Ionics (2013) 19:933–940
DOI 10.1007/s11581-012-0816-7

ORIGINAL PAPER

Selective and sensitive voltammetric sensor based on modified multiwall carbon nanotubes paste electrode for simultaneous determination of L-cysteine and folic acid

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Received: 29 July 2012 / Revised: 22 September 2012 / Accepted: 6 October 2012 / Published online: 17 October 2012

Abstract This paper reports the selective and sensitive voltammetric determination of L-cysteine in the presence of folic acid using ethynylferrocene modified carbon nanotubes paste electrode in 0.1 M phosphate buffer solution (pH 7.0). Using square wave voltammetry, we could measure L-cysteine and folic acid in one mixture independently from each other by a potential difference of about 410 mV for the first time. Square wave voltammetric peak current of L-cysteine and folic acid increased linearly with their concentrations in the ranges of 0.2–250.0 and 1.0–500.0 $\mu mol L^{-1}$, respectively. The detection limits of 0.07 and 0.6 $\mu mol L^{-1}$ were achieved for L-cysteine and folic acid, respectively. The proposed voltammetric sensor was successfully applied to the determination of L-cysteine and folic acid in real samples.

Keywords L-Cysteine · Folic acid · Carbon nanotubes paste electrode · Voltammetry

Introduction

L-Cysteine (2-amino-3-mercapto propanoic acid), a sulfur-containing amino acid, and its derivatives have attracted especial attention because of its involvement in many important biological processes, and its chemical activity in the formation of complexes with various ionic species and biomolecules [1]. L-Cysteine is a highly significant bioactive compound, and is known to be an active site in the catalytic function of certain enzymes known as cysteine proteases and in many other peptides and proteins. Numerous chemical and instrumental techniques for detection of L-cysteine have been reported [2–4]. However, most of them suffer from difficulty imposed in sample preparation, the need for derivatization or the lack of sufficient sensitivity, all of which limit their utility [5]. Compared to other options, the electroanalytical methods have the advantages of simplicity and high sensitivity [6–12].

The folic acid is chosen as the analyte for this research because it is an electroactive component of considerable biological importance. It has long been recognized as part of the vitamin B complex found in some enriched foods and vitamin pills. It is usually employed in the treatment or prevention of megaloblastic anaemia during pregnancy, childhood and other clinical situations often associated with alcoholism and liver diseases [13]. Several methods have been proposed for the determination of folic acid (as a single species) in real samples, including LC/MS/MS [14], HPLC [15], capillary electrophoresis [16], voltammetry [17,18] and chemiluminescence method [19].

Results shows cysteine and folic acid are effective on age [20]. On other hand, cysteine is effective on the folic acid conjugation [21]. Therefore, simultaneous determination of these compounds is very important.

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 fields, such as energy conversion and storage [25,26], chemical actuators [27,28] and chemical sensing [29–33].

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To our knowledge, no study has reported the electrocatalytic and simultaneous determination of L-cysteine and folic acid by using modified multiwall carbon nanotubes paste electrodes. Thus, in this study, we described initially the preparation and suitability of an ethynylferrocene modified carbon nanotubes paste electrode (ETFMCNTPE) as a new electrode in the electrocatalysis oxidation and determination of L-cysteine in a buffer solution. Then, the analytical performance of the modified electrode in quantification of L-cysteine in the presence of folic acid was evaluated. We also evaluated the analytical performance of the modified electrode for voltammetric determination of L-cysteine and folic acid in real samples such as serum, tablet, water and urine samples.

Experimental

Reagents

L-Cysteine and folic acid were purchased from Sigma, and all others were acquired from Merck (Darmstadt, Germany), which were used as received. Double distilled water was used throughout.

A stock of 1.0×10^{-3} molL^{-1} L-cysteine solution was prepared daily by dissolving 0.012 g L-cysteine in water, and the solution was diluted to 100 mL with water in a 100-mL volumetric flask. The solution was kept in a refrigerator at 4 °C in the dark. More dilute solutions were prepared by serial dilutions with water. Folic acid stock solution, 1.0×10^{-3} molL^{-1}, was prepared by dissolving 0.015 g of the reagent in 1.0 mmolL^{-1} NaOH in a 100-mL volumetric flask. Phosphate buffer (sodium dihydrogen phosphate and disodum monohydrogen phophate plus sodium hydroxide, 0.1 molL^{-1}) solutions with different pH values were used.

Spectrally pure graphite powder (particle size, <50 μm) from Merck and multiwall carbon nanotubes synthesis according to previous report procedure were used as the substrate for the preparation of the carbon paste electrode [34]. High viscosity paraffin (d=0.88 kgL^{-1}) from Merck was used as the pasting liquid for the preparation of the paste electrodes.

