Sensitive Electrochemical Sensor for Determination of Methyldopa Based on Polypyrrole/Carbon Nanoparticle Composite Thin Film Made by In Situ Electropolymerization

Saeed Shahrokhian,*[a, b] *Reyhaneh-Sadat Saberi*,[a] *Zahra Kamalzadeh*[a]

[a] Department of Chemistry, Sharif University of Technology, Tehran 11155-9516, Iran
tel.: +98-21-66005718, fax: +98-21-66012983
[b] Institute for Nanoscience and Technology, Sharif University of Technology, Tehran, Iran
*e-mail: shahrokhian@sharif.edu

Received: March 26, 2011
Accepted: June 24, 2011

Abstract
A composite surface coating is prepared by electropolymerization of a mixture of pyrrole and carbon nanoparticles onto a glassy carbon electrode (GCE). The microscopic structure and morphology of the composite film is characterized by scanning electron microscopy. The modified electrode offers a considerable improvement in voltammetric sensitivity toward methyldopa (m-dopa), compared to the bare and polypyrrole-coated GCEs. A significantly enhanced anodic peak current together with a remarkable increase in sharpness of the cyclic voltammetric (CV) signals are observed for the detection of m-dopa. The effect of experimental parameters, such as scan rate and pH, are investigated by monitoring CV responses toward m-dopa. It is found that a maximum current response can be obtained at pH 3.0 under a diffusion controlled process. A wide linear dynamic range (0.2–50 μM) with a detection limit of 60 nM is achieved for m-dopa. The excellent response characteristics, e.g., high sensitivity, very good repeatability and reproducibility, and low detection limit, have made the prepared sensor suitable for the analysis of m-dopa in pharmaceutical and clinical preparations.

Keywords: Methyldopa, Carbon nanoparticles, Polypyrrole, Composite electrodes, Modified electrodes

DOI: 10.1002/elan.201100169

1 Introduction

Methyldopa (m-dopa) is an antihypertensive agent. It affects nerve centers which control blood vessels in the brain. As blood vessels relax, m-dopa relieves high blood pressure [1]. Several techniques have been reported in the literatures for the determination of m-dopa [2–4], but electrochemical methods have attracted great interests because of their simplicity, rapidness and high sensitivity in determination of m-dopa and various other analytes, without any tedious pre-treatments [5–7].

Nanostructured materials such as carbon nanoparticles (CNPs) and nanotubes, because of their unique physical and chemical properties such as high surface area, exceptional physicochemical stability, and high electrical conductivity, have been used in designing modified electrodes for electrochemical sensing [8–13]. Because of their unique properties, such as good electrical conductivity, excellent environmental stability, and ease of preparation, conducting polymers have been widely used for the development of robust, reliable and maintenance free electrochemical sensors [14–16]. Polypyrrole (PPy) is one of the most extensively used conducting polymers in the design of electrochemical sensors. This polymer has good stability, facile synthesis, higher conductivity and versatility compared to many other conducting polymers [17,18]. Further, PPy can be easily coated as thin adherent films on various metal or carbon substrates by electropolymerization from aqueous or organic solvents [14,19]. These characteristics make PPy highly suitable for various electrochemical applications for the determination of various cationic, anionic and neutral molecular species [20–23]. Recently, a variety of methods have been reported for producing composites from the combination of various conductors (carbon black, graphite powder, carbon fiber and metal nanoparticles) with various conducting and nonconducting polymers [24–29]. The goal is to achieve a synergistic effect with regards to the properties of the two components. This rapidly expanding field creates many exciting new composite materials with desirable properties, which are unknown in the parent constituent materials [30–32]. The advantages of carbon nanoparticles such as their highly effective surface area, high porosity, more adsorption capability and excellent electrical conductivity make them suitable for improving properties of composite materials for preparation of modified electrodes [33,34].

In the present work we describe the development of a PPy/CNP-modified GCE that offers substantial improvements in the kinetics and sensitivity of the voltammetric

responses toward m-dopa. Recent studies have illustrated that the response of PPy-modified electrodes can be greatly enhanced by coupling PPy with secondary conductive centers (such as metallic or carbonaceous materials) [35,36]. The PPy/CNP coated GCE described in the present work, offers marked acceleration of the m-dopa electrooxidation compared to the individual (PPy and CNP) modified electrodes. These behaviors, along with detailed characterization of the PPy/CNP-modified electrode are reported in the following sections.

2 Experimental

2.1 Apparatus

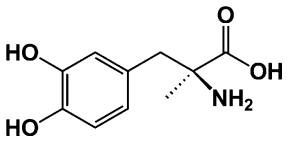
Voltammetric experiments were performed using a Metrohm Computrace analyzer model 797 VA. A conventional three electrode system was used with a working GCE electrode (unmodified or modified, $d=2$ mm), a saturated Ag/AgCl reference electrode and a Pt wire counter electrode. A digital pH/mV/Ion meter (Metrohm, 827 pH Lab) was utilized for preparation of the buffer solutions used as supporting electrolyte in the voltammetric experiments.

