Int. J. Electrochem. Sci., 8 **(2013)** 1938 - 1948

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Sensitive and Stable Voltammetric Measurements of Norepinephrine at Ionic Liquid–Carbon Nanotubes Paste Electrodes

Mahsa Ansari[1], *Sohrab Kazemi*[1*], *Mohammad A. Khalilzadeh*[1], *Hassan Karimi-Maleh*[2], *Mohammad Bagher Pasha Zanousi*[3]

[1]Department of Chemistry, Science and Research Branch, Islamic Azad University, Mazandaran, Iran
[2]Department of Chemistry, Graduate University of Advanced Technology, Haftbagh Highway, Kerman, Iran
[3] Department of Chemistry, Chalous Branch, Islamic Azad University, Chalous, Iran
[*]E-mail: sohrab_kk@yahoo.com

Received: 5 August 2012 / *Accepted:* 1 December 2012 / *Published:* 1 February 2013

The electrochemistry of norepinephrine (NE) was studied by cyclic voltammetry, chronoamperometry and square wave voltammetry (SWV) at a carbon paste electrode modified by multiwall carbon nanotubes (MWNTs) and room-temperature ionic liquid, 1-methyl-3-butylimidazolium chloride (MBIDZCl),. The cyclic voltammogram showed an irreversible oxidation peak at 0.36 V (vs. $Ag/AgCl_{sat}$), which corresponded to the oxidation of NE. Compared to common carbon paste electrode, the electrochemical response was greatly improved. Under the optimized conditions, the oxidation peak current of NE showed linear dynamic range (0.2–500 μmol L^{-1}) with a detection limit of 0.08 μmol L^{-1}, using SWV method. The proposed sensor was successfully applied for the determination of NE in real samples such as drug and urine.

Keywords: Norepinephrine, Ionic liquid, Multiwall carbon nanotubes, Voltammetry

1. INTRODUCTION

Norepinephrine is a drug belonging to the stimulants that are on the World Anti-Doping Agency's 2005 Prohibited List. It is also critical in mental disease, heart failure; DNA breaks in cardiac myoblast cells, and diabetes. Recent reports have indicated that NE enhances adhesion of human immunodeficiency virus-1 (HIV-1)-infected leukocytes to cardiac micro-vascular endothelial cells and also accelerates HIV replication via proteinkinase [1]. Therefore, it is very essential to develop fast, accurate and sensitive methods for its direct determination. Various methods, including spectrophotometry [2], capillary electrophoresis [3], and high-performance liquid chromatography [4] have been employed to the determination of NE. Because NE is an electroactive compound, its electrochemical detection has been the focus of research for electroanalytical researchers and neurochemists and some modified electrodes have been used to determine NE [5-10].

Since the discovery of carbon nanotubes (CNTs) in 1991 [11], they have received considerable attention in the fields of biotechnology and medicine due to their unique optical, magnetic, electronic and chemical properties, which differ greatly from those of the bulk material [12, 13]. There are four main advantages to the use of a nanotubes-modified electrode compared with a macroelectrode: high effective surface area, mass transfer, catalysis and control over the local microenvironment [14-20]. The catalytic properties of some nanotubes can cause a decrease in the overpotential, producing a more reversible voltammetry than that displayed by the same material in a macroelectrode form. In addition, CNTs can effectively promote electron-transfer reactions.

The better performance of the CNTs electrode compared to carbon electrodes may be due to the carbon nanotube dimensions, the electronic structure, and the topologic defects present on the tube surface [21-31].

In continuation of our studies on chemically modified electrodes [32–52], we used a novel multiwall carbon nanotubes modified carbon ionic liquid paste electrode, which utilizes 1-methyl-3-butylimidazolium chloride as a binder. The electrochemical behavior of NE at multiwall carbon nanotubes modified carbon ionic liquid electrode (MWCNTs/CILE), and at carbon paste electrode (CPE) was investigated. The results showed the superiority of MWCNTs/CILE to the other electrodes in terms of both provision of better reversibility and higher sensitivity.

2. EXPERIMENTAL

2.1. Chemicals

All chemicals used were of analytical reagent grade purchased from Merck (Darmstadt, Germany) unless otherwise stated. Doubly distilled water was used throughout.

