Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
*J. Serb. Chem. Soc.* *78* (10) 1595–1607 (2013)
*JSCS–4521*
UDC 577.112.386+544.6.076.32–039.6:544.4:543.552
*Original scientific paper*

# Electrocatalytic oxidation and determination of homocysteine at nanotubes-modified carbon paste electrode using dopamine as a mediator

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(Received 29 October 2012, revised 11 January 2013)

*Abstract*: A carbon paste electrode modified with multiwall carbon nanotubes (MWCNTPE) was prepared to study the electrocatalytic activity of dopamine (DP) in the presence of homocysteine (HCy) and it was used for the determination of HCy. The diffusion coefficient of HCy ($D$ = 6.79×10$^{-6}$ cm$^2$ s$^{-1}$), and the kinetic parameters of its oxidation, such as electron transfer coefficient ($\alpha$ = 0.46), and rate constant ($k_h$ = 7.44×10$^2$ dm$^3$ mol$^{-1}$ s$^{-1}$) were also determined using electrochemical approaches. Under the optimum pH of 5.0, the peak current of oxidation of HCy at MWCNTPE in the presence of DP occurred at a potential of about 530 mV and the results showed that the oxidation peak current of HCy at the modified carbon nanotubes electrode was higher than on the unmodified electrode. The peak current of differential pulse voltammograms of HCy solutions increased linearly in the range 3.0–600 μM HCy with a detection limit of 2.08 μM HCy. This method was also examined for determination of HCy in physiological serum and urine samples.

*Keywords*: homocysteine; dopamine; electrocatalytic effect; carbon nanotubes, carbon paste electrode; voltammetry.

## INTRODUCTION

Homocysteine (HCy), as an important sulfur-containing amino acid, plays a considerable role in many biochemical processes.[1] HCy was discovered by Butz and Du Vigneaud in 1932 by heating methionine in sulfuric acid.[2] The produced compound was found to have chemical properties similar to those of cysteine.

The concentration of HCy can be an independent risk factor for disease in human society.[3,4] Mild hyperhomocysteinemia has no signs but it provides the conditions for developed premature coronary disease in the third or fourth decade of life.[4] Thus, early determination of the HCy plasma concentration is necessary and essential. Based on clinical studies, the normal total plasma HCy concen-

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doi: 10.2298/JSC121019022M

tration range has been confirmed as 5–15 μM in the fasting state and elevated level of HCy has been classified in three range, moderate (16–30 μM) medium (30–100 μM) and severe (>100 μM).[5,6] The total plasma HCy includes, 75 % homocysteine bound to various proteins *via* disulfide bonds and the remaining 25 % free HCy includes oxidized homocysteine dimers (homocystine) or as homocysteine–cysteine hetero dimers. Only 1–2 % of HCy is in the reduced state and is distributed among different tissues, cells, and intracellular compartments. In addition, small metabolic changes of HCy affect many physiological concentrations.[7,8]

According to many researches, elevated plasma HCy concentrations are known to be a factor implicated in the appearance and progression of various diseases, such as coronary artery and cerebro vascular disease.[4,9,10]

To date, due to the importance of the determination of HCy, different techniques have been employed its measurement. The direct methods included the enzyme immunoassay (EIA),[11,12] the fluorescence polarization immunoassay (FPIA),[13–15] and the enzyme-linked immunosorbent assay (ELISA).[16,17] Gas chromatography (GC),[18–21] liquid chromatography (LC),[22,23] high-performance liquid chromatography (HPLC),[24–31] capillary electrophoresis (CPE)[28,32,33] and electrochemical methods[1,5,34–41] fall in the category of indirect methods. The advantages of electrochemical methods, such as sensitivity, selectivity, facile, high performance, low cost, high accuracy and precision, aroused the attention of many researchers.[1,5,35,41–51] Other methods such as immunoassay kits and chromatographic methods require sample preparation that consumes a long time, are expensive and necessitate the use environmentally hazardous solvents.[1,5,35]

In the present work, we applied a carbon nanotubes-modified graphite paste electrode for determination of HCy using dopamine (DP) as an electrocatalytic mediator. The nanotubes interconnect the graphite powder particles together to form a continuous conductive network. The small diameters of the nanotubes enabled the nanotubes to be homogeneously distributed in the thin electrode material and to introduce a larger surface area to react with the electrolyte. The improved electrical conductivity of the electrode was related to the high electrical conductivity of the tubes, and the function of the electrical bridge between the graphite particles. In the presence of carbon nanotubes, the anodic signal increased, which could be due to the increase in the active surface of electrode and increase the conductivity of the electrode.[52–61] Effective parameters on anodic signal were studied and a method was proposed for determination of HCy in biological serum and urine samples.

## EXPERIMENTAL

### *Reagents and apparatus*

All employed chemical were of analytical grade and purchased from Merck, Aldrich and Fluka. Homocysteine (≥95 %) and DP (99 %) were purchased from Aldrich.

Spectrally pure graphite powder (particle size <50 μm) from Merck and multiwall carbon nanotubes (> 90 %, MWCNTs, *d*×l, 100–70 nm×5–9 μm) from Fluka were used as the substrate for the preparation of the carbon paste electrode as a working electrode (WE). High viscosity paraffin ($\rho$ = 0.88 kg $L^{-1}$) from Merck was used as the pasting liquid for the preparation of the paste electrodes.

