Int. J. Electrochem. Sci., 3 **(2008)** 1248 - 1257

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Bioelectrocatalysis of Methyldopa by Adsorbed Tyrosinase on the Surface of Modified Glassy Carbon with Carbon Nanotubes

Ali Mohammadi[1,2], *Abdolmajid Bayandori Moghaddam*[1,*], *Rassoul Dinarvand*[1], *Jalil Badraghi*[3,5], *Fatemeh Atyabi*[4], *Ali Akbar Saboury*[5]

[1] Medical Nanotechnology Research Centre, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, P.O. Box 14155-6451, Iran
[2] Drug and Food Control Department, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[3] Research Institute of Applied Sciences (ACECR), Shahid Beheshti University, Tehran, Iran
[4] Novel Drug Delivery Systems Lab, Pharmaceutics Department, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[5] Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
[*]E-mail: bayandori@farabi.tums.ac.ir and bayandori@gmail.com

Received: 27 August 2008 / *Accepted:* 1 September 2008 / *Published:* 4 October 2008

In this work, a method for performance of tyrosinase/single-walled carbon nanotubes/glassy carbon (TR/SWCNTs/GC) electrode, prepared by modification of GC electrode surface with SWCNTs and adsorption of tyrosinase on the SWCNT surfaces were investigated. SWCNTs were studied with the help of scanning electron microscopy (SEM). Dimensions of SWCNTs make them ideal candidates for the adsorption of proteins. Copper containing enzyme, tyrosinase, exhibited an electrical contact with electrode, because of structural alignment of enzyme on the SWCNT surfaces. This method has the requisite accuracy, sensitivity and precision and the modified electrode showed good repeatability and stability to assay methyldopa in bulk form and pharmaceutical dosage forms.

Keywords: Single-walled carbon nanotube; Methyldopa; Bioelectrochemistry; Nanotechnology

1. INTRODUCTION

Immobilization of biomolecules on carbon nanotubes has been prosecuted in the past, actuated by prospects of using nanotubes as new types of materials for the investigation of direct electron transfer in redox proteins [1-4]. Recent years have witnessed significant interest in applications of novel nanomaterials [5] with motivation to create new types of analytical tools for life science and biotechnology [6]. A range of nanostructure materials prepared from carbon, polymeric species, metals

and semiconductors have been widely investigated for their ability to nanofabrications [7-12]. Carbon nanotubes (CNTs) are one of the important parts of nanostructure materials that can be single-walled or multi-walled. Single-walled carbon nanotubes (SWCNTs) are molecular wires that exhibit interesting structural, mechanical, electrical and electrochemical properties [13,14]. A SWCNT is unique among solid-state materials in that every atom is on the surface.

Methyldopa is an alpha-2 receptor agonist. It reduces elevated blood pressure by relaxing and dilating blood vessels. Blood flows more freely and at a lower pressure through dilated blood vessels [15]. Various methods like spectrophotometry [16], gas chromatography [17], chemiluminescence [18], voltammetric [19] and ^{1}H NMR [20] has been described in literature for determination of methyldopa.

Tyrosinase, one important enzyme, converts tyrosine to L-DOPA. It is considered an important biocatalyst in neutral response system and in the development of some diseases, like Parkinson's disease [21]. In particular, elevated amounts of tyrosinase were observed in certain kinds of melanoma cells and its use as a marker for these melanoma cells was discussed [22]. The first publication on direct electron transfer (DET) between tyrosinase and graphite electrode appeared in 1996 [23]. Yarpolov et al. described electrochemistry of native, holo- and apoenzyme-modified graphite electrodes with the cyclic voltammetric method. Various methods have been reported for the immobilization of tyrosinase on different suitable substrates. These reports have employed conventional electrode materials as substrates, such as silver [24], glassy carbon [25,26], graphite-epoxy resin [27], gold [28], boron-doped diamond [29,30] and other materials [31-33]. Additionally, some reports related to electrochemical determination of kinetic parameters of mushroom tyrosinase [34,35].

In this work, electrochemical properties and biocatalytic activity of TR/SWCNTs/GC electrode for oxidation of methyldopa and application to methyldopa determination was exemplified.

2. EXPERIMENTAL PART

2.1. Chemical reagents

Tyrosinase (T 7755, from mushroom) [9002-10-2] was purchased from Sigma. All reagents were of analytical reagent grade, and distilled water was used throughout. Phosphate buffer solution (PBS) consisted of a potassium phosphate solution (KH_2PO_4 and K_2HPO_4 from Merck; 0.05 M total phosphate) at pH 7.0.

