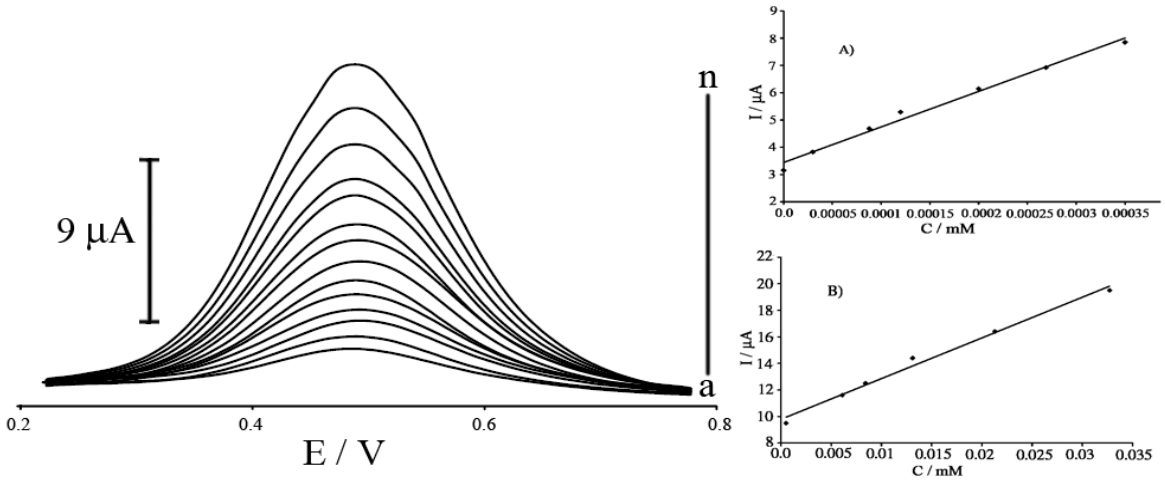

Figure 5. Square wave voltammograms of FDCCNTPE in the buffer solution (pH =10.5) containing different concentration of amoxicillin. A-N corresponds to 0, 0.03, 0.088, 0.12, 0.2, 0.28, 0.35, 0.5, 5.1, 8.4, 13.1, 213 and 32.7 µmol L^{-1} AMX. Inserts show the plots of electrocatalytic peak current as a function of AMX concentration in the range of (A) 0.03-0.35 µmol L^{-1} and (B) 0.50-33.00 µmol L^{-1}.

Table 2. Comparison of the efficiency of some methods in the determination of AMX

Methods	pH	LOD(µM)	LDR(µM)	Ref.
LC-MS/MS		0.0136	0.0136-54.7	[19]
Spectrophotometry			13.6-82	[9]
Chromatography	3.2-3.4	0.041	0.041-1.36	[15]
SWV [1]	5.2	1.06	2-25	[20]
SWV	10.5	0.0087	0.03-32	This work

[1] Square Wave Voltammetry
LOD: Limit of determination.
LOQ: Limit of qualification.

3.6. Interference studies

The influence of various substances as the potential interference compounds on the determination of AMX under the optimum conditions with 5 µmol L^{-1} AMX was studied. The

tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than ± 5% for determination of AMX. After the experiments, we concluded that, 800-fold glucose, Sucrose, Lactose, Fructose, Citric acid, 600-fold methanol, ethanol, K^+, Na^+, Cl^-, Ca^{2+}, Mg^{2+}, SO_4^{2} , Al^{3+},NH^{4+}, F^- , NO_3^- , Saturated of Starch and 300-fold urea did not interfere the determination of AMX.

3.7. Determination of AMX in real sample

Table. 3. Determination of AMX in drug and urine samples

no	sample	added(μM)	Expected (μM)	founded(μM)	Standard methods [19]	F_{ex}	F_{tab}	t_{ex}	t_{tab}
1	Tablet	0	10	9.45 ± 0.24	9.86 ± 0.92	5.6	19.0	2.7	3.8
2		20	30	30.55± 0.33	30.85± 1.33	6.8	19.0	3.1	3.8
3		40	50	50.42 ± 0.42	50.52± 0.87	7.2	19.0	3.2	3.8
4	Capsule	0	10	9.97± 0.63	10.11± 1.2	6.2	19.0	3.1	3.8
5		20	30	29.8± 0.83	30.25± 0.73	5.8	19.0	2.9	3.8
6		10	40	40.66±0.45	39.95± 0.25	3.7	19.0	1.8	3.8
7	Urine	0		<detection limit					
8		10	10	10.31 ± 0.65	10.65± 0.92	3.5	19.0	2.1	3.8
9	Urine[1]			5.62 ± 0.31	5.80± 0.85	5.6	19.0	3.2	3.8
10		10	15.6	15.52± 0.45	15.23± 0.95	4.5	19.0	2.9	3.8

Average of four replicate measurements
[b] Sampling was made after 2.0 h from a man who is sick and used AMX.

To investigate the applicability of the proposed sensor for the electrocatalytic determination of AMX in real samples, we used urine and AMX drug samples. The AMX contents were measured after sample preparation using standard addition method and results are given in Table 3. These results demonstrated the ability of FDCCNTPE for voltammetric determination of AMX in real samples with good recoveries of the spiked AMX and good reproducibility.