A 1.0×10^{-3} mol L^{-1} NE solution was prepared daily by dissolving 0.0170 g NE in water and the solution was diluted to 100 mL with water in a 100–mL volumetric flask. The solution was kept in a refrigerator at 4 ^{o}C in dark. More dilute solutions were prepared by serial dilution with buffer solution.

Phosphate buffer (sodium dihydrogen phosphate and disodium monohydrogen phosphate plus sodium hydroxide, 0.1 mol L^{-1}) solutions (PBS) with different pH values were used.

High viscosity paraffin (d = 0.88 kg L^{-1}) from Merck was used as the pasting liquid for the preparation of the carbon paste electrodes. Spectrally pure graphite powder (particle size<50 μm) from Merck and multiwall carbon nanotubes (>90% MWNT basis, $d \times l$ = (100 – 80 nm) × (5 – 9 μm) from Fluka were used as the substrate for the preparation of the electrodes.

2.2. Apparatus

Cyclic voltammetry (CV), and SWV were performed in an analytical system, Autolab with PGSTAT 302N (Eco Chemie, the Netherlands). The system was run on a PC using GPES software. A conventional three-electrode cell assembly consisting of a platinum wire as an auxiliary electrode and an Ag/AgCl/KCl_{sat} electrode as a reference electrode was used. The working electrode was either an unmodified carbon nanotubes paste electrode, or a MWCNTs/CILE.

2.3. Synthesis of multiwalled 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 [43]. In this work MWCNTs were synthesized from acetylene on a Fe:Co: $CaCO_3$ catalyst at 720 ^oC. 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 (Figure 1). The diameter, length, purity and other specifications of synthesized MWCNTs are summarized in Table 1.

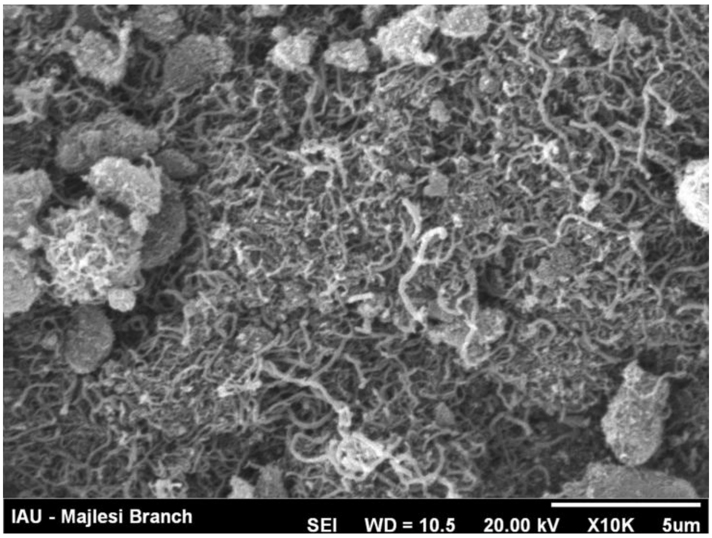


Figure 1. SEM for multi-wall carbon nanotubes.

2.4. Preparation of the modified electrode

To eliminate any metal oxide catalysts within the nanotubes, multiwall carbon nanotubes were refluxed in the 2.0 M HNO_3 for 12 h, and then washed with twice-distilled water and dried at room temperature. MWCNTPE was prepared by hand–mixing of 0.85 g of graphite powder and 0.15 g multiwall carbon nanotubes plus paraffin oil at a ratio of 70/30 (*w*/*w*) and mixed well for 45 min until a uniformly wetted paste was obtained. The paste was then packed into 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 the paste out of the tube and polishing it on a weighing paper.

Table 1. Specification of synthesized multi-walled carbon nanotubes by chemical vapor deposition method

Catalyst	Co:Fe
Color	Black
Purity	> 95%
Outside Diameter (OD)	70–90 nm
Inside Diameter (ID)	100 – 120 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

2.5. Preparation of real samples

Injection solution was prepared (0.1 mg mL^{-1}, Darou Pakhsh Company, Iran) and then 0.10 mL of the solution plus 10 mL of 0.1 mol L^{-1} buffers (pH 7.0) was used for the analysis.