Single-walled carbon nanotubes (SWCNTs) purchased from Research Institute of Petroleum Industry (Iran). The SWCNTs were prepared by chemical vapor deposition, with up to 80% yield of high quality were obtained. Removing of metallic impurity from product performed by washing in HCl. All solutions were prepared with deionized water.

2.2. Measurements

All electrochemical experiments were performed by Autolab potentiostat PGSTAT 30 (Eco Chemie B.V., Netherlands), equipped with GPES 4.9 software. A three-electrode cell was also used

[36,37], employing a glassy carbon (2 mm in diameter) electrode or modified GC electrodes, acting as the working electrodes. A platinum wire was applied as counter electrode and Ag|AgCl|KCl (sat.) was applied as the reference electrode. All potentials were reported with respect to this reference. All experiments were performed at 25 ± 2 °C.

Furthermore, the scanning electron microscopic images were recorded using a ZEISS DSM 960.

2.3. SEM image of SWCNTs

As is well known, properties of a broad range of materials and performance of different devices depend strongly on their surface characteristics. Surface chemistry could therefore be critical to physical properties of SWCNTs and their applications [38].

Figure 1 shows the scanning electron microscopic image of SWCNTs structure. They were formed as boundless tubes because of the van der Waals forces.

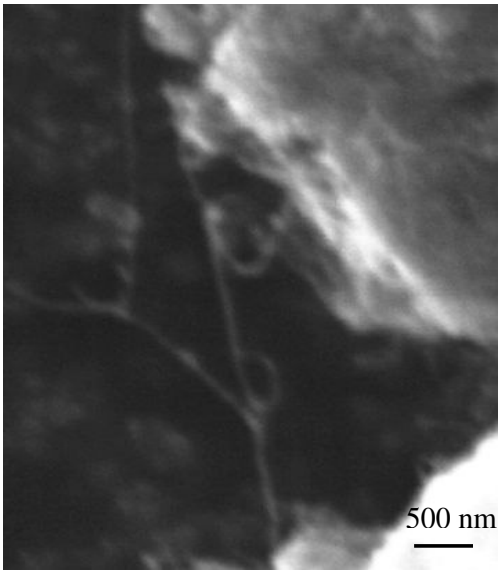


Figure 1. SEM image of the SWCNTs.

2.4. Preparation of TR/SWCNTs/GC electrode

Glassy carbon (disk, 2 mm in diameter) surface was mechanically polished with 1.0 and 0.3 μm alumina and rinsed thoroughly before use. The electrode was then sonicated in ethanol to remove adsorbed particles. Voltammograms were applied in 0.5 M H_2SO_4 between -0.5 and 1.1 V with a 200 mV/s scan rate until the repetitive cyclic voltammograms (CVs) were obtained. N,N-dimethylformamide (DMF) solution, in which SWCNTs dispersed (1 mg/mL) and sonicated, typically consisted of 10 μL from the solution placed on GC electrode and dryed. Afterwards, the SWCNTs/GC

electrode was placed into a fresh PBS including 3 mg/mL tyrosinase (pH 7.0, 3-5 °C) for 16 h (protocol for TR/SWCNTs/GC electrode). Afterwards, the modified electrode was washed in deionized water and placed in PBS (PH 7.0) at a refrigerator (3-5 °C), before being employed in electrochemical measurements as working electrode.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of TR/SWCNTs/GC electrode

Figures 2(a) and (b) show the comparative CVs for two electrodes; (a) GC in PBS and (b) GC in PBS containing 3 mg/mL of tyrosinase at 40 mV/s. These figures were noticed that there were no voltammetric responses at bare GC electrode in both PBS and PBS containing 3 mg/mL of tyrosinase, which indicated that GC electrode was electroinactive in studied potential windows.