Apparatus

Voltammetric measurements were carried out using a computerized potentiostat/galvanostat (Autolab PGSTAT101, 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. A three-electrode assembly was employed for the experiments in a 50-mL glass cell containing an Ag/AgCl/KCl_{sat} electrode as reference electrode, a platinum wire counter electrode and ETFMCNTPE as working electrode. All of the potentials were measured and reported vs. Ag/AgCl/KCl_{sat} reference electrode. The pH of the buffer solutions was controlled adjusted with a Metrohm pH meter.

Preparation of the working electrode

Thirty milligrams of ethynylferrocene was hand mixed with 770 mg of graphite powder and 200 mg of carbon nanotubes in a mortar and pestle. Using a syringe, 0.5 g of paraffin was added to the mixture and mixed well for 50 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. The unmodified carbon paste electrode (CPE) was prepared in the same way without adding ethynylferrocene and carbon nanotubes to the mixture.

Result and discussion

Synthesis of ethynylferrocene

To a solution of lithium diisopropylamide (33.5 mmol) in tetrahydrofuran (THF) was added a solution of 7.0 g (30.5 mmol) acetylferrocene in 25 ml THF at −78 °C under argon atmosphere. The resulting mixture was stirred at −78 °C for 1.0 h. Then, 4.6 ml (31 mmol) of diethylchlorophosphate was added into the above mixture. After 1 h, the reaction was allowed to gradually warm to room temperature and stirred for 2 h. After addition of 62 mmol lithium diisopropylamide in THF at −78 °C, the reaction mixture was under stirring for 3 h and then poured into ice water. The mixture was extracted with CH_2Cl_2. After removal of solvents, the crude product was purified by chromatography in a silica gel column (*n*-pentane/CH_2Cl_2, 3:1) to give ethynylferrocene (5.5 g, 85 %). ^{1}H-NMR (400 MHz, $CDCl_3$), δ=4.45 (*pseudo t*, 2H), 4.21 (s, 5H), 4.19 (*pseudo t*, 2H), 2.71 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ=82.54 (C), 73.46 (CH), 71.70 (CH), 69.99 (CH), 68.65 (CH), 63.85 (C).

SEM characterization

Figure 1 shows typical SEM images of different electrodes. It can be seen that on the surface of CPE (Fig. 1a), the layer of irregular flakes of graphite powder was present and isolated with each other. After multiwall carbon nanotubes (MWNTs) were added to the carbon paste, it can be seen that the MWNTs were distributed on the electrode with special three-dimensional structure (Fig. 1b), indicating that the MWNTs were successfully modified on the CNTPE. In addition, it can be clearly seen that the mediator dispersed homogeneously in modified electrode (Fig. 1c).

