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Electrochemical and Catalytic Investigations of Homocysteine Using Multiwall Carbon Nanotubes Paste Electrode as a Sensor and Methyldopa as a Mediator

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ABSTRACT

A sensitive and selective electrochemical sensor for the determination of homocysteine (HCy) was developed using a modified multiwall carbon nanotubes paste electrode (MWCNTPE) in the presence of methyldopa (MDOP) as a mediator. This modified electrode showed very efficiency electrocatalytic activity for the anodic oxidation of HCy. Under the optimized conditions, the electrocatalytic peak current showed linear relationship with HCy concentration in the range of 0.1 – 800.0 μmol L^{-1} with a detection limit of 0.06 μmol L^{-1} HCy. The relative standard deviations for six successive assays of 1.0 and 15.0 μmol L^{-1} HCy were 2.1% and 2.6%, respectively. Finally, the modified electrode was examined as a selective, simple and precise new electrochemical sensor for the determination of HCy in serum and urine samples.

KEYWORDS: Homocysteine, Carbon paste electrode, Multiwall carbon nanotubes, Sensor

1. INTRODUCTION

Homocysteine is a non-protein amino acid. It is a homologue of the amino acid cysteine, differing by an additional methylene (-CH_2-) group. Homocysteine can be recycled into methionine or converted into cysteine with the aid of B-vitamins. While detection of high levels of homocysteine has been linked to cardiovascular disease, lowering homocysteine levels may not improve outcomes [1]. Therefore, determination of this compound is very important in biological sample such as urine and serum. Several methods have been proposed for the determination of HCy that include gas chromatography–mass spectroscopy (GC/MS) [2], HPLC (or capillary electrophoresis) with fluorescent [3], laser-induced fluorescent [4], mass spectrometric [5] and electrochemical methods [6-8].

Electrochemical methods have shown significant advantages in the analysis of different compounds in biological and pharmaceutical samples. These advantages are mainly attributable to the simplicity, low cost and relatively short analysis times of these compounds as compared to chromatography and other methods [9-16]. In recent years, chemically modified electrodes have attracted large notice due to their potential applications in various analyses [17-21]. Different modified electrodes based on amperometric and voltammetric detection have been reported for the determination of HCy.[6-8] The advantages and disadvantages of the proposed method versus recently published electrochemical methods for the determination of HCy are given in Table 1.

Carbon nanotubes (CNTs) have been proved to be a novel type of nanostructure with unique structural electronic and mechanical properties and have drowned extensive since their discovery [22-24]. Research over the past decade has revealed that the CNTs constituted a new form of carbon materials that are finding striking application in many filed, such as energy conversion and storage [22,26], chemical actuators [27,28], and chemical sensing [29-32].

Table 1. Comparison of the efficiency of reported electrchemical methods in the determination of HCy.

Method	Electrode	pH	LDR (μmol L^{-1})	LOD (μmol L^{-1})	Ref.
Amperometry	Carbon paste	2.0	0.1-5.0	0.03	6
Amperometry	Glassy carbon	---	0.1-60	0.06	7
Amperometry	Carbon nanotube paste	7.4	5.0-200.0	4.6	8
SWV	Carbon nanotube paste	6.0	0.1-800	0.06	This work

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In the continuation of our recently studies concerning the preparation of chemically modified electrodes,[33-40] in the present work we describe application of multiwall carbon nanotubes paste electrode as a voltammetric sensor for determination of HCy in the presence of MDOP as a suitable mediator. Additionally, we used the modified electrode as a new and sensitive sensor for determination of HCy in different real samples such as urine and serum.