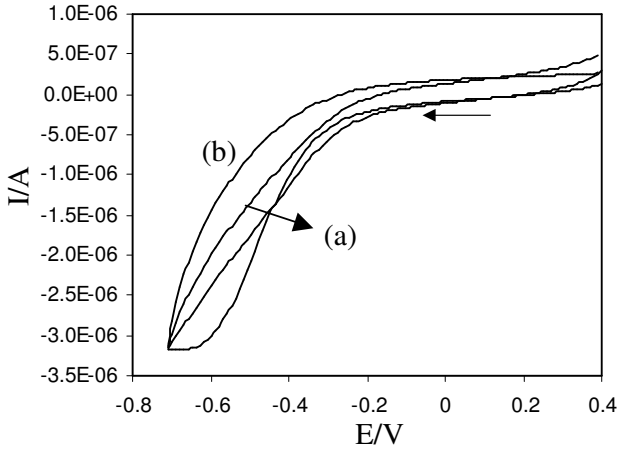


Figure 2. Comparative voltammograms of GC electrode; (a) in PBS, (b) in PBS containing 3 mg/mL tyrosinase, scan rate; 40 mV/s.

In accordance to Figure 3, tyrosinase on TR/SWCNTs/GC electrode shows a one pair of redox waves corresponds to the conversion between TR-Cu(II) and TR-Cu(I). The reductive (E_{pc}) and oxidative (E_{pa}) peaks are situated at -255 mV and 127 mV, respectively (at 100 mV/s) with the peak separation (ΔE), an indication of electron transfer rate, of 382 mV. Its formal potential, $E^{\circ'}$ (defined as the average of E_{pa} and E_{pc}), is calculated to be -64 mV with respect to the reference electrode (133 mV *vs.* NHE). Formal potential value for tyrosinase on graphite electrode was 0.550 V *vs.* SCE (0.794 V *vs.* NHE) [23]. The formal potential of tyrosinase, determined through redox titration to be about 0.60 V [39]. This value was 0.415 V *vs.* NHE for adsorbed tyrosinase at silver electrode [24]. In agreement with data in Figures 2 and 3, it is concluded that SWCNTs play a key role in direct electron transfer reactivity of tyrosinase.

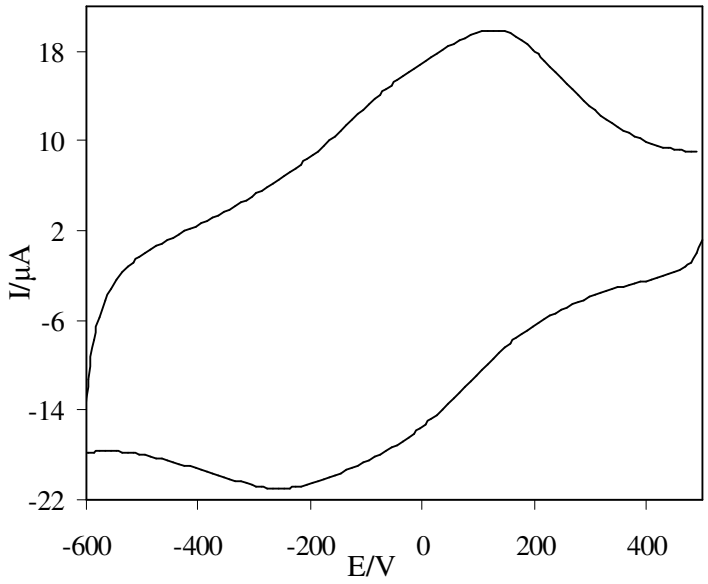


Figure 3. The recorded cyclic voltammogram in PBS for TR/SWCNTs/GC electrode, scan rate; 100 mV/s.

Integrity of immobilized tyrosinase construction and its ability to exchange electrons with SWCNT surfaces were assessed by voltammetry in fresh PBS (pH 7.0, 0.05 M) and scan rate effect on tyrosinase voltammetric behavior was studied in detail. However, Figure 4(a) depicts a well-defined pair of reduction-oxidation (redox) peaks, observed at TR/SWCNTs/GC electrode at various scan rates. The scan rate (v) and the square root scan rate ($v^{1/2}$) *vs.* i_{pa} and i_{pc} are plotted in Figures 4(b) and (c). It can be seen that the redox peak currents increased linearly with scan rate, correlation coefficient was 0.998 (i_{pc} = -204.33 v + 0.700) and 0.997 (i_{pa} = 185.68 v + 0.873), respectively. This phenomenon suggested that redox process was an adsorption-controlled and immobilized tyrosinase was stable.

3.2. Electrocatalysis of methyldopa on the TR/SWCNTs/GC electrode

In one hand, methyldopa is an important drug that works by controlling impulses along certain nerve pathways. As a result, it relaxes blood vessels so that blood passes through them more easily. This helps to lower blood pressure. In other hand, tyrosinase is bifunctional enzyme, which catalyze *o*-diphenols to related quinones [40] is also referred to diphenolase activity. Therefore, we studied the catalysis of methyldopa by using of adsorbed tyrosinase on SWCNTs for electrode response characteristics.