All experiments were performed at $25\pm1\,^{\circ}C$. Solutions were deaerated by bubbling nitrogen prior to the experiments, and the electrochemical cell was kept under a nitrogen atmosphere throughout the experiments. The morphological scanning electron microscope (SEM) images of PPy/CNP film and PPy film were obtained using a Hitachi S4160 Field Emission Scanning Electron Microscope (Hitachi Co., Japan).

2.2 Reagents

Pyrrole was purchased from Merck and purified by distillation and then kept in refrigerator before use. Carbon nanoparticle (ca. 9–18 nm diameter, Emperor 2000, Cabot Corporation) was obtained commercially and used without further purification. Sodium dodecylbenzenesulfonate (SDBS) was purchased from Fluka. Ascorbic acid (AA) and Potassium ferrocyanide ($K_4Fe(CN)_6$) were purchased from Merck. $Ru(bpy)_3Cl_2$ was synthesize in our laboratory based on a reported procedure [37]. 3-(3,4-Dihydroxyphenyl)-2-methyl-l-alanine (methyl-l-dopa) was purchased from Fluka (Scheme 1).

M-dopa tablets (250 mg) were taken from Darupakhsh pharmaceutical company. All other reagents used were of analytical grade. Phosphate-buffer solutions (0.1 M) were used for preparation of buffers of pH 3, 6, 7 and 8. Buffer solutions of pH 4 and 5 were prepared from 0.1 M acetic acid and potassium hydroxide (Fluka), using the pH-meter. All aqueous solutions were prepared in doubly distilled, deionized water. High purity nitrogen (99.999%, Roham Gas Company, Iran) was used for deaeration of the solutions.



Scheme 1. Chemical structure of m-dopa.

2.3 Preparation of PPy/CNP-Modified Electrode

Glassy carbon electrode was carefully polished with alumina slurry, rinsed with water, and sonicated in chloroform and water successively for 5 min. The electropolymerization solution was prepared by dispersing 2 mg CNPs in 5 mL 0.005 M of SDBS and 0.005 M of pyrrole aqueous solutions and sonicating for 5 min at room temperature. The PPy/CNP composite modified GCE was prepared at the optimized conditions of cycling potential between 0 and 0.8 V (3 cycles) at a scan rate 0.05 $V\,s^{-1}$ in the electropolymerization solution. The obtained modified electrode was rinsed with distilled water and subjected to electrochemical oxidation in 0.01 M sodium hydroxide solution by performing 60 successive cyclic sweeps between 0.0 V and 1 V. The resulting electrode is denoted as PPy/CNP/GCE. For comparison, a PPy/GCE was also prepared under the same conditions without adding CNP to the electropolymerization solution.

3 Results and Discussion

3.1 Characterization of PPy/CNP/GCE by SEM and CV

The surfaces of different modified electrodes were characterized by SEM technique. Figure 1 compares the SEM images of the surfaces of bare (A), PPy-modified (B), and PPy/CNP-modified (C and D) electrodes. The micrograph of the surface of PPy-modified electrode (B) shows a quite uniform coverage of the GCE surface with PPy. As expected, incorporating the CNP in the polymer matrix results in some humps and projections (Figure 1C), which are not observed in PPy/GCE (Figure 1B). Figures 1C and 1D show that CNPs have been incorporated into PPy film successfully and the polymer is grown around the nanoparticles. This is more clearly shown in Figure 1D obtained at higher magnification. The mean size of the CNPs is around 15 nm, but formation of polypyrrole film can increase the diameter of the particles at the surface of the composite. Moreover, the CNPs are distributed uniformly throughout the PPy film. The CNP sites across the polymer act as some kind of electron transfer positions by electron hopping mechanism, resulting in improved electron transfer during the electrochemical process on the electrode surface [38].