EXPERIMENTAL

Apparatus and reagents

All the voltammetric measurements were performed using an Autolab PGSTAT 302N, potentiostat/galvanostat (Utrecht, The Netherlands) connected to a three-electrode cell, Metrohm (Herisau, Switzerland) Model 663 VA stand, linked with a computer (Pentium IV, 1,200 MHz) and with Autolab software. A platinum wire was used as the auxiliary electrode. MWCNTPE and $Ag/AgCl/KCl_{sat}$ were used as the working and reference electrodes, respectively. The electrode prepared with carbon nanotubes was characterized by scanning electron microscopy (SEM) (Seron Tech. AIS 2100). A digital pH/mV-meter (Metrohm model 710) was applied for pH measurements. Spectrally pure graphite powder (particle size <50 µm) from Merck and multiwall carbon nanotubes (>90% MWCNTs basis, $d \times l$ = (90–70 nm) × (5–9 µm) from Fluka were used as the substrate for the preparation of the carbon paste electrode.

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

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

Preparation of the electrode

Graphite powder (0.900 g) was dissolved in diethyl ether and hand mixed with 0.100 g carbon nanotubes in a mortar and pestle. The solvent was evaporated by stirring. A syringe was used to add paraffin to the mixture, which was mixed well for 40 min until a uniformly wetted paste, was obtained. The paste was then packed into a glass tube. Electrical contact was made by pushing a copper wire down the glass tube into the back of the mixture. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing it on a weighing paper.

Preparation of real samples

Urine samples were stored in a refrigerator immediately after collection. Ten milliliters of each sample was centrifuged for 15 min at 1500 rpm. The supernatant was filtered using a 0.45 µm filter and then diluted five times with universal buffer solution (pH 6.0). The solution was transferred into the voltammetric cell to be analyzed without any further pretreatment. Standard addition method was used for the determination of HCy in real samples.

Optimization of MDOP concentration

The influence of MDOP concentration on the electrocatalytic oxidation peak current was studied at three different concentrations of HCy (Figures 1 (a)–1(c)) at pH 6.0, and in the range of 100.0 to 800 µmol L^{-1} MDOP.

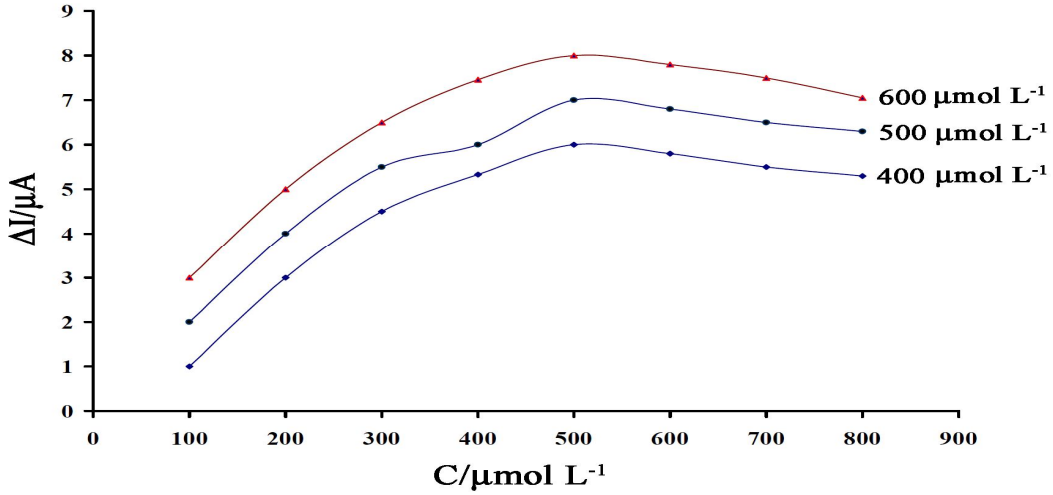


Fig. 1. Effect of MDOP concentration on the anodic peak current of HCy. Scan rate 20 mVs^{-1}; pH 6.0.

The results showed that by increasing the concentration of MDOP up to 500 μmol L^{-1} the peak current increased, whereas higher concentrations of MDOP caused a slight decrease on the magnitude of peak current, which may be due to the formation of MDOP aggregates. Therefore, 500 μmol L^{-1} MDOP concentration was selected for further studies.

