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Electrocatalytic determination of cysteine on the multi-wall carbon nanotubes glassy carbon electrode using a homogenous mediator

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Abstract

The electrocatalytic oxidation of cysteine on multi-wall carbon nanotubes glassy carbon electrode (MWCNT-GCE) in phosphate buffer solution in pH (4.0) has been characterized using cyclic voltammetry, chronoamperometry and differential pulse voltammetry. The results showed that the catalytic current of 1,5-bis(3,4-dihydroxy phenyl)penta-1,4-dien-3-one (BPO) depended on the concentration of cysteine. Although cysteine itself showed a very poor electrochemical response at the glassy carbon electrode, the response could be greatly enhanced by using BPO as a mediator, which enabled a sensitive determination of cysteine. The kinetics parameters of this process were calculated, the apparent catalytic rate constant (k'_h) and electron transfer (α) were 867 $M^{-1}s^{-1}$ and 0.57 respectively. The differential pulse voltammetry response of the modified MWCNT-GCE was linear against the concentration of cysteine in the range 0.5 to 30 μM the limit of detection was found to be 0.17μM.

Keywords: Electrocatalysis, cysteine, MWCNT-GCE, 1, 5-bis (3, 4-dihydroxy phenyl) penta-1, 4-dien-3-one

1. Introduction

cysteine plays an important role in biological systems and has been widely used in the medicine and food chemistry [1] .It could be used as a prospective radiation protector, antitoxin, antioxidant and free radical scavenger [2-4]. So the study of its oxidation and detection is important. Since cysteine itself lacks a strong chromophor, the determination of its concentration by absorbance measurements is very difficult [5]. Because of high over potential and slow oxidation rate of cysteine on the surface of classical solid electrodes (such as pt, Au, graphite and carbon) [6], it is necessary to electrocatalyze this reaction in electrochmical detections. Different mediators have been used for cysteine determination by using voltammetric methods [6-11]. Although many of these methods could detect small amounts but they are not selective for cysteine determination and / or have a narrow linear dynamic range. The differential pulse voltammetry is a suitable method for studying electrochemical oxidation and determination of cysteine. Carbon nanotubes(CNTs) have gained considerable attention in recent years because of their remarkable electronic and mechanical properties [12, 13]. The ability of CNTs-modified electrodes to

promote electeron-transfer reaction and resistance to surface fouling has been documented in connection to important biomolecules [14-16]. In this work we have described the catalytic behavior of BPO on MWCNT-GCE for oxidation of cysteine. There are few reports for this kind of mediators for determination of cysteine. Furthermore a new determination method for trace amount of cysteine has been investigated.

2. Experimental

2.1. Chemicals and solutions

BPO was synthesized and purified according to the following procedure: The mixture of 2 mmol acetone (0.116 g), 4.2 mmol 3,4-dihydroxy-benzaldehyde (0.580 g) and 0.3 g of 37% $BF_3.SiO_2$ was heated and stirring at 80 °C for 150 minutes. After completion of reaction, the product was dissolved in ethanol and filtered to recover the catalyst. The solvent was evaporated and the crude product was recrystallized from chloroform, and 0.530 g of 1,5-bis-(3,4-dihydroxy-phenyl)-penta-1,4-dien-3-one was recovered; an 89% yield [17].

IR (cm^{-1}): 1455 (C_6H_6), 1640 (C=C), 1678 (C=O), 3120 (C-H), 3404 (O-H).

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^{1}HNMR (400 MHZ; DMSO d_6): δ: 5.35(s,4H) 5.61(d,2H), 6.5(d,d,2H), 6.8(d,2H), 7.2(t,t,4H)

NaOH, H_3PO_4, L-serine, L-tryptophane, L-methionine, L-histidine, L- Cysteine, D-glatamic acid, L-alanine, ascorbic acid, and all other chemicals were received from Merck and used without further purification. Doubly distilled deionized water was used for preparing all of the solutions throughout the experiments. All solutions of cysteine were prepared daily fresh and deoxygenated for 300 s. Cysteine solutions were stored at 4 °C when it was not using.

