Simultaneous Voltammetric Determination of Norepinephrine and Folic Acid at the Surface of Modified Carbon Nanotube Paste Electrode

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The redox response of a modified carbon nanotube paste electrode of ferrocenemonocarboxylic acid (FMC) was investigated. Differential pulse voltammetric peak currents of norepinephrine (NE) increased linearly with its concentrations at the range of 0.52 μM to 530 μM and the detection limit (3σ) was determined to be 0.16 μM. The diffusion coefficient (D) and transfer coefficient (α) of NE were also determined. Mixture of NE and folic acid (FA) can be separated from one another by differential pulse voltammetry. These conditions are sufficient to allow determination of NE and FA both individually and simultaneously.

1. INTRODUCTION

Norepinephrine (NE) is an important catecholamine neurotransmitter in the mammalian central nervous system [1]. NE functions as a neurotransmitter, which is secreted and released by the adrenal glands and the noradrenergic neurons during synaptic transmission. It can be used for treating

myocardial infarction hypertension, bronchial asthma and organic heart disease. Extreme abnormalities of NE concentration levels may lead to the occurrence of many diseases, such as ganglia neuroblastoma, ganglion neuronal, paraganglioma, Parkinson' disease etc. So it is very necessary to develop fast, accurate and sensitive methods for the direct detection of NE. Many methods, such as high-performance liquid chromatography [2], spectrophotometry [3], ion chromatography [4] and fluorometry [5] were employed for the determination of NE. However, most of these methods have their shortcomings, such as comparatively high detection limit, time-consuming, low sensitivity and complicated process. Consequently, the electrochemical method [6,7] has attracted much attention because of its quick response, relatively high sensitivity and the ability to be miniaturized.

Folic acid (FA), is a form of the water-soluble vitamin B_9. Folate is also found in vegemite or marmite. Folate is also synthesized in bacteria. Several important nutrients serve as building blocks of a healthy pregnancy. FA is one such nutrient of great importance, especially for women planning for pregnancy. Adequate folate intake during the preconception period helps protect against a number of congenital malformations [8]. The most notable birth defects that occur from folate deficiency are neural tube defects, which result in malformations of the spine (spina bifida), skull, and brain anencephaly [9,10]. Women planning for pregnancy are advised to eat foods fortified with FA or to take supplements in addition to eating folate-rich foods to reduce the risk of some serious birth defects. Taking 400 µg of synthetic FA daily from fortified foods and/or supplements has been suggested. The Recommended Dietary Allowance (RDA) for folate equivalents for pregnant women is 600−800 µg, twice the normal RDA of 400 µg for women who are not pregnant. Recent research has shown that it is also very important for men who are planning on fathering children, reducing birth defect risks [11]. Numerous methods for the measurement of FA are available, including enzyme-linked immunosorbent assays (ELISAs) [12], liquid chromatography/tandem mass spectrometry (LC/MS/MS) [13], capillary electrophoresis (CE) [14], microemulsion electrokinetic chromatography (MEEKC) [15] and high-performance liquid chromatography (HPLC) [16]. As FA is an electroactive component, some electrochemical methods have been reported for its determination [17-19]. Comparing with other technologies, electrochemical method is more desirable because of its convenience and low cost.

Carbon nanotubes (CNTs) are new kinds of porous nanostructured carbon materials, which are promising as immobilization substances because of their significant mechanical strength, excellent electrical conductivity, high surface area and good chemical stability [20]. CNTs can be used to promote electron transfer reactions when used as electrode materials in electrochemical devices, in electrocatalysis and electroanalysis processes due to their significant mechanical strength, high electrical conductivity, high surface area, good chemical stability, as well as relative chemical inertness in most electrolyte solutions and a wide operation potential window [21-25]. Both redox mediators and CNTs exhibited excellent electrochemical performance for the fabrication of sensors or biosensors. Synergistic effects in the enhanced current response were observed when both CNTs and redox mediators were employed [26-30].

The chemical modification of electrodes using electron transfer mediators is an interesting field in analytical chemistry. One of the most important effects of any mediator is a reduction of the overpotential required for electrochemical reaction, which enhances the sensitivity (current) and selectivity of the method [31-33].

Carbon-based electrodes are among the most commonly used electrodes in voltammetric techniques because of their low cost, wide potential windows, low electrical resistances and versatility of chemical modification. Many types of carbon-based electrodes, such as glassy carbon, carbon composites and carbon pastes, are used as modified electrodes [34-38].