RESULTS AND DISCUSSION

Characteristics of the MWCNTPE

Figure 2 shows SEM images for MWCNTPE and CPE. As can be seen at a surface of CPE (Fig. 2A), the layer of irregularly flakes of graphite powder was present and isolated with each other. After multiwall carbon nanotubes (MWCNTs) added to carbon paste, it can be seen that MWCNTs were distributed on the surface of electrode with special three-dimensional structure (Fig. 2B), indicating that the MWCNTs were successfully modified on the MWCNTPE.

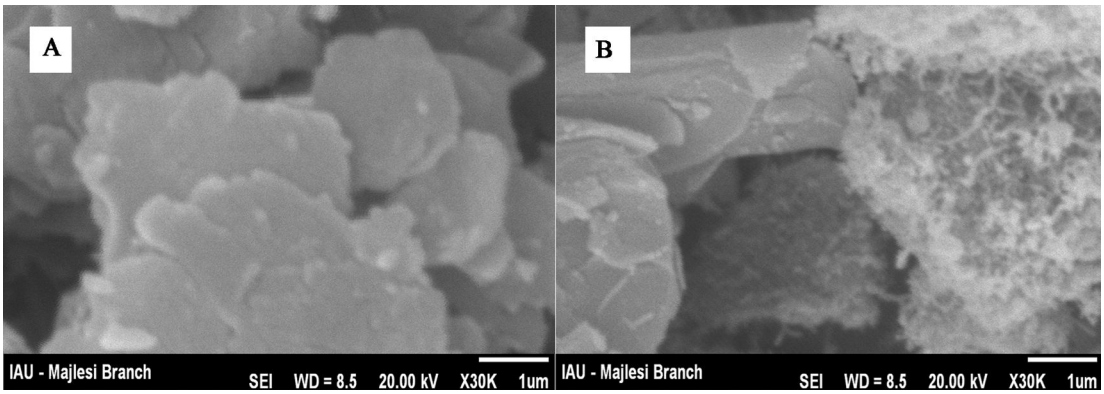


Fig. 2 SEM images of CPE (A) and MWCNTPE (B).

The active surface areas of the modified electrodes are estimated according to the slope of the I_p vs. $\nu^{1/2}$ plot for a known concentration of $K_2Fe(CN)_6$, based on the Randles–Sevcik equation [41]:

$$I_p = 2.69 \times 105n^{3/2}AD_R^{1/2}\nu^{1/2}C_0 \qquad (1)$$

where I_{pa} refers to the anodic peak current, n the electron transfer number, A the surface area of the electrode, D_R the diffusion coefficient, C_0 the concentration of $K_2Fe(CN)_6$ and ν is the scan rate. For 1.0 $mmolL^{-1}$ $K_2Fe(CN)_6$ in 0.10 mol L^{-1} KCl electrolyte with n=1 and $D_R = 7.6\times10^{-6}$ cms^{-1} and from the slope of the I_{pa}–$\nu^{1/2}$ relation, the microscopic areas were calculated. The active surface areas were equal to 0.048 and 0.092 cm^2 for CPE, MWCNTPE. The result shows that the presences of MWCNTPE cause increasing the active surface of the electrode.

Electrocatalytic oxidation of HCy

The voltammetric behavior of the MDOP in the buffer solution (pH 6.0) is shown in Fig. 3a. The cyclic voltammetric responses for the electrochemical oxidation of 500 μmol L^{-1} of HCy at MWCNTPE (curve c), and at the carbon paste electrode (curve b), in the presence of mediator, curves d and e are as c, b respectively, without MDOP. As can be seen, the anodic peak potentials for the oxidation of HCy at MWCNTPE and CPE in the presence of mediator (curve c and b) are about 515 mV. Note that HCy at the surface of MWCNTPE (without the mediator), curve d, and at the surface of CPE (without the mediator), curve e, could oxidized at potentials of 720 and 740 mV, respectively. Similarly, when we compared the oxidation of HCy at the surface of MWCNTPE (curve c) and CPE with mediator (curve b), it was observed that a dramatic enhancement of the anodic peak current occurred at MWCNTPE *vs.* the value obtained with CPE. In other words, the data obtained clearly show that the combination of MWCNTPE and the mediator (MDOP) definitely improve the characteristics of the electrode for the oxidation of HCy.