An interesting feature of the PPy/CNP film electrode which was discovered by CV, is its diverse interaction with analytes having different charges. Compared to the bare GCE, the electrochemical response of PPy/CNP/GCE reduces in the presence of the negatively charged analytes like $Fe(CN)_6^{4-}$ (Figure 2A), but considerably in-

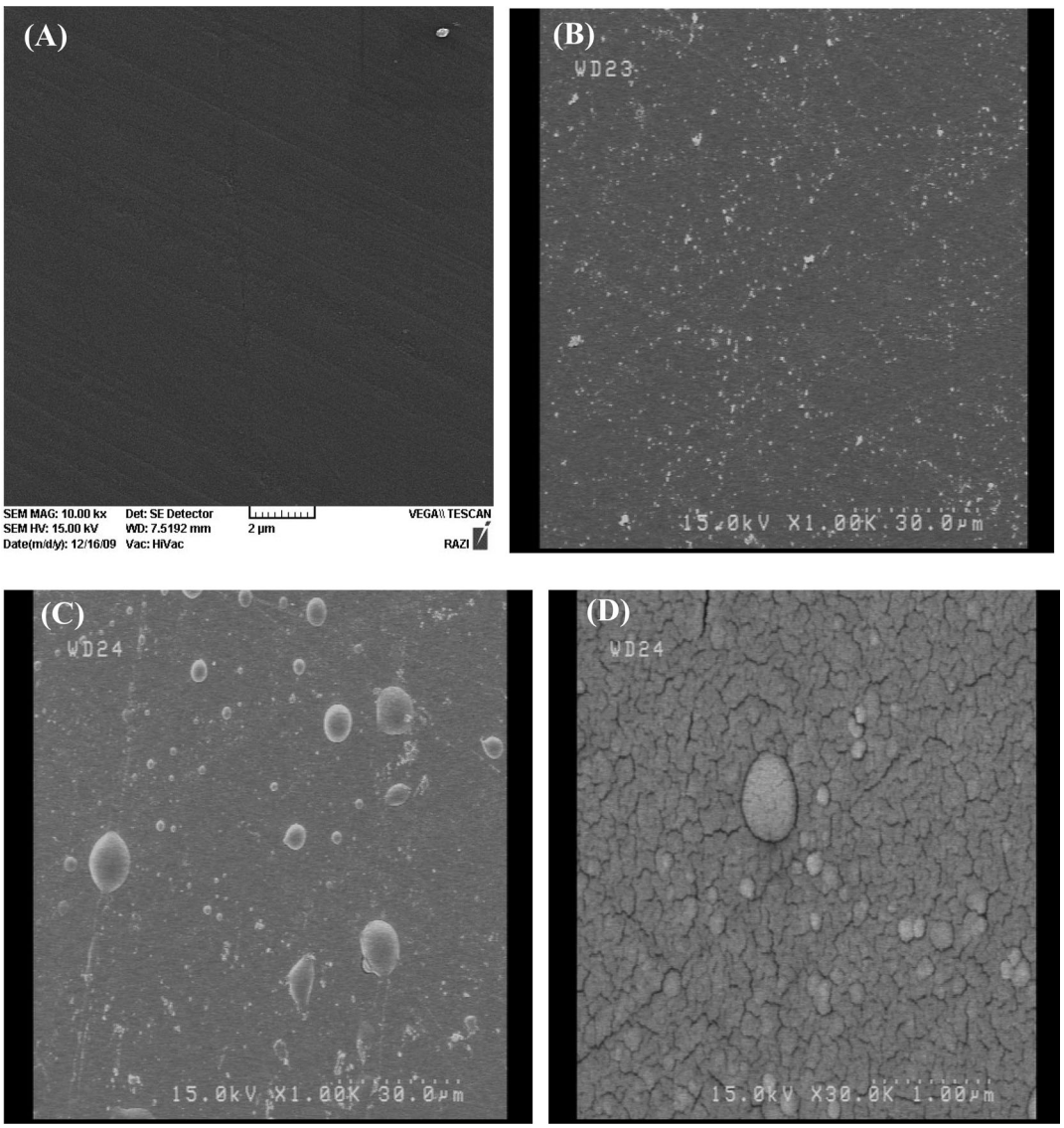


Fig. 1. SEM images of various GCEs: (A) bare electrode, (B) PPy film coated electrode, (C) and (D) PPy/CNP composite film coated electrode with different resolutions.

creases when a positively charged analyte such as $Ru(PPy)_3^{2+}$ is present (Figure 2B). This can be related to electrostatic interaction between the negative charge density of the modified film and the analytes, as it has been discussed in the literature for over-oxidized polypyrrole [39].

3.2 Electrochemical Behavior of m-Dopa on the Surface of PPy/CNP/GCE

Figure 3 displays CVs at the bare (a), PPy-modified (b) and PPy/CNP-modified (c) glassy-carbon electrodes in the presence of 10 μM m-dopa in 0.1 M phosphate buffer solution of pH 3.0, recorded at a scan rate of 100 $mV\,s^{-1}$. As can be seen, all the electrodes display defined oxidation peaks for m-dopa. The oxidation of m-dopa is very weak on the surface of the bare GCE, but a considerable increase in the oxidation peak current is observed on the PPy-modified electrode. However, the electrode modified with PPy/CNP, shows relatively higher sensitivity in comparison to the PPy-modified GCE. Higher sensitivity (30 times with respect to GCE) for the oxidation of m-dopa at PPy/CNP/GCE indicates the important role of the incorporated CNPs in improving the kinetics of the electron transfer process. Such accelerated response is attributed to a synergistic enhancement of PPy and CNPs, rather than a combination of the individual surface area effects. A very weak reduction peak is observed in the reverse scan at all three electrodes, indicating that the electrochemically generated product involved in a chemical reaction and the resulted product could not be readily reduced in the potential range studied. Such a behavior has been previously reported for other catechol amines with similar functional groups, e.g. dopamine [40], epinephrine [41] and l-dopa [42].