Universal buffer solutions (0.04 M acetic acid, boric acid, phosphoric acid and 0.2 M sodium hydroxide) contain the 0.1 M potassium chloride with different pH values were prepared to examine and provide the optimum pH value.

All aqueous solutions were prepared from deionized water by passing through a Milli-Q water purification system (resistance >18 MW $cm^{-1}$).

The homocysteine and DP solutions, $1.0\times10^{-2}$ M, were prepared daily by dissolving 0.0068 g homocysteine, respectively, 0.0095 g dopamine in the buffer solution and then diluting to 5 ml in two volumetric flasks. The solution was kept in a refrigerator at 4 °C in the dark. More dilute solutions were prepared by serial dilution of these solutions with water.

All electrochemical experiments including cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed using a computerized potentiostat/galvanostat (Autolab PGSTAT101, Eco Chem. Utrecht, The Netherlands). A Pentium IV computer controlled all settings and data processing of the system. All the electrochemical studies were performed at 25±1 °C. The system was connected to a three-electrode cell assembly consisting of a 50 mL glass cell containing an Ag/AgCl electrode as the reference electrode (RE), a platinum wire counter electrode and a MWCNTPE working electrode. All of the measured potentials are reported *vs*. the Ag/AgCl reference electrode. The pH of the solutions was controlled with a Metrohm pH meter (model 827). The electrode prepared with carbon nanotubes was characterized by scanning electron microscopy (SEM) (Seron Tech. AIS 2100).

*Preparation of the modified electrode (MWCNTPE)*

A mixture of 0.10 g carbon nanotubes (CNT) and 0.9 g graphite powder was hand mixed using a pestle and mortar. To achieve a uniform mixture, diethyl ether was added to the mixture, the mixture was mixed again, and the diethyl ether allowed adequately to evaporate. Paraffin oil, about of 0.4 g, was added to the mixture and mixed well for 20 min until a uniformly wetted paste was obtained. The prepared paste was inserted into a glass tube (internal radius 2.2 mm) with a copper wire in it for electrical connection. A fresh surface was obtained after pushing out an excess the paste and polishing it on a weighing paper.

For comparison, a uniform carbon paste electrode (CPE) was obtained in the same way without addition of CNT to the mixture.

*Electrochemical procedure*

The MWCNTPE as working electrode was located in an electrochemical cell containing 10 ml of buffer solution (universal pH 5 and 0.10 M KCl) including 400 μM DP to record the voltammetric measurements. The voltamogramms were recorded with a scan rate 20 mV $s^{-1}$ to give the blank signal from initial to final potentials of 0.1 mV to 0.7 mV *vs*. the Ag/AgCl reference electrode. Subsequently, different amounts of HCy solution were added to the cell and the same procedure was repeated to give the analytical signal. Differential pulse voltammetry (DPV) curves were recorded with a pulse height and pulse width of 50 mV and 0.005 s, respectively, using the same procedure to obtain blank and analytical signals. The difference between the blank and the analytical signal was obtained as a net peak current that was proportional to the concentration of HCy. Calibration curves were constructed by plotting the net peak current *vs*. concentration of HCy.

*Preparation of real sample*

Urine sample was stored in a refrigerator (at 4 °C) immediately after collection. Ten milliliters of the sample was centrifuged for 15 min at 1500 rpm and the supernatant solution was filtered through a 0.45 μm filter. Then, the solution was diluted ten times with buffer solution (pH 5.0 and 0.10 M KCl) and was transferred into the electrochemical cell, where it was analyzed without any further pretreatment. The standard addition method was used for the determination of HCy in real samples.

Physiological serum solutions (0.9 % NaCl) were analyzed directly after 1:1 diluted with buffer solution (pH 5 and 0.10 M KCl) without any further preparation.

## RESULTS AND DISCUSSION

### *SEM characterization of electrodes*

The SEM image of the CPE and MWCNTPE has been shown in the Fig. 1. As can be seen, multiwall carbon nanotubes were dispersed to the carbon paste matrix and were caused increasing the surface area of the active sites of the electrode.

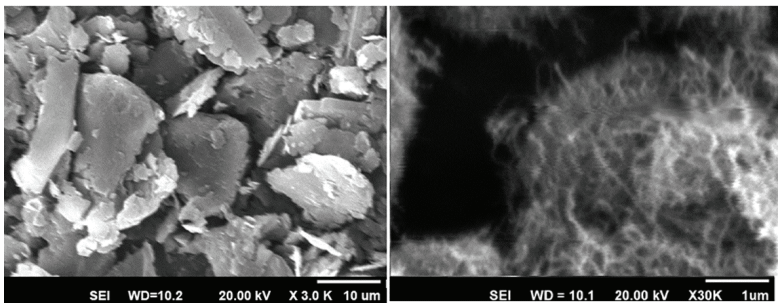



Fig. 1. SEM Image of A) CPE and B) MWCNTPE.