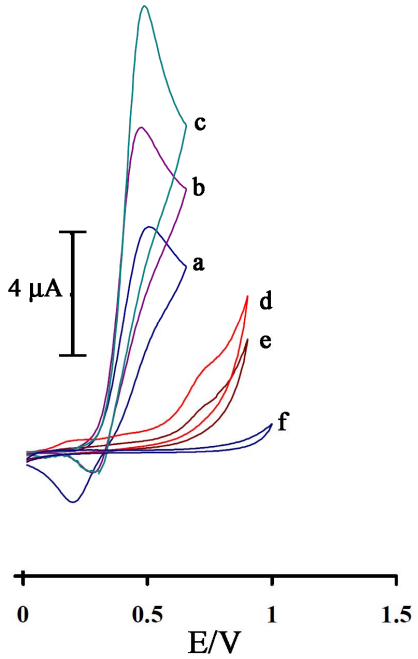


Fig. 3 Cyclic voltammograms of 500 µmol L^{-1} MDOP at the surface of MWCNTPE in 0.1 mol L^{-1} PBS (pH 6.0) at a scan rate of 20 mV s^{-1} in the absence (a) and in the presence of 500 µmol L^{-1} HCy (c). (b) as (c) for the carbon paste electrode. (d) as (c) and (e) as (b) for the unmodified electrode (and in the absence of MDOP). (f) Cyclic voltammogram MWCNTPE in 0.1 mol L^{-1} PBS (pH 6.0).

The effect of scan rate on the electrocatalytic oxidation of 800 µmol L^{-1} HCy at the modified electrode was investigated by linear sweep voltammetry (Fig. 4 inset). 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 (I_p) against the square root of scan rate ($v^{1/2}$), in the range 5–25 mV s^{-1}, was constructed, which was found to be linear, suggesting that at sufficient overpotential the process is diffusion rather than surface controlled (Fig. 4).

