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ORIGINAL PAPER

Electrocatalytic oxidation of glutathione at carbon paste electrode modified with 2,7-*bis* (ferrocenyl ethyl) fluoren-9-one: application as a voltammetric sensor

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Abstract Electrooxidation of glutathione (GSH) was studied at the surface of 2,7-*bis* (ferrocenyl ethyl) fluoren-9-one modified carbon paste electrode (2,7-BFEFMCPE). Cyclic voltammetry (CV), double potential-step chronoamperometry, and differential pulse voltammetry (DPV) were used to investigate the suitability of this ferrocene derivative as a mediator for the electrocatalytic oxidation of GSH in aqueous solutions with various pH. Results showed that pH 7.00 is the most suitable pH for this purpose. At the optimum pH, the oxidation of GSH at the surface of this modified electrode occurs at a potential of about 0.410 V versus Ag|AgCl|KCl_{sat}. The kinetic parameters such as electron transfer coefficient, $\alpha = 0.61$, and rate constant for the chemical reaction between GSH and redox site in 2,7-BFEFMCPE, $k_h = 1.73 \times 10^3$ cm^3 mol^{-1} s^{-1}, were also determined using electrochemical approaches. Also, the apparent diffusion coefficient, D_{app}, for GSH was found to be 5.0×10^{-5} cm^2 s^{-1} in aqueous buffered solution. The electrocatalytic oxidation peak current of GSH showed a linear dependence on the glutathione concentration, and linear calibration curves were obtained in the ranges of 5.2×10^{-5} M to 4.1×10^{-3} M and 9.2×10^{-7} M to 1.1×10^{-5} M with cyclic voltammetry and differential pulse voltammetry methods, respectively. The detection limits (3σ) were determined as 1.4×10^{-5} M and 5.1×10^{-7} M for the CV and DPV methods, respectively. This method was also examined as a selective, simple, and precise new method for voltammetric determination of GSH in real sample such as hemolysed erythrocyte.

Keywords 2,7-*bis* (Ferrocenyl ethyl) fluoren-9-one · Glutathione · Electrocatalysis · Cyclic voltammetry · Differential pulse voltammetry · Chronoamperometry · Hemolysed erythrocyte

1 Introduction

Glutathione (L-γ-glutamyl-L-cysteinylglycine), which is present in virtually all mammalian tissues, provides reducing capacity for several reactions and plays an important role in detoxification of hydrogen peroxide, other peroxide, and free radicals [1]. Synthesis and degradation of glutathione are controlled by reaction of the γ-glutamyl cycle; a decrease in blood reduced glutathione (GSH) has been reported in patients affected by deficiencies of the enzymes involved in the synthesis of glutathione [1]. However, platelet activation may play a significant role in pathogenesis of atherosclerosis and thrombosis [2]. One of the factors that enhance platelet activation is lipid peroxides [3], and increased lipid peroxides in plasma have been shown in some pathological states [4]. An essential part of the antioxidative system that prevents accumulation of lipid peroxides is glutathione (GSH), found in virtually all cell types [5, 6]. Study shows that in cells total glutathione can be free or bound to proteins; measurement of free glutathione 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 diagnosis of γ-glutamyl cycle disorders.

As a consequence of the widespread involvement of GSH in many biological functions, much effort has been

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invested to develop sensitive and selective methods for its detection. Many chemical and instrumental techniques have been reported for the determination of GSH, which are divided into titrimetry [7], spectrophotometry [8, 9], spectrofluorimetry [10–12], high-performance liquid chromatography [13, 14], capillary zone electrophoresis [15], proton nuclear magnetic resonance (^{1}H NMR) [16], and enzymatic methods [17]. However, most of them suffer from difficulties of sample preparation, the necessity for derivatization, or lack of sufficient sensitivity, all of which limit their utility. Compared with the other techniques, electroanalytical methods have the advantages of simplicity, low expense, and high sensitivity. The electron transfer reaction of GSH at bare common electrodes such as carbon, platinum, gold, and silver is a slow process that requires a high overpotential, except for the mercury electrode [18, 19]. Mercury is obviously not ideal for use as a sensor due to its toxicity. In addition, strong adsorption of GSH at the surface of noble-metal electrodes makes GSH detection unsatisfactory, resulting in serious blocking and fouling of these electrodes [20].

