J Solid State Electrochem (2010) 14:1415–1423
DOI 10.1007/s10008-009-0978-z

ORIGINAL PAPER

Voltammetric measurement of trace amount of glutathione using multiwall carbon nanotubes as a sensor and chlorpromazine as a mediator

Ali A. Ensafi · M. Taei · T. Khayamian · H. Karimi-Maleh · F. Hasanpour

Received: 5 August 2009 / Revised: 25 October 2009 / Accepted: 14 November 2009 / Published online: 2 December 2009

Abstract In this work, we propose chlorpromazine as a new mediator for the rapid, sensitive, and highly selective voltammetric determination of glutathione (GSH) using multiwall carbon nanotubes paste electrode (MWCNTPE). The experimental results showed that the carbon nanotubes paste electrode has a highly electrocatalytic activity for the oxidation of GSH in the presence of chlorpromazine as a mediator. Cyclic voltammetry, double potential step chronoamperometry, and differential pulse voltammetry (DPV) are used to investigate the suitability of chlorpromazine at the surface of MWCNTPE as a mediator for the electrocatalytic oxidation of GSH in aqueous solutions. It is shown that chlorpromazine can catalyze the oxidation of GSH in an aqueous buffer solution to produce a sharp oxidation peak current at about +0.70 versus Ag/AgCl as a reference electrode. Kinetic parameters such as electron transfer coefficient and catalytic reaction rate constant, k/h, are also determined. Using DPV and under the optimum conditions at pH 4.0, the electrocatalytic oxidation peak current of GSH shows a linear dependence on GSH concentration in the GSH concentration range of 0.3 to 18.3 μM. The detection limit (3σ) is determined to be 0.16 μM. The relative standard deviation for 1.5 and 5.0 μM GSH are found to be 3.7% and 2.5%, respectively. The proposed method may, thus, also be used as a novel, selective, simple, and precise method for the voltammetric determination of GSH in such real samples as hemolyzed erythrocyte.

A. A. Ensafi (✉) · M. Taei · T. Khayamian · H. Karimi-Maleh · F. Hasanpour
Department of Chemistry, Isfahan University of Technology, Isfahan 84156-83111, Iran
e-mail: Ensafi@cc.iut.ac.ir

Keywords Chlorpromazine · Electrocatalysis · Glutathione determination · Voltammetric methods

Introduction

Glutathione (GSH) is a tripeptide that contains an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side chain. An antioxidant, GSH protects cells against such toxins as free radicals [1]. Thiol groups are kept in a reduced state at a concentration of approximately ~5 mM in animal cells. GSH reduces any disulfide bond formed within cytoplasmic proteins to cysteines by acting as an electron donor. In the process, GSH is converted to its oxidized form, glutathione disulfide. GSH is found almost exclusively in its reduced form since the enzyme reverting it from its oxidized form, glutathione reductase, is constitutively active and inducible upon oxidative stress. In fact, the ratio of reduced GSH to oxidized GSH within cells is often used scientifically as a measure of cellular toxicity [2]. The biosynthesis pathway for GSH is found in some bacteria, like cyanobacteria and proteobacteria, but is missing in many other bacteria. Most eukaryotes synthesize GSH, including humans, but some do not, such as Leguminosae, Entamoeba, and Giardia. The only archaea that make GSH are halobacteria [3]. Study shows that the total GSH in cells can be free or bound to proteins. Measurement of free GSH in blood samples indicates the status of cells in relation to its protective role against oxidative and free radical-mediated cell injury. Moreover, GSH measurement is important for the diagnosis of *c*-glutamyl cycle disorders. Because of the widespread involvement of GSH in many biological functions, much effort has been invested in developing sensitive and selective methods for its detection.

Several methods have been proposed for the determination of GSH that include titrimetry [4], spectrophotometry [5, 6], spectrofluorimetry [7–9], high-performance liquid chromatography [10, 11], capillary zone electrophoresis [12], proton nuclear magnetic resonance (^{1}H-NMR) [13], enzymatic methods [14], flow injection analysis with chemiluminescence detection [15], and electrochemical methods [16–21]. The comparison of the proposed method for GSH determination with the other electrochemical methods reported in the literature is given in Table 1. As shown, the proposed method compares well with the reported methods.

Nanotechnology is nowadays sharing knowledge, tools, techniques, and information on electrochemistry and electroanalysis with other fields [22]. Carbon nanotubes represent one of the commonly used building blocks of nanotechnology. We, therefore, proposed on the basis of previous work [21–25], chlorpromazine as a mediator for the rapid, sensitive, and highly selective voltammetric determination of GSH on the surface of a multiwall carbon nanotubes paste electrode (MWCNTPE). The results showed that the catalytic current depends on the concentration of GSH. Cyclic voltammetry (CV) and double potential step chronoamperometry are employed to establish the electrocatalytic behavior of chlorpromazine. The proposed method is selective, sensitive, and fast for the determination of GSH in real samples such as hemolyzed erythrocyte.

Experimental

Reagents and solutions

All chemicals were of analytical reagent grade and used without any purification unless stated otherwise. All the solutions were made up by doubly distilled water.

High-viscosity paraffin (density = 0.88 kg l^{-1}) from Merck was used for the preparation of the carbon paste electrode. Graphite powder (particle diameter = 0.1 mm) was from Fluka, and MWCNTs was brought from Iran's Research Institute of Petroleum Industry (synthesized by chemical vapor deposition) with a diameter of 8–15 nm, a length of 50 μm, and a purity of 95%.

A 1.0×10^{-2} M GSH solution was prepared daily by dissolving 0.307 g GSH in water, and the resulting 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 dilution with water.

A 1.0×10^{-2} M chlorpromazine solution was prepared daily by dissolving 0.089 g chlorpromazine in water. The solution was diluted to 25 ml with water in a 25-ml volumetric flask.

Universal buffer solutions (boric acid, phosphoric acid, and acetic acid plus sodium hydroxide [0.44 M]) with different pH values were prepared and used to study the influence of pH.