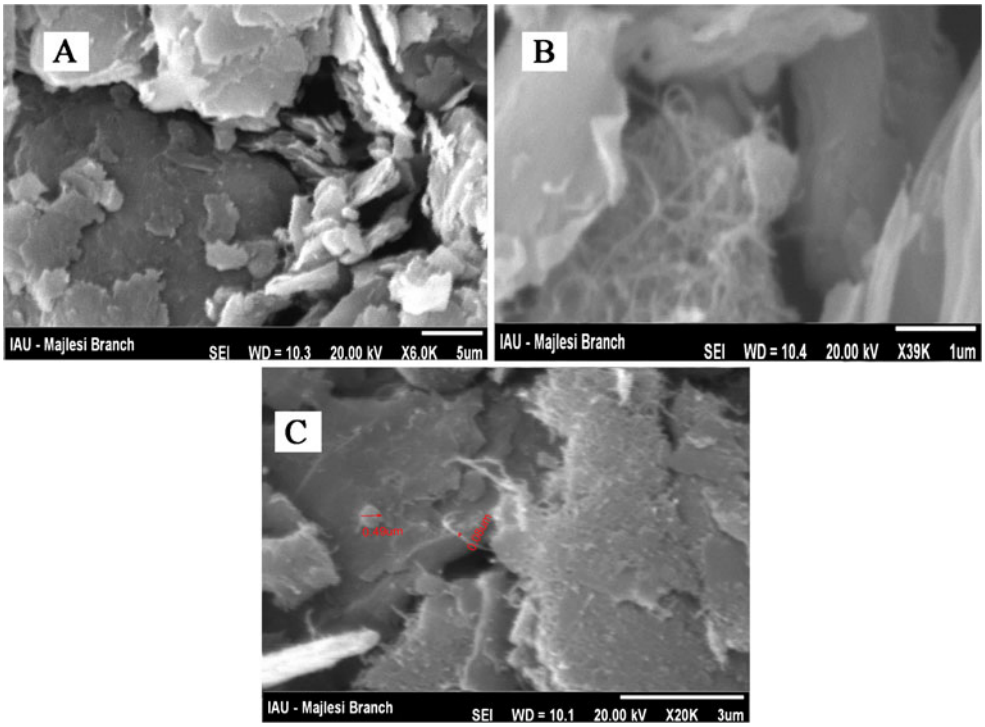


Fig. 1 SEM image of **a** CPE, **b** unmodified CNTPE and **c** ETFMCNTPE

Electrochemistry of mediator

Cyclic voltammetry was employed for the investigation of the electrochemical properties of the ETFMCNTPE in a pure buffered aqueous solution (pH 7.0). The cyclic voltammogram (Fig. 2) exhibits an anodic and corresponding cathodic peaks with E_{pa}=0.5 V and E_{pc}=0.4 V vs. Ag/AgCl/KCl$_{sat}$. The experimental results showed well-defined and reproducible anodic and cathodic peaks related to the ethynylferrocene/ethynylferrocenium redox couple with quasi-reversible behaviour because the peak separation potential, $\Delta E_p=(E_{pa}-E_{pc})$, was greater than the 59/nmV expected for a reversible system. In addition, the result obtained from cyclic voltammetry of this modified electrode in various buffered solutions did not show any shift in the anodic and cathodic peak potentials. Therefore, the electrochemical behaviour of the redox process of ethynylferrocene/ethynylferrocenium in the ETFMCNTPE is independent of the pH of the aqueous solution. The capability of the electrode for the generation of a reproducible surface was examined by cyclic voltammetric data obtained in the optimum solution pH from four separately prepared ETFMCNTPE (Table 1). The calculated RSDs for various parameters accepted as the criteria for a satisfactory surface reproducibility were 1–4 %.

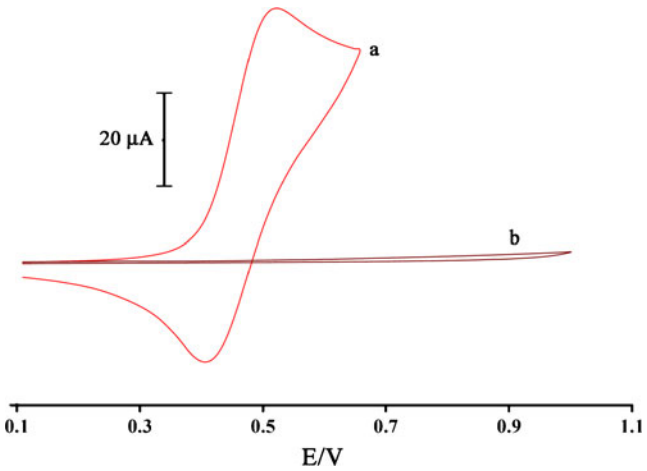


Fig. 2 Cyclic voltammograms of *a* ETFMCNTPE and *b* bare CPE in 0.1 M PBS (pH 7.0) at a scan rate of 35 mVs^{-1}

Catalytic effect

Figure 3 depicts the cyclic voltammetric responses from the electrochemical oxidation of 800 µmolL^{-1} L-cysteine at ETF modified CPE (ETFMCPE) (curve b), ETFMCNTPE (curve c), CNTPE (curve d) and bare CPE (curve e). As can be seen, the anodic peak potential for the oxidation of L-cysteine at ETFMCNTPE (curve c) and ETFMCPE (curve b) is about 470 mV, while at the CNTPE (curve d) peak

Table 1 Cyclic voltammetric data obtained for constructed ETFMCNTPE in 0.1 M PBS (pH 7.0) at 35 mVs^{-1}

E_{pa} (V)[a]	E_{pc} (V)	$E_{1/2}$ (V)	E_p(V) Δ	I_{pa} (µA))	I_{pc} (µA)
0.5±1.5	0.4±1.4	0.35±1.5	0.10±1.4	48.74±2.2	−21.44±2.3

All the '±' values are RSD% (n=4)

[a] Versus Ag/AgCl/KCl (3.0 M) as reference electrode

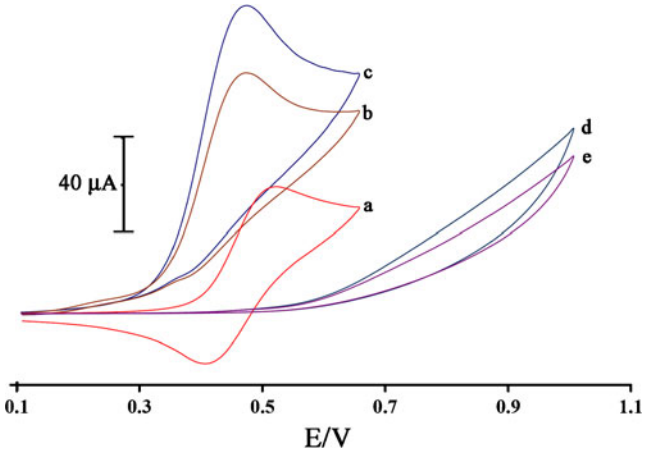


Fig. 3 Cyclic voltammograms of *a* the buffer solution at ETFMCNTPE, *b* 800 μmolL^{-1} L-cysteine at ETFMCPE, *c* 800 μmol L^{-1} cysteine at ETFMCNTPE, *d* 800 μmolL^{-1} L-cysteine at CNTPE and *e* 800 μmolL^{-1} L-cysteine at unmodified CPE. Conditions: 0.1 molL^{-1} PBS (pH 7.0) and scan rate of 35 mVs^{-1}