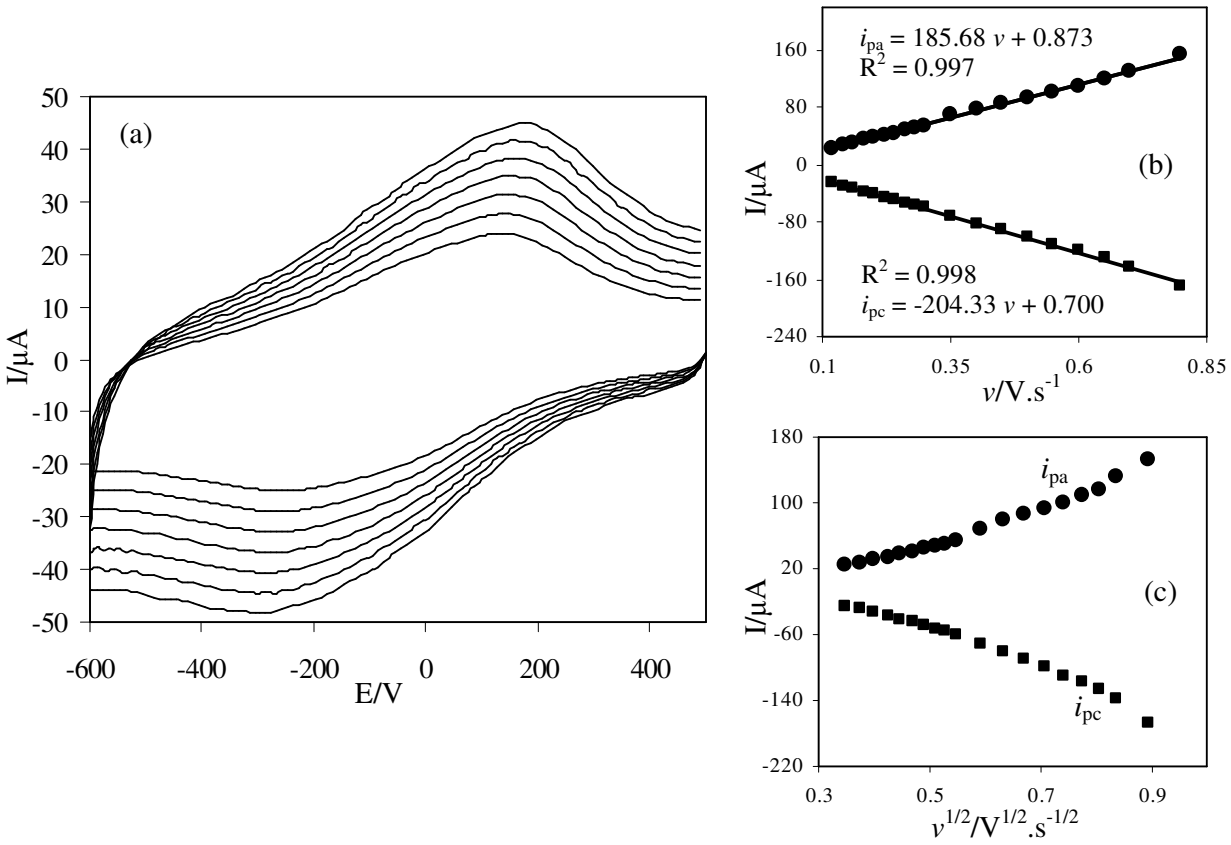
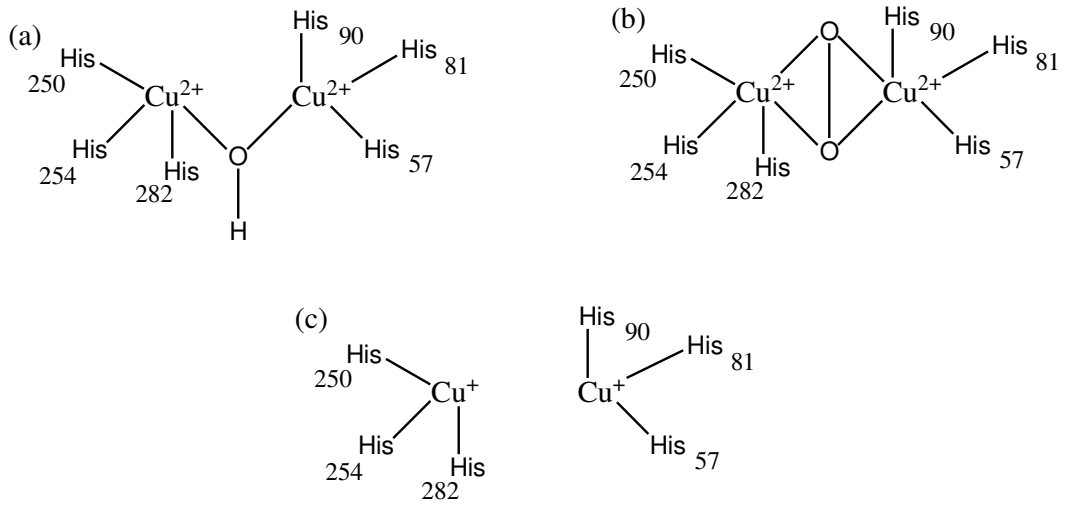