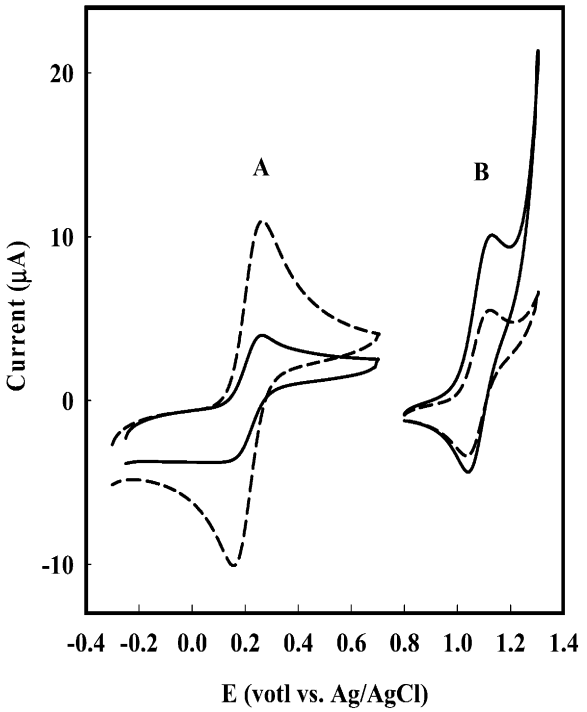


Fig. 2. CVs of bare GCE (-----) and PPy/CNP/GCE (—) in, (A) 1 mM of $K_4Fe(CN)_6$ and (B) 1 mM $Ru(bpy)_3Cl_2$ in 0.1 M KCl. Scan rate, 100 mV s^{-1}.

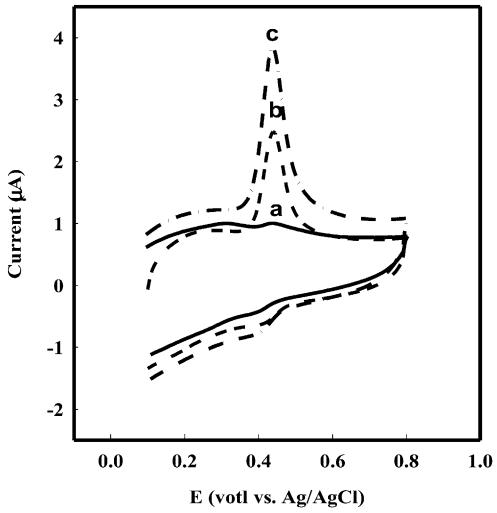


Fig. 3. Cyclic voltammograms of 10 μM m-dopa on the surface of various electrodes; bare GCE (—), PPy/GCE (----) and PPy/CNP/GCE (-·-·-·-). Potential scan rate, 100 mV s^{-1}; supporting electrolyt, 0.1 M phosphate buffer solution of pH 3.

3.3 The Effects of pH and Scan Rate

The effect of pH of the buffer solution on the electrochemical behavior of m-dopa was investigated in the range from 3.0 to 8.0 by CV method (Figure 4A). A linear negative shift was observed for the variation of the anodic peak potential of m-dopa by increasing pH with a slope of about −55 mV per pH unit based on Equation 1:

$$E_{p,a}\ (\text{mV}) = 605.6 - 55.20\ \text{pH} \quad (R^2 = 0.9943,\ n = 6) \quad (1)$$

These results indicate that equal numbers of electrons and protons participate in the electrode process of m-dopa. In agreement with a previous report [5], the anodic peak current of m-dopa reaches its maximum value at pH 3.0. Therefore, all voltammetric determinations were performed in phosphate buffer solution of pH 3.0, as the supporting electrolyte.

The effect of the potential scan rate on the electrochemical mechanism of m-dopa was investigated by CV method in 0.1 M phosphate buffer solution of pH 3.0 containing 10 μM m-dopa (Figure 4). The results showed a linear relationship between anodic peak current, $i_{p,a}$, and square root of scan rate, $v^{1/2}$, ($i_{p,a}$ (μA) = 0.309 $v^{1/2}$ − 1.014 (v in mV s^{-1}), with a slope of 0.309 μA mV^{-1} s$^{1/2}$ and a regression coefficient (R^2) of 0.9995 in the range of 25–400 mV s^{-1}. No considerable cathodic (reverse) peak was observed for m-dopa in the reverse potential sweep. The results confirm an irreversible diffusive mechanism for the electrochemical oxidation of m-dopa on the surface of PPy/CNP/GCE.