Apparatus

Voltammetric measurements were carried out using a Metrohm instrument (Herisau, Switzerland), Model 797 VA, connected to a three-electrode cell. A carbon nanotubes paste electrode, a platinum wire, and an Ag/AgCl electrode were used as the working electrode, the auxiliary electrode, and the reference electrode, respectively.

For chronoamperometric study, an Autolab PGSTAT 12, 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 the Autolab software was used.

A pH meter (Corning, Model 140) with a double-junction glass electrode was used to check the pH levels of the solutions.

Table 1 Comparison of the efficiency of some modified electrodes in the determination of GSH

Electrode	Modifier	pH	Limit of detection (μM)	Linear dynamic range (μM)	Reference
Carbon paste	2,7-BFEF	7.0	0.5	0.92–11	[16]
Carbon paste	FC	7.0	2.1	2.2–3,000	[17]
GC	Well-aligned/carbon nanotubes	7.0	0.2	0.4–16.4	[18]
GC	PQQ/PPy	8.4	13.2	–	[19]
Carbon paste	TTF-TCNQ	7.0	0.30	5–340	[20]
EPPGE	–	7.0	2.7	10–80	[21]
MWCNTPE	Chlorpromazine	4.0	0.16	0.3–18.3	This work

2,7-BFEF 2,7-bis(ferrocenyl ethyl)fluoren-9-one, *FC* ferrocene, *PQQ/PPy* pyrroloquinoline quinine into polypyrrole, *TTF–TCNQ* tetrathiafulvalene–tetracyanoquinodimethane, *EPPGE* edge-plane pyrolytic-graphite electrode

Electrode preparation

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.

Recommended procedure

The MWCNTPE was polished with a white and clean paper. To prepare a blank solution, 9.0 ml of the buffer solution (boric acid, phosphoric acid, acetic acid/sodium hydroxide, 0.10 M, pH 4.0) and 1.0 ml of chlorpromazine (0.010 M) solution were transferred into an electrochemical cell. The initial and final potentials were adjusted to +0.40 and +0.90 V versus Ag/AgCl, respectively. The differential pulse voltammogram (DPV) was recorded with a pulse height of 50 mV and a pulse width of 5 mV to give the blank signal and labeled as I_{pb}. Then, different amounts of GSH solutions were added to the cell using a micropipette, and the DPV was recorded again to get the analytical signal (I_{ps}; final GSH concentrations varied over 0.3–18.3 μM). This net signal ($I_{ps} - I_{pb}$) was proportional to the GSH concentrations. The calibration curve was constructed by plotting the net peak current versus the GSH concentration.

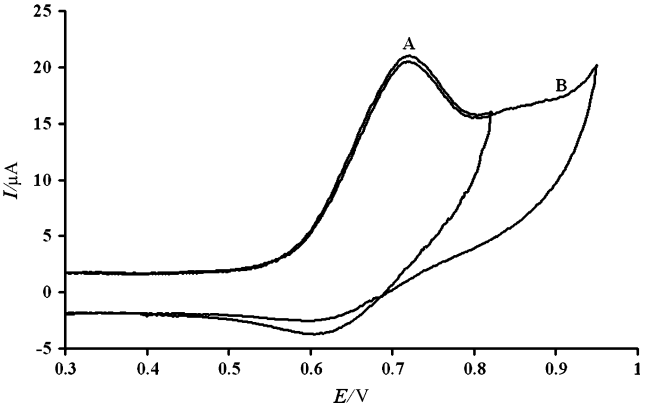


Fig. 2 Cyclic voltammograms of 1.0 mM chlorpromazine at the surface of MWCNTPE in 0.04 M universal buffer (pH 4.0) at a scan rate of 100 mV s^{-1}. *A* For a potential range of 0.30 to 0.80 V; *B* for a potential range of 0.30 to 0.95 V

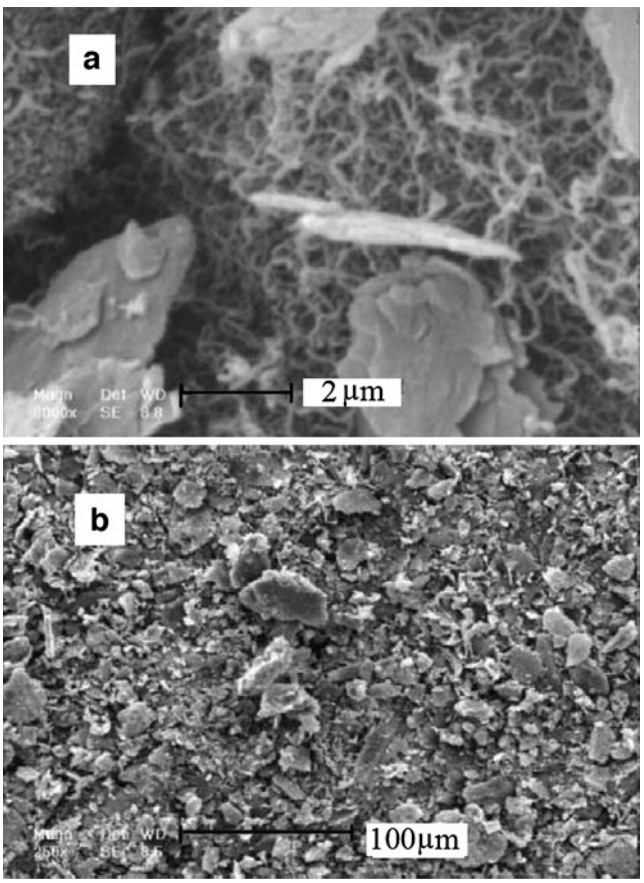
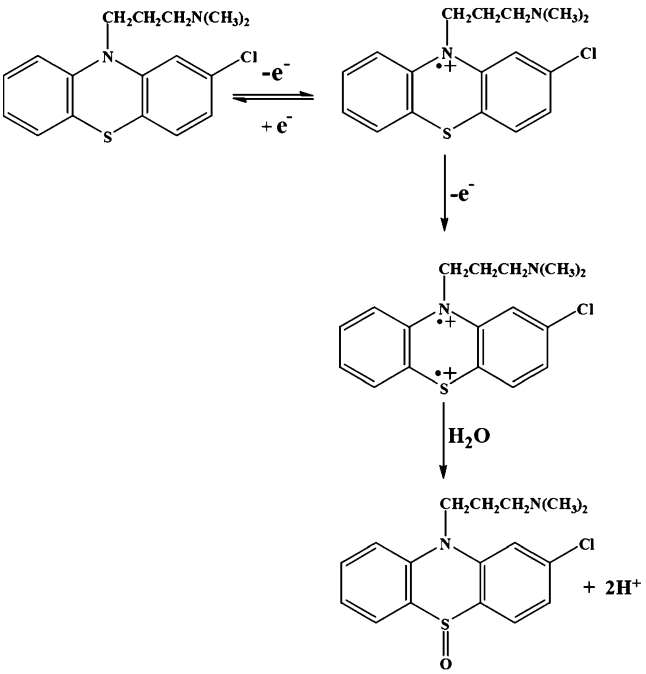