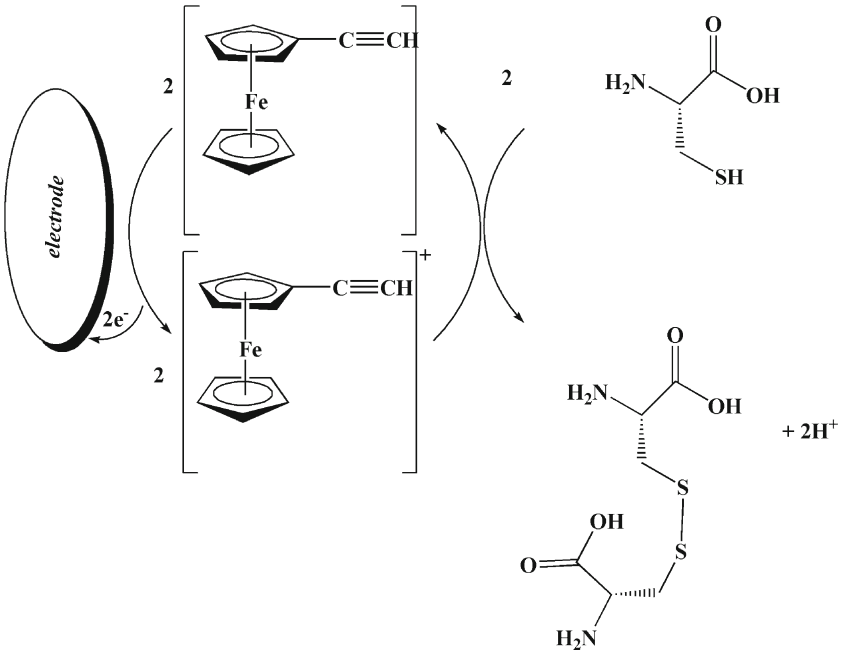
potential is about 850 mV, and at the bare CPE peak potential is about 880 mV for L-cysteine (curve e). From these results, it is concluded that the best electrocatalytic effect for L-cysteine oxidation is observed at ETFMCNTPE (curve c). For example, the results show that the peak potential of L-cysteine oxidation at ETFMCNTPE (curve c) shifted by about 380 and 410 mV towards the negative values compared with that at a CNTPE (curve d) and bare CPE (curve e), respectively. Similarly, when we compared the oxidation of L-cysteine at the ETFMCNTPE (curve c) and ETFMCPE (curve b), there is a dramatic enhancement of the anodic peak current at ETFMCNTPE relative to the value obtained at the ETFMCPE. In other words, the data obtained clearly show that the combination of CNTs and mediator definitely improves the characteristics of L-cysteine oxidation. The ETFMCNTPE in 0.1 M PBS (pH 7.0) without L-cysteine in solution exhibits a well-behaved redox reaction (curve a); upon the addition of 800 μmolL^{-1} L-cysteine, there is a dramatic enhancement of the anodic peak current (curve c). Based on these results, we propose an EC catalytic mechanism [35–38] (Scheme 1) to describe the electrochemical oxidation of L-cysteine at ETFMCNTPE.

Figure 4 (inset) shows the linear voltammetric peaks potential of ETFMCNTPE at scan rates ranging from 2 to 20 mVs^{-1} at pH 7.0 containing 400 μmolL^{-1} L-cysteine. We observed a linear variation of the peak current with the square root of scan rate ($\nu^{1/2}$; Fig. 4). This result clearly indicates a diffusion-controlled electro-oxidative process.

In order to obtain information on the rate-determining step, a Tafel plot was developed for ETFMCNTPE using the data derived from the raising part of the current–voltage curve (Fig. 5). The slope of the Tafel plot is equal to $2.3RT/n(1-\alpha)F$ which comes up to 0.1125 V decade^{-1}. Assuming $n=1$, then $\alpha=0.47$.