In the present work, we described initially the preparation and suitability of a ferrocenemonocarboxylic acid modified carbon nanotube paste electrode (FMCMCNPE) as a new electrode in the electrocatalysis and determination of NE in an aqueous buffer solution. Then we evaluated the analytical performance of the modified electrode in quantification of NE in the presence of FA. Finally, in order to demonstrate the catalytic ability of the modified electrode in the electrooxidation of NE and FA in real samples, we examined this method for the voltammetric determination of NE and FA in pharmaceutical preparations.

2. EXPERIMENTAL

2.1. Apparatus and reagents

All the cyclic voltammetric measurements were performed using a BHP 2063[+] Electrochemical Analysis System, Behpajooh, Iran potentiostat/galvanostat. An Ag / AgCl / KCl 3 M, a platinum wire, and a FMCMCNPE were used as reference, auxiliary and working electrodes, respectively. A digital pH/mV meter (Metrohm model 710) was applied for pH measurements. NE, FA, carbon nanotubes, graphite powder, paraffin oil and reagents were analytical grade from Merck. The buffer solutions were prepared from ortho phosphoric acid and its salts in the pH range 2.0-12.0.

2.2. Preparation of the electrode

Modified carbon nanotube paste electrodes were prepared by dissolve 0.01 g of FMC in diethyl ether and hand mixing with 89-times its weight of graphite powder and 10-times its weight of carbon nanotubes with a mortar and pestle. The solvent was evaporated by stirring. A 70:30 (w/w) mixture of FMC spiked carbon nanotubes powder and paraffin oil was blended by hand mixing for 20 min until a uniformly-wetted paste was obtained. The paste was then packed into the end of a glass tube (*ca.* 3.0 mm *i.d.* and 8 cm long). Electrical contact was made by inserting a copper wire into the glass tube at 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, prepared in the same way without adding FMC and carbon nanotube to the mixture and was used for comparison purposes.

3. RESULTS AND DISCUSSION

3.1. Electrochemistry of FMCMCNPE

Cyclic voltammetry was employed for investigation the electrochemical properties of FMCMCNPE in a pure buffered aqueous solution (pH 7.0). The cyclic voltammogram exhibits an

anodic and corresponding cathodic peaks with E_{pa}=0.36 V and E_{pc}=0.23 V vs. Ag / AgCl / KCl 3 M. The electrode capability for the generation of a reproducible surface was examined by cyclic voltammetric data obtained in optimum solution pH from five separately prepared FMCMCNPEs. The calculated RSDs for various parameters accepted as the criteria for a satisfactory surface reproducibility (1 – 4%). This degree of reproducibility is virtually the same as that expected for the renewal or ordinary carbon paste surface.

3.2. Electro-catalytic oxidation of NE

The utility of the modified electrode for oxidation of NE was evaluated by cyclic voltammetry. The cyclic voltammetric responses of a bare carbon-paste electrode in 0.1M phosphate buffer (pH 7.0), without and with NE, are shown in Fig. 1 (curves c and d, respectively).
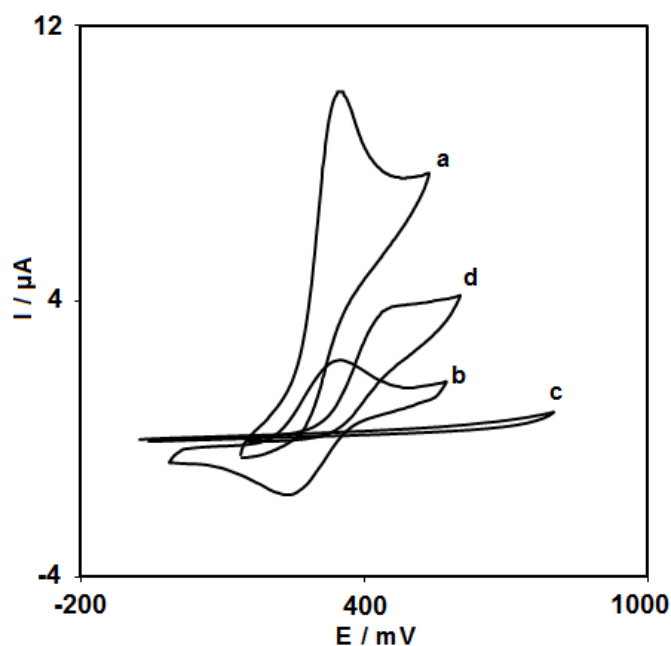

Figure 1. Cyclic voltammograms of FMCMCNPE at 10 mV s^{-1} in 0.1M phosphate buffer (pH 7.0): (a) In the presence and (b) in the absence of 0.25 mM NE; (c) and (d) for an unmodified carbon-paste electrode in the absence and presence of 0.25 mM NE, respectively.