Fig. 1 SEM image of **a** MWCNTPE and **B** CPE



Scheme 1 Mechanism of redox behavior of chlorpromazine

Preparation of real samples

For the determination of GSH in human erythrocyte, human whole blood was obtained from the Isfahan University Hospital, and erythrocytes were separated from the whole blood by removing the plasma. Human whole blood (2.0 ml) was firstly centrifuged for 10 min at 3,000 rpm. The supernatant (plasma) was then discarded, and the rest was mixed with 5 ml 0.9% NaCl solution. The solution was centrifuged for another 5 min at 3,000 rpm, and the supernatant was again discarded. The washing procedure with NaCl solution was repeated three times in order to remove the plasma almost completely. The erythrocyte pellets were hemolyzed with water (1:1; *v*/*v*). For protein precipitation, the hemolysate was mixed with 5-sulfosalysilic acid (10%; *m*/*v*) with a 2:1 (*v*/*v*) ratio. This mixture was centrifuged in the same way previoulsy described. Then, the supernatant was divided into two parts: one to be used for the chemiluminescence determination [16] and the other for the proposed electrochemical method.

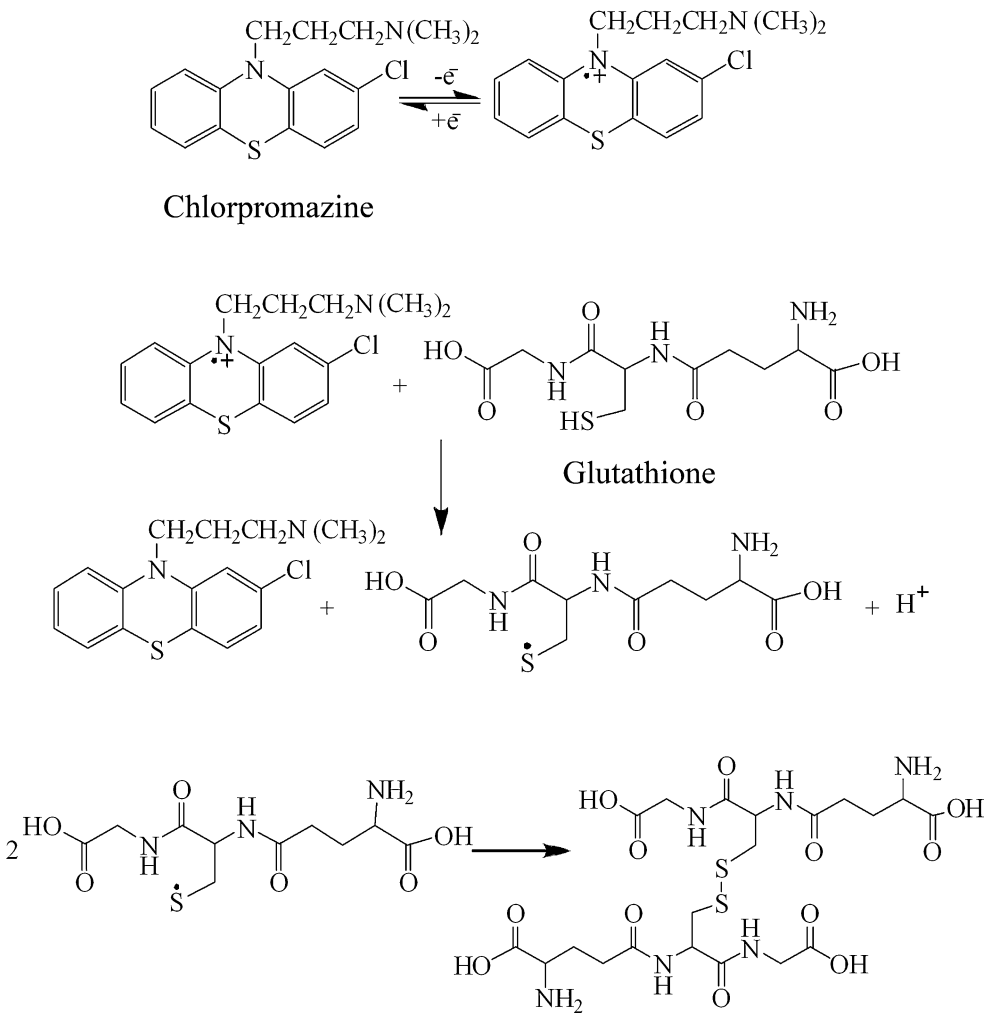
Results and discussion

Scanning electron microscopy characterization of MWCNTs

Figure 1 displays a typical morphology of the MWCNTPE (a) and the carbon paste electrode without carbon nanotubes (b) characterized by scanning electron microscopy (SEM). As shown in Fig. 1, the multiwall carbon nanotubes did not change the morphology of the carbon paste electrode but made it more compact. However, it can be clearly seen that the multiwall carbon nanotubes dispersed homogeneously.