Urine samples were stored in refrigerator immediately after collection. Ten milliliters of the sample was centrifuged for 20 min at 1500 rpm. The supernatant was filtered out using a 0.45 µm filter and then diluted 3–times with the PBS (pH 7.0). The solution was transferred into the voltammetric cell to be analyzed without any further pretreatment. The standard addition method was used for the determination of NE in real samples.

3. RESULTS AND DISCUSSION

3.1. Characteristics of the MWCNTs/CILE

The active surface areas of the modified electrodes are estimated according to the slope of the I_P versus $v^{1/2}$ plot for a known concentration of $K_4Fe(CN)_6$, based on the Randles–Sevcik equation:

$$I_p = 2.69\times10^5 n^{3/2} A D^{1/2} v^{1/2} C_O \qquad (1)$$

where I_{pa} is the anodic peak current, n is the electron transfer number, A is the surface area of the electrode, D_R is the diffusion coefficient, C_0 is the concentration of $K_4Fe(CN)_6$. and ν is the scan rate. For 1.0 mmol L^{-1} $K_4Fe(CN)_6$ in 0.10 mol L^{-1} KCl electrolyte with n = 1 and $D_R = 7.6 \times 10^{-6}$ cm s^{-1} and from the slope of the I_{pa}–$\nu^{1/2}$ relation, the microscopic areas were calculated. They were 0.25, and 0.09 cm^2 for MWCNTs/CILE, and CPE, respectively. The results show that the presence of CNTs and IL together increases the active surface of the electrode.

Fig. 2 (curves a & b) showed the electrochemical responses of MWCNTs/CILE, and CPE in 500 µmol L^{-1} NE in PBS solution (pH 7.0), respectively. At MWCNTs/CILE, and CPE, NE showed an irreversible oxidation peak, with oxidation peak potential (E_{pa}) of 0.36 V, and 0.45 V, respectively. However, the peak current of NE at MWCNTs/CILE was much larger than that at the CPE; it was about 2.8 times larger than CPE by cyclic voltammetry. Thus, the modified electrode exhibited a catalytic activity toward the oxidation of NE. This further testified the superiority of MWCNTs/CILE to CPE and indicated that the use of ILs and MWNTs as modifier facilitated the electron transfer between NE and electrode.

The influence of potential scan rate (ν) on I_p of 500 µmol L^{-1} NE at MWCNTs/CILE was studied by linear sweep voltammetry at various sweep rates. Results show, the peak currents of NE grow with the increase in scan rates and there are good linear relationships between the peak currents and square root of the scan rate ($\nu^{1/2}$). The results also show that the action is mass transfer controlled at sufficient over-potentials.

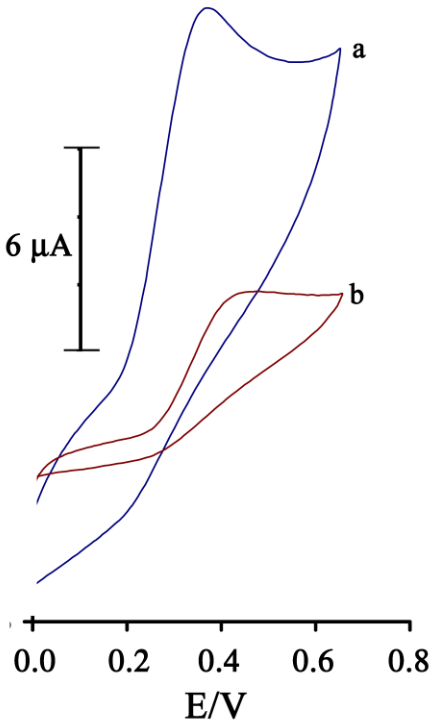


Figure 2. Cyclic voltammograms of (a) MWCNTs/CILE, and (b) CPE in the presence of 500 µmol L^{-1} NE at pH 7.0, respectively.

In addition, with increasing the potential scan rate, the oxidation peak potential gradually shifts towards more positive potentials, suggesting a kinetic limitation in the reaction of NE at a surface of modified electrode. To obtain further information on the rate-determining step, a Tafel plot was developed for the NE at a surface of MWCNTs/CILE using the data derived from the rising part of the current–voltage curve (Fig. 3). The slope of the Tafel plot is equal to $2.3RT/n(1-\alpha)F$, which comes up to 0.1712 V decade^{-1}. We obtained α as 0.8.