Figures 1a and b show cyclic voltammograms of modified electrode in the buffer solution with 250 μM of NE and without NE, respectively. The results show that the sensor produces a large anodic peak current in the presence of NE without a cathodic counterpart (Fig. 1, curve a). That the current observed is associated with NE oxidation and not the oxidation of modifier is demonstrated by comparing the current in Fig. 1 (curve b, without NE) with the one in the presence of NE in Fig. 1 (curve a). It is apparent that the anodic current associated with the surface-attached materials is significantly less than that obtained in the solution containing NE. At the surface of a bare electrode, NE was oxidized around 480 mV. As can be seen, the electroactivity of NE on the modified electrode

was significant (Figs. 1 curve a), with strongly defined peak potential, around 360 mV vs. Ag/AgCl electrode. Thus, a decrease in overpotential and enhancement of peak current for NE oxidation are achieved with the modified electrode. Such a behavior is indicative of an EC´ mechanism [39].

The effect of scan rate on the electrocatalytic oxidation of 250 µM NE at the modified electrode was investigated by linear sweep voltammetry (Fig.2.A).
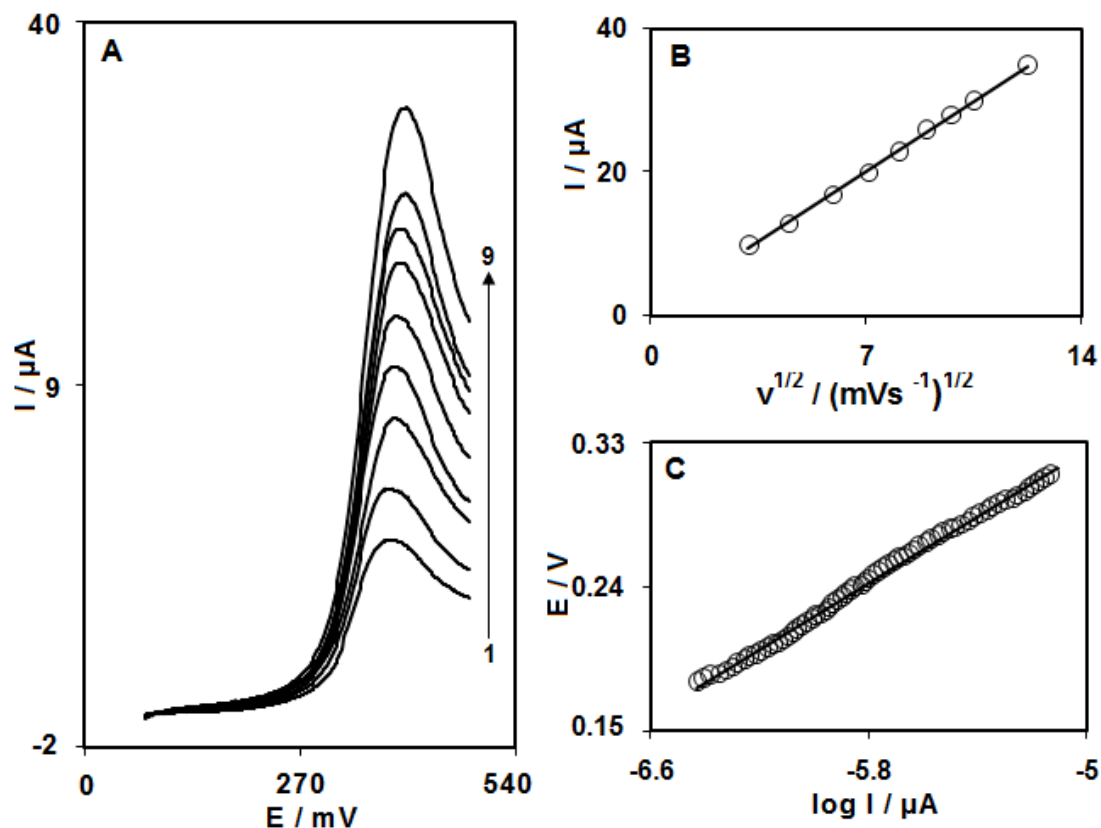
Figure 2. (A) Linear sweep voltammograms of the FMCMCNPE in the presence of 250 µM NE at various scan rates; The numbers 1-9 correspond to 10, 20, 35, 50, 65 80, 95, 110 and 150 mVs^{-1} scan rates, respectively. (B) The variation of the anodic peak currents vs. $v^{1/2}$. (C) Tafel plot derived from the rising part of voltammogram recorded at a scan rate 10 mVs^{-1}