2.2. Apparatus

The electrochemical experiments were carried out using Sama 500 electroanalyzer system (Isfahan, IRAN) and a personal computer was used for data storage and recording. Amperometry experiments were performed by using Potentiostate-Galvanostate Autolab PGSTAT 302N with Nova software. The cell configuration contained a glassy carbon electrode with a diameter of 2.0 mm as a working electrode, a pt wire as an auxiliary electrode and a silver/silver chloride electrode (saturated KCl) as the reference electrode. All the potentials were recorded versus the reference electrode. A Metrohm 691 pH/ ion meter was also used for pH measurements.

2.3. Preparation of the electrode

The glassy carbon electrode, GCE was first polished with 0.05 μm alumina in water slurry using a polishing cloth and then rinsed with double distilled water. The fabrication of the MWCNT modified GCE is described as follows. With the aid of ultrasonic agitation, 1 mg of purified MWCNTs was dispersed in 1 mL of *N*, *N*-dimethyl formamide (DMF). To modify the GCE with MWCNTs, 3 μL of the MWCNTs suspension was cast onto the GC electrode and the solvent was allowed to dry at room temperature. The surface area of MWCNT modified GCE was estimated to be 0.0612 Cm^2 by plotting cyclic voltammograms of $K_3[Fe(CN)_6]$ (1.0 mM) at various scan rates and using Randles-Sevcik equation [18].

3. Results and discussion

3.1. Electrochemical properties of BPO at MWCNT modified GCE

To the best of our knowledge there is no prior report on the electrochemical properties and, in particular, the electrocatalytic activity of BPO in phosphate buffer. Therefore, we studied the electrochemical behavior of 1.0 mM BPO in 0.1 M phosphate buffer solution (pH 4.0) using cyclic voltammetry (Fig.1). Cyclic voltammogram of BPO in the buffer solution exhibited an anodic and corresponding cathodic peak, whereas cyclic voltamograms of MWCNT-GCE in supporting electrolyte showed no anodic and cathodic peaks. Figure 1 shows the cyclic voltammograms of the electrode at various scan rates (ν = 20–800 mV/s). The experimental results showed well-defined and

reproducible anodic and cathodic peaks for modifier redox couple with quasi-reversible behavior, with peak potential separation ($\Delta Ep = Epa–Epc$) [19] of 105 mV. As Fig. 1 shows the peak potential difference (ΔE_p) is more than 59/n mV and increases slightly with increasing scan rate. This indicates that the system is not totally reversible. In the quasi-reversible systems, we have:

$$E_p - E_{p/2} = \Delta(\Lambda, \alpha) RT/F = 26 \Delta(\Lambda, \alpha) \text{ mV} \quad [19]$$
(1)

$$\Lambda = K° \left(\frac{RT}{DvF}\right)^{1/2} \qquad [19]$$

(2)