Electrochemistry of the mediator

Cyclic voltammogram of 1.0 mM chlorpromazine at MWCNTPE is shown in Fig. 2. Figure 2b shows a voltammogram of chlorpromazine in a wide range of potential with two different anodic peak currents where



Scheme 2 The role of chlorpromazine on the oxidation of GSH

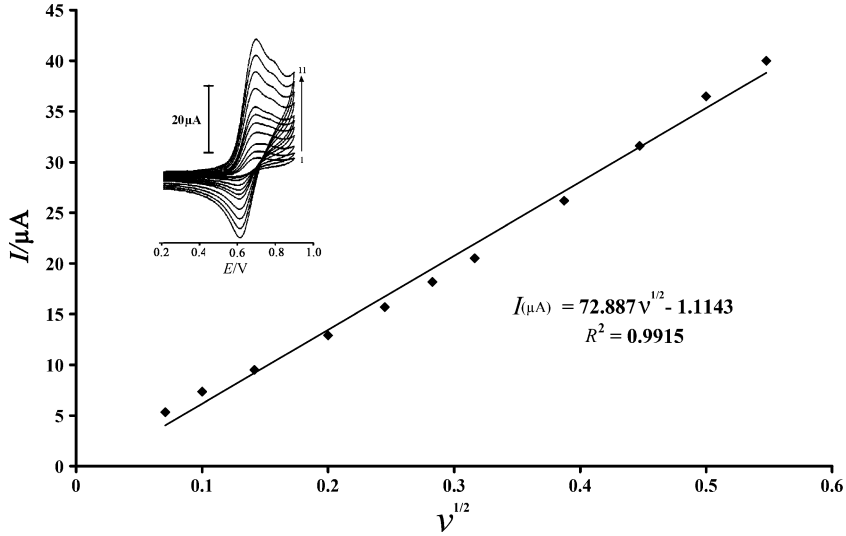


Fig. 3 **a** Cyclic voltammograms of 1.0 mM chlorpromazine at various scan rates as *1* 5, *2* 10, *3* 20, *4* 40, *5* 60, *6* 800, *7* 100, *8* 150, *9* 200, *10* 250, and *11* 300 mV s^{-1} in 0.04 M buffer solution (pH 4.0). **b** Plot of I_{pa} versus $\nu^{1/2}$ for the oxidation of chlorpromazine at the surface of MWCNTPE

the first one is reversible (around 0.70 V), and the second one (around 0.85 V) is irreversible [26]. Therefore, the first oxidation peak has a potential to act as a mediator for oxidation reaction. In the first step, the electrochemical oxidation of the substances occurs at the nitrogen atom, while the second wave comprises the formation of the sulfoxide as shown in Scheme 1 [26–28]. The role of chlorpromazine as a mediator in the GSH oxidation at the surface of MWCNTPE is shown in Scheme 2 [26, 27]. As shown in Scheme 2, chlorpromazine was oxidized directly at the surface of the modified electrode. Then, the oxidized form of chlorpromazine reacts with GSH to oxidize GSH.

Figure 3 (inset) shows the cyclic voltammograms of chlorpromazine at MWCNTPE in the universal buffer (pH 4.0) at various scan rates. These cyclic voltammograms were used to examine the variation of the peak current versus the sweep. The plots of the anodic peak currents against the sweep rate show that the I_p values vary linearly with $\nu^{1/2}$ at all scan rates.

Electrocatalytic oxidation of GSH

The voltammetric behavior of chlorpromazine in the buffer solution (pH 4.0) is shown in Fig. 4. The cyclic voltammetric responses for the electrochemical oxidation of 500 μM of GSH at MWCNTPE are shown in curve b (Fig. 4) and at the carbon paste electrode in curve c (Fig. 4), both in the presence of the mediator (chlorpromazine). Curves d and f (Fig. 4) are the same as curves b and c (Fig. 4), respectively, but only without chlorpromazine. Curve e (Fig. 4) shows the cyclic voltammogram of the buffer solution (pH 4.0) at the surface of carbon paste electrode. As can be seen, the anodic peak potentials for the oxidation of GSH at MWCNTPE in the presence of the mediator (curve b) is about 700 mV, whereas this potential is 700 mV when using carbon paste electrode with chlorpromazine (curve c). On the other hand, GSH oxidation (without chlorpromazine) does not take place at the surface of MWCNTPE and/or carbon paste electrode up to +1.10 V. Similarly, when we compared the oxidation of GSH at the surface of MWCNTPE (Fig. 4b) and the carbon paste electrode with the mediator (Fig. 4c), an enhancement of the anodic peak current was found to occur at MWCNTPE versus Ag|AgCl|KCl_{sat}. In other words, the data obtained clearly show that the combination of MWCNTPE and the mediator (chlorpromazine) definitely improve the characteristics of the electrode for the oxidation of GSH.