Figure 4. (a) The CVs of TR/SWCNTs/GC electrode in PBS at various scan rates, from inner to outer; 120, 140, 160, 180, 200, 220 and 240 mV/s. The relationship between the peak currents (i_{pa}, i_{pc}) *vs.* (b) the scan rates and (c) the square root of scan rates.

Chemical and spectroscopic studies of tyrosinase have indicated that its binuclear copper active site can be prepared in several forms: *met*-tyrosinase, *oxy*-tyrosinase, and *deoxy*-tyrosinase [41-43]. Most of enzymes in a freshly prepared sample (resting tyrosinase) are in *met*-tyrosinase form, unable to bind O_2. Only a small fraction is present in the sample as *oxy*-tyrosinase [44]. The binuclear copper center in *met*- and *oxy*-tyrosinase states is Cu^{2+} Cu^{2+}, while it is Cu^{1+} Cu^{1+} for *deoxy*- tyrosinase state (Figure 5).

Integrity of TR/SWCNTs/GC electrode ability to methyldopa catalysis has been assessed by differential pulse voltammetry (DPV). DPV waves for various concentrations of methyldopa in PBS (pH 7.0, 0.05 M) are demonstrated in Figure 6(a). A response for methyldopa (catalytic current versus methyldopa concentration) obtained in PBS is shown in Figure 6(b) that were linear over the concentration range of 0.1 – 5 μM. Three correlation coefficients of R1 = 0.998, R2 = 0.996 and R3 = 0.997 with %R.S.D. values ranging from 1.4–3.2% across the concentration range studied were obtained following linear regression analysis. Typically, the regression equation for calibration curve

was found to be y = 1.796x + 0.144. Detection limit (3σ) of electrode towards methyldopa was found to be 0.02 μM.



(d)

MSLIATVGPTGGVKNRLNIVDFVKNEKFFTLYVRSLELLQAKEQHDYSSFFQLAGI**H**GLP
FTEWAKERPSMNLYKAGYCT**H**GQVLFPTW**H**RTYLSVLEQILQGAAIEVAKKFTSNQTDWV
QAAQDLRQPYWDWGFELMPPDEVIKNEEVNITNYDGKKISVKNPILRYHFHPIDPSFKPY
GDFATWRTTVRNPDRNRREDIPGLIKKMRLEEGQIREKTYNMLKFNDAWERFSNHGISDD
QHANSLESV**H**DDI**H**VMVGYGKIEGHMDHPFFAAFDPIFWLH**H**TNVDRLLSLWKAINPDVW
VTSGRNRDGTMGIAPNAQINSETPLEPFYQSGDKVWTSASLADTARLGYSYPDFDKLVGG
TKELIRDAIDDLIDERYGSKPSSGARNTAFDLLADFKGITKEHKEDLKMYDWTIHVAFKK
FELKESFSLLFYFASDGGDYDQENCFVGSINAFRGTAPETCANCQDNENLIQEGFIHLNH
YLARDLESFEPQDVHKFLKEKGLSYKLYSRGDKPLTSLSVKIEGRPLHLPPGEHRPKYDH
TQARVVFDDVAVHVIN

Figure 5. (a) *met*-tyrosinase, (b) *oxy*-tyrosinase, (c) *deoxy*-tyrosinase, Tyrosinase-Agaricus bisporus, (d) sequence information of tyrosinase, UniProtKB /Swiss-Prot entry, O42713|TYRO_AGABI Tyrosinase-Agaricus bisporus (common mushroom). Emphatic symbols related to His_{57}, His_{81}, His_{90}, His_{250}, His_{254} and His_{282}, respectively.