### *Electrochemical behavior of dopamine (DP) on MWCNTPE*

The electrochemical behavior of DP on MWCNTPE in buffer solution (pH 5.0 and 0.10 M KCl) was characterized by cyclic voltammetry. The cyclic voltammogram (Fig. 2) of DP showed well-defined and reproducible anodic and cathodic peaks with a peak separation potential of $\Delta E_p = E_{pa} - E_{pc} = 620$ mV. This value was greater than the value of $59/n$ mV for reversible behavior and was indicative of quasi-reversible behavior. The influence of potential scan rate (20 to 500 mV $s^{-1}$) on the anodic peak current of DP was studied by cyclic voltammetry. The peak currents of DP grew with increasing scan rate and the anodic peak current increased linearly with square root of the scan rate ($I = 0.986v^{1/2} - - 0.534$, $R^2 = 0.995$). Such a dependence shows that the oxidation reaction of DP on the MWCNTPE is diffusion-controlled (Fig. 2).[62]

### *pH Effect*

As the oxidation of DP is accomplished with the exchange of two electrons and two protons, the dependence of the redox reaction of DP on pH could be con-

cluded. The voltammetric response of DP showed that with increasing pH of the solution, the peak potential shifted to less positive potentials. In addition, the effect of pH solution on the electrocatlytic oxidation of HCy in the presence of DP at the surface of MWCNTPE was investigated using cyclic voltammetry. The optimum pH was investigated by varying the pH from 3.0 to 6.0. As can be seen in Fig. 3, maximum peak current was obtained at pH 5.0. Hence, this pH was selected as optimum pH for further experiments.

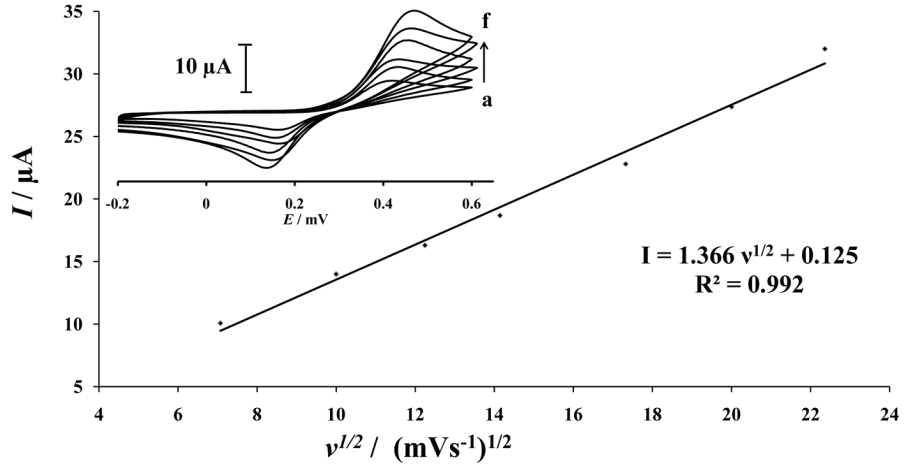



Fig.2. Plot of $I$ *vs.* $v^{1/2}$ for 400 µM DP at the MWCNTPE. Inset: cyclic voltammograms at various scan rates of a) 50, b) 100, c) 150, d) 200, e) 300 and f) 400 mV $s^{-1}$ in the universal buffer (pH 5).

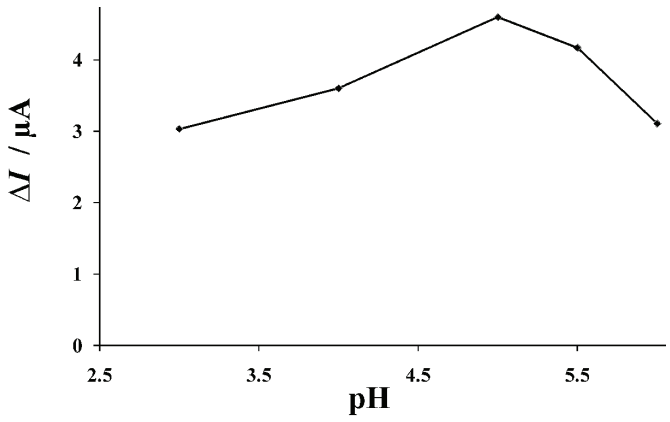



Fig. 3. Effect of pH on the electrocatalytic current.

*Optimization of the dopamine (DP) concentration*

The effect of DP concentration on the peak currents was studied by varying its concentration in the range of 100–600 µM at pH 5.0. The electrocatalytic

response was optimized and the maximum value of the oxidation peak current was achieved by increasing the DP concentration up to 400 μM. This value was selected as the optimum concentration for further experiments.

*Electrocatalytic oxidation of HCy on MWCNTPE*

HCy, as an oxidizable compound, can be detected by electrochemical methods based on its anodic oxidation.[8] The voltammetric response of HCy at MWCNTPE was investigated in presence and absence of DP in pH 5.0 buffer solution using cyclic voltammetry at a scan rate of 20 mV $s^{-1}$. The recorded cyclic voltammograms of HCy in the absence of DP did not show a significant redox peak for HCy (Fig. 4, curve d). Moreover, a pair of well-defined redox peaks was observed as the voltammetric behavior of DP (400 μM) in the absence of HCy (Fig. 4, curve c). Upon the addition of 500 μM HCy, the anodic peak current increased sharply and no cathodic current was observed in the reverse scan (Fig. 4, curve a). Therefore, HCy can be detected in the presence of DP potential about 0.53 V *vs*. Ag/AgCl at the surface of a MWCNTPE. As can be seen, the oxidation of HCy on a CPE in presence of DP (Fig. 4, curve b) has a lower peak current compare to that on the MWCNTPE. In the other words, the presence of multiwall carbon nanotubes increased the conductivity and effective surface area of the electrode for the oxidation of HCy.[53,54] Based on the the obtained information, the electrocatalytic mechanism showen in Scheme 1 is suggested for the oxidation of HCy. In the first step, DP is oxidized on the surface of the MWCNTPE. Then, the oxidized form of DP is reduced by HCy, whereby HCy is converted to its oxidized form.[8]