In order to solve the problems mentioned above, much work has been done to develop chemically modified electrodes for determination of GSH. A series of modified electrodes have been used based on enzymes [21, 22], organometallic compounds [23, 24], organic compounds [25], tetrathiafulvalene-tetracyanoquinodimethane (TTF–TCNQ) complex [26], etc. [27]. Recently, electrochemical determination of thiols was reviewed by Compton and coworkers [28]. However, some authors have emphasized the instability of the attached materials on the electrodes as a problem arising in the utilization of chemically modified electrodes (CMEs) [29]. It seems that the incorporation of electrocatalysts into the electrode matrix can, partly, help to solve these problems. Carbon paste electrodes (CPEs), due to their ease and speed of construction, obtaining a new reproducible surface, low residual current compared with other solid electrodes, porous surface, compatibility with various types of modifiers, chemical inertness and compatible with molecules and biological species, and low cost have been widely used as a suitable matrix for preparation of CMEs [30–33].

Ferrocene and its derivatives, due to their good stability in solution, rapid response to many electroactive substances, pH independence, stability in both oxidized and reduced forms, unreactiveness with oxygen, regeneration at low potential, and fast electron transfer, are the most successful mediators. In this paper, we suggest a voltammetric sensor for GSH determination based on the use of 2,7-*bis* (ferrocenyl ethyl) fluoren-9-one as a mediator into the carbon paste electrode matrix. Therefore, the suitability of 2,7-BFEFMCPE in the electrocatalysis and determination of glutathione are discussed based on results of cyclic voltammetry, double potential step chronoamperometry, and differential pulse voltammetry.

2 Experimental

2.1 Materials

The solvent used for the electrochemical studies was twice-distilled water. Buffer solution was prepared from orthophosphoric acid and its salts in the pH range 3.00–9.00. High-viscosity paraffin (density 0.88 g cm^{-3}) from Fluka was used as the pasting liquid for the carbon paste electrode. Graphite powder (particle diameter 0.1 mm) from Merck was used as the working electrode (WE) substrate. The 2,7-*bis* (ferrocenyl ethyl) fluoren-9-one was prepared by a reported procedure [34]. Glutathione was obtained from Fluka and was used as received. All other reagents were of analytical grade.

2.2 Working electrode

A 1% (w/w) 2,7-*bis* (ferrocenyl ethyl) fluoren-9-one spiked carbon powder was made by dissolving the given quantity of 2,7-*bis* (ferrocenyl ethyl) fluoren-9-one in diethyl ether and hand-mixing with 99 times its weight of graphite powder with a mortar and pestle. The solvent was evaporated by stirring a 1:1 (w/w) mixture of 1% 2,7-*bis* (ferrocenyl ethyl) fluoren-9-one spiked carbon powder and paraffin oil was blended by hand-mixing and the resulting paste was inserted in the bottom of a glass tube (internal radius 3.0 mm). The electrical connection was implemented by a copper wire lead fitted into a glass tube. A carbon paste electrode without 2,7-*bis* (ferrocenyl ethyl) fluoren-9-one was used as a blank to determine background current.

2.3 Apparatus

The electrochemical experiments were carried out using a potentiostat/galvanostat (BHP 2061-C Electrochemical Analysis System, Behpajooh, Iran) coupled with a Pentium IV personal computer connected to a HP laserjet 6L printer, and experiments were performed in a three-compartment cell. A platinum wire was used as the auxiliary electrode. Ag|AgCl|KCl$_{sat}$ (Metrohm) and 2,7-BFEFMCPE were used as the reference and working electrodes, respectively. A pH-meter (Ion Analyzer 250, Corning) was used to read the pH of the buffered solutions. Spectrometric measurements were performed with ultraviolet/visible (UV/Vis) spectrophotometer JASCO V-570 (PMT) for recording the absorption spectra.

2.4 Preparation of real sample

For determination of GSH in human erythrocyte, human whole blood was obtained from Isfahane University Hospital and erythrocytes were separated from whole blood by removing the plasma. Human whole blood (2 ml) was firstly centrifuged for 10 min at 3,000 rpm. The supernatant (plasma) was discarded and the rest was mixed with 5 ml 0.9% NaCl. The solution was centrifuged for another 5 min at 3,000 rpm and the supernatant (diluted plasma) 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 hemolysed with water (1:1 v/v). For protein precipitation, the hemolysate was mixed with 5-sulfosalysilic acid (10% w/v) in the ratio 2:1 (v/v). This mixture was centrifuged in the same condition described above. Then supernatant was divided into two parts for spectrophotometric and electrochemical measurements.

