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Voltammetric determination of glutathione in haemolysed erythrocyte and tablet samples using modified-multiwall carbon nanotubes paste electrode

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A chemically modified electrode was prepared by incorporating *p*-aminophenol into multiwall carbon nanotubes paste matrix. Cyclic voltammetry, square wave voltammetry, double potential step chronoamperometry, and electrochemical impedance spectroscopy were used to investigate the electrochemical behaviour of glutathione at the chemically modified electrode prepared. According to the results, *p*-aminophenol multiwall carbon nanotubes paste electrode (*p*-APMWCNTPE) showed high electrocatalytic activity for glutathione oxidation, producing a sharp oxidation peak current at about +0.285 *vs* Ag/AgCl reference electrode at pH 5.0. Chronoamperometry was also used to determine glutathione's catalytic rate constant and diffusion coefficient at *p*-APMWCNTPE. The square wave voltammetric peak current of glutathione increased linearly with glutathione concentration in the range of 2.0×10^{-7} – 1.0×10^{-4} mol L^{-1} with a detection limit of 9.0×10^{-8} mol L^{-1}. The method was also successfully employed as a selective, simple, and precise method for the determination of glutathione in haemolysed erythrocyte, tablet, and urine samples. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: glutathione; carbon nanotubes paste electrode; *p*-Aminophenol; electrocatalytic method; voltammetry

Introduction

Glutathione (GSH) is the most abundantly found non-protein thiol in living organisms with numerous important roles in protein and DNA synthesis, transport, catabolism, and metabolism.[1] It provides living cells with protection against toxicity, hypoxia, or mutagenicity, and the effects of many carcinogens.[2] Changes in its concentration levels are possible indications of certain diseases such as premature arteriosclerosis, occlusive vascular, leukaemia, diabetes, acquired immunodeficiency syndrome (AIDS), and cataract, among others.[3] Studies have shown that the total GSH present in cells may be either free or bound to proteins. The amount of free GSH in blood is indicative of cell protection against oxidative and free radical-mediated cell injury. In addition, GSH levels in blood samples help in diagnosis of c-glutamyl cycle disorders. Its precise determination is, therefore, of utmost importance for diagnostic purposes.

A number of methods have been proposed for the determination of GSH that include titrimetry,[4] spectrophotometry,[5,6] spectrofluorimetry,[7–9] high performance liquid chromatography (HPLC),[10,11] capillary zone electrophoresis,[12] proton nuclear magnetic resonance (^{1}H NMR),[13] enzymatic method,[14] flow injection analysis,[15] and electrochemical methods.[16–18] Since the 'rediscovery' of CNTs by Iijima in 1991, electrochemical sensing based on carbon nanotubes (CNTs) has grown into a fully fledged research field.[19] The extraordinary electrochemical features of CNTs make them suitable for use in Faradaic processes. Compared with these electrochemical methods, the proposed method enjoys such advantages as better sensitivity, higher linear dynamic range, and lower limit of detection (LOD) while it is free from many potential interfering substances. Table 1 compares the proposed method with the voltammetric ones reported (using modified electrode) for the determination of GSH. *p*-Aminophenol has been used as a suitable mediator for the determination of thioguanine,[20] pencillamine,[21] mercaptopurine,[22] and cysteine plus tryptophan.[23] The electrocatalytic behaviour of *p*-aminophenol for oxidation of organic compounds depends on solution pH and the type of analyte used. This prevents the interference of many compounds in the determination of GSH in real samples, especially in haemolysed erythrocyte, with good selectivity. This is basically due to the removal of potential interfering compounds from the haemolysed erythrocyte. The present study investigates the application of *p*-aminophenol (*p*-AP) as a mediator for electrocatalytic determination of GSH using voltammetric methods. As haemolysed erythrocyte is used for the analysis of GSH, selectivity is not affected by many of the potential interfering compounds.[15,16] In order to demonstrate the catalytic ability of the modified electrode in the electrooxidation of GSH in real samples, the method was employed for the voltammetric determination of glutathione in haemolysed erythrocyte and urine samples.