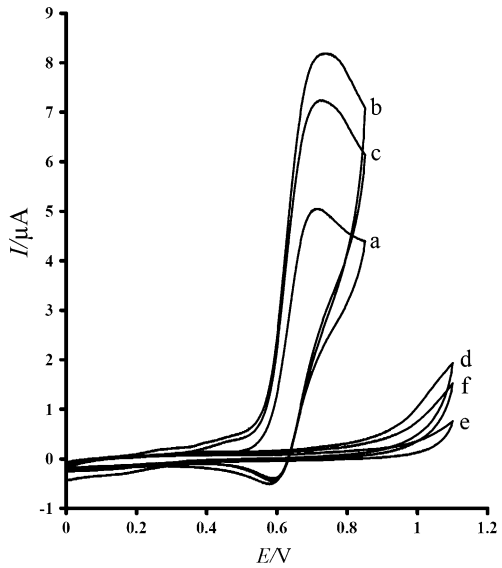


Fig. 4 Cyclic voltammograms of 1.0 mM chlorpromazine at the surface of MWCNTPE in 0.04 M universal buffer (pH 4.0) at a scan rate of 15 mV s^{-1} in the absence (*a*) and in the presence (*b*) of 500.0 μM GSH. *c* as *b* for the carbon paste electrode. *f* as *c* and *d* as *b* for the unmodified electrode (and in the absence of chlorpromazine). *e* For the buffer solution at the surface of unmodified electrode (carbon paste electrode)

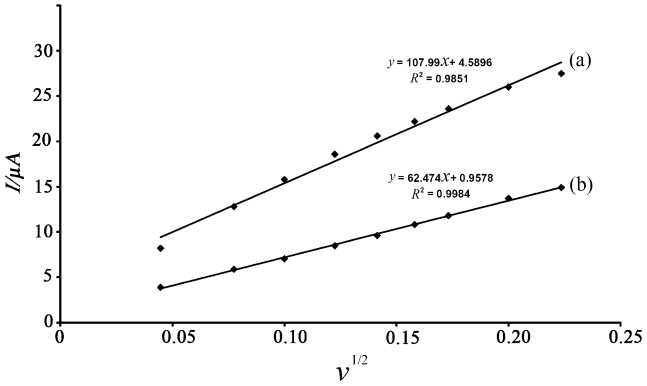


Fig. 5 Variation of electrocatalytic current (I_p) with the square root of scan rate for 7.5 μM (*a*) and 1.5 μM (*b*) GSH in the presence of 1.0 mM chlorpromazine at the surface of MWCNTPE

Figure 5 shows the relationship between the anodic peak current (I_{pa}) and the square root of the scan rate ($\nu^{1/2}$) for two different concentrations of GSH using MWCNTPE. The results confirm that the anodic peak currents increase linearly with the square root of the scan rate suggesting that at a sufficient overpotential, the reaction is mass transfer controlled. The regression equations for 1.5 and 7.5 μM of GSH are $I_p = 107.99X + 4.5896$ with $R^2 = 0.9851$ and $I_p = 62.474X + 0.9578$ with $R^2 = 0.9984$, respectively. On the other hand, at scan rates higher than 15 mV s^{-1}, the electrocatalytic reaction does not favorably occur and the reduction peak begins to increase. A possible interpretation is that the overall electrochemical oxidation of GSH at the MWCNTPE in the presence of the mediator might be controlled by the cross-exchange process between GSH and the redox site of chlorpromazine as well as by the diffusion of GSH to the surface of the electrode.

In order to obtain information on the rate-determining step, a Tafel plot was developed for MWCNTPE in the presence of the mediator using the data derived from the raising part of the current–voltage curve (Fig. 6, inset A). The slope of the Tafel plot is equal to $n(1-\alpha)F/2.3RT$ which comes up to 11.274 per decade. We obtained $n\alpha$ equal to 0.34. Assuming $n = 1$, then $\alpha = 0.34$.

Influence of pH

In order to optimize the electrocatalytic response to GSH oxidation, we investigated the effects of pH on the electrocatalytic oxidation of GSH in 0.04 M universal buffer solutions with various pH values (2.0 < pH < 5.5) at the surface of MWCNTPE in the presence of the mediator using CV. The effects of pH on both peak currents and peak potentials were assessed through examining the electrode response in the buffer solutions. The results showed that the maximum electrocatalytic current was obtained at pH 4.0. At higher pH values (pH > 5.0), the peak currents of GSH decreased because chlorpromazine was precipitated from the solution. Therefore, pH 4.0 was chosen as the optimum pH for the determination of GSH at the surface of the MWCNTPE in the presence of chlorpromazine.

Optimization of chlorpromazine concentration

The influence of chlorpromazine concentration on the peak currents was studied in the chlorpromazine concentration range 0.05–4.0 mM at pH 4.0. The results showed that by increasing chlorpromazine concentration up to 1.0 mM the net peak current increased, whereas any further increase in chlorpromazine concentration caused a decrease in the magnitude of the peak current. This is due to the fact that at higher concentration of chlorpromazine electropolymerization of chlorpromazine occurred at the surface of the electrode [29]. Therefore, 1.0 mM was selected as the optimal chlorpromazine concentration.

Chronoamperometric studies

Double potential step chronoamperometry was also employed to investigate the electrochemical behavior of

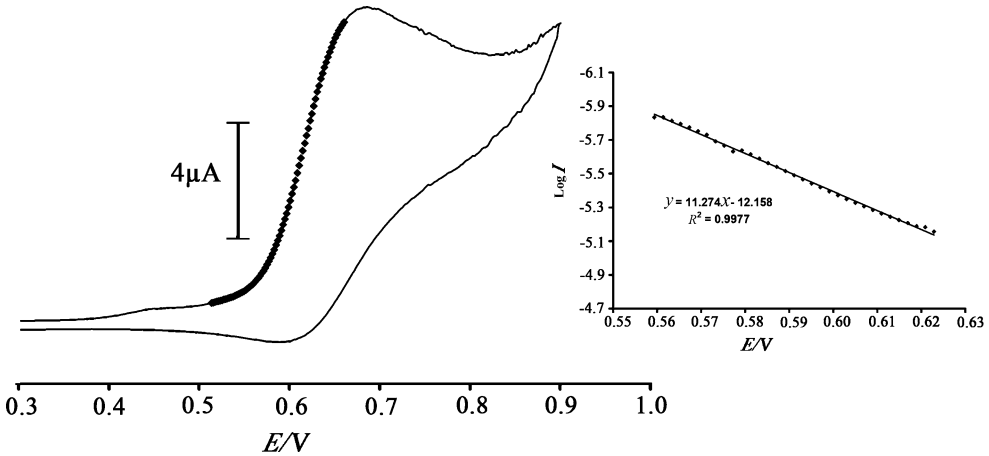


Fig. 6 Tafel plot 1.0 mM chlorpromazine at the surface of MWCNTPE in 0.04 M universal buffer (pH 4.0) at a scan rate of 15 mV s^{-1} in the presence of 0.40 mM GSH