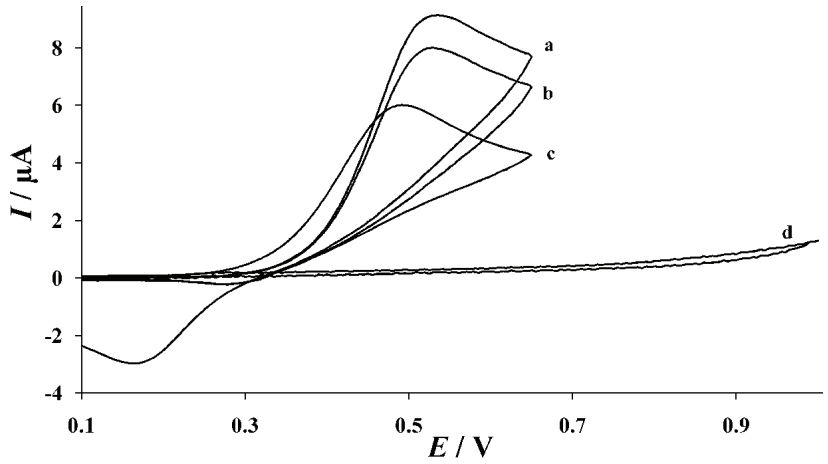
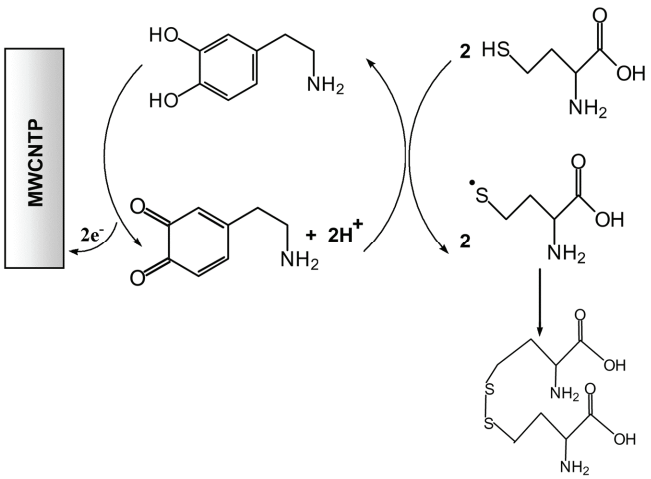



Fig.4. Cyclic voltammograms recorded at a scan rate of 20 mV $s^{-1}$ in universal buffer solution pH 5.0. a) 400 μM DP at the MWCNTPE in the presence of 500 μM HCy, b) as a) but at the CPE, c) 400 μM DP at the MWCNTPE, d) 500 μM HCy at the MWCNTPE in the absence of DP.




Scheme 1. Electrocatalytic mechanism for the oxidation of HCy at the surface of the modified electrode.

Cyclic voltammograms of 500 μM HCy in presence of 400 μM DP at pH 5.0 were recorded in the potential range −0.1 to 0.6 V at various sweep rates. With increasing scan rate from 5 to 40 mV $s^{-1}$, the peak currents of HCy increaed (Fig. 5, inset). The results showed, there was a linear relationship between the peak current and the square root of the scan rate ($v^{1/2}$) with the regression equation:

$$I\,(\mu A) = 1.621 v^{1/2}\,(\text{mV s}^{-1})^{1/2} + 0.820$$
$$(R^2 = 0.997)$$

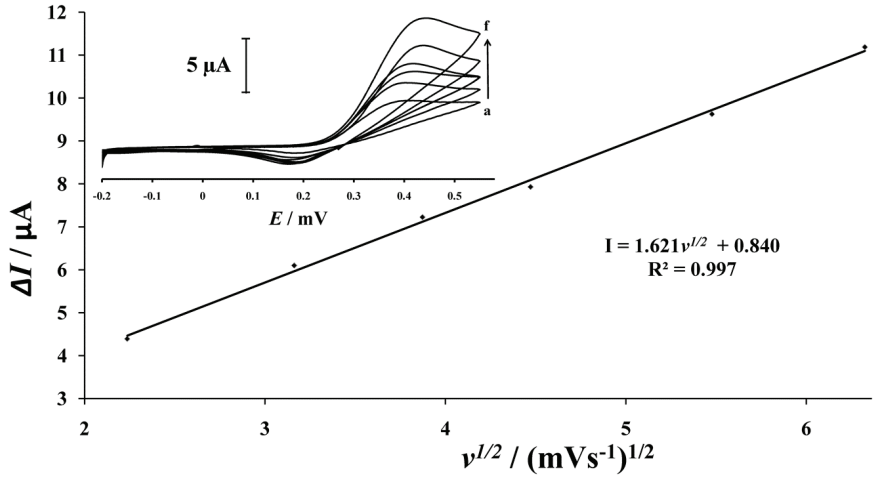



Fig. 5. Plot of $I$ *vs.* $v^{1/2}$ for 500 μM HCy in the presence of 400 μM DP at the MWCNTPE. Inset: cyclic voltammograms recorded at various scan rates: a) 5, b) 10, c) 15, d) 20, e) 30 and f) 40 mV $s^{-1}$ in the universal buffer (pH 5.0).