For spectrophotometric measurements, the Ellman reference method [35] was performed, which is based on the reaction of glutathione and 5,5′-dithio-*bis*-(2-nitrobenzoic acid), (DTNB), Ellman's reagent, generating 2-nitro-5-mercapto-benzoic acid. This was monitored spectrophotometrically at 412 nm. For electrochemical measurement, 100 µL hemolysed erythrocyte sample was diluted in 25 ml water.

3 Results and discussion

3.1 Electrochemical behavior of 2,7-BFEFMCPE

We have recently constructed 2,7-BFEFMCPE by incorporation of 2,7-*bis* (ferrocenyl ethyl) fluoren-9-one into carbon paste matrix and studied its electrochemical properties in buffered aqueous solution by cyclic voltammetry. Its cyclic voltammograms exhibits an anodic (E_{pa} = 0.37 V) and corresponding cathodic peaks with E_{pc} = 0.27 V versus Ag|AgCl|KCl_{sat} related to the Fc/Fc^+ redox couple with quasireversible behavior [36]. Also, the obtained result shows that the redox process of Fc/Fc^+ in 2,7-*bis* (ferrocenyl ethyl) fluoren-9-one is independent of the aqueous solution pH.

3.2 pH effect on GSH electrochemical behavior at 2,7-BFEFMCPE

It is well known that the electrochemical behavior of GSH is dependent on the pH value of the aqueous solution, whereas the electrochemical properties of the Fc/Fc^+ redox couple are independent of pH. Therefore, we studied the electrochemical behavior of GSH in buffered solution

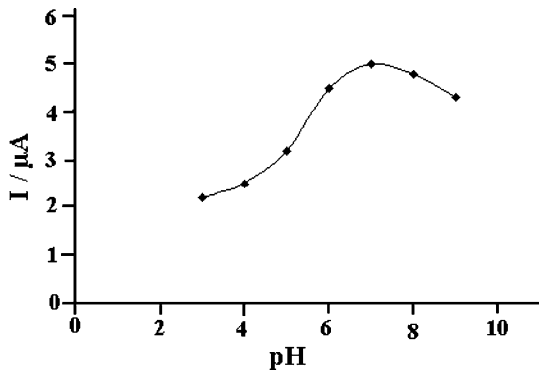

Fig. 1 Current–pH curve for electrooxidation of 0.3 mM GSH in 0.1 M phosphate buffer solution at the surface of 2,7-BFEFMCPE at scan rate 20 mV s^{-1}

(0.1 M phosphate) with various pHs ($3.00 < pH < 9.00$) at the surface of 2,7-BFEFMCPE by cyclic voltammetry. Figure 1 shows the variation of I_{pa} versus pH for GSH oxidation at the surface of this modified electrode. As can be seen, the maximum electrocatalytic current was obtained at pH 7.00. Therefore, pH 7.00 was chosen as the optimum pH for electrocatalytic oxidation of GSH at 2,7-BFEFMCPE and all electrochemical experiments were done at this pH.

3.3 Electrocatalytic oxidation of glutathione at 2,7-BFEFMCPE

Cyclic voltammograms obtained for modified and unmodified carbon paste electrode in a phosphate buffer solution (pH 7.00) in presence (0.3 mM) and absence of GSH are shown in Fig. 2. Oxidation of GSH at the bare

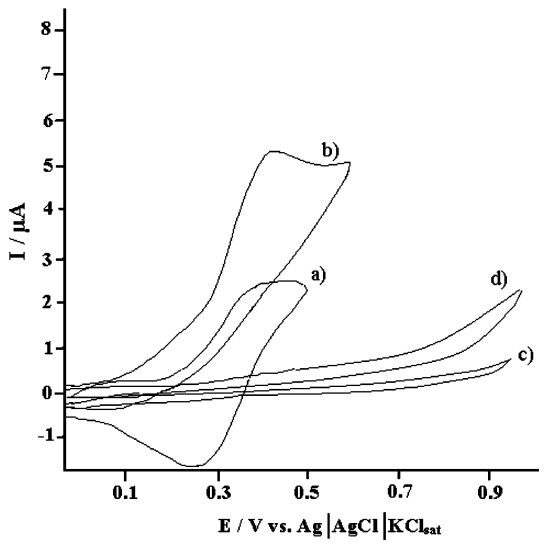

Fig. 2 Cyclic voltammograms of (a) 2,7-BFEFMCPE in 0.1 M phosphate buffer solution (pH 7.00) at scan rate 20 mV s^{-1} and (b) as (a) in presence of 0.3 mM GSH, (c) as (a), and (d) as (b), at the surface of CPE