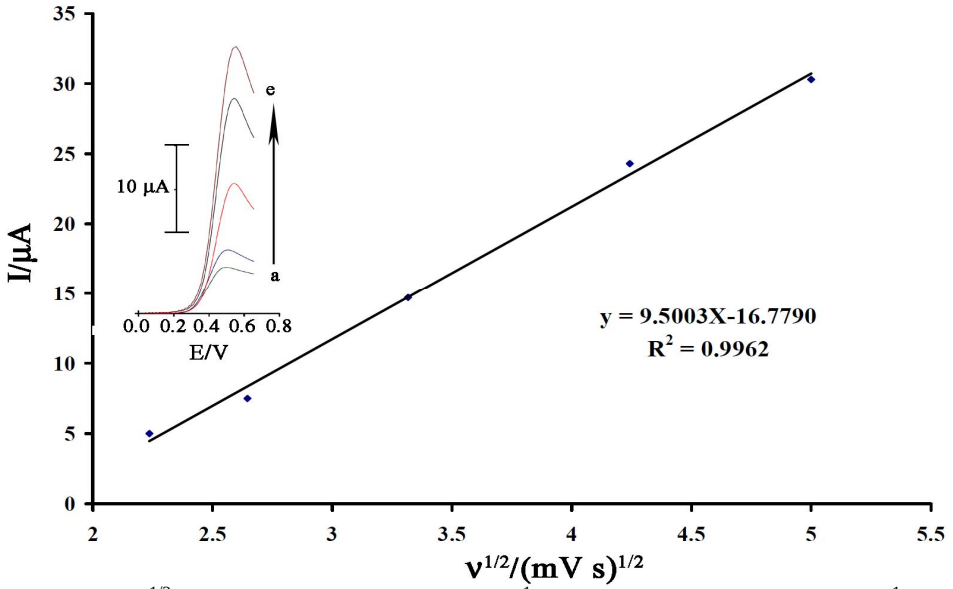


Fig. 4. Plot of I_{pa} versus $v^{1/2}$ for the oxidation of 800 µmol L^{-1} HCy in the presence 500 µmol L^{-1} MDOP at the surface of MWCNTPE. Inset) Cyclic voltammograms of 800 µmol L^{-1} HCy in the presence 500 µmol L^{-1} MDOP at various scan rates as a) 5, 2) 8, 3) 11, 4) 18 and 5) 25 mV s^{-1} in 0.1 M PBS (pH 6.0).

To obtain further information on the rate determining step, a Tafel plot was developed for the HCy at MWCNTPE in the presence of the mediator using the data derived from the rising part of the current–voltage curve (Fig. 5). The slope of the Tafel plot is equal to $n(1-\alpha)F/2.3RT$, which comes up to 9.2327 V decade^{-1}. We obtained n_α as 0.45. Assuming n=1, then α=0.45.

In order to obtain an estimation of the rate constant of the catalytic oxidation (k'_h) of HCy, chronoamperometric method was applied to the system (Fig. 6A). The rate constant for the chemical reaction between MDOP and HCy (k_h) is determined according to the method of Galus [16]:

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

where I_C is the catalytic current of MDOP in the presence of HCy and I_L is the limiting current in the absence of HCy. From the slope of I_C/I_L versus $t^{1/2}$ for two different concentrations of HCy, the average value of k_h was calculated to be 2.322×10^3 M^{-1} sec^{-1} (Fig. 6B). This value of rate constant explains the sharp catalytic peak observed for the oxidation of HCy at the surface of MWCNTPE in the presence of mediator.

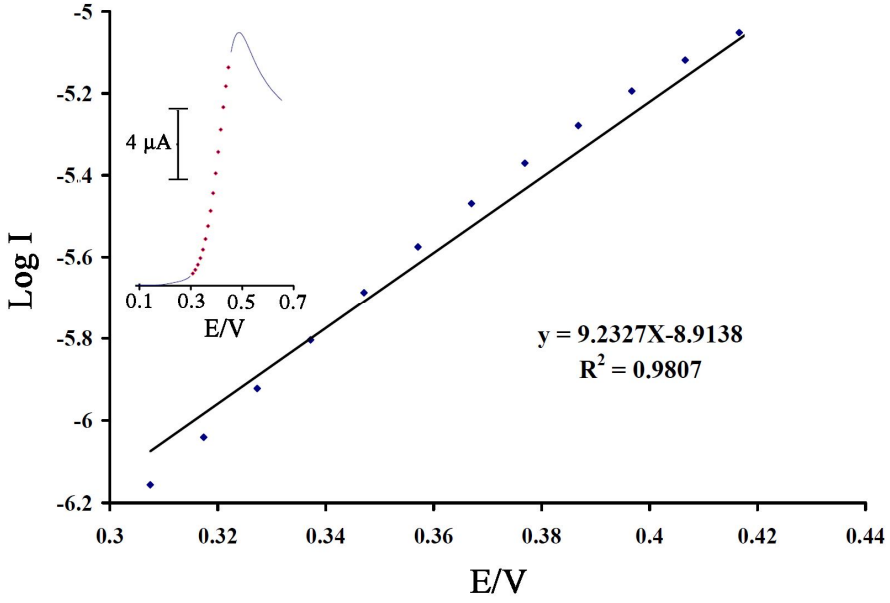


Fig. 5. Tafel plot of 600 µmol L^{-1} MDOP at the surface of MWCNTPE in 0.1 mol L^{-1} PBS (pH 6.0) at a scan rate of 20 mV s^{-1} in the presence of 600 µmol L^{-1} HCy.