Precision of the assay was investigated with respect to both repeatability and reproducibility. Repeatability was investigated by analysis six replicate samples of each of 0.1, 2 and 5 μM standards where mean concentrations were found to be 0.11, 2.05 and 5.14 with associated %R.S.D. values of 2.8, 1.6 and 0.74%, respectively. Inter-day precision was assessed by analysis the same three concentrations over 3 consecutive days, resulting in mean concentrations of methyldopa of 0.12, 2.07 and 4.86 μM and associated %R.S.D. of 3.45, 1.8 and 0.85%, respectively. The ruggedness of method was assessed by comparison of intra- and inter-day assay results for methyldopa that has been performed by two analysts. The %R.S.D. values for intra - and inter – day assays of methyldopa

performed in same laboratory by two analysts did not exceed 4%, thus indicating the ruggedness of method.

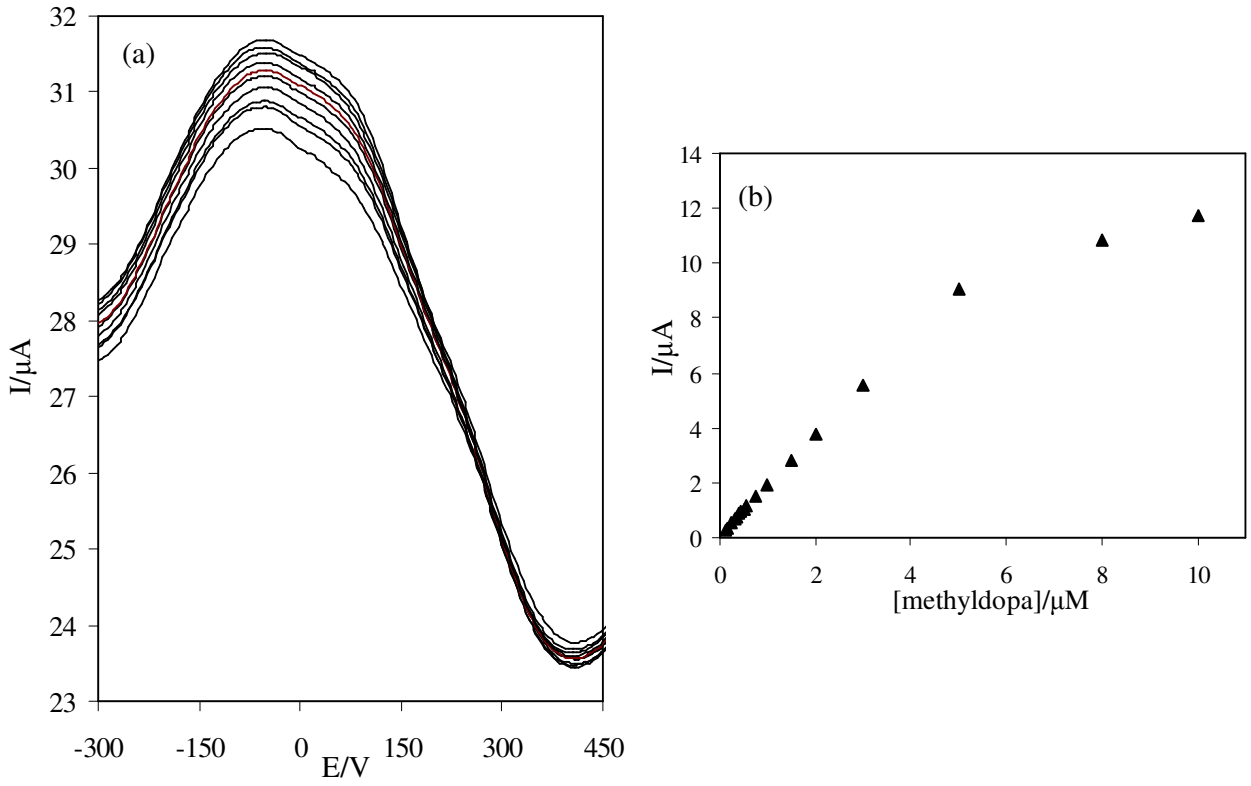


Figure 6. (a) Differential pulse voltammograms corresponding to the electrochemical responses of TR/SWCNTs/GC electrode, generated by different concentrations of methyldopa in PBS from inner to outer; 0, 0.1, 0.14, 0.22, 0.3, 0.35, 0.4, 0.45, 0.5 and 0.55 µM. The step potential and modulation amplitude values of 5 mV and 25 mV, respectively. (b) Calibration curve corresponding to the electrochemical analysis of DPVs for various concentrations of methyldopa.