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Table 1. Comparison of efficiencies of electrochemical methods in the determination of GSH

Electrode	Modifier	pH	LOD ($\mu mol\ L^{-1}$)	LDR ($\mu mol\ L^{-1}$)	Sensitivity ($\mu A\ \mu mol^{-1}\ L$)	Reference
Carbon paste	2,7-BFEF	7.0	0.50	0.92–11	1.997	16
Carbon paste	FC	7.0	2.10	2.2–3000	0.017	17
MWCNTPE	Chlorpromazine	4.0	0.16	0.3–18.3	0.541	18
Glassy carbon	Well-aligned/carbon nanotubes	7.0	0.20	0.4–16.4	0.010	27
Glassy carbon	PQQ/PPy	8.4	13.2	–	–	28
Carbon paste	TTF-TCNQ	7.0	0.30	5–340	–	29
EPPGE	–	7.0	2.7	10–80	–	30
Carbodiimide matrix	Glutathione peroxidase	7.8	15	19–140	–	31
Glassy carbon	Horseradish peroxidase	7.0	0.03	0.04–90	0.024	32
p-APMWCNTPE	*p*-Aminophenol	5.0	0.09	0.2–100	0.442	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;
MWCNTPE multiwall carbon nanotubes paste electrode.

Experimental

Chemicals

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

A $1.0 \times 10^{-2}\ mol\ L^{-1}$ GSH solution was prepared daily by dissolving 0.307 g GSH 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 and in the dark. More dilute solutions were prepared by serial dilution with water.

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

Multiwall carbon nanotubes (>90% MWCNT basis, d × l = (110–70 nm) × (5–9 μm), spectrally pure graphite powder (particle size < 50 μm) and high viscose paraffin oil (density = $0.88\ kg\ L^{1}$) from Fluka were used for the preparation of the electrodes.

Apparatus

Square wave voltammetry (SWV), cyclic voltammetry, and chronoamperomtry were performed in an electro analytical system, Micro-Autolab, potentiostat/galvanostat connected to a three-electrode cell, Metrohm Model 663 VA stand linked with a computer (Pentium IV, 1200 MHz) and with micro-Autolab software as stated before.[22,23] Impedance spectroscopy was performed in a system using an Autolab PGSTAT 12, potentiostat/galvanostat connected to a three-electrode cell, Metrohm Model 663 VA stand. The system was run on a PC using GPES and FRA 4.9 software. For impedance measurements, a frequency range of 100 kHz to 1.0 Hz was employed. The AC voltage amplitude was 5 mV, and the equilibrium time was 10 min. A conventional three-electrode cell assembly consisting of a platinum wire as an auxiliary electrode and an Ag/AgCl (KCl_{sat}) electrode as a reference electrode was used. The working electrode was either a carbon paste electrode (CPE), an unmodified carbon nanotubes paste electrode (CNTPE), or *p*-aminophenol–modified multiwall carbon nanotubes paste electrode (*p*-APMWCNTPE).

Scanning electron microscopy (SEM), Philips Model XLC, was used to characterize the electrodes. A pH-meter (Corning, Model 140) with a double-junction glass electrode was used to check the pH values of the solutions.

Preparation of the modified electrode

The percentages of multiwall carbon nanotubes and *p*-aminophenol to graphite in the modified electrode were optimized as described before.[23] The procedure used for the preparation of the electrode was as follows:[22,23] 10 mg of *p*-AP was hand mixed with 890 mg of graphite powder and 100 mg of carbon nanotubes in a mortar and pestle. Using a syringe, 0.50 g of paraffin was added to the mixture and mixed well for 40 min until a uniformly wetted paste was obtained (70:30 (*w/w*)). 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 *p*-AP and carbon nanotubes to the mixture.

Preparation of real samples

Human whole blood samples were obtained from the Isfahan University Health Centre. Erythrocytes were separated from whole blood samples by removing the plasma. The sample thus obtained (2.0 ml) was first centrifuged for 10 min at 3000 rpm. The supernatant (plasma) was discarded and the rest was mixed with 5 ml of 0.9% NaCl solution. The solution was centrifuged for another 5 min at 3000 rpm and the supernatant (diluted plasma) was again discarded. The washing procedure with NaCl solution was repeated three times in order to remove almost all the plasma.

Erythrocyte pellets were haemolysed with water (1:1, *v/v*). For protein precipitation, the haemolysate was mixed with 5–sulfosalysilic acid (10%, *m/v*) at a ratio of 2:1 (*v/v*). The mixture obtained was centrifuged under the same conditions described above. Then, the supernatant was divided into two parts, one for spectrophotometric determination and the other for use with the proposed electrochemical method. For spectrophotometric measurement of the Ellman, a reference method [24] was used based on the reaction of GSH with DTNB (Ellman's reagent),

generating 2-nitro-5-mercapto-benzoic acid. Absorbance was monitored spectrophotometrically at 412 nm.

Urine samples were stored in a refrigerator immediately after collection. Ten millilitres of each sample was centrifuged for 15 min at 1500 rpm. The supernatant was filtered using a 0.45 μm filter and then diluted five times with PBS (pH 5.0). The solution was transferred into the voltammetric cell to be analyzed without any further pretreatment. Standard addition method was used for the determination of GSH in real samples.