3.4 Analytical Characteristics

Under the optimized experimental conditions, the anodic peak currents of differential pulse voltammograms (DPVs) were proportional to m-dopa concentration in the range of 0.2–50 μM (Figure 5) with the following regression equation:

$$i_{p,a}\ (\mu\text{A}) = 0.1296\ C\ (\mu\text{M}) + 0.1214 \quad (2)$$

The detection limit was estimated to be 60 nM (based on $S/N = 3$).

A series of six repetitive measurements in 10 μM of m-dopa at the same PPy/CNP/GCE yielded repeatable current responses with a relative standard deviation (*RSD*) of 1.73 % (mean peak current of 54 μA; not shown). The reproducibility of 5 independent composite PPy/CNP films generated by electropolymerization at the same chemical and instrumental parameters was found to be 5.95 % *RSD*.

A standard addition protocol was adopted to assess the recovery of m-dopa from the commercial tablets (250 mg). In this regard, a known amount of the powered tablet was spiked with different concentrations of m-dopa standard solutions and diluted with phosphate buffer solution of pH 3. Analysis of the corresponding standard addition plots show that the matrix of the m-dopa tablet does not have any significant interference effect on the electrochemical response of m-dopa (Fig. 5C). The resulted amount of m-dopa content was 240 mg per tablet, indicating adequate accuracy of the proposed method.

The recovery study of m-dopa spiked to a human blood serum sample was also investigated (Figure 6). A sample of blood serum was diluted 25 fold with phosphate buffer

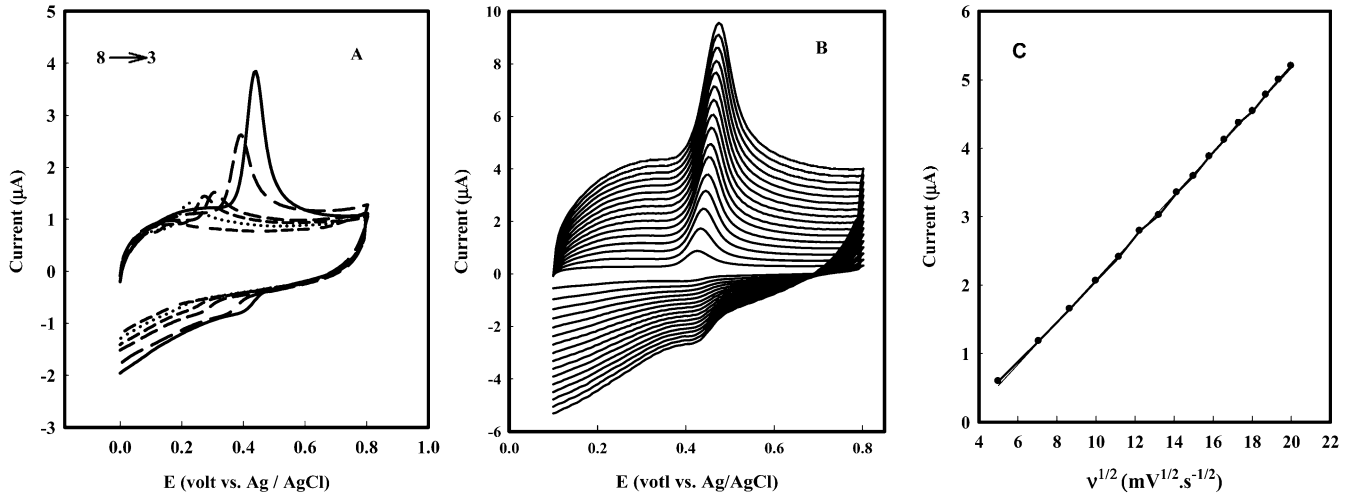


Fig. 4. CVs of 10 μM m-dopa at PPy/CNP/GCE (A) in buffer solutions of various pH (from 3 to 8), (B) at various potential scan rates from 25 to 400 mV s^{-1} and (C) plot of I_{pa} *vs.* $v^{1/2}$.