$E_p - E_{p/2}$ is found to be 69 mV at scan rate of 0.01 V s^{-1}. Using Eq. (1) and From Fig. 1, Δ is calculated to be 2.6. From the variation of Δ with Λ and α, Λ is obtained to be 0.224. With the substitution of a scan rate of 0.01 V s^{-1} and a modifier diffusion coefficient of $D = 1.2 \times 10^{-6}$ cm^2 s^{-1} (see chronoamperometry) in Eq. (2), the standard rate constant for the modifier, K° was found to be 1.53×10^{-4} cm s^{-1} which is compatible with the rate constants of quasi-reversible reactions ($2 \times 10^{-5} \times v^{1/2} < K° < 0.3 \times v^{1/2}$) [19]. These CVs were used to examine the variation of the peak currents versus the potential scan rates. The plot of the peak current versus $v^{1/2}$ for BPO solution at MWCNT-GCE is linear with a correlation coefficient of 0.9753 and 0.960 for anodic and cathodic branches, respectively (Fig.1 (inset)). The linearity of the ($I-v^{1/2}$) plot represents that the modifier diffuses to the electrode surface; in another word the modifier does not adsorb to the electrode surface [8]. In order to get information about the rate determining step, the Tafel plot was developed from data of the rising part of the cyclic voltammogram of BPO solution at scan rate of 10 mVs^{-1} (Fig. 2). By assuming that only one electron is transferred in rate determining step and using the slope of Tafel plot (0.2145 V $decade^{-1}$) the electron transfer coefficient was concluded.
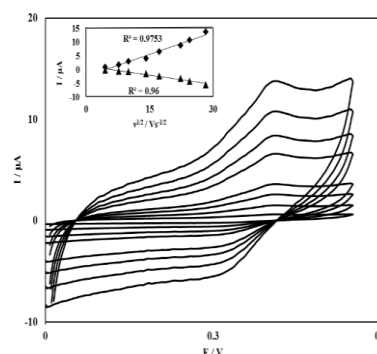

Fig 1. Cyclic voltammograms of a MWCNT-GCE in 0.1 M phosphate buffer (pH 4.0) and 1.0 mM BPO solution at various scan rates: 20, 60, 100, 200, 300, 500, 600 and 800 mVs^{-1}. Inset shows variations of Ip versus the square root of scan rate.

3.2. Electrocatalysis of cysteine oxidation

Preliminary experiments for elucidation of the catalytic activity of the BPO at MWCNT-GCE toward cysteine were performed using cyclic voltammetry (CV). Cyclic voltammograms were recorded at MWCNT-GCE in absence and presence of BPO in phosphate buffer solution. The processes associated with the electrochemical oxidation of cysteine are typically illustrated in Fig. 3.
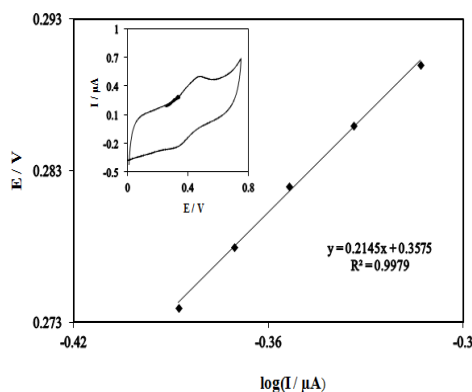

Fig 2. The Tafel plot that is plotted from rising part of cyclic voltammogram of 1mM BPO at scan rate of 10 mVs^{-1}. Inset shows the cyclic voltammogram of 1.0 mM of BPO solution in 0.1 M phosphate buffer (pH 4.0).

Curve b in Fig. 3 shows the cyclic voltammogram of cysteine at the surface of GCE and curve c shows cyclic voltammogram of BPO at GCE. As curve b shows cysteine is oxidized at 0.9 V whereas modifier is oxidized at 0.4 V (curve c of Fig. 3). Curve e in Fig. 3 shows that in the presence of BPO, cysteine is oxidized at 0.4 V. Comparison of curve b and e shows that in the presence of BPO cysteine is oxidized at the potential more negative than in absence of BPO (At potential 0.4 V; about 500 mV easier than in absence of BPO). In this work, with increasing analyte concentration, BPO oxidation peak current increased and its reduction peak current decreased. In another word oxidation of cysteine is kinetically slow therefore, its oxidation needs about 500 mV overpotential, but in the presence of modifier its oxidation is done at 0.4 V. So in the presence of BPO a large anodic peak current was observed and the overpotential was obviously decreased for cysteine oxidation. On the other hand, as curve c and d in Fig 3 shows cyclic voltammograms of modifier looks peak shape but cyclic voltammogram of modifier in the presence of cysteine reaches to a limit current (curve e and f in Fig. 3). These evidences demonstrate that the oxidation of cysteine in the presence of BPO follows an EC catalytic mechanism. Curve f in the figure shows oxidation of cysteine in the presence of BPO at MWCNT–GCE. Comparison of curve e and f shows that oxidation peak current of cysteine in the presence of BPO at GCE-MWCNT is 1.94 times of anodic peak current on GCE. It shows that MWCNTs improves the GCE surface more effectively for cysteine oxidation.
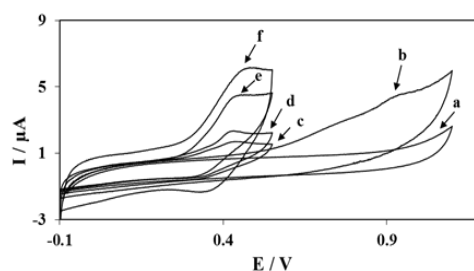