Fig. 6C shows the double-potential step chronocolougrams for the mediator in the absence and presence of different concentration of HCy at a surface of MWCNTPE. The results show that forward and backward potential step chronocoloumetry in a blank buffer solution yields very symmetrical chronocolougrams. These had about an equal charge consumed for both oxidation and reduction of the redox system in the mediator at a surface of MWCNTPE. However, in the presence of HCy, the charge value associated with forward chronocoloumetry was significantly greater than that observed for backward chronocoloumetry. This behavior is typically expected for electrocatalysis at chemically modified electrodes [42].

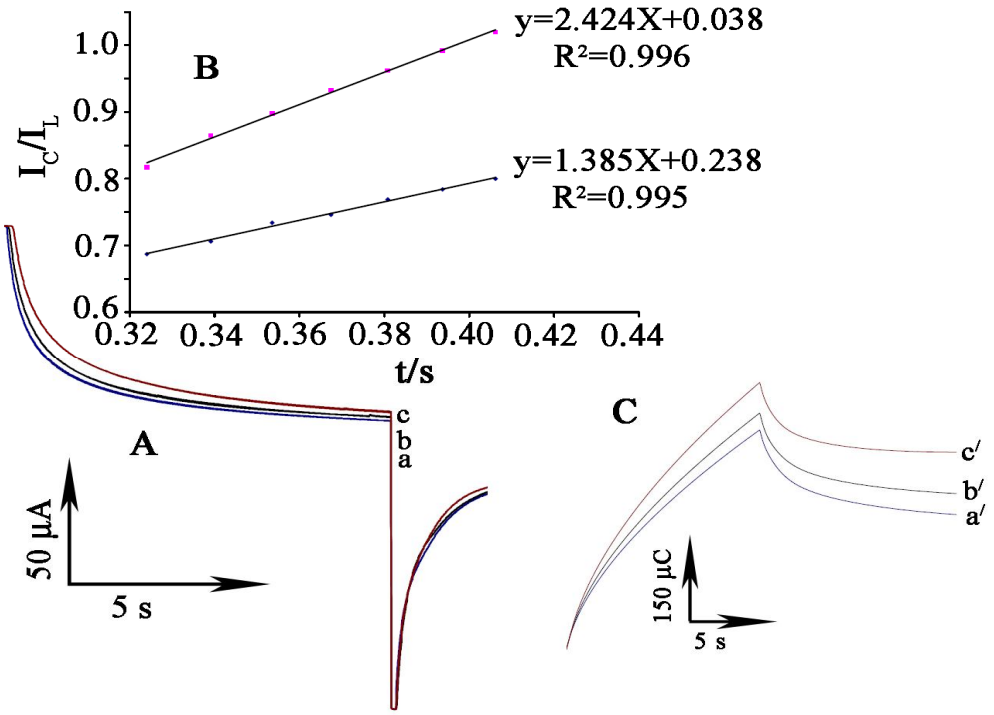


Fig. 6. A) Chronoamperograms obtained at the MWCNTPE in the absence a) and in the presence of b) 400 and c) 600 µmol L^{-1} HCy in a buffer solution (pH 6.0). B) Dependence of I_c/I_L on the $t^{1/2}$ derived from the chronoamperogram data. C) The charge-time curves a') for curve (a); b') for curve (b) and c') for curve (c).

Electrochemical impedance spectroscopy studies

Electrochemical impedance spectroscopy (EIS) was also employed as a powerful method to investigate the oxidation of HCy at MWCNTPE in the presence of 500 µmol L^{-1} MDOP. Figure 7 represents Nyquist diagrams of the imaginary impedance (Z_{im}) versus the real impedance (Z_{re}) of the EIS obtained at the modified electrode recorded at 0.48 V dc-offset in the absence (curve a) and the presence of 500 µmol L^{-1} HCy (curve b) in 0.1 M PBS with pH 6.0.