Double potential step chronoamperometry was employed to investigate the electrochemical behaviour of an aqueous buffered solution (pH 7.0) containing various concentrations of L-cysteine at ETFMCNTPE by setting the working electrode potential at 0.3 V (at the first potential step) and 0.7 V (at the second potential step). For an electroactive material (L-cysteine in this case) with a diffusion coefficient of D, the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation. Experimental plots of I vs. $t^{-1/2}$ were employed, with the best fits for

Scheme 1 Proposed response mechanism of the sensor based on ethynylferrocene for the catalytic electro-oxidation of L-cysteine



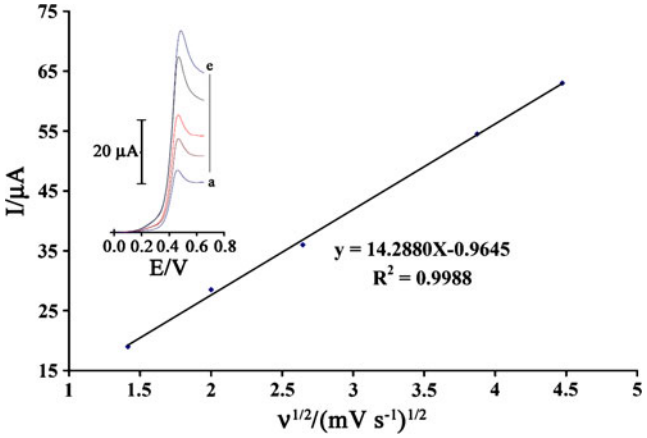


Fig. 4 Plot of I_{pa} versus $\nu^{1/2}$ for the oxidation of L-cysteine at ETFMCNTPE. *Inset* linear sweep voltammograms of 400 μmolL^{-1} L-cysteine at various scan rates: *a* 2, *b* 4, *c* 7, *d* 15 and *e* 20 mVs^{-1} in 0.1 molL^{-1} PBS (pH 7.0)

different concentrations of GSH. The slopes of the resulting straight lines were then plotted vs. L-cysteine concentration (not shown). From the resulting slope and Cottrell equation, the mean value of the D was found to be 1.6×10^{-4} cm^2s^{-1} as near as to the reported value in the same condition [2].

However, we determined catalytic reaction rate constant k_h for L-cysteine using Galuse method [39]:

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

The above equation can be used to calculate the rate constant of the catalytic process k_h. Based on the slope of the I_C/I_L versus $t^{1/2}$ plots, k_h can be obtained for a given L-cysteine concentration. From the values of the slopes, an average value of k_h was found to be $k_h=5.53\times10^3$ $mol^{-1}s^{-1}$.

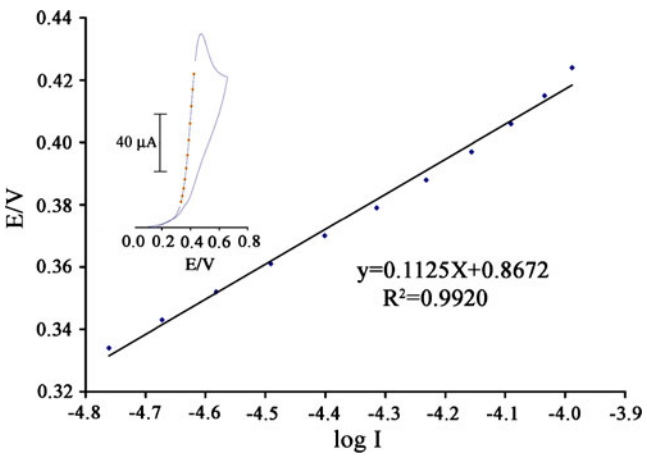


Fig. 5 Tafel plot for ETFMCNTPE in 0.1 M PBS (pH 7.0) with a scan rate of 35 mVs^{-1} in the presence of 800 μmolL^{-1} L-cysteine

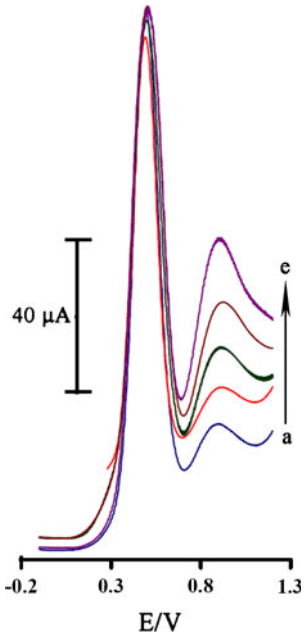


Fig. 6 Square wave voltammograms of L-cysteine and folic acid under optimum conditions; 5.0 μmolL^{-1} L-cysteine with folic acid concentrations of: *a* 40.0, *b* 60.0, *c* 90, *d* 140 and *e* 200 μmolL^{-1}

Simultaneous determination of L-cysteine and folic acid

SWV method was used to determine the concentration of L-cysteine and folic acid. The results show two linear segments with different slopes for L-cysteine concentration, namely, for 0.3–5.0 μmolL^{-1} of L-cysteine, the regression equation was I_p (microampere)=$(3.451\pm0.381)C_{\text{L-cysteine}}+(131.800\pm1.457)$ (r^2=0.995, n=5), while for 5.0–600.0 μmolL^{-1} of L-cysteine, the regression equation was I_p (microampere)=$(0.251\pm0.043)C_{\text{L-cysteine}}+(150.730\pm1.347)$ (r^2=0.997, n=7). The decrease of sensitivity (slope) in the second linear range is likely due to kinetic limitations. The regression equation for folic acid in the range of 5.0–600.0 μmolL^{-1} was I_p (microampere)=$(0.301\pm0.003)C_{\text{folic acid}}+(25.984\pm0.859)$ (r^2=0.998, n=11), where C is micromoles per litre concentration of L-cysteine or folic acid, and I_p is the peak current.