The oxidation peak potential shifts with increasing scan rates toward a more positive potential, confirming the kinetic limitation of the electrochemical reaction. Also, a plot of peak height (Ip) against square root of scan rate ($v^{1/2}$), in range of 10– 150 mVs^{-1}, was constructed, which was found to be linear, suggesting that at sufficient overpotential the process is diffusion rather than surface-controlled (Fig.2 B).

Fig. 2.C, shows a Tafel plot that was drawn from data of the rising part of the current–voltage curve recorded at a scan rate of 10 mVs^{-1}. This part of voltammogram, known as Tafel region [39], is affected by electron transfer kinetics between NE and FMC, assuming the deprotonation of substrate as a sufficiently fast step. In this condition, the number of electron involved in the rate determining step

can be estimated from the slope of Tafel plot. A slope $0.1039 V decade^{-1}$ is obtained indicating a one electron transfer to be rate limiting assuming a transfer coefficient of $\alpha = 0.43$.

3.3. Chronoamperometric studies

The catalytic oxidation of NE by a modified electrode was also studied by chronoamperometry (Fig. 3). Chronoamperometric measurements of different concentrations of NE at modified electrode were done by setting the working electrode potential at 400 mV.
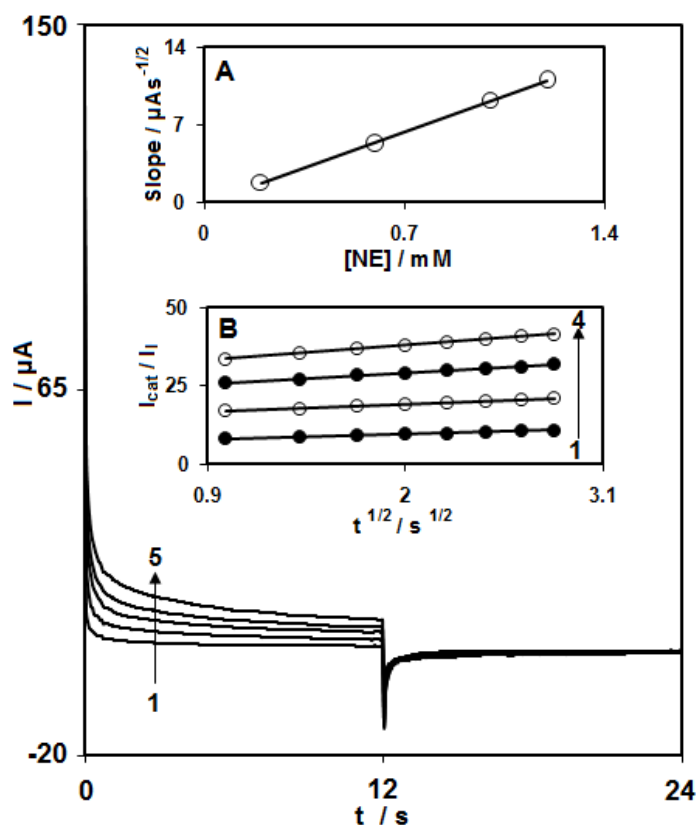
Figure 3. Chronoamperograms obtained at FMCMCNPE in 0.1 M phosphate buffer solution (pH 7.0) for different concentration of NE. The numbers 1–5 correspond to 0.0, 0.2, 0.6, 1.0 and 1.2 mM of NE. Insets: (A) plot of the slope of the straight lines against the NE concentration. (B) Dependence of I_{cat}/I_l on $t^{1/2}$ derived from the data of chronoamperograms.