Tablet solution was prepared by completely grinding and homogenizing five tablets of glutathione, labelled 100 mg per tablet (Chongqing Yaoyou Pharmaceutical Co., Ltd., Chongqing, China). Then, 10 mg of each tablet powder was accurately weighed and dissolved in 100 ml water by ultrasonication. After mixing completely, the mixture was filtered on an ordinary filter paper, 10 ml of which was subsequently transferred into a 100-ml volumetric flask and diluted to the mark with water. Then, 1.0 ml of the solution plus 4.5 ml of the buffer (pH 5.0) was used for analysis using the standard addition method.

Recommended procedure

p-APMWCNTPE was polished with a white and clean paper. To record the blank signal, 10.0 ml of the buffer solution (PBS, pH 5.0) was transferred into the electrochemical cell. The initial and final potentials were adjusted to −0.10 and +0.50 V *vs* Ag/AgCl, respectively. Square wave voltammogram was recorded with an amplitude potential of 50 mV and a frequency of 15 Hz, respectively. The blank signal was labelled as I_b. Then, different amounts of GSH were added to the cell using a micropipette and the SWVs were recorded again to get the analytical signal (I_s). Calibration curves were constructed by plotting catalytic peak currents *vs*. GSH concentrations.

Results and discussion

SEM characterization of *p*-APMWCNTPE

A typical morphology of MWCNTs paste electrode (a) and the modified multiwall carbon nanotubes paste electrode (b) characterized by SEM are shown in Figure 1. As shown in Figure 1, *p*-AP spread on the modified electrode and did not change the morphology of MWCNTs, as already explained.[22,23] In addition, it can be clearly seen that MWCNTs and *p*-AP dispersed on the paste.

Electrochemistry of the mediator

The electrochemical behaviour of *p*-APMWCNTPE at pH = 5.0 was investigated using cyclic voltammetry. Figure 2 (inset) shows the cyclic voltammograms of the modified electrode at different scan rates in 0.1 mol L^{-1} phosphate buffer (pH 5.0). The cyclic voltammogram exhibits an anodic peak at the forward scan, which is related to the oxidation of $p\text{-AP}_{(Red)}$ to form $p\text{-AP}_{(Ox)}$. Reduction of $p\text{-AP}_{(Ox)}$ to $p\text{-AP}_{(Red)}$ occurs during the reverse scan (cathodic current peak). A pair of reversible peaks was observed at $E_{pa} = 0.285$ V and $E_{pc} = 0.185$ V. Half-wave potential ($E_{1/2}$) and ΔE_p were 0.235 V and 0.095 V, respectively. The peak separation potential, ΔEp ($E_{pa} - E_{pc}$), was greater than the expected one (59/*n* mV) for a reversible system. The same behaviours were observed for *p*-AP at pH 6.0,[23] pH 7.0,[22] and pH 9.0,[21] with different values of ΔE_p. This suggests a quasi reversible behaviour in an aqueous medium.[21–23] Also, the plot of the anodic peak current was linearly dependent on $u^{1/2}$ with a correlation coefficient of 0.9915 at all scan rates (Figure 2). This behaviour confirms the electrocatalytic behaviour of the system and indicates that the nature of the redox process is diffusion controlled.

Catalytic effect

Figure 3 depicts the cyclic voltammetric responses of different paste electrodes in the blank or in 500 μmol L^{-1} GSH solution. Figure 3 (curve e) shows the CV response of the blank solution

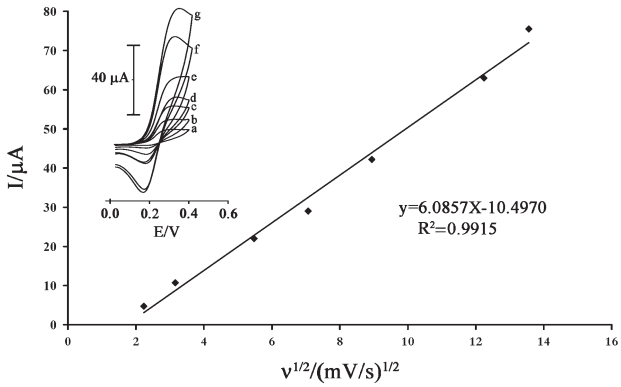


Figure 2. Plots of the anodic peaks current of *p*-APMWCNTPE *vs* $\nu^{1/2}$ (from cyclic voltammograms, inset). Inset shows cyclic voltammograms of *p*-APMWCNTPE in 0.1 mol L^{1} PBS at pH 5.0 at various scan rates of a) 5; b) 10; c) 30; d) 50; e) 80 f) 150; and g) 185 mV s^{1}.

