

ELECTROCHEMICAL DETERMINATION OF THIOLS AT SINGLE-WALL CARBON NANOTUBES AND PQQ MODIFIED ELECTRODES

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1. ABSTRACT

The electrocatalytic oxidation of thiols has been observed at a glassy carbon (GC) electrode coated with a single-wall carbon nanotube (SWNT) film. Fourteen thiols including L-cysteine (CySH) and glutathione were tested using the SWNT/GC electrode, and the cyclic voltammetry (CV) showed that each thiol was oxidized at much less positive potential than those at other electrodes such as bare GC and diamond electrodes. The SWNT/GC electrode was also modified with pyrroloquinoline quinone (PQQ) which showed a further improvement of the catalytic behavior of the SWNT/GC electrode: e.g. the oxidation peak current of CySH was observed at 0.27 V vs. Ag/AgCl in pH 7.5 phosphate buffer. The amperometric responses at these electrodes showed a linear relationship with the substrate concentration in a 10^{-6} - 10^{-3} M range and 10^{-6} - 10^{-7} M detection limits for several thiols including CySH, L-homocysteine, N-acetyl-L-cysteine, L-penicillamine and glutathione. These electrodes show a response time of 2-3 s and storage stabilities over 3 weeks. A PQQ/SWNT/GC electrode has been successfully applied for the assay of both L-cysteine and N-acetyl-L-cysteine in the dietary supplement.

2. INTRODUCTION

Since the discovery of carbon nanotubes (CNTs), including multi-wall carbon nanotubes (MWNTs) (1) and single-wall carbon nanotubes (SWNTs) (2), there has been growing interest in using CNTs in chemical and biochemical sensing (3-14) and nanoscale electrode devices during the past decade due to their remarkable electronic and mechanical properties, nanometer size and minimization of surface fouling onto electrochemical devices. CNTs behave as a metal or as a semiconductor,

and CNT-modified electrodes have better conductivity than graphite (15,16) and show a superior performance compared with such electrodes as Au, Pt and other carbon electrodes. An improved electrochemical behavior of dopamine (17), NADH (18), and cytochrome c (19) has been observed at the CNT modified electrodes. When used as electrochemical sensors, CNTs show the ability to greatly promote the electron-transfer between enzymes or other molecules and electrodes (20), and the CNT modified electrodes show direct electrochemical responses with analytes. These attractive electrochemical features make CNTs, in particular SWNTs, suitable for important sensing applications for chemicals and biomolecules.

Thiols are important marker molecules in biological systems, and an accurate determination of the change in thiol levels provides critical insight into proper physiological functions and can be used in the diagnosis of disease states (21,22). Many electrochemical methods have been developed for the analysis of thiols (23-25). However, the direct detection of these compounds is very difficult because the direct oxidation of thiols at solid electrodes is slow and requires a potential of at least 1.0 V to proceed. Earlier indirect methods were based on the interaction of a thiol with a mercury electrode to form mercury thiolate complexes (23). Platinum and gold electrodes have also been used for the anodic detection of sulfur compounds (24,25), but the surface oxide formation at platinum and gold electrodes complicates analytical applications (26). Boron-doped diamond electrodes (BDD) have been applied for the detection of thiols due to its wide potential window in aqueous solution and low background current, but applied potentials are still very high ($> +0.7$ V vs. Ag/AgCl) (27,28). Bare carbon electrodes show very

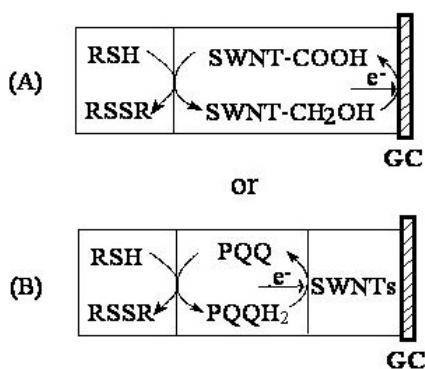


Figure 1. Electron transfer from substrate thiols to the electrode surface on (A) SWNT/GC and (B) PQQ/SWNT/GC electrodes.

poor activity for thiols determination (29) and much efforts have been devoted toward using chemically modified electrodes with inorganic electrocatalysts for detection of thiols, but has been limited in many cases by the fact that their response decreases with time (30,31). Inoue and Kirchhoff (32,33) recently reported that organic thiols can be detected at a GC electrode modified with pyrroloquinoline quinone (PQQ) in polypyrrole (PPy) film. This type of thiol sensor can detect thiols at less positive potentials due to the catalytic oxidation of thiols with PQQ, but its response time is relatively slow because the catalytic oxidation of thiols proceeds in the PPy matrix instead of the electrode surface.