CPE occurred irreversibly near the anodic limiting current (curve d); in absence of GSH no peaks appears (curve c). The anodic peak that is observed for 2,7-BFEFMCPE in absence of GSH increases greatly in 0.3 mM GSH solution, while the corresponding cathodic peak disappeared on the reverse scan (curves a and b). Therefore, the Fc^+ electrogenerated at the 2,7-BFEFMCPE surface undergoes a catalytic reduction by GSH back to Fc, which can then be electrochemically reoxidized to produce an enhancement in the anodic current. The process could be expressed as follows:

$$Fc \rightarrow Fc^+ + e^- \qquad : E \qquad (1)$$

$$GSH_{red} + Fc^+ \rightarrow GSH_{ox} + Fc \qquad : C' \qquad (2)$$

Therefore, oxidation of GSH at the surface of 2,7-BFEFMCPE is performed at about 0.410 V versus $Ag|AgCl|KCl_{sat}$. Compton and co-workers reported the response of an edge-plane pyrolytic graphite electrode in the direct oxidation of thiol moieties such as homocysteine, *N*-acetylcysteine, cysteine and glutathione. They explored and found that this electrode to be electrocatalytic producing a reduction in the overpotential while having enhanced signal-to-noise characteristics compared to glassy carbon and basal plane pyrolytic graphite electrodes [37]. Then, the electrooxidation of glutathione was occurred at 0.65 V versus saturated calomel electrode at the surface of an edge-plane pyrolytic graphite electrode and this electrode has electrocatalytic ability for GSH electrooxidation. Therefore, 2,7-BFEFMCPE has more electrocatalytic effect than that of an edge-plane pyrolytic graphite electrode.

In order to obtain information on the rate-determining step a Tafel slope (b) for a totally irreversible diffusion-controlled process was determined using the following equation [38]:

$$E_P = b/2\log\ v + \text{constant} \qquad (3)$$

based on Eq. 3, the slope of E_p versus log v is $b/2$, where b indicates Tafel slope. The slope of E_p versus log v plot was found to be 0.048 V in this work (Fig. 3), thus $b = 2 \times 0.048 = 0.096$ V. This slope value indicates an electron transfer process, which is the rate-limiting step under the assumption of a transfer coefficient (α) of 0.61.

Also, the values of αn_α (where α is the transfer coefficient and n_α is the number of electrons involved in the rate-determining step) were calculated for the oxidation of glutathione at pH 7.00 at both modified and unmodified CPEs according to the following equation [39]:

$$\alpha n_\alpha = 0.048/\left(E_P - E_{P/2}\right), \qquad (4)$$

where $E_{P/2}$ is the potential corresponding to $I_{P/2}$. The values for αn_α were found to be 0.25 and 0.61 for oxidation of GSH at the surface of the CPE and 2,7-BFEFMCPE, respectively. These values clearly show that not only is the overpotential for GSH oxidation reduced at the surface of 2,7-BFEFMCPE, but also the rate of the electron transfer process is greatly enhanced. This phenomenon is thus confirmed by the larger I_{pa} value recorded during cyclic voltammetry at 2,7-BFEFMCPE.

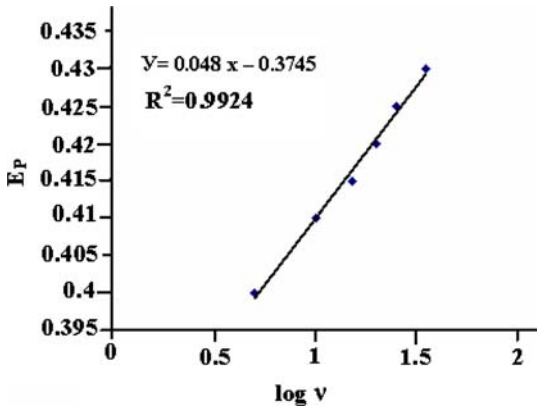

Fig. 3 Peak potential, E_{pa} dependence on log v for oxidation of GSH at the surface of 2,7-BFEFMCPE