In chronoamperometric studies, we have determined the diffusion coefficient, D, of NE. The experimental plots of I versus $t^{-1/2}$ with the best fits for different concentrations of NE were employed. The slopes of the resulting straight lines were then plotted versus the NE – concentrations (Fig. 3A), from whose slope and using the Cottrell equation [39]

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \qquad (1)$$

we calculated a diffusion coefficient of 2.96×10^{-6} cm^2 s^{-1} for NE .

Also, chronoamperometry can also be employed to evaluate the catalytic rate constant, k, for the reaction between NE and the FMCMCNPE according to the method of Galus [40]:

$$I_C / I_L = \pi^{1/2} \gamma^{1/2} = \pi^{1/2} (kC_b t)^{1/2} \qquad (2)$$

where t is the time elapsed and C_b is the bulk concentration of NE. 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 versus $t^{1/2}$ plot; k can be obtained for a given NE concentration. Such plots were obtained from the chronoamperograms in Fig. 3.B. From the values of the slopes an average value of k was found to be k = 3.84×10^3 M^{-1}s^{-1}.

### 3.4. Electrocatalytic Determination of NE

The electrocatalytic peak current of NE oxidation at the surface of the modified electrode can be used for determination of NE in solution. Therefore, differential pulse voltammetry experiments were performed using modified electrode in phosphate buffer solution containing various concentration of NE. The results show the electrocatalytic peak current of NE oxidation at the surface of modified electrode was linearly dependent on the NE concentrations. The mediated oxidation peak currents of NE at the surface of a modified electrode were proportional to the concentration of the NE within the ranges 5.2×10^{-7} M– 5.3×10^{-4} M (with a correlation coefficient of 0.9988) in the differential pulse voltammetry. The detection limits (3σ) wad 1.6×10^{-7} M.

3.5. Simultaneous determination of NE and FA at FMCMCNPE

One of the main objectives of the present study was the development of a modified electrode capable of the electro-catalytic oxidation of NE and separation of the electrochemical responses of NE and FA. The utilization of the modified electrode for the simultaneous determination of NE and FA was demonstrated by simultaneously changing the concentrations of NE and FA. The differential pulse voltammetric results show two-well-defined anodic peaks (Fig. 4), while the bare carbon paste electrode only gave an overlapped and broad oxidation peak. Fig. 4 insets A and B show the dependence of differential pulse voltammetric peak currents on the concentration of NE and FA respectively.

3.6. Determination of NE in NE injection

In order to assess the applicability of the proposed electrode, an attempt was made to determine NE in NE injection using the standard addition method. The potentials were controlled between 0.15 and 0.45 V for scan rate of 10 mVs^{-1}. The average determination results of NE in the injection were 1.03 mg mL^{-1}, which were quite corresponding to the value that was given by injection specification (1.0 mg mL$^{-1)}$. (Table 1).
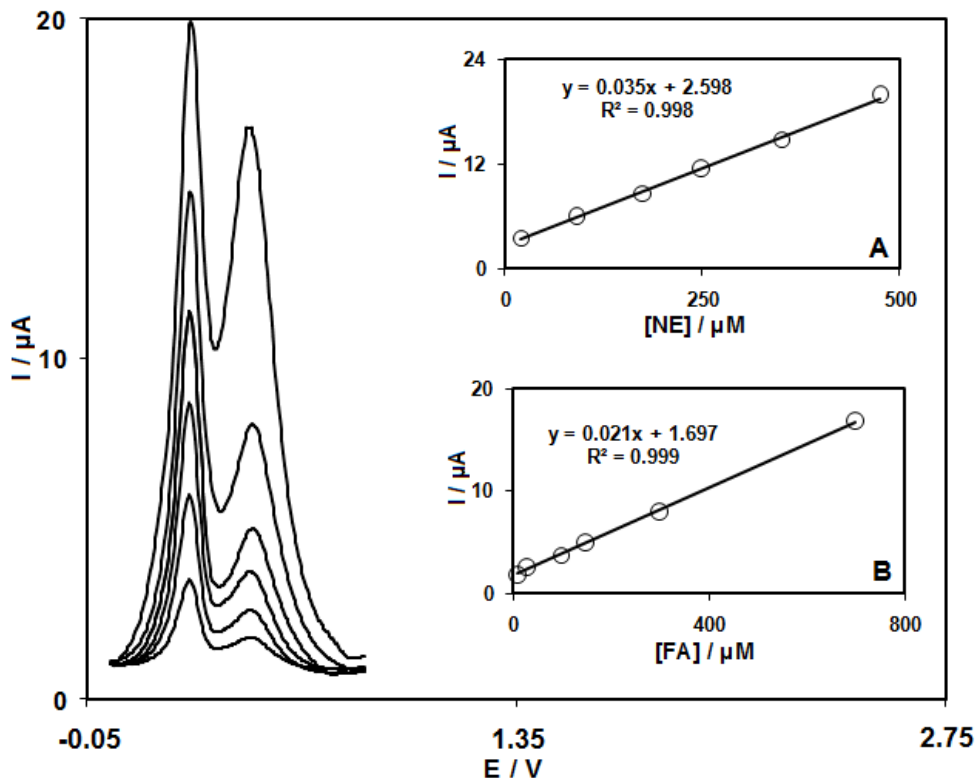
Figure 4. Differential pulse voltammograms of FMCMCNPE in 0.1M PBS (pH 7.0) containing different concentrations of NE and FA (from inner to outer) mixed solutions of 20+10, 90+30, 175+100, 250+150, 350+300 and 475+700 respectively, in which the first value is concentration of NE in µM and the second value is concentration of FA in µM. Insets: (A) Plot of the peak currents as a function of NE and (B) FA concentrations respectively.