In this paper, we utilized the catalytic nature of SWNT modified electrodes for the detection of thiols, which provided a significant decrease in the overpotential for the oxidation of thiols. The SWNT-modified GC electrode showed the catalytic oxidation of thiols as well as a fast response time with thiols. The SWNT/GC electrode was also coated with a thin PQQ film which further helped to improve the catalytic behavior of the SWNT/GC electrode for the thiol determination. Nafion solution (mixed with DMF) used for solubilizing SWNTs, helped to minimize the interference of ascorbic acid and uric acid for the thiol determination. Nafion is a good cation-exchanger with the advantage of thermal stability, chemical inertness, and mechanical strength, and has been mostly used for modifying electrodes for restricting the transport of anionic species (34,35). Wang and his coworkers reported the use of Nafion to solubilize CNTs for the preparation of CNT fiber microelectrodes (11).

A likely mechanism for the detection of thiols at both SWNT/GC and PQQ/SWNT/GC electrodes is shown in figure 1. Fourteen thiols including L-cysteine and glutathione were tested, and the cyclic voltammetry showed that each thiol was oxidized at much less positive potential than those at other electrodes such as bare GC and diamond electrodes. Amperometric studies at both SWNT/GC and PQQ/SWNT/GC electrodes for the detection of L-cysteine, L-homocysteine, N-acetyl-L-cysteine, L-penicillamine and glutathione electrodes with respect to dynamic range, detection limit and sensitivity are described. A PQQ/SWNT/GC electrode has successfully applied for the

assay of both L-cysteine and N-acetyl-L-cysteine in the dietary supplement.

3. MATERIALS AND METHODS

3.1. Apparatus

The electrochemical studies were carried out in a single-compartment glass cell equipped with a saturated Ag/AgCl reference electrode, a platinum wire as the auxiliary electrode, and glassy carbon (GC) electrode (Cypress, 1.5 mm dia), SWNT/GC electrode, or PQQ/SWNT/GC electrode as the working electrode. Cyclic voltammetry and amperometric measurements were performed by using a CHI 440 electrochemical workstation (CH Instruments) under nitrogen atmosphere.

3.2. Reagents

SWNTs were obtained from Carbon Nanotechnologies Inc. (Houston, TX). The thiols including L-cysteine, L-penicillamine, N-acetyl-L-cysteine, L-homocysteine, and glutathione were obtained from Sigma. Uric acid, L-ascorbic acid, and pyrroloquinoline quinone (PQQ) (> 97% purity) were also obtained from Sigma. All other chemicals were of analytical reagent grade and used as received. All solutions were prepared with Milli-Q water (Millipore).

3.3. Preparation of SWNT/GC and PQQ/SWNT/GC electrodes

SWNTs were shorted and functionalized as described previously (36,37). In brief, 10 mg of SWNTs was dispersed in 10 ml of 3:1 v/v solution of concentrated sulfuric acid (98%) and concentrated nitric acid (70%) and sonicated for 6 hours. The shorted SWNTs were filtered and washed with Milli-Q water until the pH was nearly neutral. The formation of carboxyl groups was monitored by FT-IR spectroscopy (Nicolet model Nexus 670). The presence of the characteristic C=O stretching band of carboxylic groups at 1710 cm⁻¹ in the FT-IR spectrum strongly suggested the formation of open-ended and carboxyl-terminated SWNTs after the oxidation treatment (38,39). The shorted SWNTs (3 mg) were dispersed in 5 mL of dimethylformamide (DMF) to give a 0.6 mg/mL black suspension, which also contained 0.5% (v/v) Nafion. GC electrodes were polished successively with 1, 0.3, and 0.05 micron alumina suspensions (Buehler). Then they were sonicated in purified water and ethanol for 5 min, respectively. The cleaned GC electrode was coated by casting a 5 × 10⁻⁶ L of the suspension of SWNTs (0.6 mg/mL) and was dried at room temperature (22 ± 2°C).

For the preparation of PQQ/SWNT/GC electrodes, PQQ was first dissolved in 1 mL water to give a 1.5 mM PQQ solution, and then a few drops of 0.1 M KOH were added in order to dissolve PQQ completely. The SWNT/GC electrode was coated by casting a 3 × 10⁻⁶ L of PQQ solution (1.5 mM) and was dried at room temperature.