Table 2 Interference study for the determination of 10.0 μmolL^{-1} L-cysteine under optimized conditions

Species	Tolerant limits ($W_{\text{Substance}}/W_{\text{L-cysteine}}$)
Glucose, sucrose, lactose, fructose	1,000
K^+, ClO_4^-, Na^+, Cl^-, CO_3^{-2}, Ca^{2+}, Mg^{2+}, SO_4^{2-}	800
Tryptophan, leucine, L-threonine, L-phenylalanine, glycine, methionine, alanine, valine	500
Urea, thiourea, uric acid	200
Starch	Saturation

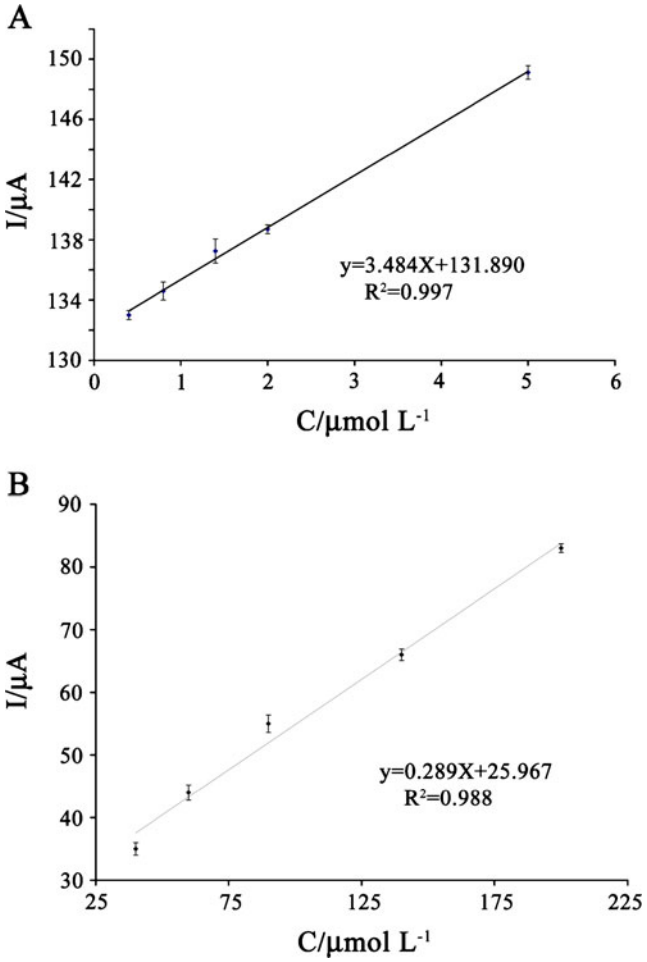


Fig. 7 **a** Plot of Ip vs. L-cysteine concentrations (*data points from left to right*): 0.4, 0.8, 1.4, 2.0 and 5.0 μmolL^{-1}. **b** Plot of Ip vs. folic acid concentrations (*data points from left to right*): 40.0, 6.0, 90.0, 140.0, and 200.0 μmolL^{-1} in 0.1 μmolL^{-1} PBS (pH 7.0) at the surface of the modified electrode

The plot of peak current vs. L-cysteine concentration consisted of two linear segments with slopes of 3.451 and 0.251 μA/(μmol/L) in the concentration ranges of 0.3–5.0 and 5.0–600.0 μmol/L, respectively. The decrease in sensitivity (slope) of the second linear segment is likely due to kinetic limitation. On other hand, the responses were linear with folic acid concentration in the range from 1.0 to 500.0 μmolL^{-1}, and the current sensitivity was 0.301 μA/(μmolL^{-1}). The detection limit was determined at 0.07 μmolL^{-1} L-cysteine and 0.6 μmol L^{-1} folic acid according to the definition of $Y_{LOD}=Y_B+3\sigma$, respectively.

Table 4 Precision (intra- and inter-day) in standard solutions of L-cysteine

Concentrations (μM)	Intra-day (n=3) Mean response ±SD	CV (%)	Inter-day (n=3) Mean response ±SD	CV (%)
0.7	0.321±0.005	1.425	0.352±0.006	0.946
1.0	1.421±0.102	1.983	1.378±0.122	2.043
50	3.522±0.145	3.742	3.589±0.274	3.389
300	6.493±0.381	4.221	6.590±0.489	4.555

The main objective of the present work was to develop a modified electrode that is capable of both electro-catalytic oxidation of L-cysteine and separation of the electrochemical responses of L-cysteine and folic acid. Results show, at unmodified CNTPE, the peak potential of L-cysteine and folic acid overlapped with each other. On the other hand, at the modified electrode, these compounds have two well-separated peak potential (with a 410 mV separation of the peaks; Fig. 6). Therefore, the modifier has a critical role, and it is necessary for the determination of L-cysteine and folic acid simultaneously.