Table 1. The application of FMCMCNPE for simultaneous determination of NE and FA in NE injection.

Sample No.	NE Added (µM)	NE Found (µM)	Rec. (%)	RSD (%)	FA Added (µM)	FA Found (M)	Rec. (%)	RSD (%)
1	0	1.5	-	-	0	0	-	-
2	2	3.59	102.57	3.2	10	9.89	98.9	1.9
3	4	5.34	97.09	2.2	15	15.27	101.8	2.3
4	6	7.39	98.53	1.7	20	20.56	102.8	1.3
5	8	9.67	101.79	3.1	25	24.48	97.92	3.3

4. CONCLUSION

This work demonstrates the construction of a carbon nanotube paste electrode chemically modified by incorporationof FMC. The electrochemical behavior of FMC has been studied by cyclic

voltammetry and chronoamperometry in both the absence and presence of NE. The results show that the oxidation of NE is catalyzed at pH 7.0, where the peak potential of NE is shifted by −120 mV at the surface of the FMCMCNPE. The FMCMCNPE exhibits highly electrocatalytic activity for oxidation of NE and FA. The modified electrode displays improved selectivity for voltammetric measurement of NE and FA in solutions containing two analytes.

References

1. R.P.H. Nikolajsen, A.M. Hansen, *Anal. Chim. Acta* 449 (2001) 1.
2. M.E.P. Hows, L. Lacroix, C. Heidbreder, A.J. Organ, A.J. Shah, *J. Neurosci. Methods* 138 (2004) 123.
3. M. Zhu, X.M. Huang, J. Li, H.X. Shen, *Anal. Chim. Acta* 357 (1997) 261.
4. C.L. Guan, J. Ouyang, W.R.G. Baeyens, *Talanta* 50 (2000) 1197.
5. L.P. Lu, S.Q. Wang, X.Q. Lin, *Anal. Chim. Acta* 519 (2004) 161.
6. M. Mazloum-Ardakani, H. Beitollahi, B. Ganjipour, H. Naeimi, *Int. J. Electrochem. Sci.* 5 (2010) 531.
7. M. Mazloum-Ardakani, H. Beitollahi, M. K. Amini, F. Mirkhalaf, M. Abdollahi-Alibeik, *Sens. Actuators B* 151 (2010) 243.
8. J. Mulinare, J.F. Cordero, J.D. Erickson, R.J. Berry, *J. Am. Med. Assoc.* 260 (1988) 3141.
9. A.Milunsky, H. Jick, S.S. Jick, C.L. Bruell, D.S. MacLaughlin, K.J. Rothman, W. Willett, *J. Am. Med. Assoc.* 262 (1989) 2847.
10. Medical News: Extra Folate for Men May Reduce Birth Defects – In Primary Care, Diet & Nutrition from MedPage Today.
11. D. Hoegger, P. Morier, C. Vollet, D. Heini, F. Reymond, J.S. Rossier, *Anal. Bioanal. Chem.* 387 (2007) 267.
12. B.C. Nelson, K.E. Sharpless, L.C. Sander, *J. Chromatogr. A* 1135 (2006) 203.
13. S.L. Zhaoa, H.Y. Yuan, C. Xie, D. Xiao, *J. Chromatogr. A* 1107 (2006) 290.
14. M.S.A. Pradoa, C.A. Silvaa, M.F.M. Tavaresa, K.D. Altriab, *J. Chromatogr. A* 1051 (2004) 291.
15. A.Rodriguez-Bernaldo de Quiros, C. Castro de Ron, J. Lopez-Hernandez, M.A. Lage-Yusty, *J. Chromatogr. A* 1032 (2004) 135.