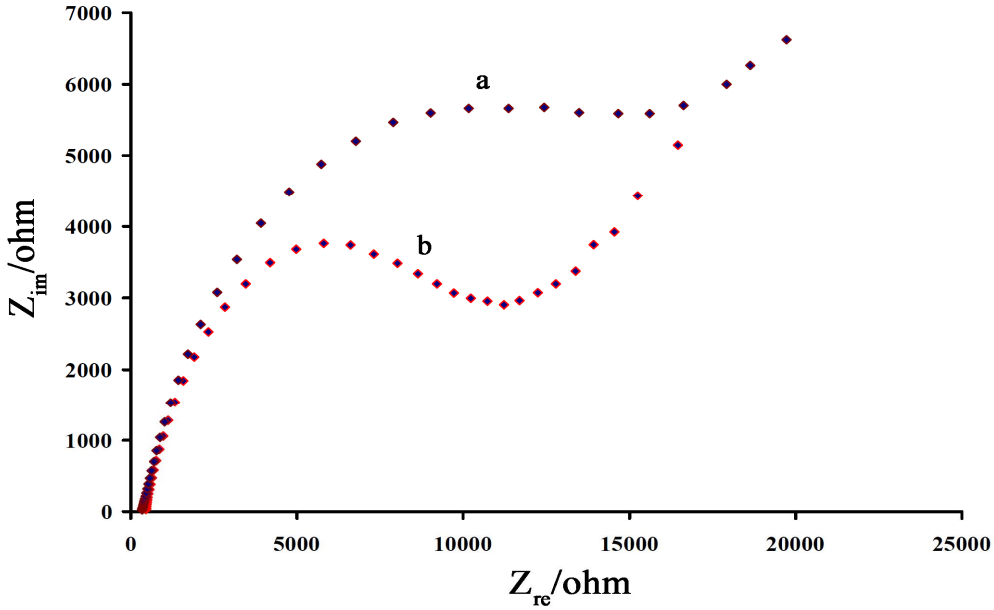
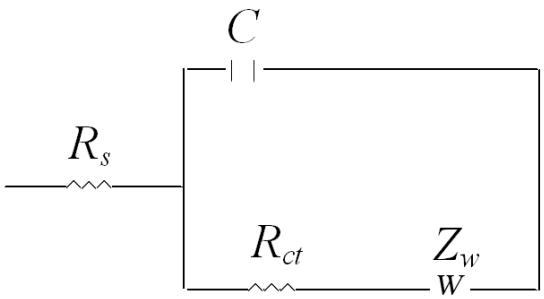


Fig. 7. Nyquist diagrams of 500 µmol L^{-1} MDOP on the MWCNTPE in the absence (a) and presence of (b) 500 µmol L^{-1} HCy (pH 6.0). Bias is 0.48 V with E_{ac} = 5 mV and frequency range of 10 kHz to 1 Hz.

In the absence of HCy, the Nyquist diagram comprises a depressed semicircle at the high frequencies which can be related to the combination of charge transfer resistance of MDOP electrooxidation and the double-layer capacitance, followed by a straight line with the slope of near 45°. The latter is due to the occurrence of mass transport process via diffusion.

The equivalent circuit compatible with the Nyquist diagram recorded in the absence and the presence of HCy is depicted in scheme 1. In this circuit, R_s, C and R_{ct} represent solution resistance, a capacitance for the double-layer. W is a finite-length Warburg short-circuit term coupled to R_{ct}, which accounts for the Nernstian diffusion. In the presence of HCy, the diameter of the semicircle is decreased, confirming the electrocatalytic ability of the mentioned electrocatalyst for oxidation of HCy. This is due to the instant chemical reaction of HCy with the $MDOP_{(ox)}$ species.