3.4 Chronoamperometric studies

Subsequently, the chronoamperometric behaviors of unmodified and modified CPEs were examined in absence and presence of GSH by setting the working electrode potential at 0.6 V (the first potential step) and 0.2 V (the second potential step) versus $Ag|AgCl|KCl_{sat}$ (Fig. 4A). As can be seen, there is no net cathodic current corresponding to the reduction of mediator in presence of glutathione when the potential is stepped from 0.60 V to 0.2 V versus $Ag|AgCl|KCl_{sat}$. However, in presence of GSH, the charge value associated with forward chronoamperometry is greater than that observed for backward chronoamperometry (Fig. 4B, c′). The linearity of the electrocatalytic current versus $t^{-1/2}$ indicates that the current must be controlled by diffusion of GSH from bulk solution toward surface of electrode. Therefore the slope of this linear plot can be used to estimate the apparent diffusion coefficient, D_{app}, of GSH. The mean value of D_{app} was found to be 5.0×10^{-5} $cm^2\ s^{-1}$. Therefore, the results show that mediator at the surface of 2,7-BFEFMCPE can catalyze oxidation of GSH.

The rate constant for the chemical reaction between GSH and redox sites in 2,7-BFEFMCPE, k_h, can be evaluated by chronoamperometry according to the method described in [40]:

$$I_C/I_L = \pi^{1/2}\gamma^{1/2} = \pi^{1/2}(k_h C_b t)^{1/2}, \qquad (5)$$

where I_C is the catalytic current of 2,7-BFEFMCPE in the presence of GSH and I_L is the limited current in the absence of GSH, γ is the argument of the error function, C_b

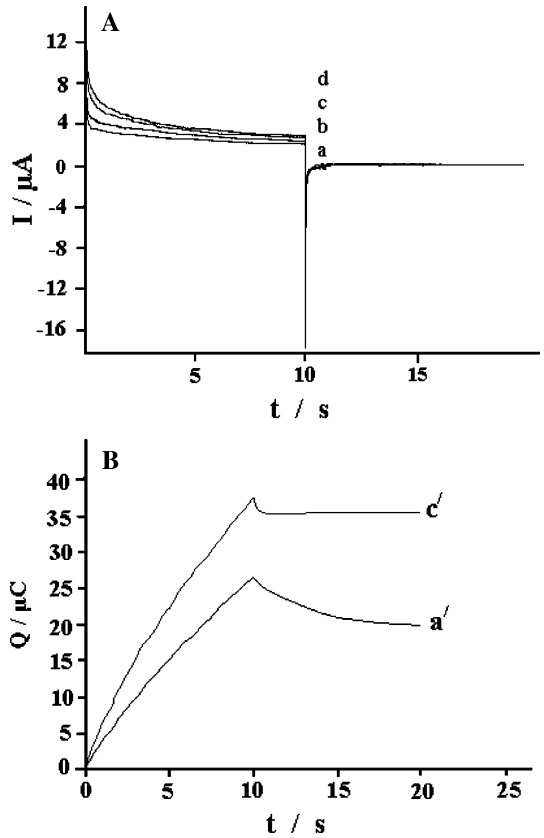

Fig. 4 **A** Chronoamperograms obtained at 2,7-BFEFMCPE in absence (a) and presence of (b) 1.0 mM, (c) 3.0 mM, and (d) 4.0 mM of GSH in 0.1 M phosphate buffer solution (pH 7.00). First and second potential steps were 0.60 and 0.20 V versus Ag|AgCl|KCl_{sat}. **B** Charge−time curves: (a′) for curve (a) and (c′) for curve (c)

is the bulk concentration of GSH (mol cm^{-3}), and k_h and t are the catalytic rate constant (cm^3 mol^{-1} s^{-1}) and time elapsed (s), respectively. Equation 5 can be used to calculate the rate constant of the catalytic process, k_h. The value of k_h can be simply calculated for a given concentration of substrate from the slope of I_C/I_L versus $t^{1/2}$. Therefore, the calculated value of k_h is 1.73×10^3 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 catalytic oxidation of GSH at 2,7-BFEFMCPE.