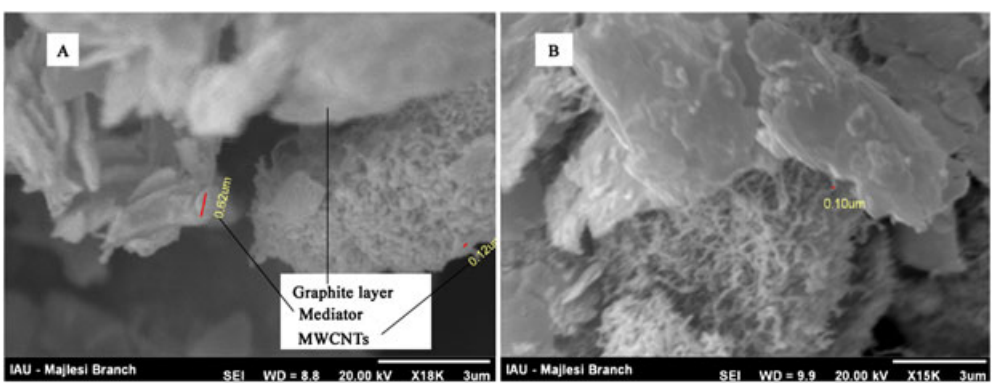


Figure 1. SEM image of (A) *p*-APMWCNTPE, and (B) unmodified MWCNTPE.

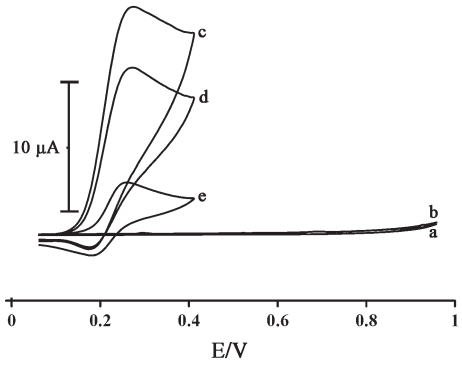


Figure 3. Cyclic voltammograms of: (a) CPE in a solution containing 500 µmol L^{-1} GSH in 0.1 mol L^{-1} PBS (pH 5.0). b) as (a) at the surface of CNPE. c) Cyclic voltammogram of *p*-APMWCNTPE in the presence of 500 µmol L^{-1} GSH in 0.1 mol L^{-1} PBS (pH 5.0). d) as (c) at the surface of *p*-APCPE. e) Cyclic voltammogram of *p*-APMWCNTPE in the absence of GSH (blank solution). Condition: scan rate of 10 mV s^{-1}.

(pH 5.0) at the surface of *p*-APMWCNTPE. Figure 3 (curve c) shows the electrochemical oxidation of 500 µmol L^{-1} GSH at *p*-APMWCNTPE while in Figure 3, curves d, b, and a depict the CV responses of 500 µmol L^{-1} GSH solution at *p*-aminophenol modified CPE (*p*-APCPE), at CNPE, and at unmodified CPE, respectively. As can be seen, the anodic peak potentials for the oxidation of GSH at both *p*-APMWCNTPE and *p*-APCPE (curves c and b) are about 285 mV. On the other hand, GSH oxidation (without the mediator) does not take place at the surface of CNPE and/or CPE up to +0.98 V. Similarly, when we compared the oxidation of GSH at the surface of *p*-APMWCNTPE (Figure 3, curve c) and at *p*-APCPE (Figure 3, curve d), an enhancement of the anodic peak current was found to occur at *p*-APMWCNTPE. In other words, the data obtained clearly show that the combination of carbon nanotubes and the mediator definitely improve the characteristics of the electrode for the oxidation of GSH. Based on these results, the following catalytic diagram (EC′ catalytic mechanism) describes the voltammetric response of the electrochemical oxidation of GSH at *p*-APMWCNTPE (Scheme 1). The same behaviour was observed in the case of the modified electrode for the determination of cysteine [23] at pH 6.0, for 6-thioguanine [21] and 6-mercaptopurine[22] at pH 9.0.

Figure 4 shows the catalytic oxidation peak potential of *p*-AP gradually shifting towards more positive potentials with increasing scan rate, suggesting a kinetic limitation in the reaction between the redox site of the *p*-AP and GSH. However, the oxidation currents change linearly with the square root of the scan rate (Figure 4), suggesting that the reaction is mass transfer controlled at sufficient over-potentials. The results show that the overall electrochemical oxidation of GSH at the modified electrode might be controlled by the cross-exchange process between GSH and the redox site of the *p*-AP and by the diffusion of GSH.