This equation confirms that the process is diffusion controlled (Fig. 5). In addition, by increasing in the scan rate and following the shift of the oxidation peak potential towards more positive potential, the kinetic limitation of the electrochemical reaction was verified.[63]

In order to obtain information on the rate determining step, Tafel plot was obtained from raising part of the current–voltage curve at scan rate of 20 mV $s^{-1}$ (Fig. 6). The slope of the Tafel plot was equal to $2.3RT/n(1-\alpha)F$, which was 0.108 V $decade^{-1}$. Using this data and assuming $n = 1$, then $\alpha = 0.46$.

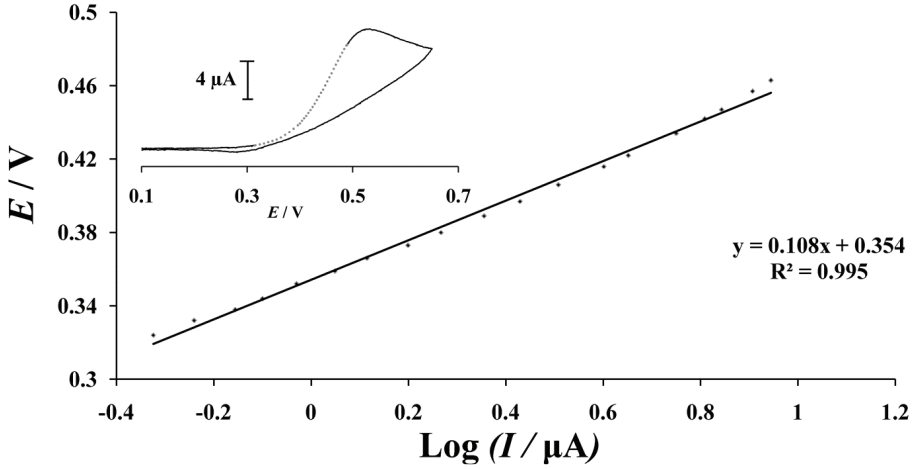



Fig. 6. Tafel plot for 500 µM HCy in the presence of 400 µM DP (pH 5.0) at the MWCNTPE.

*Chronoamperometric study*

The electrochemical response of HCy at the MWCNTPE was investigated using various concentrations of HCy by chronoamperometry. The chronoamperograms were obtained in buffered solution by employing and adjusting a double-step potential from 0.3 (first step) to 0.7 V (second step) *vs*. Ag/AgCl (Fig. 7). The linearity of the electrocatalytic current *vs.* $t^{-1/2}$ indicates that the current must have been under diffusion control. The inset in Fig. 7 shows the linear relationships between $I$ *vs*. $t^{-1/2}$ for different concentrations of HCy. The Cottrell Equation is given by:

$$I = \frac{nFAc\sqrt{D}}{\sqrt{\pi t}} \quad (1)$$

where $I$ is the current (A), $n$ the number of electrons involved in the process, $F$ is the Faraday constant, $A$ is the area of the electrode ($cm^2$), $c$ is the initial concentration of HCy (mol $cm^{-3}$), $D$ is the diffusion coefficient ($cm^2$ $s^{-1}$) and $t$ is time (s).

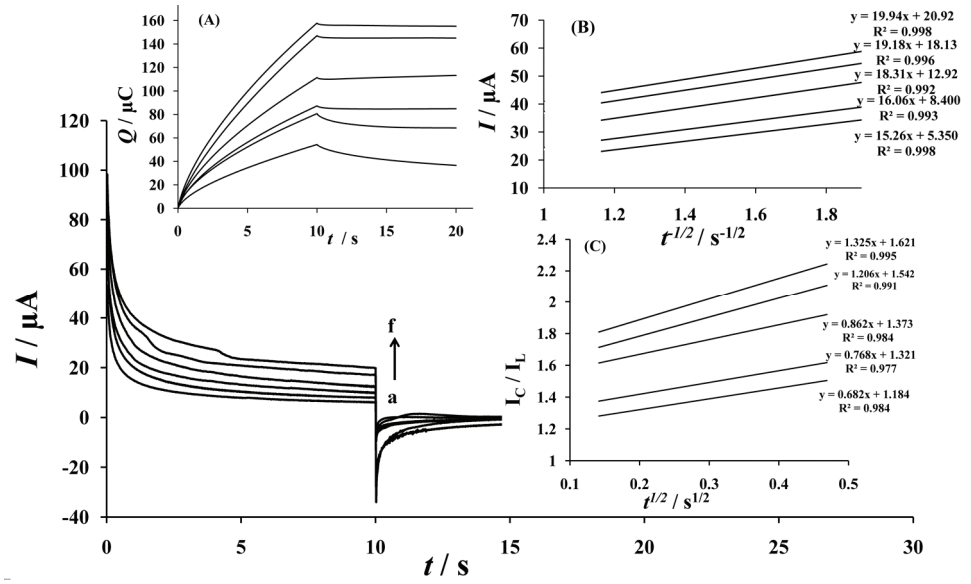



Fig.7. Chronoamperograms obtained at MWCNTPE in the absence (a), and in the presence of b) 100, c) 300, d) 700, e) 900 and f) 1000 µM HCy in buffer solution (pH 5.0). Inset (A): charge–time curves; inset (B): the Cottrell plot for data from the chronoamperograms; inset (C): dependence of $I_C/I_L$ on $t^{1/2}$ derived from the chronoamperogramic data.