16. H. Beitollahi, M. Mazloum Ardakani, B. Ganjipour, H. Naeimi, *Biosens. Bioelectron.* 24 (2008) 362.
17. F. Xiao, C. Ruan, L. Liu, R. Yan, F. Zhao, B. Zeng, *Sens. Actuators B* 134 (2008) 895.
18. M. Mazloum-Ardakani, H. Beitollahi, M. A. Sheikh-Mohseni, H. Naeimi, N. Taghavinia, *Appl. Catal. A: Gen.* 378 (2010) 195.
19. H. Beitollahi, H. Karimi-Maleh, H. Khabazzadeh, *Anal. Chem.* 80 (2008) 9848.
20. M. Zidan, W.T. TAN, Z. Zainal, A. H. Abdullah, J. Kheng Goh, *Int. J. Electrochem. Sci.,* 5 (2010) 501.
21. J.B. Raoof, M. Jahanshahi, S. Momeni Ahangar, *Int. J. Electrochem. Sci.* 5 (2010) 517.
22. H. Ma, L. Zhang, Y. Pan, K. Zhang, Y. Zhang, *Electroanalysis* 20 (2008) 1220
23. G.P. Keeley, M.E.G. Lyons, *Int. J. Electrochem. Sci.* 4 (2009) 794.
24. F. Faridbod, M. R. Ganjali, M. Pirali-Hamedani, P. Norouzi, *Int. J. Electrochem. Sci.* 5 (2010) 1103.
25. J. J. Gooding, *Electrochim. Acta* 50 (2005) 3049.
26. N. Rastakhiz, A. Kariminik, V. Soltani-Nejad, S. Roodsaz, *Int. J. Electrochem. Sci.* 5 (2010) 1203.
27. M.R. Akhgar, M. Salari, H. Zamani, A. Changizi, H. Hosseini- Mahdiabad, *Int. J. Electrochem. Sci.* 5 (2010) 782.
28. J.U. Yan, L.Y. Feng, R.W. Zhong, *J. Serb. Chem. Soc.* 70 (2005) 277

29. H. Yaghoubian, H. Karimi-Maleh, M.A. Khalilzadeh, F. Karimi, *Int. J. Electrochem. Sci.* 4 (2009) 993.
30. J.B. Raoof, R. Ojani, H. Beitollahi, *Int. J. Electrochem. Sci.* 2 (2007) 534.
31. M.T. Shreenivas, B.E. Kumara Swamy, U. Chandra, S. Sharath Shankar, J.G. Manjunatha, B.S. Sherigara, *Int. J. Electrochem. Sci.* 5 (2010) 774.
32. F. Wu, G. Zhao, X. Wei, Z. Yang, *Microchim. Acta* 144 (2004) 243.
33. M. R. Ganjali, H. Ganjali, M. Hosseini, P. Norouzi, *Int. J. Electrochem. Sci.* 5 (2010) 967.
34. M. R. Ganjali, F. Faridbod, A. Divsalar, A. A. Saboury, P. Norouzi, G. Rezaei Behbehani, S. Abdolahzadeh, *Int. J. Electrochem. Sci.* 5 (2010) 852.
35. Q. Shen, X. Wang, *J. Electroanal. Chem.* 632 (2009) 149.
36. S. Reddy, B.E. Kumara Swamy, U. Chandra, B.S. Sherigara, H. Jayadevappa, *Int. J. Electrochem. Sci.* 5 (2010) 10.
37. B.N. Chandrashekar, B.E. Kumara Swamy, M. Pandurangachar, S. Sharath Shankar, O. Gilbert, J.G. Manjunatha, B.S. Sherigara, *Int. J. Electrochem. Sci.* 5 (2010) 578.
38. A.J. Bard, L.R. Faulkner, Electrochemical Methods: Fundamentals and Applications, second edn., Wiley, New York 2001.
39. Z. Galus, Fundamentals of Electrochemical Analysis, Ellis Horwood, New York 1976.