Fig 3. Cyclic voltammograms of (a) 0.1 M phophate buffer solution (pH 4.0) at GCE; (c) as (a) in 0.1 mM BPO; (e) as (c) in presence of 0.1 mM cysteine (b) as (a) in presence of 0.1 mM cysteine; (d) as (c) at MWCNT-GCE, and (f) as (d) in presence of 0.1 mM cysteine; scan rate 100 mVs^{-1}.

3.3. Influence of variables

The influence of variables such as pH, BPO concentration and scan rate was studied on the sensitivity. The effect of pH on the oxidation signal of cysteine at MWCNT-GCE was investigated by cyclic voltammetry in 0.1 M buffer solutions at various pH values, ranging from 2.0 to 10.0. As can be seen in Fig. 4, the formal potential of the redox couple, taken as the average of anodic and cathodic peak potentials, was pH-dependent, with a slope of 56.0 mV / pH unit in a wide range from 2.0 to 8.0, which is close to the anticipated Nernstian value of 59 mV with an equal number of electrons and protons. Two linear segments were found with slope values of -56.1 mV/pH and -25.0 mV/pH in the pH ranges of 2.0–8.0 and 8.0–10.0, respectively. The intersection of these two linear segments indicates the pK$_a$ of BPO [20]. In order to optimize the electrocatalytic response of the modified electrode for cysteine oxidation, we investigated the effect of pH on the electrocatalytic oxidation of cysteine at a 1 mM cysteine solution. It can be seen that the oxidation peak current of cysteine increased from pH 2.0 to 4.0, and then decreased at higher pH (4.0 to 10.0) (Fig.4. Inset). Therefore, a pH of 4.0 was selected for further investigations. The effect of BPO concentration, on the anodic peak current of cysteine, was studied for the concentration range of 0.01-1.6 mM for BPO, with the different concentrations of cysteine at pH 4.0. The investigations were studied for three concentrations of cysteine (0.1, 0.2 and 0.3 mM). The results showed that by increasing BPO concentration up to 1.0 mM the peak current increased, whereas higher concentration of BPO showed a constant magnitude of peak current. Therefore, 1.0 mM BPO was selected as the optimal mediator concentration. The overall mechanism for the mediated reaction involves the following two steps: (i) in the first step, BPO undergoes a four electron oxidation and loses four protons to form the positively charged species in solutions with pH greater than 3.0 and then (ii) electrocatalytic reaction between the oxidized form of BPO and cysteine, as follows:

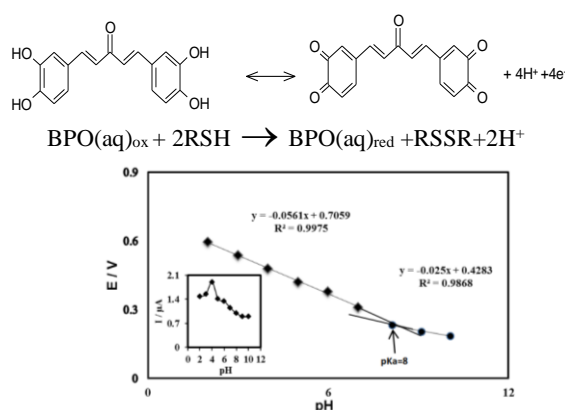$$BPO(aq)_{ox} + 2RSH \longrightarrow BPO(aq)_{red} + RSSR + 2H^+$$

Fig. 4. The variation of oxidation peak potential of cysteine versus pH in presence of 1.0 mM BPO. Inset shows the effect of pH on the electrocatalytic oxidation peak current of 1.0 mM cysteine.

3.4. Effect of scan rate and Tafel plot

Fig. 5 shows the cyclic voltammograms of a BPO at MWCNT–GCE at various scan rates obtained in 0.1 M phosphate buffer solution (pH 4.0) containing 0.01 mM cysteine. The oxidation current of cysteine increased linearly with the square root of scan rate (inset A of Fig.5), suggesting that the oxidation reaction is mass transfer controlled. In addition, a plot of normalized current (Ip / $v^{1/2}$) versus sweep rate decreases gradually and then will be constant (inset B of Fig. 5), in another word it exhibits the characteristic shape, typical for an EC$_{cat.}$ process [21]. It confirms that oxidation of cysteine in presence of BPO at GCE-MWCNT shows an EC catalytic mechanism. The Tafel plot was drawn using the data from the rising part of the current–voltage curves at scan rate 20 mVs^{-1} (inset C of Fig. 5). By assuming one electron in the rate determining step of the electrode process and using the Tafel plot slope of 0.1255 (V/decade)$^{-1}$ a charge transfer coefficient of α = 0.57 is obtained between modifier and cysteine.
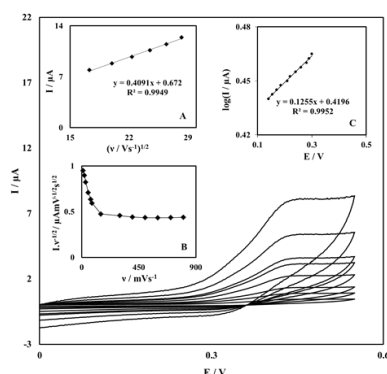

Fig. 5. Cyclic voltammograms of one MWCNT-GCE in 0.1 M phosphate buffer (pH 4.0) containing 0.1 mM cysteine and 1 mM BPO at different scan rates. The curves from down to up correspond to scan rates of 10, 20, 30, 50, 70, 80, 150, 300 mVs^{-1}, respectively. Insets: (A) variation of the electrocatalytic currents versus the square root of scan rate. (B) Variation of the scan rate normalized current (Ip /$v^{1/2}$) versus scan rate. (C) The Tafel plot from the rising part of the cyclic voltammogram of 0.1 mM cysteine in presence 1 mM BPO in phosphate buffer (pH 4.0) at MWCNT-GCE; at scan rate 20 mVs^{-1}.

3.5. chronoamperometry methods

The chronoamperometric behavior of MWCNT–GCE in phosphate buffer solution containing BPO was examined in the absence and in the presence of cysteine. Chronoamperometric measurements of different concentrations of cysteine solutions, were done by setting the working electrode potential at 650 mV [19].