3.5 Electrocatalytic determination of glutathione

The electrocatalytic peak current of GSH oxidation at the surface of 2,7-BFEFMCPE can be used for determination of GSH in solution. Therefore, CV and DPV experiments were performed using 2,7-BFEFMCPE in phosphate buffer solution containing various concentrations of GSH. The results show that the electrocatalytic peak current for the oxidation of GSH on 2,7-BFEFMCPE is linearly dependent on the concentration of GSH. The range of this linearity depends on the amount of mediator in the electrode matrix. The mediated oxidation peak currents of GSH on 1% 2,7-BFEFMCPE were proportional to the concentration of the substrate in the ranges of 5.2×10^{-5} M to 4.1×10^{-3} M (with a correlation coefficient of 0.9973) and 9.2×10^{-7} M

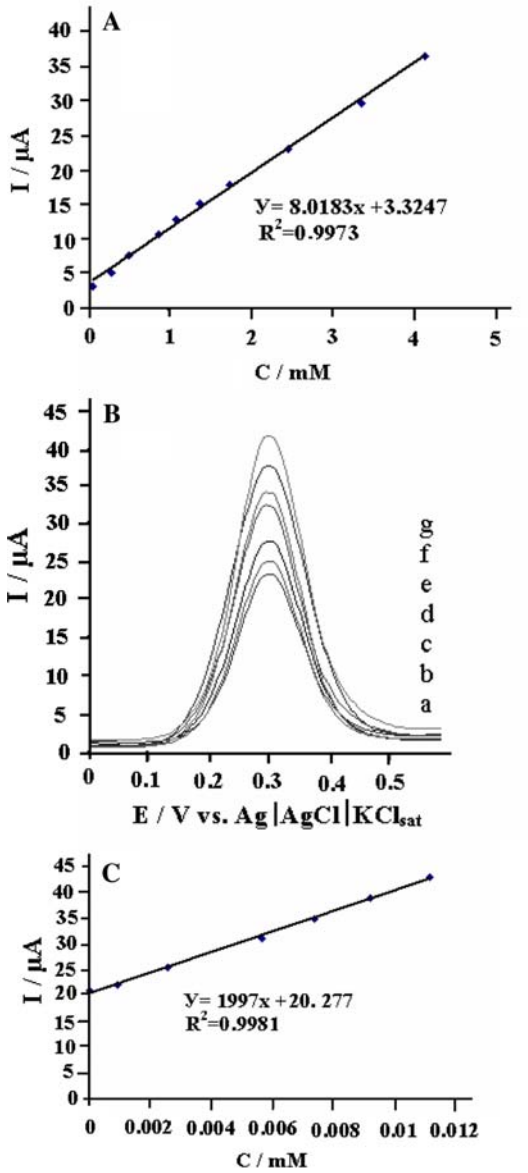

Fig. 5 **A** Plot of electrocatalytic peak currents from cyclic voltammograms of GSH at various concentrations: (a) 0.0519 mM, (b) 0.2800 mM, (c) 0.4900 mM, (d) 0.8600 mM, (e) 1.0700 mM, (f) 1.3600 mM, (g) 1.7300 mM, (h) 2.4500 mM, (i) 3.3500 mM, and (j) 4.1300 mM at the surface of 2,7-BFEFMCPE in 0.1 M phosphate buffer solution (pH 7.00) at scan rate 20 mV s^{-1}. **B** Differential pulse voltammograms at 2,7-BFEFMCPE in (a) absence and presence of (b) 0.00092 mM, (c) 0.00256 mM, (d) 0.00566 mM, (e) 0.00738 mM, (f) 0.00920 mM, and (g) 0.01100 mM GSH in 0.1 phosphate buffer solution (pH 7.00). **C** Plot of electrocatalytic peak current (from DPV of **B**) versus GSH concentration

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

Electrode	Modifier	pH	LOD (μM)	LDR (μM)	Reference
GC	Well-aligned/CNT	7.00	0.2	0.4–16.4	[41]
GC	PQQ/PPy[a]	8.42	13.2	–	[42]
Carbon paste	TTF–TCNQ[b]	7.00	0.3	5–340	[43]
Edge-plane pyrolytic graphite electrode	–	7.00	2.7	10–80	[37]
Carbon paste	2,7-BFEF[c]	7.00	0.5	0.92–11	This work

[a] Pyrroloquinoline quinine into polypyrrole

[b] Tetrathiafulvalene (TTF)–tetracyanoquinodimethane (TCNQ)

[c] 2,7-*bis* (ferrocenyl ethyl) fluoren-9-one

Table 2 Determination of GSH in human erythrocyte

Sample	Electrochemical method (mM)[a]	Spectrophotometric method (mM)[a]	F_{exp}	t_{exp}
1	0.95 ± 0.07	1.10 ± 0.09	3.51	3.30
2	1.06 ± 0.10	1.23 ± 0.07	2.25	2.23
3	1.12 ± 0.09	1.07 ± 0.12	2.84	0.96

[a] Result at 95% confidence limits, obtained from replicated determination per sample

Theoretical values for $t = 4.30$ and $F = 19$ ($P = 0.05$)

to 1.1×10^{-5} M (with a correlation coefficient of 0.9981) for CV and DPV, respectively (Fig. 5). The detection limits, LOD (3σ) were 1.4×10^{-5} M and 5.1×10^{-7} M for the CV and DPV methods, respectively. These values are comparable to the values obtained by other research groups (Table 1). Thus, the catalytic oxidation of glutathione can readily be applied for determination of GSH.