4. CONCLUSIONS

It was presented that SWCNTs were useful for tyrosinase entrapment. After the demonstration of an excellent behavior towards tyrosinase redox by recommended electrode, direct electron transfer of tyrosinase on TR/SWCNTs/GC electrode was achieved. The reconstitutions of enzyme tyrosinase on SWCNTs provide a general example to orient enzymes on electrode supports. The electrical properties of CNTs mediate charge transport between the redox site of biocatalyst and electrode. Analytical characteristics of TR/SWCNTs/GC, including linear rang and detection limit are described. The biosensor exhibited good performance in terms of reusability, operational stability and fabrication simplicity.

ACKNOWLEDGEMENTS
The financial support provided by Research Affairs of Tehran University of Medical Sciences is gratefully acknowledged.

References

1. S. C. Tsang, J. J. Davis, M. L. H. Green, H. A. O. Hill, Y. C. Leung, P. J. Sadler, *J. Chem. Soc. Chem. Commun.* (1995) 2579.
2. S. S. Tsang, Z. Guo, Y. K. Chen, M. L. H. Green, H. A. O. Hill, T. W. Hambley, P. J. Sadler, *Angew. Chem. Int. Ed.* 36 (1997) 2198.
3. Z. Guo, P. J. Sadler, S. C. Tsang, *Adv. Mater.* 10 (1998) 701.
4. F. Balavoine, P. Schultz, C. Richard, V. Mallouh, T. W. Ebbeson, C. Moskowski, *Angew. Chem. Int. Ed.* 38 (1999) 1912.
5. M. R. Nabid, M. Golbabaee, A. Bayandori Moghaddam, A. R. Mahdavian, M. M. Amini, *Polym. Comp.* (2008)) doi: 10.1002/pc.20628.
6. X. Gao, L. Yang, J. A. Petros, F. F. Marshall, J. W. Simons, S. Nie, *Science* 293 (2001) 1289.
7. F. Atyabi, F. A. Moghaddam, R. Dinarvand, M. J. Zohuriaan-Mehr, G. Ponchel, *Carbohyd. Polym.* 74 (2008) 59.
8. A. Bayandori Moghaddam, M. Kazemzad, M.R. Nabid, H.H. Dabaghi, *Int. J. Electrochem. Sci.* 3 (2008) 291.
9. A. Bayandori Moghaddam, T. Nazari, *Int. J. Electrochem. Sci.* 3 (2008) 768.
10. X. Gao, Y. Cui, R. M. Levenson, L. W. Chung, S. Nie, *Nature Biotechnol.* 22 (2004) 969.
11. A. Bayandori Moghaddam, M. R. Ganjali, R. Dinarvand, A. A. Saboury, T. Razavi, A. A. Moosavi-Movahedi, P. Norouzi, *Biophys. Chem.* 129 (2007) 259.
12. A. Bayandori Moghaddam, M. R. Ganjali, R. Dinarvand, S. Ahadi, A. A. Saboury, *Biophys. Chem.* 134 (2008) 25.
13. M. S. Dresselhaus, G. Dresselhaus, P. C. Eklund, "Science of Fullerenes and Carbon Nanotubes" Academic Press, New York, 1996.
14. H. Dai, *Phys. World* 13 (2000) 43.
15. A. M. Sambrook, R. C. Small, *Anaesth. & Int. Care Med.* 9 (2008) 128.
16. O. Fatibello, I. da Cruz Vieira, *Analyst* 122 (1997) 345.
17. C. Sharma, S. Mohanty, S. Kumar, N. J. Rao, *Analyst* 121 (1996) 1963.
18. C. Funan, Z. Zhujun, Z. Yingxue, H. Deyongl, *Anal. Bioanal. Chem.* 382 (2005) 211.
19. K. D. Kozminski, D. A. Gutman, V. Davilla, D. Sulzer, A. G. Ewing, *Anal. Chem.* 70 (1998) 2123.
20. Z. Talebpour, S. Haghgoo, M. Shamsipur, *Anal. Chim. Acta* 506 (2004) 97.
21. Y. Xu, A. H. Stokes, W. M. Freeman, S. C. Kumer, B. A. Vogt, K. E. Vrana, *Mol. Brain. Res.* 45 (1997) 159.
22. N. Kounalakis, J. S. Goydos, *Curr. Oncol. Rep.* 7 (2005) 377.
23. A. I. Yaropolov, A. N. Kharybin, J. Emneus, G. Marko-Varga, L. Gorton, *Bioelectrochem. Bioenerg.* 40 (1996) 49.
24. B. Ye, X. Zhou, *Talanta* 44 (1997) 831.
25. E. Dempsey, D. Diamond, A. Collier, *Biosens. Bioelectron.* 20 (2004) 367.
26. V. C. Sanz, M. L. Mena, A. Gonzalez-Cortes, P. Yanez-Sedeno, J. M. Pingarron, *Anal. Chim. Acta* 528 (2005) 1.
27. J. Wang, F. Lu, D. Lopez, *Analyst* 119 (1994) 455.
28. S. Campuzano, B. Serra, M. Pedrero, F. J. M. Villena, J. M. Pingarron, *Anal. Chim. Acta* 494 (2003) 187.
29. J. P. McEvoy, J. S. Foord, *Electrochim. Acta* 50 (2005) 2933.
30. Y. L. Zhou, R. H. Tian, J. F. Zhi, *Biosens. Bioelectron.* 22 (2007) 822.