From the Cottrell Equation and the obtained slope values for different concentrations of HCy, the diffusion coefficient could be calculated and was found to be $6.79\times10^{-6}$ cm$^2$ s$^{-1}$.[62]

In addition, the chronoamperometry method was applied to obtain the rate constant of the catalytic oxidation ($k_h$) of HCy. For this purpose, the Galus Method was used:

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

where $I_C$ is catalytic current of HCy in the presence of DP at the MWCNTPE, $I_L$ is the limiting current in the absence of HCy, and $t$ is the time elapsed (s). Based on the slope of the $I_C/I_L$ *vs*. $t^{1/2}$ plots, $k$ could be obtained for different concentrations of HCy. The average value of $k_h$ was calculated to be $7.44\times10^2$ dm$^3$ mol$^{-1}$ s$^{-1}$.

*Analytical features of the method*

Differential pulse voltammetry method (DPV) was used for preparation of the calibration plot. The DP voltammograms were recorded for the determination of HCy in the buffer solution (pH 5.0 and 0.10 M KCl). The electrocatalytic current of MWCNTPE increased with increasing HCy concentration in the pre-

sence of 400 μM DP. The results showed that there is a linear dynamic range of 3.0–600 μM for HCy.

$$I\,(\mu A) = 0.008 c_{HCy} + 0.873,\ R^2 = 0.997$$

The limit of detection was 2.08 μM and the relative standard deviations (*RSD*) for six replicated analyses of 200 and 400 μM HCy at 20 mV $s^{-1}$ were 1.15 and 2.3 %, respectively.

*Interference study*

To evaluate the ability of proposed method for determination of HCy in real samples, such as urine, the interference effects due to some potential interfering species that are commonly found in urine, were studied under the optimum conditions with 400 μM HCy at pH 5.0. The tolerance limit was taken as the maximum concentration of foreign substances that caused no more than ±5 % relative error in the determination of HCy. Some cations may form complexes with HCy or DP and disrupt or effect on electrocatalytic mechanism, but no interference was observed for the following cations: $Pb^{2+}$, $Ni^{2+}$, $Ba^{2+}$and $Ca^{2+}$ (1000 fold) or $Fe^{2+}$ (100 fold). The results are presented in Table I.

TABLE I. Interference study

| Tolerance limit ($M/M_{HCy}$) | Species |
|---|---|
| 1000 | $Pb^{2+}$, $NO_3^{2-}$, $Ni^{2+}$ , $SO_4^{2-}$, $Ba^{2+}$, $Cl^-$, $Ca^{2+}$, glucose, methionine, phenylalanine, ethanol, fructose, lactose, sucrose, urea |
| 100 | $Fe^{2+}$ |
| 50 | Citric acid |
| 10 | Uric acid |
| 1 | Ascorbic acid, L-cysteine |

*Determination of HCy in real samples*

The utility of the proposed sensor for the electrocatalytic determination of HCy was investigated in physiological serum solution and urine samples. The centrifuged urine sample and physiological serum were diluted ten and two times, respectively, with buffer and then various amounts of HCy were added to the physiological serum and urine samples. The recovery of HCy was obtained using the standard addition method. The results are given in Table II, from which it could be seen the proposed method had good recoveries of the spiked HCy.

TABLE II. Determination of HCy in real samples

| No. | Biological sample | HCy added μM | Expected μM | Found±*SD* (*n* = 3) μM | Recovery % |
|---|---|---|---|---|---|
| 1 | Serum | – | – | < Detection limit | |
| 2 | | 50.0 | 50.0 | 50.6±1.1 | 101.2 |
| 3 | | 100.0 | 150.0 | 148.9±1.2 | 99.3 |

TABLE II. Continued

| No. | Biological sample | HCy added μM | Expected μM | Found±*SD* (*n* = 3) μM | Recovery % |
|---|---|---|---|---|---|
| 4 | Urine | – | – | < Detection limit | – |
| 5 | | 50.0 | 50.0 | 48.7±0.6 | 97.4 |
| 6 | | 50.0 | 100.0 | 102.2±0.7 | 102.2 |
| 7 | | 100.0 | 200.0 | 198.6±0.8 | 99.3 |

## CONCLUSIONS

In this paper, DP was proposed as a homogeneous mediator for determination of HCy by electrochemical oxidation at a multiwall carbon nanotubes graphite paste electrode (MWCNTPE). The electrochemical behavior of HCy was studied using cyclic voltammetry, differential pulse voltammetry and chronoamperometry in a buffer solution (pH 5.0 and 0.10 M KCl). The peak current of HCy oxidation was at 0.53 V on the MWCNTPE in the presence of DP, while in the absence of DP oxidation of HCy did not occur at the surface of the carbon nanotube graphite paste electrode. The kinetic parameters of the electrocatalytic process and the diffusion coefficients of HCy in an aqueous solution were determined. The proposed method has a good linear dynamic range and was applied for the determination of HCy in biological serum and urine samples.

*Acknowledgments.* The authors gratefully acknowledge the National Foundation for Iranian Elites, the research councils of Islamic Azad University, Falavarjan branch, and Payame Noor University, Iran, for their support of this work.