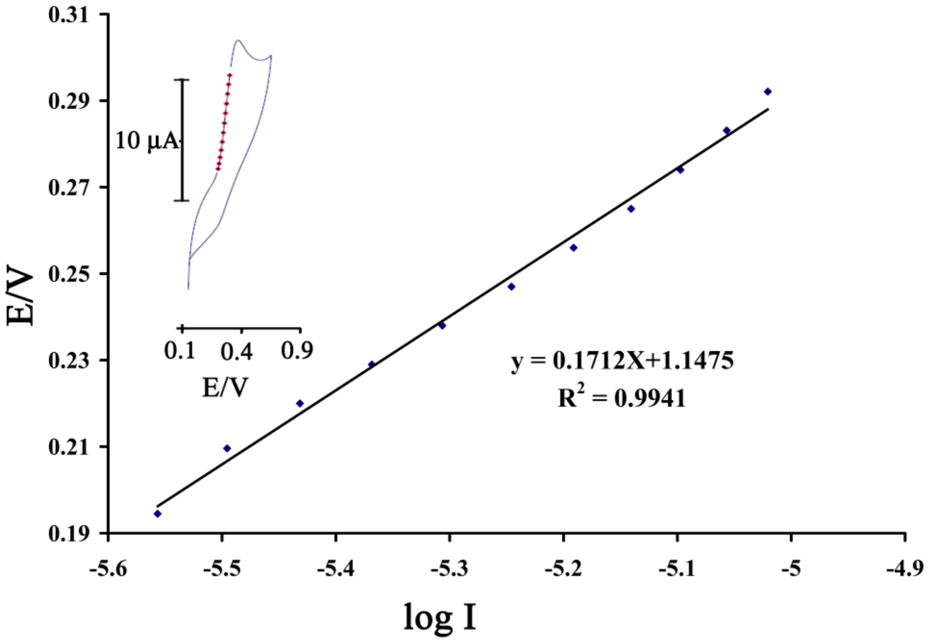
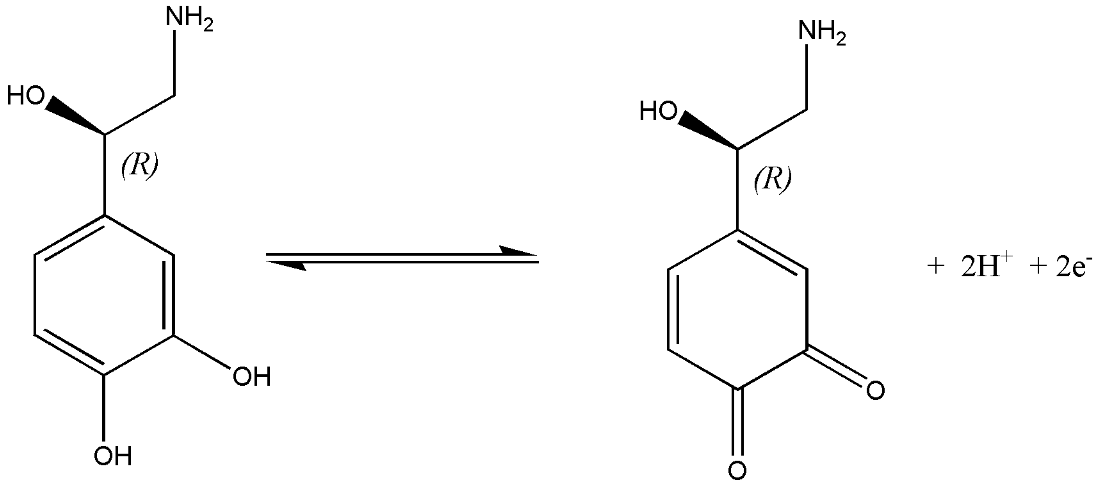


Figure 3. Tafel plot for MWCNTs/CILE in 0.1 M PBS (pH 7.0) with a scan rate of 40 mV s^{-1} in the presence of 500 μmol L^{-1} NE.