3.6 Determination of glutathione in real sample

In order to demonstrate the electrocatalytic oxidation of GSH in real samples, we examined this ability in the voltammetric determination of GSH in human erythrocyte and the result was compared with spectrophotometric method which is usually used for glutathione determination. The results are listed in Table 2. The results were obtained for concentration of glutathione in three different hemolysed erythrocyte samples by means of both methods.

These experiments demonstrated the ability of 2,7-BFEFMCPE for voltammetric determination of GSH with high electrocatalytic effect and good reproducibility.

3.7 Interference studies

Study of interference on the electrocatalysis signal is useful to set up the sample preparation with the goal of minimizing their effect. In this work, interferences were considered to be common foreign species and the compounds that are structurally related to GSH and present in blood samples. For the interference study the signal for 100 μM concentration of GSH was recorded. A foreign species was considered not to interfere if it caused a relative error of less than 3% in the analytical signal of GSH. The tolerance ratios were as following: 800-fold for K^+, Na^+, PO_4^{3-}, Ca^{2+}, Zn^{2+}, Mg^{2+}, NH_4^+, NO_3^-, SO_4^{2-}, Cl^-, glucose, sucrose, lactose, fructose, glycine, and valine; 400-fold for phenylalanine, leucine, alanine, Fe^{+2}, Fe^{+3}; and 100-fold for trptophan. However equal amount of cysteine and ascorbic acid interfered in the GSH electrocatalytic signal.

Although ascorbic acid and cysteine show interference, they are not present at significant level in hemolysed erythrocyte sample. To investigate the effect of these two interferences, a concentration of 100 μM GSH was recorded and the obtained signal was compared with those of mixture of GSH and interfering compound in the ratio of 100:1 for cysteine and 25:1 for ascorbic acid. These ratios were chosen since these interferences are not found in blood at higher concentration [44, 45]. At these ratios, the results showed no significant interference from these two compounds. Moreover, interference from ascorbic acid can be minimized by using ascorbic oxidase enzyme, which exhibits high selectivity to oxidation of ascorbic acid, if necessary.

In human blood, more than 99.5% of GSH was localized in erythrocyte and 97% of cysteine was in plasma [46]. Furthermore the GSH content is higher than 90% of total thiol-containing compound in blood, therefore thiol compounds in whole blood can be regarded as GSH [47].

Therefore, this method is a selective, simple, and precise new method for voltammetric determination of GSH in real samples such as hemolysed erythrocytes.

4 Conclusions

This work demonstrates the construction of a chemically modified carbon paste electrode by incorporation of 2,7-*bis* (ferrocenyl ethyl) fluoren-9-one as a modifying species into carbon paste matrix. The reported experimental results demonstrate this modified electrode and show the electrocatalytic ability on oxidation of GSH in aqueous solution with optimum pH of 7.00, and that oxidation of GSH occurred at potential of about 0.410 V versus Ag|AgCl| KCl_{sat}. The mediated oxidation current of GSH at the 2,7-BFEFMCPE was used for the determination of GSH in aqueous solution. Finally, the electrocatalytic oxidation of GSH at the surface of this modified electrode can be employed as a new method for voltammetric determination of glutathione in real samples such as human erythrocytes.