## ИЗВОД

## ЕЛЕКТОКАТАЛИТИЧКА ОКСИДАЦИЈА И ОДРЕЂИВАЊЕ ХОМОЦИСТЕИНА НА ЕЛЕКТРОДИ ОД УГЉЕНИЧНЕ ПАСТЕ МОДИФИКОВАНОЈ ДОПАМИНОМ КАО МЕДИЈАТОРОМ

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Електрода од угљеничне пасте која је модификована вишеслојним угљеничним наноцевима припремљена је за испитивање електрокаталитичке активности допамина у присуству хомоцистеина и коришћена за одређивање хомоцистеина у раствору. На основу електрохемијских мерења одређен је коефицијент дифузије хомоцистеина ($D$ = = $6{,}79\times10^{-6}$ $cm^2$ $s^{-1}$) и кинетички параметари за његову оксидацију, као што су коефицијент прелаза ($\alpha = 0.46$) и константа брзине ($k_h$ = $7{,}44\times10^2$ $dm^3$ $mol^{-1}$ $s^{-1}$). При оптималној вредности pH 5,0 максимум струје оксидације хомоцистеина на електроди од угљеничне пасте модификованој допамином постиже се на потенцијалу од око 530 mV. Запажено је да је тај максимум виши него на немодификованој електроди. Струјни максимум за оксидацију хомоцистеина одређен диференцијалном пулсном волтаметријом повећавао се линеарно у опсегу концетрације 3,0–600,0 μM са границом детекције 2,08 μM хомоцистеина. Ова метода је такође тестирана за одређивање хомоцистеина у физиолошком серуму и у узорцима урина.

(Примљено 29. октобра 2012, ревидирано 11. јануара 2013)

REFERENCES


1. S. Timur, D. Odaci, A. Dincer, F. Zihnioglu, A. Telefoncu, L. Gorton, *Sensors Actuators, B* **125** (2007) 234
2. L. W. Butz, V. Du Vigneaud, *J. Biol. Chem*. **99** (1932) 135
3. A. A. Z. Sanjay Kaul, P. K. Shah, *J. Am. Coll. Cardiol.* **48** (2006) 914
4. G. N. Welch, J. Loscalzo, *N. Engl. J. Med*. **338** (1998) 1042
5. L. Agüí, C. Peña-Farfal, P. Yáñez-Sedeño, J. M. Pingarrón, *Talanta* **74** (2007) 412
6. G. Zhang, D. Liu, S. Shuang, M. M. F. Choi, *Sensors Actuators, B* **114** (2006) 936
7. T. G. Guilliams, *JANA* **7** (2004) 11
8. F. Gholami-Orimi, F. Taleshi, P. Biparva, H. Karimi-Maleh, H. Beitollahi, H. R. Ebrahimi, M. Shamshiri, H. Bagheri, M. Fouladgar, A. Taherkhani, *J. Anal. Methods Chem.* 2012 (2012), Article ID 902184, doi: 10.1155/2012/902184
9. H. S. Collaboration, *JAMA* **288** (2002) 2015
10. J. Perla-Kajan, T. Twardowski, H. Jakubowski, *Amino Acids* **32** (2007) 561
11. A. D. Paramonov, S. V. Moiseev, V. V. Fomin, M. V. Kopeleva, L. I. Stankevich, A. I. Martynov, N. A. Mukhin, *Terapevt. Arkh.* **76** (2004) 67
12. A. Khosrowbeygi, H. Ahmadvand, *Bangladesh Med. Res. Counc. Bull.* **37** (2011) 106
13. J. Wan, Y. Deng, J. Guo, G. Xiao, D. Kuang, Y. Zhu, Y. Duan, G. Wang, *Exp. Mol. Pathol.* **91** (2011) 411
14. C. Huang, L. Zhang, Z. Wang, H. Pan, J. Zhu, *Acta Cardiol*. **66** (2011) 773
15. Y. J. Lai, W. L. Tseng, *Talanta* **91** (2012) 103
16. C. Sanli, D. Oguz, R. Olgunturk, F. S. Tunaoglu, S. Kula, H. Pasaoglu, O. Gulbahar, A. Cevik, *Pediatr. Cardiol.* **33** (2012) 1323
17. M. Y. Alsagaff, M. Thaha, M. Aminuddin, R. M. Yogiarto, M. Yogiantoro, Y. Tomino, *J. Int. Med. Res.* **40** (2012) 340
18. F. Wang, L. Han, Y. Yang, X. Gu, J. Ye, W. Qiu, H. Zhang, Y. Zhang, X. Gao, Y. Wang, *J. Inherit. Metab. Dis.* **33** Suppl. 3 (2010) 435
19. Z. Svagera, D. Hanzlikova, P. Simek, P. Husek, *Anal. Bioanal. Chem.* **402** (2012) 2953
20. J. Kaluzna-Czaplinska, M. Michalska, J. Rynkowski, *Nutr. Res.* **31** (2011) 318
21. J. Kaluzna-Czaplinska, M. Michalska, J. Rynkowski, *Acta Biochim. Pol.* **58** (2011) 31
22. S. Zhang, F. Huang, J. Zhao, L. Wen, F. Zhou, P. Yang, *Talanta* **58** (2002) 451
23. Y. Rao, M. McCooeye, Z. Mester, *Anal. Chim. Acta* **721** (2012) 129
24. S. Tokgz, C. Bulgu, E. C. Akir, M. K. Erbul, T. Kutluay, *Turk. J. Med. Sci.* **33** (2003) 71
25. A. Khan, M. I. Khan, Z. Iqbal, Y. Shah, L. Ahmad, S. Nazir, D. G. Watson, J. A. Khan, F. Nasir, Ismail, *Talanta* **84** (2011) 789
26. O. Chailapakul, W. Siangproh, B. V. Sarada, C. Terashima, T. N. Rao, D. A. Tryk, A. Fujishima, *Analyst* **127** (2002) 1164
27. C. M. Pfeiffer, D. L. Huff, E. W. Gunter, *Clin. Chem.* **45** (1999) 290
28. K. Kuśmierek, G. Chwatko, R. Głowacki, P. Kubalczyk, E. Bald, *J. Chromatogr., B* **879** (2011) 1290
29. M. Zhang, C. M. Pfeiffer, *Clin. Chim. Acta* **340** (2004) 195
30. A. A. Ghanem, S. M. Mady, H. E. El Awady, L. F. Arafa, *Curr. Eye Res.* **37** (2012) 712
31. M. Lopez-Alarcon, A. Chavez-Negrete, I. Montalvo-Velarde, J. Maldonado-Hernandez, V. S. Vital-Reyes, *Cir Cir* **79** (2011) 432
32. S. A. Pasas, N. A. Lacher, M. I. Davies, S. M. Lunte, *Electrophoresis* **23** (2002) 759
33. A. Zinellu, S. Sotgia, B. Scanu, E. Pisanu, M. Sanna, S. Sati, L. Deiana, S. Sengupta, C. Carru, *Talanta* **82** (2010) 1281
34. Z. Chen, Y. Zu, *J. Electroanal. Chem.* **624** (2008) 9