Chronoamperometry study

For determination of the diffusion coefficient and the catalytic reaction rate constant of GSH, double potential step chronoamperometry was used with *p*-APMWCNTPE. Figure 5A shows the current-time curves of *p*-APMWCNTPE by setting the electrode potential at 180 mV (first step) and 250 mV (second step) for different GSH concentrations. As

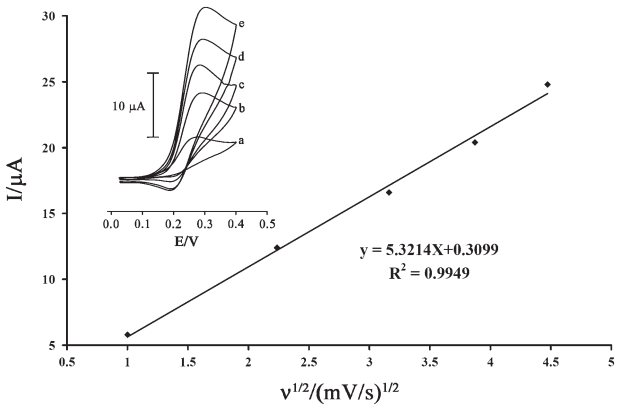
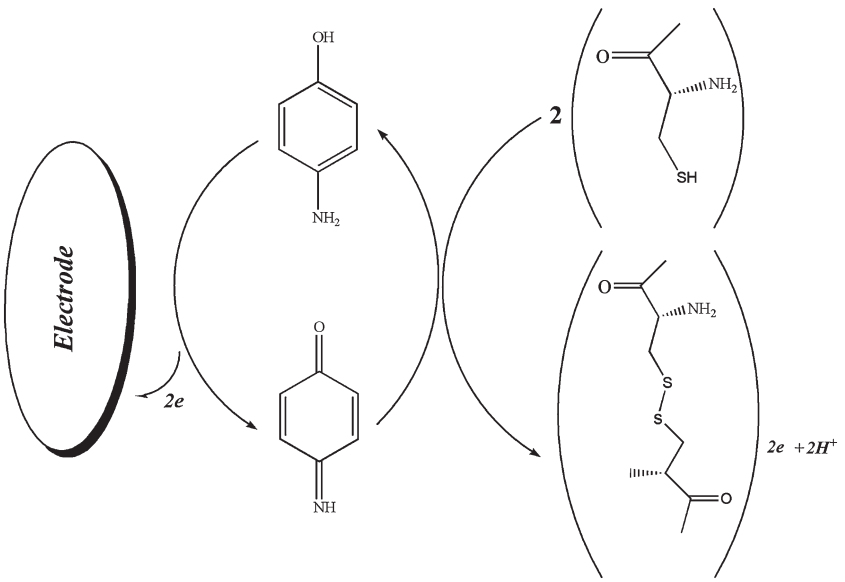


Figure 4. Plot of I_{pa} *vs.* $\nu^{1/2}$ for the oxidation of GSH at *p*-APMWCNTPE. Inset A) Cyclic voltammograms of 500 µmol L^{-1} GSH at various scan rates of a) 1.0; b) 5.0; c) 10.0; d) 15, and e) 20 mV s^{-1} in 0.1 mol L^{-1} PBS (pH 5.0).



Scheme 1. Proposed mechanism for the oxidation of GSH at the surface of the modified electrode.