References

1. Meister A, Larsson A (1989) The metabolic bases on inherited disease, 6th edn. McGraw-Hill, New York
2. Ganda OP (1980) Diabetes 29:29
3. Mickel HS, Horbar J (1973) Lipids 9:68
4. Clavind J, Hartman S, Clemmensen J (1952) Acta Pathol Microbiol Scand 30:1
5. Fujii S, Dale GL, Beutler E (1984) Blood 63:1096
6. Meister A (1981) Trends Biochem Sci 23:1
7. Nagendra P, Yathirajan HS, Rangappa KS (2002) J Indian Chem Soc 79:602
8. Raggi M, Nobile L, Giovannini AG (1991) J Pharm Biomed Anal 9:1037
9. Kamata K, Takahashi M, Terajima K, Nishijima M (1995) Analyst 120:2755
10. Liang SC, Wang H, Zhang ZM, Zhang X, Zhang HS (2002) Anal Chim Acta 451:211
11. Zhang JY, Hu ZD, Chen XG (2005) Talanta 65:986
12. Kaňďár R, Žáková P, Lotková H, Kučera O, Červinková Z (2007) J Pharm Biomed Anal 43:1382
13. Katrusiak AE, Paterson PG, Kamencic H, Shoker A, Lyon AW (2001) J Chromatogr B Biomed Appl 758:207
14. Xu F, Wang L, Gao M, Jin J (2002) Anal Bioanal Chem 372:791
15. Causse E, Malatray P, Calaf R, Chariots P, Candito M, Bayle C, Valdiguie P, Salvayre R, Couderc F (2000) Electrophoresis 21:2074
16. Rabenstein DL, Brown DW, McNeil CJ (1985) Anal Chem 57:2294
17. Satoh I, Arakawa S, Okamoto A (1988) Anal Chim Acta 214:415
18. Jin WR, Zhan X, Xian L (2000) Electroanalysis 12:858
19. Banica FG, Fogg AG, Moreira JC (1995) Talanta 42:227
20. Tang H, Chen J, Nie L, Tao S, Kuang Y (2006) Electrochim Acta 51:3046
21. Compagnone D, Federici G, Scarciglia L (1993) Biosen Bioelectron 8:257
22. Huang TH, Kuwana T, Warsinke A (2002) Biosen Bioelectron 17:1107
23. Wang Sh, Ma H, Li J, Chen X, Bao Z, Sun Sh (2006) Talanta 70:518
24. El-Kosasy AM, Shehata AM, Hassan NY, Fayed AS, El-Zeany BA (2005) Talanta 66:746
25. Gong X, Li HL (2000) J Electrochem Soc 147:238
26. Calvo-Marzal P, Chumbimuni-Torres KY, HöeKubota LT (2006) Clin Chim Acta 371:152
27. White PC, Lawrence NS, Davis J, Compton RG (2002) Electroanalysis 14:89
28. Gracheva S, Digga A, Livingstone C, Davis J (2004) Electroanalysis 17:205
29. Pham MC, Dubois JE (1986) J Electroanal Chem 199:153
30. Almeida CMVB, Giannetti BF (2002) Electrochem Commun 4:985
31. Raoof JB, Ojani R, Ramine M (2007) Electroanalysis 19:597
32. Liu L, Song J (2006) Anal Biochem 354:22
33. Kozan JVB, Silva RP, Serrano SHP, Lim AWO, Angnes L (2007) Anal Chim Acta 591:200
34. Hosseinzadeh R, Ph.D Thesis (1999) Justus-Liebig-Universitate, Giessen, Germany
35. Ellman GL (1959) Archives of Biochem Biophy 82:70
36. Raoof JB, Ojani R, Beitollahi H, Hossienzadeh R (2006) Electroanalysis 18:1193
37. Moore RR, Banks CE, Compton RG (2004) Analyst 129:755
38. Pournaghi-Azar MH, Razmi-Nerbin H (2000) J Electroana. Chem 488:17
39. Takeuchi ES, Murray R (1985) J Electroanal Chem 189:49
40. Galus Z (1976) Fundamentals of Electrochemical analysis. Ellis Horwood, New York
41. Tang H, Chen J, Nie L, Yao S, Kuang Y (2006) Electrochem Acta 51:3046
42. Inoue T, Kirchhof JR (2000) Anal Chem 72:5755
43. Calvo-Marzal P, Chumbimuni-Torres KY, Hoehr NF, Kubota LT (2006) Clin Chemi Acta 371:152
44. Westerman MP, Zhang Y, McConnell JP, Chezick PA, Neelam R, Freels S, Feldman LS, Allen S, Baridi R, Feldman LE, Fung LWM (2000) Amer J Hemato 65:174
45. Reid ME, Badaloo A, Forrester T, Jahoor F (2006) Am J Physiol Endocrinol Metab 291:3
46. Mills BJ, Lang CA (1996) Biochem Pharmacol 52:401
47. Jocelyn PC (1973) Biochemistry of SH Group. Academic Press, London