Chronoamperograms obtained for different concentration of cysteine are plotted in Fig. 6. In chronoamperometric studies, the diffusion coefficient of cysteine was determined in phosphate buffer solution. The Cottrell equation describes relationship between diffusion coefficient and bulk concentration [19, 21].

$$I = nFAD^{1/2}Cb / \pi^{1/2}t^{1/2} \qquad (3)$$

Where, D and C_b are diffusion coefficient (cm^2 s^{-1}) and the bulk concentration (mol/cm^3) for cysteine, respectively. The level of Cottrell current, for curves in Fig.6, increased with increasing of cysteine concentration.
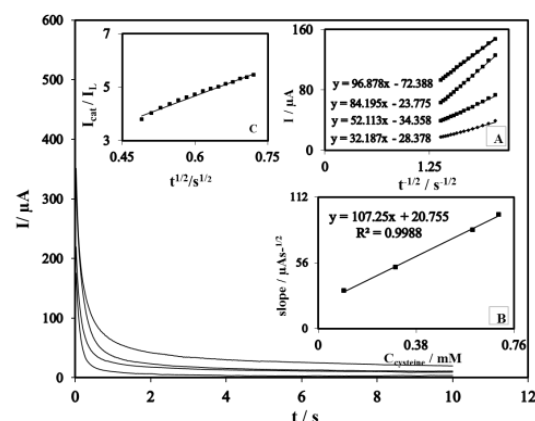

Fig 6. Chronoamperograms for different concentrations of cysteine in presence of BPO at MWCNT-GCE (Chronoamperograms from down to up correspond to 0.1, 0.3, 0.6 and 0.7 mM of cysteine). Inests: (A) plots of I *vs.* t$^{-1/2}$ from the data of chronoamperograms for different concentrations of cysteine. (B) Plot of the slope of straight lines against the cysteine concentration. (C) The plot of I$_{cat}$/I$_L$ vs. t$^{-1/2}$ for 0.2 mM cysteine at optimum condition to evaluate k.

Inset A of Fig. 6 shows the plot of I *vs.* t$^{-1/2}$, under the diffusion control. The slopes of the resulting straight lines from the inset B of Fig. 6 was plotted versus the cysteine concentration. From slope of this line, the diffusion coefficient of cysteine was calculated to be 4.15×10^{-6} cm^{2}s^{-1}. We have also used the chronoamperometric method to evaluate the catalytic rate constant, k/M^{-1}s^{-1}, for reaction between BPO and cysteine using Galus method [10].

$$I_c / I_l = \gamma^{1/2}[\pi^{1/2}erf(\gamma^{1/2}) + \exp(-\gamma)/\gamma^{1/2}] \qquad (4)$$

where I$_c$ is the catalytic current of BPO in presence of cysteine at MWCNT–GCE, I$_l$ is the limited current

in the absence of cysteine and $\gamma = kC_b t$ (C is the bulk concentration of cysteine) is the argument of the error function. In the cases where γ exceeds from 2, the error function is almost equal to 1 and the above equation can be reduced to:

$$I_c / I_l = \pi^{1/2} \gamma^{1/2} = \pi^{1/2} (kC_b t)^{1/2} \tag{5}$$

Where t/s is the time elapsed. The above equation can be used to calculate the catalytic rate constant $(k/M^{-1}s^{-1})$ of the catalytic process. Based on the slope of the I_c/I_L versus $t^{1/2}/s^{1/2}$ plot, k can be obtained for a given cysteine concentration. This plot is shown in inset C of Fig. 6. From the values of the slopes, the average of the catalytic rate constant is obtained for catalytic oxidation of cysteine in presence of BPO at MWCNT–GCE surface. The catalytic rate constant $(k/M^{-1}s^{-1})$ of catalytic reaction was calculated to be k= 867 $M^{-1}s^{-1}$

3.6. Differential pulse voltammetry Investigations

Since differential pulse voltammetry (DPV) has a much higher current sensitivity than cyclic Voltammetry, it was used to estimate the limit of detection and the linear range of cysteine. In addition the charging current contribution to the background current, which is a limiting factor in the analytical determination, is negligible in the DPV mode. The effects of increasing the concentration of cysteine on the voltammograms are presented in Fig. 7. The figure shows differential pulse voltammograms for cysteine in the concentration ranges of 0.5-30 μM. Also, as can be seen in inset A of Fig. 7 peak current is linear versus cysteine concentration in the wide range of concentration (0.5-30 μM cysteine) with a correlation coefficient of 0.9732. The detection limit for cysteine using $3s_{bl}/m$ was obtained to be 0.17 μM.