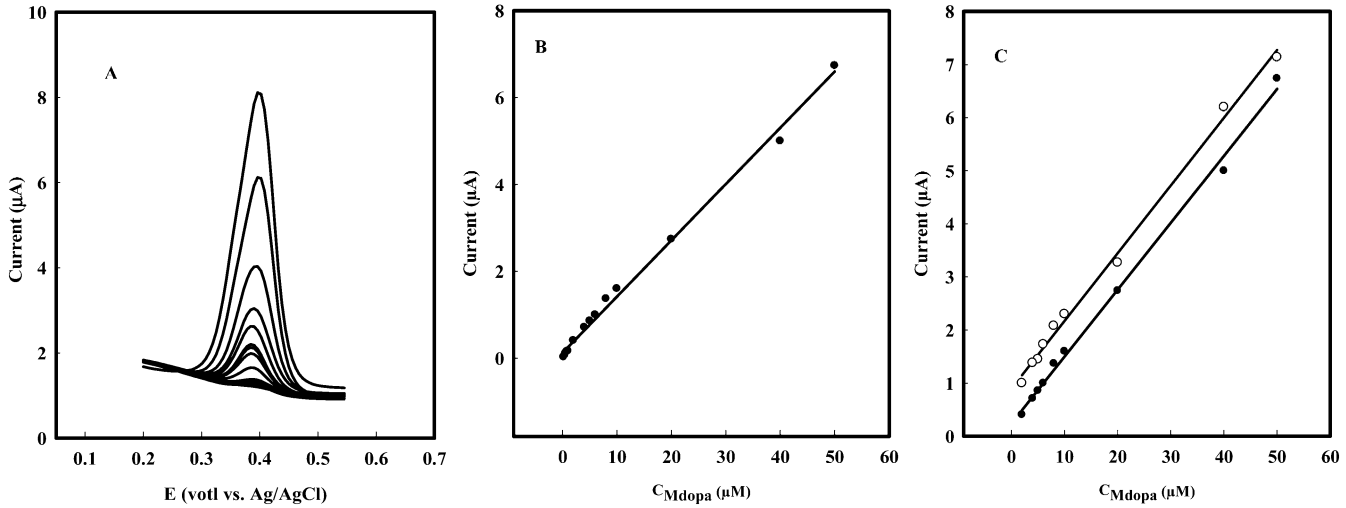


Fig. 5. (A) DPVs of 0.1 M phosphate buffer solution containing various concentrations of m-dopa; from bottom to top: 0.2, 0.4, 0.5, 0.6, 0.8, 1, 2, 4, 5, 6, 8, 10, 20, 40 and 50 μM. Pulse amplitude 50 mV. (B) Corresponding linear calibration curve of peak current versus m-dopa concentration. (C) Linear calibration curve of the anodic peak current versus m-dopa (●) added to 0.1 M phosphate buffer solution of pH 3.0, (○) added to m-dopa tablet in solution buffered at pH 3.0.

solution of pH 3, and the measurement of m-dopa was performed by standard addition method. A separate peak for the oxidation of uric acid (UA) was also observed in DPV of spiked plasma samples. Complete separation of the oxidation peaks of m-dopa and UA shows the ability of the PPy/CNP/GCE electrode for determination of m-dopa in clinical applications.

In most electrochemical determinations of m-dopa and other catechol amines, ascorbic acid (AA) is considered as a serious interference, due to overlapping voltammetric responses. Figure 7 shows the oxidation peaks of ascorbic acid at the surface of different electrodes: bare GCE, PPy/GCE and PPy/CNP/GCE. As can be seen, PPy/CNP/GCE shows a good performance in resolving AA oxidation peak from m-dopa. Therefore, the proposed film modified electrode can be successfully applied for the determination of m-dopa in clinical and pharmaceutical preparations in the presence of AA. The voltammetric signals show better sensitivity and sharpness for m-dopa compared to a previous report in this area [5]. The resulting recoveries for real samples of m-dopa are presented in Table 1.

4 Conclusions

A composite film of CNPs and PPy was synthesis electrochemically at the surface of GCEs. Characterization of this electrode by SEM shows incorporation of CNPs into the composite film at the surface of the electrode. We

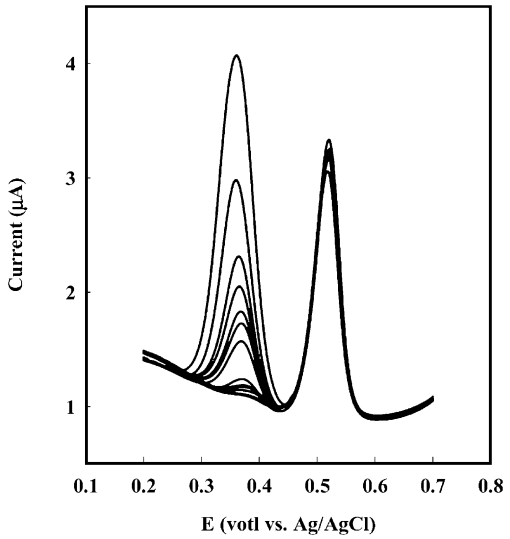


Fig. 6. DPVs of 0.1 M phosphate buffer solution containing constant amount of blood serum and various concentrations of m-dopa at PPy/CNP/GCE; from bottom to top: 0.4, 0.5, 0.6, 0.8, 1, 2, 4, 5, 6, 8, 10, 20 and 40 μM. Pulse amplitude, 50 mV.