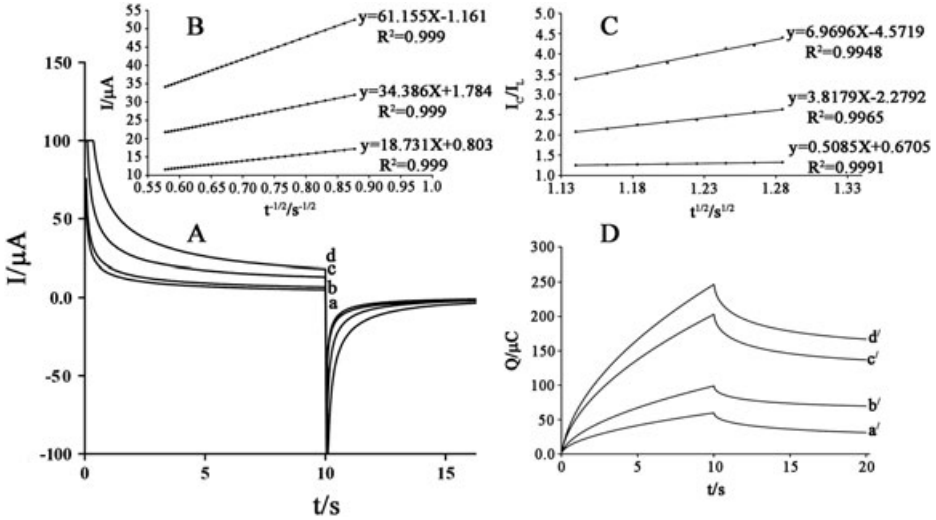


Figure 5. (A) Chronoamperograms obtained at *p*-APMWCNTPE a) in the absence and in the presence of b) 100, c) 600, and d) 800 μmol L^{-1} GSH at pH 5.0. (B) Current–$t^{-1/2}$ plot for the data from the chronoamperograms. (C) Dependence of I_C/I_L on $t^{1/2}$ derived from the chronoamperograms data. (D) The charge-time curves a′) for curve (a); b′) for curve (b); c′) for curve (c); and d′) for curve (d).

can be seen, there is no net anodic current corresponding to the oxidation of the mediator in the presence of GSH. On the other hand, the forward and backward potential step chronoamperometry for the mediator in the absence of GSH shows symmetrical chronoamperogram with an equal charge consumed for the reduction and oxidation of the *p*-AP at the surface of unmodified CPE (Figure 5D, a′). On the other hand, the charge value associated with forward chronoamperometry in the presence of GSH is significantly greater than that observed for backward chronoamperometry (Figure 5D, b′–d′). The linearity of the electrocatalytic current *vs* $u^{1/2}$ shows that the current is controlled by GSH diffusion from the bulk solution towards the surface of the electrode, leading to a near Cottrellian behaviour. A plot of I *vs.* $t^{-1/2}$ for different concentrations of GSH at the surface of *p*-APMWCNTPE yields straight lines (Figure 5B) with different slopes which can be used to estimate the diffusion coefficient of GSH (D) in the ranges of 100 to 800 μmol L^{-1}. The mean value of D for GSH was found to be 6.0×10^{-4} cm^2 s^{-1}.

The rate constant for the chemical reaction between GSH and redox sites in *p*-APMWCNTPE, k_h, can be evaluated by chronoamperometry according to the method of Galus:[25]

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

where I_C is the catalytic current of GSH at *p*-APMWCNTPE, I_L is the limited current in the absence of GSH, and t is the time elapsed (s). Equation (1) can be used to calculate the rate constant of the catalytic process k_h. Based on the slope of the I_C/I_L *vs.* $t^{1/2}$ plots (Figure 5C), k_h can be obtained for a given GSH concentration. Based on the values of the slopes, the average value of k_h was found to be equal to 9.3×10^3 mol L^{-1} s^{-1}. The value of k_h explains the sharp feature of the catalytic peak observed for catalytic oxidation of GSH at the surface of *p*-APMWCNTPE.

Electrochemical impedance spectroscopy studies

Figure 6 shows Nyquist diagrams of imaginary impedance (Z_{im}) *vs* real impedance (Z_{re}) of the EIS obtained at the modified electrode recorded at 0.150 V dc-offset in the absence (curve a) and in the presence of 500 μmol L^{-1} GSH (curve b) at pH 5.0, respectively. In the absence of GSH, the Nyquist diagram comprises a depressed semicircle at high frequencies, which may be related to the combination of charge transfer resistance of *p*-AP electrooxidation and the double-layer capacitance followed by a straight line with a slope of nearly 45°. The latter is due to the occurrence of mass transport process via diffusion. In the presence of GSH, the diameter of the semicircle decreases, confirming the electrocatalytic capability of the mentioned electrocatalyst for oxidation of GSH. This is due to the instant chemical reaction of GSH with the high-valence *p*-AP species. The catalytic reaction of oxidation of GSH that occurred via the participation of *p*-AP species virtually caused an increase in the surface concentration of low valence

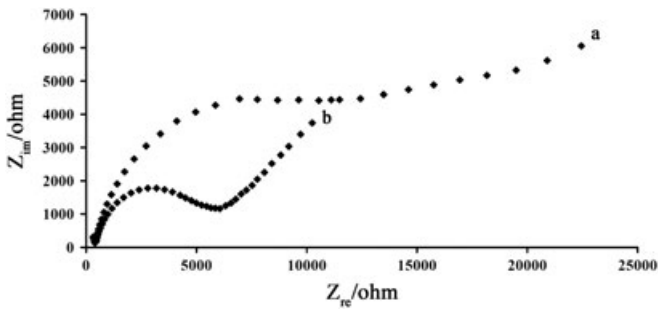


Figure 6. Nyquist diagrams of *p*-APMWCNTPE a) in the absence, and b) in the presence of 500 μmol L^{-1} GSH.