Analytical experiments were carried out by varying either the folic acid concentration in the presence of 5.0 μmolL^{-1} cysteine in a 0.1 molL^{-1} phosphate buffer (pH 7.0; Fig. 6). It can be noted that the responses to L-cysteine at modified electrode were relatively independent of folic acid responses. On other hand, current sensitivities towards L-cysteine in the absence and in the presence of folic acid were found to be 3.451±0.381 μA/(μmolL^{-1}; in the absence of folic acid) and 3.484±0.381 μA/(μmolL^{-1}; in its presence; Fig. 7a). The sensitivities towards folic acid in the absence

Table 3 Concentration values obtained from the proposed and published methods for L-cysteine and folic acid analysis in real samples

Sample	Proposed method L-cysteine	Published method [2]	Proposed method Folic acid	Published method [18]
1 Urine (μmolL^{-1})	<LOD	<LOD	<LOD	<LOD
2	5.33±0.3	5.21±0.35	15.45±0.3	15.74±0.85
3	10.32±0.58	9.89±0.22	20.52±0.65	19.75±0.86
4 Water	0.95±0.08	1.04±0.05	5.35±0.42	5.65±0.71
5	15.33±0.37	15.69±0.72	39.85±0.32	40.55±0.61
6 Tablet (folic acid)	–	–	9.85±0.45	9.94±0.59
8	–	–	30.44±0.45	29.65±0.73
9 Serum	5.15±0.20	5.32±0.41	40.25±0.44	40.56±0.68

and presence of L-cysteine were found to be 0.301± 0.445 μA/(μmolL^{-1}; in the absence of L-cysteine) and 0.289±0.328 μA/(μmolL^{-1}; in its presence; Fig. 7b).

It is interesting to note that the sensitivities of the modified electrode towards L-cysteine in the absence and presence of folic acid were virtually the same, which indicates that the oxidation processes of L-cysteine and folic acid at the modified electrode are independent and that simultaneous or independent measurements of the two compounds are, therefore, possible without any interference. However, results show that there is not any important interference in the determination of L-cysteine using this modified electrode.

Interference study

Analytical selectivity is one of the important parameters that affect the accuracy of the analysis. In order to evaluate the selectivity of the proposed method for the determination of L-cysteine, the influence of various foreign species on the determination of 10.0 μmolL^{-1} L-cysteine was investigated. The tolerance limit was taken as the maximum concentration of the foreign substances, which caused an approximately ±5 % relative error in the determination. The results are shown in Table 2.

Real sample analysis

In order to evaluate the applicability of the proposed modified electrode in real sample analysis, it was used for the determination of L-cysteine and folic acid in urine, serum, tablet and water samples using standard addition method. In addition, electrochemical methods [2,18] were used for the analysis of the analytes to confirm the accuracy of the proposed method. The results presented in Table 3 indicate that the modified electrode retained its efficiency for the determination of L-cysteine and folic acid in real samples with satisfactory results.

Stability and reproducibility

The repeatability and stability of the ETFMCNTPE were investigated using square wave voltammetric measurements of 10.0 μmolL^{-1} L-cysteine. The relative standard deviation (RSD%) for five successive assays of L-cysteine was 1.8 %. When using five different electrodes, the RSD% for seven measurements of 10.0 μmol L^{-1} L-cysteine was 2.5 %. When the modified electrode was stored in the laboratory, the response of the modified electrode retained 96 % of its initial response value after a week and 93 % after 45 days. These results indicate that ETFMCNTPE has good stability and reproducibility.

Validation parameters

The SWV method using the modified electrode was applied as a very sensitive and selective method with submicromolar detection limits and high precision for the determinations of L-cysteine in a wide concentration range. Repeatability (intra-day) for the proposed sensor was tested with each three SWV of three sample solutions containing lower, middle and higher linear range. Intermediate precision (inter-day) of the method was evaluated by considering lower, middle and higher concentrations in the linear range in 3 days. The results, which are shown in Table 4, represent excellent precisions for the determination of L-cysteine using modified electrode in the presence of a mediator. The accuracy of the proposed method was studied by recovery experiments in lower, middle and higher concentrations in the linear range (0.3–600 μmolL^{-1}). The results showed very good recoveries between 98.5 and 103.5 % with a mean RSD of 1.4 %. The selectivity of the method was assessed by adding known quantities of standard solution to the tablet solutions and human urine samples. In view of the resultant SWV, no interferences were found in the potential range for the peak of L-cysteine during the analysis.