3.4. Amperometric detection of thiols

The amperometric detection of each thiol was carried out by measuring the current response at constant potentials for both SWNT/GC electrode (+0.42 V) and

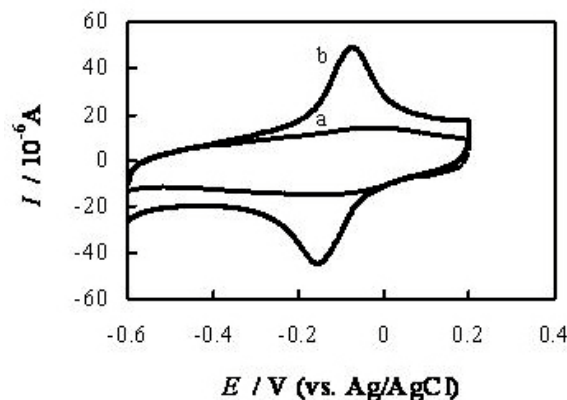


Figure 2. Cyclic voltammograms of (a) SWNT/GC electrode and (b) PQQ/SWNT/GC electrode in phosphate buffer (0.05 M, pH 7.0) containing 0.05 M KCl. Scan rate: 100 mV/s.

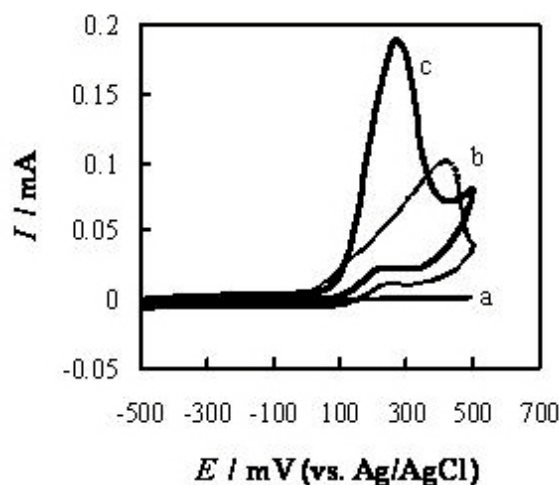


Figure 3. Cyclic voltammograms of 1 mM CySH at (a) bare GC, (b) SWNT/GC, and (c) PQQ/SWNT/GC electrodes in phosphate buffer (0.05 M, pH 7.5) containing 0.5 M KCl. Scan rate: 50 mV/s.

PQQ/SWNT/GC electrode (+0.27 V) in 0.05 M phosphate buffer (pH 7.5). Several different concentrations of each thiol were prepared for the amperometric detection of thiols. In the electrochemical cell initially contained 3 mL phosphate buffer, successive injection of 5×10^{-5} L of each thiol solution was carried out via a gas-tight syringe. The response for each thiol concentration was measured at least in triplicate. The current response (I) was plotted versus the analyte concentration, where I is defined as the difference between the steady-state current before and after the injection of analyte solutions. The amperometric response to various non-thiol compounds was also measured and compared with that of L-cysteine in order to evaluate the selectivity of the sensors.

A dietary supplement tablet (500 mg each of both L-cysteine and N-acetyl-L-cysteine per tablet, General Nutrition Corp., Pittsburgh, PA) was weighed and then was ground thoroughly. A 2.0 mg portion of the powdered

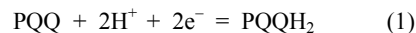
sample was dissolved in 3 mL of 0.05 M phosphate buffer (pH 7.5), filtered through Whatman filters (0.2 micron pore size) to remove undissolved impurities, and finally analyzed with a PQQ/SWNT/GC sensor.

4. RESULTS AND DISCUSSION

4.1. Electrochemical behavior of SWNT/GC and PQQ/SWNT/GC electrodes

Cyclic voltammograms of both bare GC electrode and SWNT/GC electrode were recorded in 0.05 M phosphate buffer (pH 7.5) containing 0.05 M KCl. Broad cathodic and anodic peaks of SWNTs were observed at -0.092 V and 0.042 V (vs. Ag/AgCl), respectively. These peak potentials are close to those reported (-0.126 and -0.024 V vs. SCE in pH 6.9 Britton Robinson buffer at 15°C) by Luo *et al.* (3) and the observed redox currents should correspond to the reduction and reoxidation of the carboxylic acid group on the surface of the functionalized SWNTs, which is a four-electron, four-proton reaction (3). The background current of the SWNT/GC electrode was very high (approximately 2.6 times of the redox peak currents of SWNTs) which may be due to the large capacitance of the SWNTs. Cyclic voltammograms of the $\text{Fe}(\text{CN})_6^{4-}/\text{Fe}(\text{CN})_6^{3-}$ pair recorded at a bare GC electrode and a SWNT modified electrode showed that the peak currents of the pair at the SWNT/GC electrode is almost 8.5 times as large as those observed at the bare GC electrode. It is clear that the SWNT film on the GC electrode surface is driving the electron transfer reaction faster than any other carbon electrode surfaces (40) and the surface area of the SWNT/GC electrode is much larger than that of the bare GC electrode. The effective surface area of the SWNT/GC electrode was estimated to be $(0.15 \pm 0.03) \text{ cm}^2$ from the cyclic voltammograms recorded in 5 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ containing 0.05 M KCl solution (41).