have demonstrated that PPy/CNP/GCEs have much higher electrochemical activity toward the oxidation of m-dopa compared to PPy-modified electrode. The observed improvement is attributed to the synergistic enhancement rather than a combination of surface area of the constituents. CV investigations proved that the surface of the modified electrode has a negative charge density. The modified electrode was applied for the determination of m-dopa without any interference from AA and UA. Excellent features like, wide linear range, low detection limit, high reproducibility and repeatability, and good recovery are promising for the successful application of this sensor for the determination of m-dopa in pharmaceutical and clinical preparations.

Acknowledgements

The authors gratefully acknowledge the support of this work by the Research Council and the Center of Excellence for Nanostructures of Sharif University of Technology, Tehran, Iran. They are also grateful to Prof. *Mohammad K. Amini* (Department of Chemistry, University of Isfahan) for his valuable suggestions.

References

[1] M. Hochadel, *The AARP Guide to Pills*, Sterling, London **2005**, pp. 615–616
[2] P. R. S. Ribeiro, J. A. Gomes Neto, L. Pezza, H. R. Pezza, *Talanta* **2005**, *67*, 240.
[3] S. M. T. Shaikh, D. H. Manjunatha, K. Harikrishna, K. C. Ramesh, R. Sudhir Kumar, J. Seetharamappa, *J. Anal. Chem.* **2008**, *63*, 637.
[4] S. F. Li, H. L. Wu, Y. J. Yu, Y. N. Li, J. F. Nie, H. Y. Fu, R. Q. Yu, *Talanta* **2010**, *81*, 805.
[5] M. B. Gholivand, M. Amiri, *Electroanalysis* **2009**, *21*, 2461.

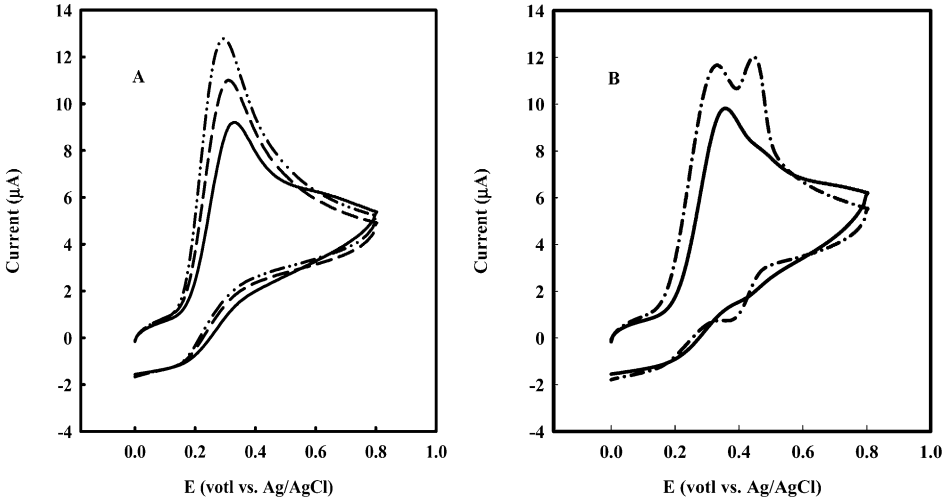


Fig. 7. CVs of (A) 1 mM AA, and (B) 1 mM AA in the presence of 0.01 mM m-dopa in phosphate buffer solution of pH 3.0 at the surface of bare GCE (—), PPy/GCE (- - - -) and PPy/CNP/GCE (-···-···-). Potential scan rate, 100 mV s^{-1}.

Table 1. Determination of m-dopa in real samples.

Sample	m-Dopa added (μM)	m-Dopa found (μM)	Recovery (%)
Blood serum	3	2.89	96.6
Blood serum	9	8.55	95
Tablet	1.5	1.44	96.2