The limit of detection, linear range, optimum pH for catalytic effect, and peak potential shift of some modified electrodes against proposed electrode for the determination of cysteine are listed in Table 1. As can be seen from the table, the limit of detection of the proposed method is lower than other methods.
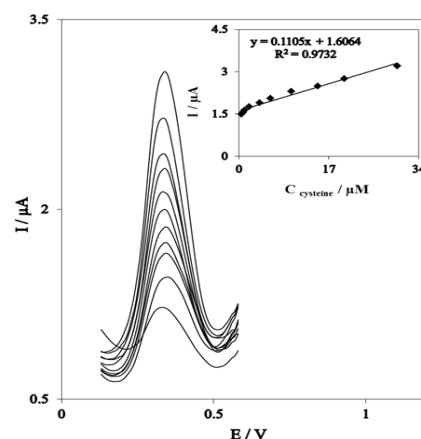

Fig 7. Differntial pulse voltammograms of in 0.1 M phosphate buffer solution (pH 4.0) containing BPO and different concentrations of cysteine at MWCNT–GCE. From down to up correspond to 0.5, 0.8, 1.0, 2.0, 5.0, 7.0,10.0 and 30.0 μM cysteine. Inset shows the plot of the electrocatalytic peak current as function of cysteine concentration.

3.7. Effects of chemical interferences

Methionine is one of the two sulfur–containing proteinogenic amino acids. Some other available amino acids (L-tryptophane, L- Serine, L-histidine, D-glutamic acid, L-alanine) were checked for interference studies. These amino acids up to molar ratio of 500 had no interference effect on oxidation peak current of cysteine. Also compounds such as glucose, urea and ascorbic acid that may be present in biological matrix were checked. Among them only ascorbic acid had serious interference effects.

3.8. Determination of cysteine in real sample

This method was found to work well under the laboratory conditions. To assess the applicability of this method in real samples, an attempt was made to determine cysteine in tablet acetyl cysteine. So five solutions from the appropriate amounts of acetyl cysteine tablet were prepared and analyzed by proposed method.

Table 1. Comparison of the efficiency of some modified electrodes used for determination of cysteine.

Method	Electrode	Peak potential shift (mV)	pH	Dynamic range (μM)	Detection limit (μM)	Ref.
CA	Q-GCE	400	2.0	0.8-10	0.16	[22]
CV	5ADB-PE	500	7.0	0.4-900	0.2	[23]
DPV	N-CPE	200	5.0	2-10000	1	[24]
CV	Cu–CoHCF-CPE	320	2.0	6-1000	5	[25]
DPV	BPO- MWCNT – GCE	500	4.0	0.5-30	0.17	This work

The results of cysteine determination, summarized in Table 2, show an average recovery of 99%, indicating that the constituents of the tablet acetyl cysteine do not significantly interfere for the determination of cysteine. Therefore, the method seems to be promising for the determination of cysteine in tablet acetyl cysteine sample.

Table 2. Determination of cysteine in tablet acetyl cysteine sample (n=5).

No.	Actual amount(μM)	Found amount(μM)	Recovery(%)
1	5	4.8	96
2	10	10.5	105
3	15	15.3	102
4	20	19.7	98
5	25	24	96

4. Conclusions

Cysteine is a sulfur containing amino acid that has a high oxidation over potential on MWCNT–GCE. BPO shows an excellent electro catalytic behavior toward cysteine oxidation in the phosphate buffer (pH 4.0) solution, with an over–potential of about 500 mV less than that on a bare GCE. This technique offers a number of advantages over other electrochemical methods especially in its simplicity and detection limit. The applicability of method was shown by determination of cysteine in tablet acetyl cysteine.

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