Figure 2 shows cyclic voltammograms of a SWNT/GC electrode (a) and a PQQ/SWNT/GC electrode (b) in phosphate buffer (pH 7.0). PQQ contains an *o*-quinone moiety that exhibits an efficient, pH-dependent and reversible electron transfer between its oxidized and reduced forms (42):



The cathodic and anodic peak potentials were observed at -0.085 and -0.125 V, respectively. The midpoint potential ($E_{1/2}$) of -0.11 V is the same as that of PQQ in polypyrrole (PPy) film on Pt electrode in phosphate buffer of pH 7.0 (43). Both anodic and cathodic peak currents were nearly identical and increased linearly with the scan rate (lower than 0.2 V/s) indicating an ideal two-electron reversible process. The difference between the anodic and cathodic peak potentials was small and independent of scan rate at low scan rates, indicating PQQ was immobilized on the SWNT/GC electrode with an "ideal" monolayer (44).

4.2. Cyclic voltammetry of L-cysteine at SWNT/GC and PQQ/SWNT/GC electrodes

Figure 3 shows cyclic voltammograms of 1 mM CySH in phosphate buffer (pH 7.5) at (a) bare GC, (b)

Table 1. Oxidation peak potentials of CySH at different electrodes

Electrode	pH	E_{pa} (V vs. Ag/AgCl)	Reference
SWNT/GC	7.5	0.425	This work
PQQ/SWNT/GC	7.5	0.272	This work
BDD	~9.0	0.62	27
PQQ/PPy/GC	8.4	0.30 ^a	33
CoPC/GP	2.43	0.77	48
Fe(II)/NP/GP	6.8	0.85	49
PBE/PIG	3.0	0.79	50
DAR/FeTsPc/GC	H ₂ SO ₄ ^b	0.945	51
BDD(OM) ^c	10.0	0.735	52

BDD, boron-doped diamond; PPy, polypyrrole; CoPC, cobalt phthalocyanine; GP, graphite paste; NP, nitroprusside; PIG, araffin impregnated graphite, PBF, Prussian Blue; DAR, diazo-resins; FeTsPc, iron(III)tetrakisulfophthalocyanine; a, based on the hydrodynamic voltammogram, authors used 300 mV to perform amperometric determination of cysteine; b, 0.05 M H₂SO₄; c, the oxidation of cysteine by electrogenerated octacyanomolybdate.

Table 2. E_{pa} values of thiols at SWNT/GC and PQQ/SWNT/GC electrodes

Thiol	Molecular Formula	E_{pa} (V vs. Ag/AgCl) ¹	
		SWNT/GC	PQQ/SWNT/GC
L-cysteine	HSCH ₂ CH ₂ CH(NH ₂)COOH	0.425 ± 0.08	0.272 ± 0.06
L-homocysteine	HSCH ₂ CH(NH ₂)COOH	0.451 ± 0.06	0.282 ± 0.04
N-acetyl-L-cysteine	HSCH ₂ CH(NHCOCH ₃)COOH	0.427 ± 0.02	0.275 ± 0.04
Penicillamine	(CH ₃) ₂ C(SH)CH(NH ₂)COOH	0.423 ± 0.03	0.301 ± 0.05
Glutathione	HOOCCH(NH ₂)CH ₂ CH ₂ NH-CH(CH ₂ SH)CONHCH ₂ COOH	0.478 ± 0.08	0.321 ± 0.06
Cystamine	(CH ₃ CH ₂ NH ₂) ₂ S	× ²	×
3-mercaptopropanesulfonic acid	(CH ₃ CH ₂ NH ₂) ₂ S	0.412 ± 0.02	0.350 ± 0.08
3-mercaptopropionic acid	HSCH ₂ CH ₂ COOH	0.418 ± 0.01	0.278 ± 0.02
3-mercapto-1-propanol	HSCH ₂ CH(OH)CH ₂ OH	0.448 ± 0.03	0.282 ± 0.05
2-mercaptoethanol	HSCH ₂ CH ₂ OH	0.423 ± 0.02	0.275 ± 0.03
2-mercaptoethyl sulfide	(CH ₃ CH ₂ CH ₂) ₂ S	×	×
2-mercaptoethyl ether	(HSCH ₂ CH ₂) ₂ O	0.425 ± 0.02	0.285 ± 0.04
4-mercapto-1-butano	HS(CH ₂) ₃ CH ₂ OH	0.429 ± 0.04	0.279 ± 0.03
2-aminoethanethiol	HSCH ₂ CH ₂ NH ₂	0.458 ± 0.02	0.310 ± 0.01