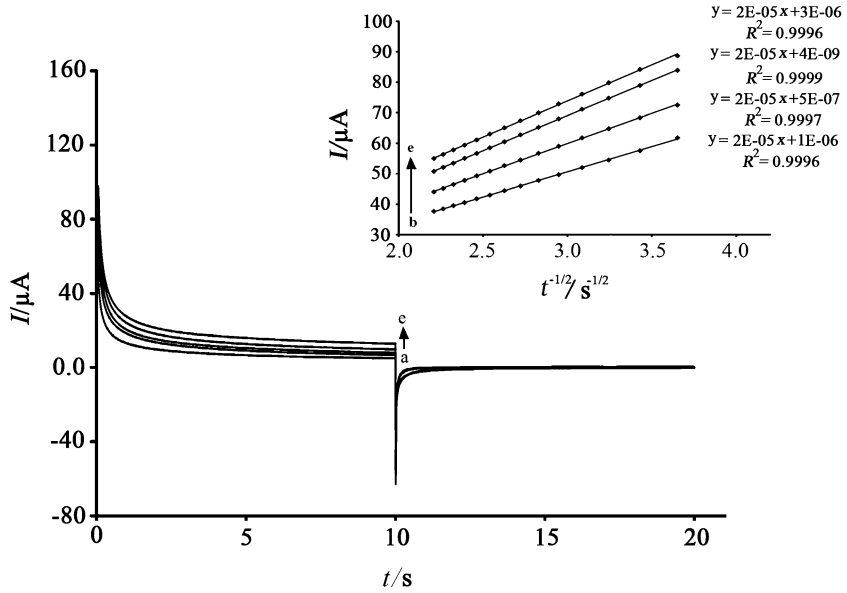


Fig. 7 Chronoamperograms obtained at the MWCNTPE in the absence (*a*) and in the presence of *b* 0.075, *c* 0.150, *d* 0.350, and *e* 0.500 mM GSH in the buffer solution (pH 4.0). *Inset* Cottrell's plot for the data from the chronoamperograms

aqueous buffered solutions (pH 4.0) containing various concentrations of GSH at MWCNTPE in the presence of the mediator by setting the working electrode potential at 0.40 V (at the first potential step) and 0.85 V (at the second potential step) versus Ag|AgCl|KCl_{sat} (Fig. 7). As can be seen, there is no net cathodic current corresponding to the reduction of the mediator in the presence of GSH when the potential is stepped from 0.40 to 0.85 V. The linearity of the electrocatalytic current versus $\nu^{1/2}$ indicates that this current is controlled by the diffusion of GSH from the bulk solution toward the surface of the electrode, causing a near-Cottrellian behavior. Therefore, the slope of the linear region of the Cottrell's plot can be used to estimate the diffusion coefficient of GSH. A plot of I versus $t^{-1/2}$ for the MWCNTPE with the mediator in the presence of GSH gives a straight line, the slope of which can be used to estimate the diffusion coefficient of GSH (D) in the range 75–500 μM (Fig. 7, inset). The mean value of D was found to be 1.75×10^{-4} cm^2 s^{-1}.

The rate constant for the chemical reaction between GSH and redox sites in MWCNTPE, k_h, can be evaluated by chronoamperometry according to the method described in [30–32]:

$$I_C/I_L = \gamma^{1/2}\left[\pi^{1/2}\mathrm{erf}\left(\gamma^{1/2}\right) + \exp\left(-\gamma/\gamma^{1/2}\right)\right] \quad (1)$$

where I_C is the catalytic current of MWCNTPE in the presence of GSH, I_L is the limited current in the absence of GSH, and $\gamma = k_h C_b t$ (C_b is the bulk concentration of GSH) is the argument of the error function. In cases where γ exceeds 2, the error function is almost equal to 1 and the above equation can be reduced to:

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

where k_h and t are the catalytic rate constant (in cubic centimeters per mole per second) and the time elapsed (in seconds), respectively. Equation 2 can be used to calculate the rate constant of the catalytic process, k_h. Having measured the catalytic current, i.e., I_C, it is possible to carry out the electrode process under identical conditions

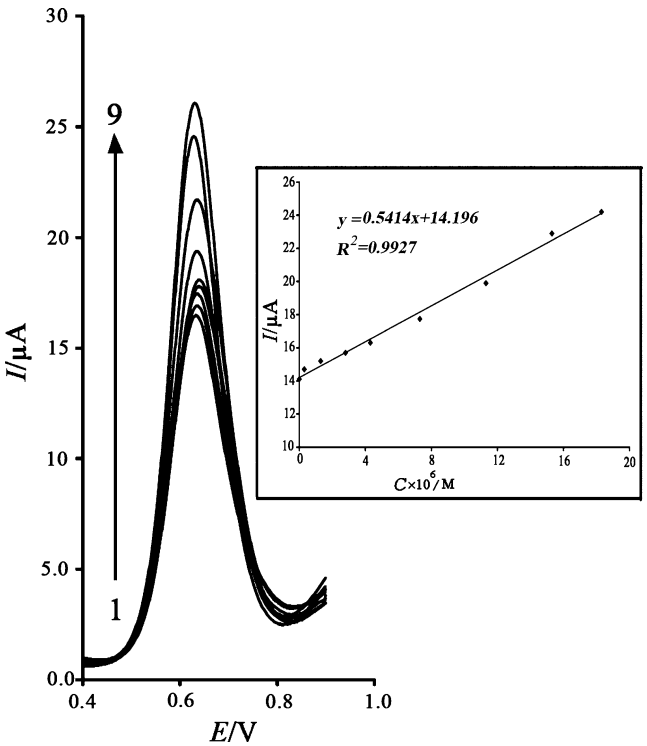


Fig. 8 Differential pulse voltammograms at the MWCNTPE with 1.0 mM chlorpromazine for *1* 0.0, *2* 0.3, *3* 1.3, *4* 2.8, *5* 4.3, *6* 7.3, *7* 11.3, *8* 15.3, and *9* 18.3 μM GSH in 0.04 M universal buffer solution (pH 4.0). *Inset* plot of the electrocatalytic peak current for DPV vs. GSH concentration