Chronoamperometry results obtained for the various concentrations of NE solution using MWCNTs/CILE are shown in Figure 4A.The plot of current signal (I) versus $t^{-1/2}$ recorded for NE solution at various concentrations using MWCNTs/CILE (Fig. 4B) gives straight lines that, given the basis of Cotrell's equation, indicate the current observed is controlled by the diffusion of NE. Diffusion coefficient (D) of NE in solution was then calculated using the plot of slopes of straight lines versus NE concentration. Calculated D for NE is 5.5×10^{-6} cm^2 s^{-1} assuming n = 2 and the electrode surface area (A) of 0.25 cm^2.

3.2. Influence of pH

Cyclic voltammetry was used to study the influence of solution pH on the response of NE over the pH range of 4.0– 8.0. The results showed that the anodic peak potential shifts negatively with increasing the solution pH, indicating that the electrocatalytic oxidation of NE at the MWCNTs/CILE is a proton involved reaction (see scheme 1).



Scheme 1. The mechanism for electrooxidation of norepinephrine

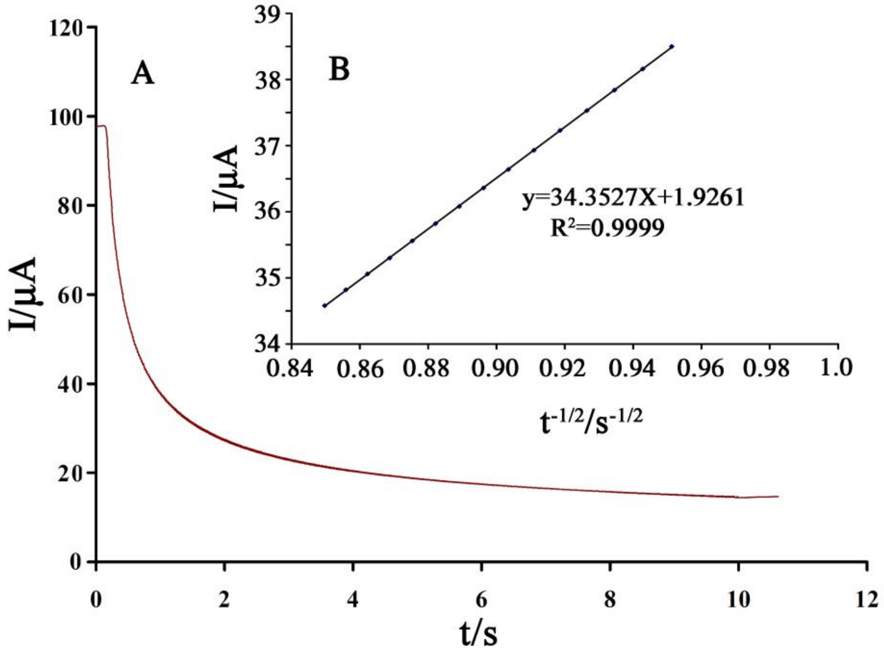


Figure 4. Chronoamperograms obtained at MWCNTs/CILE in the presence of 500 μmol L^{-1} NE in the buffer solution (pH 7.0). Inset: Cottrell's plot for the data from the chronoamperogram.

The relationship between the anodic peak potential and pH fitted into a regression equation is: E_{pa}(mV) = 0.058 pH + 0.87, with a correlation coefficient of 0.9932. The slope (–58.0 mV pH^{-1} units) over the studied pH range is very close to the theoretical value of –59 mV. It indicates that equal electrons and protons contribute to the electrocatalytic oxidation of NE at MWCNTs/CILE [5]. The peak currents are also changed with pH. The largest anodic current also appeared at pH 7.0 (Fig. 5), and so, it was selected throughout the experiments.