31. E. Burestedt, A. Narvaez, T. Ruzgas, L. Gorton, J. Emneus, E. Dominguez, G. Marko-Varga, *Anal. Chem.* 68 (1996) 1605.
32. F. Daigle, D. Leech, *Anal. Chem.* 69 (1997) 4108.
33. Z. Liu, B. Liu, , J. Kong, J. Deng, *Anal. Chem.* 72 (2004) 707.
34. G. Rivas, V. M. Solis, *Bioelectrochem. Bioenerg.* 29 (1992) 19.
35. E. S. Forzani, G. A. Rivas, V. M. Solis, *J. Electroanal. Chem.* 461 (1999) 174.
36. H. H. Dabaghi, A. Bayandori Moghaddam, M. Kazemzad, R. Dinarvand, F. Aryanasab, M. R. Nabid, *J. Appl. Electrochem.* 38 (2008) 409.
37. A. Bayandori Moghaddam, F. Kobarfard, A. R. Fakhari, D. Nematollahi, S. S. H. Davarani, *Electrochim. Acta* 51 (2005) 739.
38. P. Collins, K. Bradley, M. Ishigami, A. Zettle, *Science* 287 (2000) 1801.
39. N. Makino, P. McMahill, H. S. Mason, *J. Biol. Chem.* 249 (1974) 6062.
40. K. Lerch, *Mol. Cell. Biochem.* 52 (1983) 125.
41. R. L. Jolley, L. H. Evans, H. S. Mason, *Biochem. Biophys. Res. Commun.* 46 (1972) 878.
42. N. Makino, H. S. Mason, *J. Biol. Chem.* 248 (1973) 5731.
43. R. L. Jolley, L. H. Evans, N. Makino, H. S. Mason, *J. Biol. Chem.* 249 (1974) 335.
44. J. R. Ros-Martinez, J. N. Rodriguez-Lopez, R. V. Castellanos, F. C. Canovas, *Biochem. J.* 294 (1993) 621.
45. T. Nakaminami, S.-I. Ito, S. Kuwabata, H. Yoneyama, *Anal. Chem.* 71 (1999) 1068.
46. S. Tembe, M. Karve, S. Inamdar, S. Haram, J. Melo, S. F. D'Souza, *Anal. Biochem.* 349 (2006) 72.
47. S. Tembe, S. Inamdar, S. Haram, M. Karve, S. F. D'Souza, *J. Biotechnol.* 128 (2007) 80.
48. Y.-F. Li, Z.-M. Liu, Y.-L. Liu, Y.-H. Yang, G.-L. Shen, R.-Q. Yu, *Anal. Biochem.* 349 (2006) 33.
49. J. Yu, S. Liu, H. Ju, *Biosens. Bioelectron.* 19 (2003) 509.