Table 2 Concentration values obtained from the proposed and CL method for GSH analysis in normal subject

Sample	Proposed method (mM)	Chemiluminescence method (mM)	$F_{exp.}$	$F_{tab.(0.05);2,2}$	S_{pooled}	$t_{exp.}$	$t_{tab.(98\%)}$
1	1.17 ± 0.03	1.20 ± 0.05	3.0	19	0.042	0.86	3.80
2	1.00 ± 0.02	1.13 ± 0.06	9.9	19	0.046	3.40	3.80
3	0.88 ± 0.02	0.90 ± 0.05	4.3	19	0.037	0.65	3.80
4	1.40 ± 0.03	1.41 ± 0.07	5.1	19	0.054	0.226	3.80

The plus–minus sign shows the standard deviation with three replicates determination

but in the absence of GSH in order to determine I_L. From the slope of the I_C/I_L–$t^{1/2}$ plot, the value of k_h can be simply calculated for a given concentration of the substrate. The calculated value of k_h is 2.5×10^2 cm^3 mol^{-1} s^{-1} using the slope of the I_C/I_L–$t^{1/2}$ plot. This value of k_h also explains the sharp feature of the catalytic peak observed for the catalytic oxidation of GSH at the surface of MMWCNTPE.

Electrocatalytic determination of GSH

Since DPV has a much higher current sensitivity than CV, it was used for the determination of GSH (Fig. 8). A pulse height of 50 mV and a pulse width of 5 mV were selected in order to get the best sensitivity under specific conditions. The electrocatalytic peak current of GSH oxidation at the surface of MWCNTPE in the presence of chlorpromazine can be used for the determination of GSH in solution. The results show that the electrocatalytic peak current of GSH oxidation at the surface of MWCNTPE is linearly dependent on GSH concentration and that the range of this linearity depends on the amount of the mediator at the surface of the electrode (Fig. 8, inset). The oxidation peak currents of GSH at the surface of MWCNTPE were proportional to the concentration of GSH within the range 0.30–18.3 μM in differential pulse voltammetric method with a regression equation of $I_p = 0.5414(\pm 0.0070)C_{GSH} + 14.196(\pm 0.0914)$ $(r^2 = 0.9927,\ n = 9)$ where Ip and C_{GSH} are the current (in microampere) and concentration (in micromolars) of the GSH, respectively (Fig. 8).

The detection limit was determined at 0.16 μM GSH according to the definition $Y_{LOD} = Y_B + 3\sigma$.

The repeatability and reproducibility of any sensors are two of their important characteristic features, both of which were studied in this work. Prior to using MWCNTPE for the electrocatalytic oxidation of GSH in the presence of chlorpromazine at pH 4.0, the rate of loss of electrochemical activity for the electrode was investigated. We have prepared one MWCNTPE for the analysis of a series of seven solutions of 60.0 μM GSH in the presence of 1.0 mM chlorpromazine at pH 4.0. The results showed that the electrode response was nearly constant (with a relative standard deviation of 3.8%) for the first three experiments, whereas for the next four measurements, the electrode response decreased to 91% of the expected value. Therefore, the surface regeneration of MWCNTPE is necessary after each of the three experiments.

The relative standard deviations obtained for five replicate measurements of 1.5 and 5.0 μM GSH were 3.7% and 2.5%, respectively.

Interference studies

The influence of various substances as compounds, potentially interfering with the determination of GSH, was studied under the optimum conditions using 1.5 μM GSH. The potentially interfering substances were chosen from the group of substances commonly found with GSH in pharmaceuticals and/or in biological fluids. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error of less than ±5% for the determination of GSH. After the experiments, we found that 1,000-fold Mg^{+2}, Ca^{+2}, SO_4^{-2}, Br^-, K^+, NO_3^-, ClO_4^-, and glycine; 600-fold glucose, sucrose, lactose, fructose, and valine; 500-fold aspartic acid, urea, and methionine; and saturation of starch solution did not interfere with the determination of GSH. However, greater amounts of 20-fold cysteine and 50-fold ascorbic acid did

Table 3 Concentration values obtained from the proposed and CL method for GSH analysis in diabetes

Sample	Proposed method (mM)	Chemiluminescence method (mM)	$F_{exp.}$	$F_{tab.(0.05);2,2}$	S_{pooled}	$t_{exp.}$	$t_{tab.(98\%)}$
1	0.81 ± 0.04	0.79 ± 0.05	1.56	19	0.045	0.54	3.80
2	0.64 ± 0.03	0.70 ± 0.06	4.00	19	0.047	1.55	3.80
3	0.75 ± 0.02	0.69 ± 0.04	4.00	19	0.032	2.32	3.80
4	0.70 ± 0.02	0.73 ± 0.05	6.25	19	0.038	0.96	3.80

The plus–minus sign shows the standard deviation with three replicates determination

cause interference in the GSH electrocatalytic signal. Despite their interference, ascorbic acid and L-cysteine are not present at significant levels in the hemolyzed erythrocyte sample. To investigate the effects of these two interfering species, a concentration of 1.0 μM GSH was used and its peak current was recorded to compare the obtained signal with those of a mixture of GSH and ascorbic acid plus L-cysteine. The results showed that 20-fold cysteine and 50-fold ascorbic acid did not affect the selectivity. Higher ratios of these compounds are not normally found in the blood [33, 34]. In the human blood, more than 99.5% of the GSH was localized in the erythrocyte and 97% of the cysteine was in the plasma [35]. Furthermore, the GSH content was higher than 90% of the total thiol-containing compounds in the blood; therefore, the thiol compounds in the whole blood can be regarded as GSH [36].