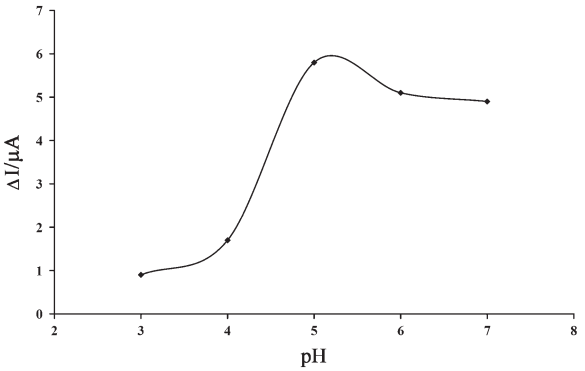


Figure 7. Current–pH curve for electro-oxidation of 300 μmol L^{-1} GSH at *p*-APMWCNTPE with a scan rate of 20 mV s^{-1}.

species of electrocatalyst, and the charge transfer resistance declined, depending on the concentration of GSH in the solution.

Influence of pH

In order to optimize the electrocatalytic response of the sensor to GSH oxidation, we investigated the effect of solution pH on the electrocatalytic oxidation of GSH in 0.1 mol L^{-1} PBS solutions with different pH values ($3.0 < pH < 7.0$) using *p*-APMWCNTPE. The influence of pH on both peaks current and peaks potential were assessed by examining the electrode responses in the buffer solutions. The variation of I_{pa} *vs.* pH is shown in Figure 7. The results show that maximum electrocatalytic current was obtained at pH 5.0. In more acidic media ($pH < 5$), the protonation of the –NH group of *p*-AP in *p*-APMWCNTPE causes a decreasing electrocatalytic behaviour. In addition, the stability of GSH decreases in basic solutions. Therefore, a pH value of 5.0 was chosen as the optimum value for the determination of GSH at *p*-APMWCNTPE.

Table 2. Interference study for the determination of 5.0 μmol L^{-1} GSH under the optimized conditions

Species	Tolerance limits ($W_{Substance}/W_{GSH}$)
ClO_4^-, NO_3^-, Na^+, Br^-, Ca^{2+}, Mg^{2+}, SO_4^{2-}	1000
Glucose, Sucrose, Fructose, Lactose,	800
Lysine, Alanine, Phenyl Alanine, Valine, Methionine, Glycine, Tryptophan	500
Urea, Thiourea	400
Starch	Saturation
Ascorbic acid, Cysteine	10

Electrocatalytic determination of GSH

Since SWV has a much higher current sensitivity than cyclic voltammetry, it was used for the determination of GSH. SWV (with an amplitude potential of 50 mV and a frequency of 15 Hz) was used to estimate the lower range of detection and the linear calibration range of GSH determination. The results showed two linear segments with different slopes for GSH concentrations: the regression equation was $Ip(\mu A) = 0.4429C_{GSH} + 0.5246$ ($r^2 = 0.9974$, $n = 6$) for 0.2 4.3 μmol L^{-1} GSH, and it was $Ip(\mu A) = 0.0995C_{GSH} + 0.8231C_{GSH}$ ($r^2 = 0.9993$, $n = 8$) for 4.3 100.0 μmol L^{-1} GSH, where C_{GSH} is the concentration of GSH in μmol L^{-1}.

Detection limits were obtained as 0.09 μmol L^{-1} GSH according to the definition of $Y_{LOD} = Y_B + 3\sigma$.[26] The detection limit, linear dynamic range, and sensitivity for GSH with *p*-APMWCNTPE observed are comparable and even better than those obtained from using several modified electrodes (Table 1).

Stability and reproducibility

The repeatability and stability of *p*-APMWCNTPE were investigated using 10 μmol L^{-1} GSH. The relative standard deviation (RSD%) for ten successive assays of GSH was 2.1%. When four different electrodes were used, the RSD% for five measurements of 10 μmol L^{-1} GSH was 2.6%. When the modified electrode was stored in the laboratory, the response of the modified electrode retained 94% of its initial response value after a week and 92% after 25 days. These results indicate that *p*-APMWCNTPE has a good stability and reproducibility. Because *p*-aminophenol has little solubility in water solution, a new surface was, therefore, obtained by pushing an excess of the paste out of the tube and polishing it on a weighing paper after each three experiments. In addition, we checked the stability of the modified electrode when stored in the buffer solution. The results showed that the response of the modified electrode retained 90% of its initial value after 24 h but decreased to 82% of its initial response value after 56 h.