Scheme 1. Equivalent circuit for the system.

The catalytic reaction of HCy oxidation causes an increase in the surface concentration of $MDOP_{(Red)}$, and the charge transfer resistance becomes low, depending on the concentration of MDOP in the solution. This behavior is consistent with the result of cyclic voltammetry and chronoamperometry (Figures 3 and 6).

Calibration plot and limit of detection

Square wave voltammetry (SWV) method was used to determine the concentration of HCy. The plot of peak current vs. HCy concentration consisted of two linear segments with slopes of 0.070 and 0.001 μA μmol L^{-1} in the concentration ranges of 0.1 to 20.0 μmol L^{-1} and 20.0 to 800.0 μmol L^{-1}, respectively. The decrease in sensitivity (slope) of the second linear segment is likely due to kinetic limitation. The detection limit (3σ) of HCy was found to be 0.06 μmol L^{-1}.

Interference studies

Interference studies were carried out with several chemical substances prior to the application of the proposed method for the assay of HCy in urine and serum. The influence of various substances as potential interference compounds on the determination of 2.0 μmol L^{-1} HCy under the optimum conditions was studied. Tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than 5% for determination of Hcy. The results are given in Table 2, which shows the peak current of HCy is not affected by all conventional cations, anions, and organic substances.

Table 2. Interference study for the determination of 2.0 μmol L^{-1} HCy under the optimized conditions.

Species	Tolerance limits (W/W)
Li^+, Br^-, NO_3^- , Hystidine, Alanine, Phenyl alanine, Methionine, Glycine, Methanol, Ethanol, Tryptophan, SCN^-,SO_4^{2-}, Br^-, L-Theronine, L-isoleucin, Glucose , Fructose, Lactose , Sucrose, Urea; L_Orinthime, Ca^{+2}	1000
Starch	Saturation
Cysteine	2

Determination of HCy in real samples

In order to evaluate the applicability of the proposed method for the determination of HCy in real samples, its utility was tested by determining HCy in serum and in urine samples. The standard addition method was used for measuring HCy concentrations in real samples. The proposed method was also compared with a published method,[7] the results of which are given in Table 3. The results indicate that the determination of HCy using the electrode is effective and can be applied for their detection of HCy in real samples.

Table 3. Determination of HCy in real samples (*n*=3).

Sample	Added (μmol L^{-1})	Expected (μmol L^{-1})	Founded (μmol L^{-1})	Founded (Published method) (μmol L^{-1}) [7]	F_{ex}	F_{tab}	t_{ex}	t_{tab}
Urine 1	---	<LOD	<LOD	<LOD	---	---	---	---
	10.00	10.00	10.42±0.56	10.79±0.85	5.0	19	1.2	3.8
	5.00	15.00	15.48±0.60	15.69±0.90	6.5	19	1.8	3.8
Urine 2	---	---	<LOD	<LOD	---	---		---
	30.00	30.00	30.85±0.90	30.98±1.05	7.5	19	2.1	3.8
	10.00	40.00	41.00±1.05	41.20±1.20	9.5	19	2.	3.8
Serum	---	---	<LOD	<LOD	---	---		---
	5.00	5.00	5.32±0.44	5.64±0.65	6.0	19	2.0	3.8
	5.00	10.00	10.35±0.41	10.90±0.90	4.5	19	1.1	3.8

LOD. limit of detection.

CONCLUSION

The experimental results reported above demonstrate that MDOP as a suitable mediator for the electrocatalytic oxidation of HCy in aqueous solution at pH 6.0. The rate constant of the catalytic reaction was estimated using chronoamperometry. Square wave voltammetry was successfully applied to the determination of HCy in the presence of an optimum concentration of mediator. Finally, this sensor was used for the determination of HCy in real samples such serum and urine using standard addition method.

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