[6] M. C. Blanco-Lopez, M. J. Lobo-Castanon, A. J. M. Ordieres, P. Tunon-Blanco, *Electroanalysis* **2007**, *19*, 207.
[7] S. S. Buduwy, Y. M. Issa, A. S. Tag-Eldin, *Electroanalysis* **1996**, *8*, 1060.
[8] P. Yanez-Sedeno, J. Riu, J. M. Pingarron, F.X Rius, *Trends. Anal. Chem.* **2010**, *29*, 939.
[9] N. Cheng, R. A. Webster, M. Pan, S. Mu, L. Rassaei, S. C. Tsang, F. Marken, *Electrochim. Acta* **2010**, *55*, 6601.
[10] L. Rassaei, M. Sillanpää, F. Marken, *Electrochim. Acta* **2008**, *53*, 5732.
[11] S. Shahrokhian, M. Ghalkhani, *Electrochem. Commun.* **2009**, *11*, 1425.
[12] M. Ghalkhani, S. Shahrokhian, *Electrochem. Commun.* **2010**, *12*, 66.
[13] S. Shahrokhian, E. Jokar, M. Ghalkhani, *Microchim. Acta* **2010**, *170*, 141.
[14] A. Ramanavičius, A. Ramanavičiene, A. Malinauskas, *Electrochim. Acta* **2006**, *51*, 6025.
[15] M. M. Chehimi, E. Abdeljali, *Synth. Met.* **2004**, *145*, 15.
[16] A. R. Zanganeh, M. K. Amini, *Electrochim. Acta* **2007**, *52*, 3822.
[17] D. Svenson, I. A. Nicholls, *Anal. Chim. Acta* **2001**, *435*, 19.
[18] L. X. Wang, X. G. Li, Y. L. Yang, *React. Funct. Polym.* **2001**, *47*, 125.
[19] A. R. Zanganeh, M. K. Amini, *Sens. Actuators B*, **2008**, *135*, 358.
[20] A. Wanekaya, O. A. Sadik, *J. Electroanal. Chem.* **2002**, *537*, 135.
[21] K. Sode, S. Ohta, Y. Yanai, T. Yamazaki, *Biosens. Bioelectron.* **2003**, *18*, 1485.
[22] M. Cortina-Puig, X. Muñoz-Berbel, M. del Valle, F. J. Muñoz, M. A. Alonso-Lomillo, *Anal. Chim. Acta* **2007**, *597*, 231.
[23] M. Heitzmann, C. Bucher, J.-C. Moutet, E. Pereira, B. L. Rivas, G. Royal, E. Saint-Aman, *Electrochim. Acta* **2007**, *52*, 3082.
[24] M. Amiri, S. Shahrokhian, E. Psillakis, F. Marken, *Anal. Chim. Acta* **2007**, *593*, 117.
[25] J. Sumfleth, X. C. Adroher, K. Schulte, *J. Mater. Sci.* **2009**, *44*, 3241.
[26] L. Rassaei, M. Sillanpää, M. J. Bonné, F. Marken, *Electroanalysis* **2007**, *19*, 1461.
[27] Y. K. Lee, K. J. Lee, D. S. Kim, D. J. Lee, J. Y. Kim, *Synth. Met.* **2010**, *160*, 814.
[28] A. Elgafy, K. Lafdi, *Carbon* **2006**, *44*, 1682.
[29] M. Knite, K. Ozols, G. Sakale, V. Teteris, *Sens. Actuators B* **2007**, *126*, 209.
[30] A. R. Hopkins, N. S. Lewis, *Anal. Chem.* **2001**, *73*, 884.
[31] K. Arshak, E. Moore, G. M. Lyons, J. Harris, S. Clifford, *Sens. Rev.* **2004**, *24*, 181.
[32] C. Li, H. Bai, G. Shi, *Chem. Soc. Rev.* **2009**, *38*, 2397.
[33] D. D.L. Chung, *J. Mater. Sci.* **2004**, *39*, 2645.
[34] M. Amiri, S. Shahrokhian, F. Marken, *Electroanalysis* **2007**, *19*, 1032.
[35] A. Mourato, J. F. Cabrita, A. M. Ferraria, A. M. Botelho do Rego, L. M. Abrantes, *Catal. Today* **2010**, *158*, 2.
[36] S. Rajesh, A. K. Kanugula, K. Bhargava, G. Ilavazhagan, S. Kotamraju, C. Karunakaran, *Biosens. Bioelectron.* **2010**, *26*, 689.
[37] H. S. Booth, *Inorganic Synthesis*, Vol. 28, McGraw-Hill, New York **1939**, pp. 338–340.
[38] S. K. De, J. R. White, *Short Fibre-Polymer Composites*, Woodhead, Cambridge **1996**, pp. 168–188.
[39] A. Witkowski, A. B. Toth, *Anal. Chem.* **1992**, *64*, 635.
[40] S. Shahrokhian, S. Bozorgzadeh, *Electrochim. Acta* **2006**, *51*, 4271.
[41] S. Shahrokhian, M. Ghalkhani, M. K. Amini, *Sens. Actuators B* **2009**, *137*, 669.
[42] S. Shahrokhian, E. Asadian, *J. Electroanal. Chem.* **2009**, *636*, 40.