Conclusion

In this study, a modified carbon nanotubes paste electrode was used for the determination of L-cysteine in the presence of folic acid. The results show that the oxidation of L-cysteine is catalyzed at pH 7.0, whereas the peak potential of L-cysteine is shifted by 380 mV to a less positive potential at the surface of the ETFMCNTPE. The detected potential difference of 410 mV between L-cysteine and folic acid is large enough to allow simultaneous determination of those compounds in mixtures without significant interferences.

Acknowledgments The authors wish to thank the Science and Research Branch, Islamic Azad University, Mazandaran for the support.

References

1. Yosypchuk B, Novotny I (2002) Talanta 56:971
2. Ensafi AA, Dadkhah-Tehrani S, Karimi-Maleh H (2011) Anal Sci 27:409
3. Dumonceaux J, Goujon C, Joliot V, Briand P, Hazan U (2001) J Virol 75:5425
4. Schrynemackers-Pitance P, Schoos-Berbette S (1987) Clin Chim Acta 166:91
5. Chwatko G, Bold E (2000) Talanta 52:509
6. Gupta VK, Singh AK, Gupta B (2006) Anal Chim Acta 575:198
7. Goyal RN, Gupta VK, Chatterjee S (2009) Biosens Bioelec 24:3562

8. Goyal RN, Gupta VK, Chatterjee S (2009) Biosens Bioelec 24:1649
9. Prasad R, Gupta VK, Kumar A (2004) Anal Chim Acta 508:61
10. Jain R, Gupta VK, Jadon N, Radhapyari K (2010) J Electroanal Chem 648:20
11. Jain R, Gupta VK, Jadon N, Radhapyari K (2010) Anal Biochem 407:79
12. Gupta VK, Jain AK, Singh LP, Khurana U (1997) Anal Chim Acta 355:33
13. Al-Shammary FJ, Al-Rashood KA, Mian NA, Mian MS (1990) Anal Profiles Drug Sub 19:221
14. Nelson BC, Sharpless KE, Sander LC (2006) J Chromatogr A 1135:203
15. Rodriguez-Bernaldo de Quiros A, Castro de Ron C, Lopez-Hernandez J, Lage-Yusty MA (2004) J Chromatogr A 1032:135
16. Zhaoa SL, Yuan HY, Xie C, Xiao D (2006) J Chromatogr A 1107:290
17. Xiao F, Ruan C, Liu L, Yan R, Zhao F, Zeng B (2008) Sens Actuators B 134:895
18. Ensafi AA, Karimi-Maleh H (2010) J Electroanal Chem 640:75
19. Zhang B, Zhao L, Lin JM (2008) Talanta 74:1154
20. Hrůza Z, Hlaváčková V, Babický A (1966) Exp Geron 2:9
21. http://www.jbc.org/content/189/2/651.full.pdf. Accessed 13 July 2012
22. Iijima S (1991) Nature 354:56
23. Goyal RN, Oyama M, Gupta VK, Singh SP, Sharma RA (2008) Sens Actuators B 134:816
24. Ajayan PM (1999) Chem Rev 99:787
25. Wang GX, Ahn J, Yao J, Lindsay M, Liu HK, Dou SX (2003) J Pow Sour 119:160
26. Che G, Lakshmi BB, Martin CR, Fisher EE (1999) Langmuir 15:750
27. Ensafi AA, Izadi M, Karimi-Maleh H (2012) Ionics. doi:10.1007/s11581-012-0705-0
28. Dai H, Hafner H, Rinzler AG, Colbert DT, Smalley RE. Nature 384:147
29. Khalilzadeh MA, Khaleghi F, Gholami F, Karimi-Maleh H (2009) Anal Lett 42:584
30. Afzali D, Karimi-Maleh H, Khalilzadeh MA (2011) Environ Chem Lett 9:375
31. Goyal RN, Gupta VK, Chatterjee S (2010) Sens Actuators B 149:252
32. Goyal RN, Gupta VK, Chatterjee S (2008) Talanta 76:662
33. Khalilzadeh MA, Karimi-Maleh H (2010) Anal Lett 43:186
34. Karimi-Maleh H, Ensafi AA, Beitollahi H, Nasiri V, Khalilzadeh MA, Biparva P (2012) Ionics 18:687
35. Asnaashariisfahani M, Karimi-maleh H, Ahmar H, Ensafi AA, Fakhari AR, Khalilzadeh MA, Karimi F (2012) Anal Meth. doi:10.1039/c2ay25418b
36. Ensafi AA, Karimi-Maleh H, Mallakpour S, Hatami M (2011) Sens Actuators B 155:464
37. Ensafi AA, Karimi-Maleh H, Mallakpour S, Rezaei B (2011) Coll Surf B 87:480
38. Khalilzadeh MA, Karimi-Maleh H, Amiri A, Gholami F, Motaghed mazhabi R (2010) Chin Chem Lett 21:1467
39. Galus Z (1976) Fundumentals of electrochemical analysis. Ellis Horwood, New York