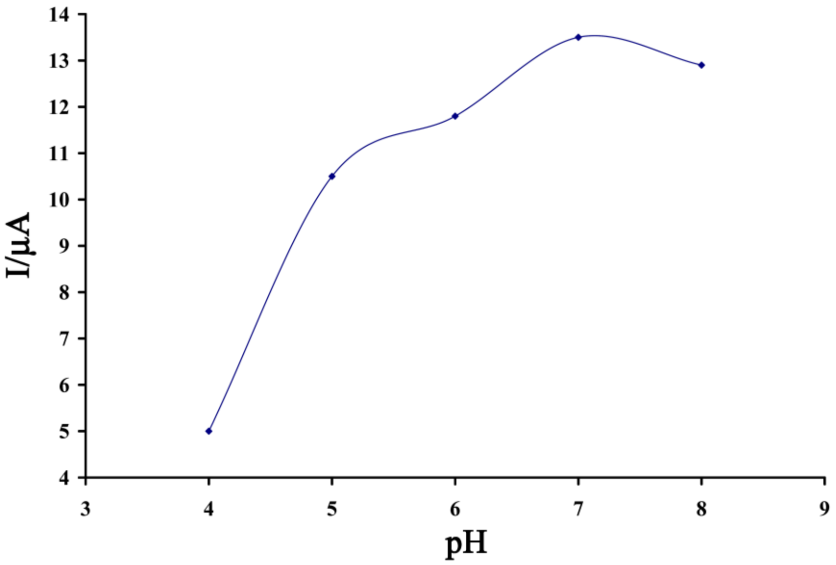


Figure 5. Current–pH curve for electro-oxidation of 500 μmol L^{-1} NE at MWCNTs/CILE with a scan rate of 50 mV s^{-1}.

3.3. Calibration plot and limit of detection

SWV method was used to prepare the calibration plot. The plot of the peak current *vs.*NE concentration consisted of two linear segments with slopes of 0.0857 and 0.0247 μA/μM in the concentration ranges of 0.2 to 30.0 μM and 30.0 to 500.0 μM NE, respectively. The detection limit (3σ) of NE was found to be 0.08 μmol L^{-1}.

3.5. Interference studies

Table 2. Interference study for the determination of 5.0 μmol L^{-1} NE under the optimized conditions.

Species	Tolerante limits ($W_{Substance}/W_{NE}$)
Glucose, Fructose, Lactose, Sucrose, Glycine,	700
Alanine, Phenylalanine, Methionine, Valine, Tryptophan, Glycine, Homocysteine,	500
Urea, Thiourea	300
Cysteine	100

* Ascorbic acid minimized by using ascorbic oxidase enzyme.

Interference studies were carried out with several chemical substances prior to the application of the proposed method for the assay of NE in urine samples and injection solution. The potential interfering substances were chosen from the group of substances commonly found with NE in pharmaceuticals and in biological fluids. The influence of various substances as potential interference compounds on the determination of 5.0 μmol L^{-1} NE under the optimum conditions was studied. Tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than 3% for the determination of NE. The results, given in Table 2, show that the peak current of NE is not affected by all conventional cations, anions, and organic substances [5].

3.6. Real sample analysis

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of NE in injection solution, serum and urine samples. Based on the repeated SWV responses (n=3) of the diluted analyte and the samples that were spiked with specified concentration of NE, measurements were made for determination of NE concentrations in the pharmaceutical, serum and urine preparations. The results are listed in Table 3. The results presented in Table 3 indicate that the modified electrode retained its efficiency for the determination of NE in real samples with satisfactory results.

Table 3. Determination of NE in drug, serum and urine samples.

Sample	Added(μmol L^{-1})	Expected(μmol L^{-1})	Founded(μmol L^{-1})	Recovery (%)
Ampoule	—	10.0	10.4±0.5	104
	10.0	20.0	20.5±0.6	102.5
	5.0	25.0	24.7±0.5	98.8
Urine	—	—	<Limit of detection	—
	40.0	40.0	40.9±1.1	102.25
	20.0	60.0	60.8±0.9	101.3
Serum	—	—	<Limit of detection	—
	15.0	15.0	14.6±0.5	97.3

±Shows the standard deviation.

7. CONCLUSION

This work describes the ability of the modified ionic liquids-multiwall carbon paste electrode for determination of NE. The voltammetric investigation demonstrates that electrooxidation of NE at the surface of MWCNTs/CILE showed very distinct characteristics which due to the presence of MWCNTs and ionic liquid layer on the surface of electrode. The proposed modified electrode presented a low detection limit and good linear range and reproducibility which make it a suitable NE sensor for practical applications.

ACKNOWLEDGEMENTS

The authors wish to thank Science and Research Branch, Islamic Azad University, for their support.