Determination of GSH in real samples

In order to demonstrate the electrocatalytic oxidation of GSH in real samples, we examined the ability of the voltammetric determination of GSH in human erythrocyte. The results were subsequently compared with a chemiluminescence method [15]. The results from the analyses of different normal and diabetes hemolyzed erythrocyte samples using both methods are given in Tables 2 and 3. Statistical analysis of the results using Student's *t* test and the variance ratio *F* test were used to compare the two methods for their accuracy and precision, which revealed the potential applicability of the proposed method.

Conclusion

The novel voltammetric sensor is developed for the determination of GSH. It can be used for real sample analysis. This mediator shows excellent catalytic effects on the oxidation of GSH. It has been found that with CV the oxidation of GSH occurs at a potential of about +0.70 V on the surface of the MWCNTPE in the presence of chlorpromazine, while the oxidation of GSH does not takes place at the surface of a carbon nanotubes paste electrode without chlorpromazine up to +1.20 V. The proposed method is sensitive to GSH levels as low as 0.16 μM. It can, therefore, be recommended as a simple and precise new sensor for the voltammetric determination of GSH in real samples such as hemolyzed erythrocyte. The method is rapid, reproducible, highly selective, and sensitive for the determination of GSH.

Acknowledgments The authors wish to thank the Isfahan University of Technology Research Council and the Center of Excellence in Sensor and Green Chemistry for their support.

References

1. Pompella A, Visvikis A, Paolicchi A, De Tata V, Casini AF (2003) Biochem Pharmacol 66:1499
2. Pastore A, Piemonte F, Locatelli M, Lo Russo A, Gaeta LM, Tozzi G, Federici G (2003) Clin Chem 47:1467
3. Newton GL, Javor B (1985) J Bacteriol 161:438
4. Nagendra P, Yathirajan HS, Rangappa KS (2002) J Indian Chem Soc 79:602
5. Raggi M, Nobile L, Giovannini AG (1991) J Pharm Biomed Anal 9:1037
6. Kamata K, Takahashi M, Terajima K, Nishijima M (1995) Analyst 120:2755
7. Liang SC, Wang H, Zhang ZM, Zhang X, Zhang HS (2002) Anal Chim Acta 451:211
8. Zhang JY, Hu ZD, Chen XG (2005) Talanta 65:986
9. Kandar R, Zakova P, Lotkova H, Kucera O, Cervinkova Z (2007) J Pharm Biomed Anal 43:1382
10. Katrusiak AE, Paterson PG, Kamencic H, Shoker A, Lyon AW (2001) J Chromatogr B Biomed Appl 758:207
11. Xu F, Wang L, Gao M, Jin J (2002) Anal Bioanal Chem 372:791
12. Causse E, Malatray P, Calaf R, Chariots P, Candito M, Bayle C, Valdiguie P, Salvayre R, Couderc F (2000) Electrophoresis 21:2074
13. Rabenstein DL, Brown DW, McNeil CJ (1985) Anal Chem 57:2294
14. Satoh I, Arakawa S, Okamoto A (1988) Anal Chim Acta 214:415
15. Ensafi AA, Khayamian T, Hasanpour F (2008) J Pharm Biomed Anal 48:140
16. Raoof JB, Ojani R, Karimi-Maleh H (2009) J Appl Electrochem 39:1169
17. Raoof JB, Ojani R, Kolbadinezhad M (2009) J Solid State Electrochem 13:1411
18. Tang H, Chen J, Nie L, Yao S, Kuang Y (2006) Electrochim Acta 51:3046
19. Inoue T, Kirchhof JR (2000) Anal Chem 72:5755
20. Calvo-Marzal P, Chumbimuni-Torres KY, Hoehr NF, Kubota LT (2006) Chem Acta 371:152
21. Moore RR, Banks CE, Compton RG (2004) Analyst 129:755
22. Merkoci A (2007) Nanobiomaterials in Electroanalysis 19:739
23. Ensafi AA, Mirmomtaz E (2005) J Electroanal Chem 583:176
24. Mirmomtaz E, Ensafi AA, Karimi-Maleh H (2008) Elecroanalysis 20:1973
25. Karimi-Maleh H, Ensafi AA, Allafchian AR (2009) J Solid State Electrochem (in press)
26. Hayen H, Karst U (2003) Anal Chem 75:483
27. Mielech-Łukasiewicz K, Puzanowska-Tarasiewicz H, Panuszko A (2008) Anal Lett 41:789
28. Blankert B, Hayen H, van Leeuwen SM, Karst U, Bodoki E, Lotrean S, Sandulescu R, Mora Diez N, Dominguez O, Arcos J, Kauffmann JM (2005) Electroanalysis 17:1501
29. Schlereth DD, Karyakin AA (1995) J Electroanal Chem 395:221
30. Galus Z (1976) Fundamentals of electrochemical analysis. Horwood, New York
31. Raoof JB, Ojani R, Karimi-Maleh H (2008) Electroanalysis 20:1259
32. Mazloum-Ardakani M, Beitollahi H, Ganjipour B, Naeimi H, Nejati M (2009) Bioelectrochemistry 75:1
33. Westerman MP, Zhang Y, McConnell JP, Chezick PA, Neelam R, Freels S, Feldman LS, Allen S, Baridi R, Feldman LE, Fung LWM (2000) Am J Hematol 65:174
34. Reid ME, Badaloo A, Forrester T, Jahoor F (2006) Am J Physiol Endocrinol Metab 291:73
35. Mills BJ, Lang CA (1996) Biochem Pharmacol 52:401
36. Jocelyn PC (1972) Biochemistry of the SH group. Academic, London