35. N. S. Lawrence, J. Davis, R. G. Compton, *Talanta* **53** (2001) 1089
36. M. K. Halbert, R. P. Baldwin, *Anal. Chim. Acta* **57** (1985) 591
37. M. Heyrovský, S. Vavřička, *Bioelectrochem. Bioenerg*. **48** (1999) 43
38. P. Kalimuthu, S. A. John, *Bioelectrochemistry* **79** (2010) 168
39. K. Gong, Y. Dong, S. Xiong, Y. Chen, L. Mao, *Biosen. Bioelectron.* **20** (2004) 253
40. E. Sharifi, A. Salimi, E. Shams, *Bioelectrochemistry* **86** (2012) 9
41. H. Han, H. Tachikawa, *Front. Biosci*. **10** (2005) 931
42. V. K. Gupta, R. Jain, K. Radhapyari, N. Jadon, S. Agarwal, *Anal. Biochem.* **408** (2011) 179
43. R. Jain, V. K. Gupta, N. Jadon, K. Radhapyari, *Anal. Biochem.* **407** (2010) 79
44. R. N. Goyal, V. K. Gupta, S. Chatterjee, *Biosensors Bioelectron.* **24** (2009) 3562
45. R. N. Goyal, V. K.Gupta, A. Sangal, N. Bachheti, *Electroanalysis* **17** (2005) 2217
46. R. Jain, V. K. Gupta, N. Jadon, K. Radhapyari, *J. Electroanal. Chem.* **648** (2010) 20
47. Y. Li, Y. Umasankar, S. M. Chen, *Anal. Biochem*. **388** (2009) 288
48. Y. Umasankar, T. Y. Huang, S. M. Chen, *Anal. Biochem*. **408** (2011) 297
49. A. P. Periasamy, Y. J. Chang, S. M. Chen, *Bioelectrochemistry* **80** (2011) 114
50. A. K. Upadhyay, T. W. Ting, S. M. Chen, *Talanta* **79** (2009) 38
51. A. P. Periasamy, Y. H. Ho, S. M. Chen, *Biosensors Bioelectron*. **29** (2011) 151
52. M. Fouladgar, M. R. Hadjmohammadi2, M. A. Khalilzadeh, P. Biparva, N. Teymoori, H. Beitollah, *Int. J. Electrochem. Sci.* **6** (2011) 1355
53. M. Fouladgar, H. Karimi-Maleh, R. Hosseinzadeh, *Ionics* **19** (2013) 665
54. M. Fouladgar, H. Karimi-Maleh, *Ionics* **19** (2013) 1163
55. M. Keyvanfard, A. A. Ensafi, H. Karimi-Maleh, *J. Solid State Electrochem*. **16** (2012) 2949
56. A. Mokhtari, H. Karimi-Maleh, A. A. Ensafi, H. Beitollahi, *Sensors Actuators, B* **169** (2012) 96
57. H. Karimi-Maleh, M. A. Khalilzadeh, Z. Ranjbarha, H. Beitollahi, A. A. Ensafi, D. Zareyee, *Anal. Methods* **4** (2012) 2088
58. H. Beitollahi, H. Khabazzadeh, H. Karimi-Maleh, A. Akbari, *Chin. Chem. Lett.* **23** (2012) 719
59. M. Roodbari Shahmiri, A. Bahari, H. Karimi-Maleh, R. Hosseinzadeh, N. Mirnia, *Sensors Actuators, B* **177** (2013) 70
60. M. Keyvanfard, R. Shakeri, H. Karimi-Maleh, K. Alizad, *Mat. Sci. Eng., C* **33** (2013) 811
61. H. Beitollahi, A. Mohadesi, S. Khalilzadeh Mahani, H. Karimi-Maleh, *Turk. J. Chem.* **36** (2012) 526
62. A. G. Bard, L. R Faulkner, *Electrochemical Methods, Fundamentals and Principles*, Wiley, New York, USA. 2001
63. M. Fouladgar, *Int. J. Electrochem. Sci.* **6** (2011) 705
64. Z. Galus, *Fundumentals of Electrochemical Analysis*, Ellis Horwood, New York, USA, 1976.

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