Table 3. Concentration values obtained from the proposed and Elman methods for GSH analysis in haemolysed erythrocyte, tablet and urine samples

Sample	Proposed method (mmol L^{-1})	Elman method (mmol L^{-1})	F_{ex}	$F_{tab,\ (0.05);2,2}$	t_{ex}	$t_{tab\ (98\%)}$
1. Haemolysed erythrocyte	2.22 ± 0.05	2.19 ± 0.09	7.5	19	3.1	3.80
2	1.31 ± 0.03	1.35 ± 0.07	6.8	19	2.9	3.80
3	0.95 ± 0.03	1.10 ± 0.08	8.9	19	3.3	3.80
4	1.83 ± 0.04	1.80 ± 0.06	5.7	19	2.5	3.80
5 Urine	Less than LOD	Less than LOD	–	–	–	–
6	0.06 ± 0.005	0.06 ± 0.008	3.7	19	1.6	3.80
7	0.10 ± 0.020	0.12 ± 0.04	5.8	19	2.1	3.80
8 Tablet	0.05 ± 0.003	0.052 ± 0.005	6.4	19	2.5	3.80
9	0.10 ± 0.02	0.11 ± 0.02	4.4	19	1.5	3.80

F_{ex} Calculated F-value; Reported F value from F-test table with 95% confidence level and 2/2 degree of freedom; t_{ex} Calculated t; $t_{tab\ (98\%)}$ Reported t value from student t-test table with 98% confidence level.

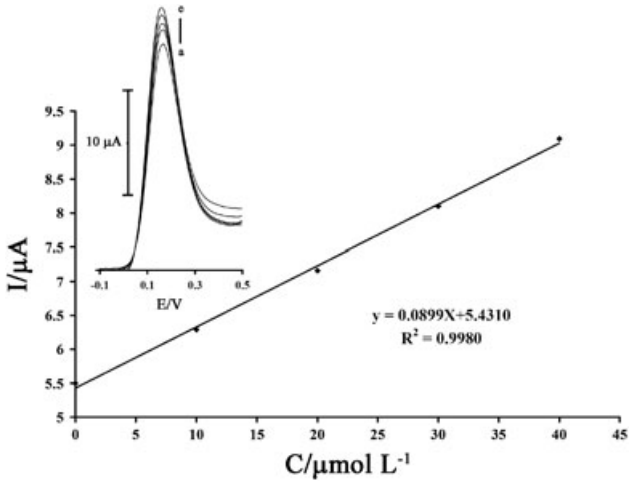


Figure 8. Square wave voltammograms of *p*-APMWCNTPE in a solution of urine sample for row No. 6 from Table 3. GSH added as a) 0.0; b) 10.0; c) 20.0; d) 30.0; and e) 40 μmol L^{-1}.

Interference studies

Under the optimum conditions described above, the influence of various substances as potential interfering compounds was studied on the determination of GSH with 5.0 μmol L^{1} GSH at pH 5.0. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than 5% for the determination of GSH. The results are given in Table 2, which shows that the peak current of GSH is not significantly affected by conventional cations, anions, and organic substances. Although ascorbic acid and cysteine show interference, they are not present at significant levels in urine and haemolysed erythrocyte samples. Moreover, interference from ascorbic acid can be minimized using ascorbic oxidase enzyme if necessary, which exhibits high selectivity to the oxidation of ascorbic acid.

Determination of GSH in real samples

In order to evaluate the applicability of the modified electrode for measuring GSH in real samples, GSH values in human erythrocyte, tablet, and urine samples were determined using the proposed method. In addition, the results were compared with those obtained from the spectrophotometric method [21] which is usually used as the standard method for glutathione determination. The results are reported in Table 3. A typical SWV for the determination of GSH in urine samples (Table 3, row No. 6) is shown in Figure 8. These experiments demonstrated the capability of the modified electrode for the voltammetric determination of GSH with high electrocatalytic effect and good reproducibility.

Conclusions

This work demonstrates the application of *p*-aminophenol for the determination of glutathione at pH 5.0 using multiwall carbon nanotubes paste electrode. The results showed that GSH oxidation is catalyzed by the mediator at pH = 5.0. The catalytic peaks current obtained by SWV was linearly dependent on GSH concentrations with a minimum of 0.09 mol L^{-1}. As shown in Table 1, the proposed voltammetric sensor has a longer dynamic range, a better LOD, and better reproducibility compared to the methods reported in the literature. Despite one of the higher sensitivity to glutathione determination reported in one study based on horseradish peroxidase (biosensor),[31] its repeatability and stability were lower than those of the proposed sensor. Current sensitivity, low detection limit, and high selectivity of *p*-APMWCNTPE for the detection of GSH prove potential sensing applications for the determination of GSH in real samples such as urine, tablet, and haemolysed erythrocyte samples.

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