References

1. D. Voet, J.G. Voet, Biochemistry, 2nd ed. Wiley, New York, 1995.
2. M. Zhu, X.M. Huang, J. Li, H.X. Shen, *Anal. Chim. Acta* 357 (1997) 261.
3. S. Wei, G. Song, J.M. Lin, *J. Chromatogr. A* 1098 (2005) 166.
4. V. Carrera, E. Sabater, E. Vilanova, M.A. Sogor, *J. Chromatogr. B* 847 (2007) 88.
5. S. Salmanpour , T. Tavana, A. Pahlavan, M.A. Khalilzadeh, A.A. Ensafi, H. Karimi-Maleh, H. Beitollahi, E. Kowsari, D. Zareyee, Mat. Sci. Eng. C 32 (2012) 1912.
6. H. Dong, S. Wang, A. Liu, J.J. Galligan, G.M. Swain, M.D. Hawley, *J. Electroanal. Chem.* 632 (2009) 20.
7. H. Beitollahi, A. Mohadesi, S. Khalilizadeh Mahani, H. Karimi-Maleh, A. Akbari, (2012) *Ionics* DOI 10.1007/s11581-012-0669-0.
8. M.R. Akhgar, H. Beitollahi, M. Salari, H. Karimi-Maleh, H. Zamani, *Anal. Methods*, 4 (2012) 259.
9. A.R. Taheri, A. Mohadesi, D. Afzali, H. Karimi-Maleh, H. Mahmoudi Moghaddam, H. Zamani, Z. Rezayati zad, *Int. J. Electrochem. Sci.*, 6 (2011) 171.
10. H. Beitollahi, H. Karimi-Maleh, H. Khabazzadeh, *Anal. Chem.* 80 (2008) 9848.
11. S. Lijima, *Nature* 345 (1991) 56.
12. H. Pan, Y.W. Zhang, V.B. Shenoy, H. Gao, *ACS Catal.*, 1 (2011) 99.
13. S. Komathi, A. I. Gopalan and K. P. Lee, *Analyst* 135 (2010) 397.
14. H. Yaghoubian, H. Karimi-Maleh, M.A. Khalilzadeh, F. Karimi, *J. Serb. Chem. Soc.* 74 (2009) 1443.
15. H. Yaghoubian, H. Karimi-Maleh, M.A. Khalilzadeh, F. Karimi, *Int. J. Electrochem. Sci.*, 4 (2009) 993.
16. M.A. Khalilzadeh, H. Karimi-Maleh, A. Amiri, F. Gholami, R. Motaghed mazhabi, *Chin. Chem. Lett.*, 21 (2010) 1467.
17. A.A. Ensafi, H. Karimi-Maleh, *Electroanalysis* 22 (2010) 2558.
18. A.A. Ensafi, M. Taei, T. Khayamian, H. Karimi-Maleh, F. Hasanpour, *J. Solid State Electrochem.* 14 (2010) 1415.
19. A.A. Ensafi, H. Karimi Maleh, *Int. J. Electrochem. Sci.*, 5 (2010) 1484.
20. A.A. Ensafi, E. Khoddami, B. Rezaei, H. Karimi-Maleh, *Coll. Surf. B* 81 (2010) 42.
21. M.A. Khalilzadeh, H. Karimi-Maleh, *Anal. Lett.*, 43 (2010) 186.
22. A.A. Ensafi, H. Karimi-Maleh, *Int. J. Electrochem. Sci.*, 5 (2010) 392.
23. J.B. Raoof, R. Ojani, H. Karimi-Maleh, *Chin. Chem. Lett.*, 21 (2010) 1462.
24. A.A. Ensafi, H. Karimi-Maleh, *J. Electroanal. Chem.*, 640 (2010) 75.
25. A.A. Ensafi, B. Rezaei, Z. Mirahmadi-Zare, H. Karimi-Maleh, *J. Braz. Chem. Soc.*, 22 (2011) 1315.
26. A.A. Ensafi, H. Karimi-Maleh, S. Mallakpour, B. Rezaei, *Coll. Surf. B* 87 (2011) 480.
27. D. Afzali, H. Karimi-Maleh, M.A. Khalilzadeh, *Environ. Chem. Lett.*, 9 (2011) 375.
28. A.A. Ensafi, M. Monsef, B. Rezaei, H. Karimi-Maleh, *Anal. Methods*, 4 (2012) 1332.
29. A.A. Ensafi, M. Izadi, H. Karimi-Maleh, *Ionics*, (2012) DOI 10.1007/s11581-012-0705-0.
30. J.B. Raoof, R. Ojani, H. Karimi-Maleh, M.R. Hajmohamadi, P. Biparva, *Anal. Methods*, 3 (2011) 2637.
31. J.B. Raoof, R. Ojani, H. Karimi-Maleh, *Bull. Chem. Soc. Ethiop.* 22 (2008) 173.
32. E. Mirmomtaz, A.A. Ensafi, H. Karimi-Maleh, *Electroanalysis* 20 (2008) 1973.
33. J.B. Raoof, R. Ojani, H. Karimi-Maleh, *Electroanalysis* 20 (2008) 1259.
34. M.A. Khalilzadeh, F. Khaleghi, F. Gholami, H. Karimi-Maleh, *Anal. Lett.*, 42 (2009) 584.