4. CONCLUSION

This work shows the ability of ferrocenedicarboxylic acid as a modifier in multi wall carbon nanotube paste electrode for electrocatalytic oxidation of AMX. It was found that under proper condition at pH= 10.5, the oxidation of amoxicillin occurred at a potential about 500 mV on the surface of the modified electrode using cyclic voltametry. The kinetic parameters of the electrocatalytic process and the diffusion coefficient of AMX in an aqueous solution were determined. Finally, the mediated oxidation peak current of AMX at the surface of FDCCNTPE was used for voltammetric determination of AMX in an aqueous solution. Therefore, the electrocatalytic oxidation

of AMX at the surface of this modified electrode can be employed as a new method for the voltammetric determination of AMX in real samples such as drugs and urine.

Acknowledgments

The authors gratefully acknolwledge Islamic Azad University, Falavarjan Branch research council for their support of this work which has been produced from Project No. 301/28102.

References

1. S. Iijima, *Nature*, 354 (1991) 56.
2. Y.P. Sun, K. Fu, Y. Lin, W. Huang, *Acc. Chem. Res*., 35 (2002) 1096.
3. P.M. Ajayan, *Chem. Rev*., 99 (1999) 1787.
4. H. Beitollahi, H. Karimi-Maleh, H. Khabazzadeh, *Anal. Chem.* 80 (2008) 9848.
5. G.P. Jin, J.B. He, Z.B. Rui, F.S. Meng, *Electrochimica Acta,* 51 (2006) 4341.
6. K.P. Gong, Y. Dong, S.X. Xiong, Y. Chen, L.Q. Mao, *Biosens. Bioelectron.* 20 (2004) 253.
7. A.Goodman-Hillman, T. Rall, A. Nier, P. Taylor, *"*The Pharmacological Basis of Therapeutics", McGraw Hill, New York, 1996.
8. J. Reynold (Ed.), Martindale: *"*The Extra Pharmacopoeia*"*, The Pharmaceutical Press, London, 1993.
9. H. Salem, G.A. Saleh, *J. Pharm. Biomed. Anal.* 28 (2002) 1205.
10. A.Pasamontes, M.P. Callao, *Anal. Chim. Acta* 485 (2003) 195.
11. H. Salem, *Anal. Chim. Acta,* 515 (2004) 333.
12. M.Q. Al-Abachi, H. Haddi, A.M. Al-Abachi, *Anal. Chim. Acta*, 554 (2005) 184.
13. A.Garc´ıa-Reiriz, P.C. Damiani, A.C. Olivieri, *Talanta*, 71 (2007) 806.
14. G.G. Mohamed, *J. Pharm. Biomed. Anal.*, 24 (2001) 561.
15. A.Aghazadeh, G. Kazemifard, *J. Pharm. Biomed. Anal*. 25 (2001) 325.
16. J.I.D. Wibawa, D. Fowkes, P.N. Shaw, D.A. Barrett, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.,* 774 (2002) 141.
17. G.Hoizey, D. Lamiable, C. Frances, T. Trenque, M. Kaltenbach, J. Denis, H.Millart, *J. Pharm. Biomed. Anal.,* 30 (2002) 661.
18. A.Wen, T. Hang, S. Chen, Z. L. Ding. Y. Tian. M. Zhang. X. Xu, *J. Pharm. Biomed. Anal.*, 48 (2008) 829.
19. B. Rezaei, S. Damiri., *Elecrtoanalysis*, 21 (2009) 1577.
20. T. Sugai, H. Yoshida, T. Shimada, T. Okazaki, H. Shinohara, S. Bandow,. *Nano Lett.* 3 (2003) 769.
21. E. Couteau, K. Hernadi, J.W. Seo, L. Thien-Nga, Cs. Miko, R. Gaal, L. Forro,. Chem. *Phys. Lett.* 378 (2003) 9.
22. A.A. Ensafi, H. Karimi-Maleh,. *Int. J. Electrochem. Sci.* 5 (2010) 392.
23. J.B. Raoof, R. Ojani, Z. Mohammadpour,. *Int. J. Electrochem. Sci.* 5 (2010) 177.
24. H. Karimi-Maleh, A.A. Ensafi, H.R. Ensafi, *J. Braz. Chem. Sco.* 20 (2009**)** 880.
25. E. S. Takeuchi, R. W. Murray, *J. Electroanal. Chem.*, 189 (1985) 49.
26. J.B. Raoof, R. Ojani, H. Karimi-Maleh., *Int. J. Electrochem. Sci.* 2 (2007) 257.
27. M.H. Pournaghi-Azar, H. Razmi-Nerbin, *J. Electroanal. Chem.*, 488 (2000) 17.
28. J.B. Raoof, R. Ojani, H. Karimi-Maleh, *Electroanalysis*, 20 **(**2008) 1259.
29. E. Mirmomtaz, A.A. Ensafi, H. Karimi-Maleh, *Electroanalysis*, 20 (2008) 1973.
30. Z. Galus, *"*Fundumentals of Electrochemical Analysis", Ellis Horwood, New York, 1976.
31. P. Daneshgar, P. Norouzi, M.R. Ganjali, A.O. Seyedlar, H. Eshraghi, *Colloid and Surface B* 68 (2009) 27.

32. J.N. Miller, J.C. Miller, "Statistics and Chemometrics for Analytical Chemistry", 4th ed., Pearson Education Ltd., Edinburgh Gate, Harlow, Essex, England, 2000.