35. J. B. Raoof, R. Ojani, H. Karimi-Maleh, *J. Appl. Electrochem.*, 39 (2009) 1169.
36. H. Karimi-Maleh, A.A. Ensafi, H.R. Ensafi, *J. Braz. Chem. Soc.*, 20 (2009) 880.
37. H. Karimi-Maleh, A.A. Ensafi, A.R. Allafchian, *J. Solid State Electrochem.*, 14 (2010) 9.
38. A.A. Ensafi, A. Arabzadeh, H. Karimi-Maleh, *Anal. Lett.*, 43 (2010) 1976.
39. A.A. Ensafi, H. Karimi-Maleh, M. Ghiaci, M. Arshadi, *J. Mater. Chem.*, 21 (2011) 15022.
40. A.A. Ensafi, H. Karimi-Maleh, S. Mallakpour, M. Hatami, *Sens. Actuators B* 155 (2011) 464.
41. M. Keyvanfard, A.A. Ensafi, H. Karimi-Maleh, *J. Solid State Electrochem.*, (2012) DOI 10.1007/s10008-011-1570-x.
42. M. Roodbari Shahmiri, A. Bahari, H. Karimi-Maleh, R. Hosseinzadeh, N. Mirnia, *Sens. Actuators B* 177 (2013) 70.
43. M. Keyvanfard, R. Shakeri, H. Karimi-Maleh, K. Alizad, *Mat. Sci. Eng. C* 33 (2013) 811.
44. B. Rezaei, N. Majidi, A.A. Ensafi, H. Karimi-Maleh, *Anal. Methods*, 3 (2011) 2510.
45. A.A. Ensafi, S. Dadkhah-Tehrani, H. Karimi-Maleh, *Anal. Sci.* 27 (2011) 409.
46. S. Kazemi, H. Karimi-Maleh, R. Hosseinzadeh, F. Faraji, *Ionics*, DOI 10.1007/s11581-012-0816-7.
47. H. Beitollah, M. Goodarzian, M.A. Khalilzadeh, H. Karimi-Maleh, M. Hassanzadeh, M. Tajbakhsh, *J. Mol. Liq.* 173 (2012) 137.
48. M. Asnaashariisfahani, H. Karimi-Maleh, H. Ahmar, A.A. Ensafi, A.R. Fakhari, M.A. Khalilzadeh, F. Karimi, *Anal. Methods*, 4 (2012) 3275.
49. H. Karimi-Maleh, M. Keyvanfard, K. Alizad, V. Khosravi, M. Asnaashariisfahani, *Int. J. Electrochem. Sci.* 7 (2012) 6816.
50. S. Mallakpour, M. Hatami, A.A. Ensafi, H. Karimi-Maleh, *J. Solid State Electrochem*. 15 (2011) 15:2053.
51. M. Arshadi, M. Ghiaci, A.A. Ensafi, H. Karimi-Maleh, S.L. Suib, *J. Mol. Catal. A* 338 (2011) 71.
52. A.A. Ensafi, H. Karimi-Maleh, S. Mallakpour, *Electroanalysis* 23 (2011) 1478.
53. E. Couteau, K. Hernadi, , J.W. Seo, L. Thien-Nga, Cs. Miko, R. Gaal, L. Forro,. *Chem. Phys. Lett.* 378 (2003) 9.