¹ Data given as mean ± SD (n = 3), ² the oxidation reaction did not occur.

SWNT/GC, and (c) PQQ/SWNT/GC electrodes. CV curves recorded at both SWNT/GC and PQQ/SWNT/GC electrodes exhibited two well-defined anodic peaks at 0.42 V and 0.27 V, respectively. Only a very low oxidation current of CySH was observed at the bare GC electrode in the potential range between -0.5 and 0.5 V due to the slow electron transfer for the oxidation of thiols at bare electrodes as previously reported (45,46). No cathodic peaks were observed on the reverse scan within the investigated potential range (-0.5 - 0.5 V), since the CySH oxidation is an electrochemically irreversible process (47). These CVs also suggested a more significant catalytic oxidation of CySH at the PQQ/SWNT/GC electrode compared with the SWNT/GC electrode. The reason for the improved catalytic behavior of the PQQ/SWNT/GC electrode may be due to that PQQ catalytically oxidizes CySH (see figure 1) at less positive potential than SWNTs shifting the oxidation peak potential (E_{pa}) of CySH by -150 mV. This should help to minimize the effects of some interfering species for the determination of CySH. Table 1 lists the anodic peak potentials (E_{pa}) of CySH on other electrodes for comparison.

The cyclic voltammograms of other thiols were also recorded at both SWNT/GC and PQQ/SWNT/GC electrodes using the same experimental condition. The results are summarized in table 2. These results indicated that the oxidation of disulfides did not take place, which was in agreement with the results of HPLC experiments which failed to show any CySH oxidation products other than disulfides (not shown).

The effects of pH on the voltammograms of CySH at both SWNT/GC and PQQ/SWNT/GC electrodes were investigated in the pH range of 3.4 - 8.5. E_{pa} values of CySH obtained at both SWNT/GC and PQQ/SWNT/GC electrodes shifted negatively with increasing solution pH having a slope of (a) -0.023 V/pH and (b) -0.041 V/pH, respectively (see figure 4). We also investigated the effects of pH on the electrochemical behavior of both SWNT/GC and PQQ/SWNT/GC electrodes in the absence of CySH. The midpoint potential of the redox reaction of SWNTs shifted negatively with the slope of -0.057 V/pH (see figure 4c), which is slightly lower than that (-0.059 V/pH)

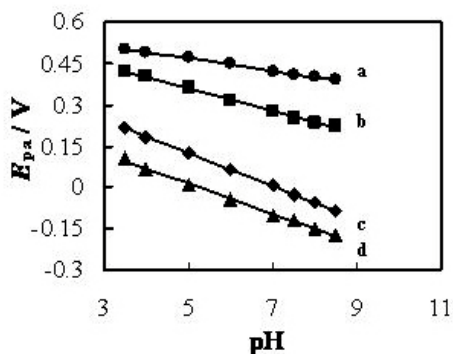


Figure 4. Effects of pH on the oxidation peak potentials of CySH (1 mM) at (a) SWNT/GC and (b) PQQ/SWNT/GC electrodes with a scan rate of 50 mV/s, and effects of pH on the midpoint potentials of (c) SWNT/GC and (d) PQQ/SWNT/GC electrodes with a scan rate of 100 mV/s.

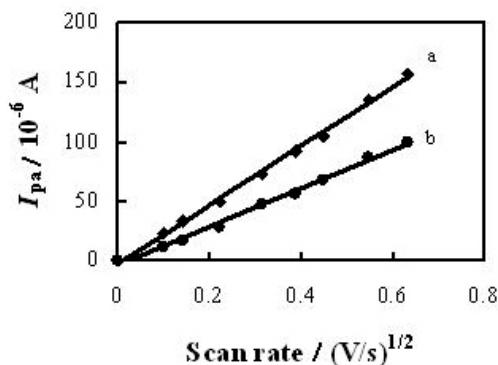


Figure 5. Effects of scan rate on the oxidation peak current of CySH (1 mM) at (a) PQQ/SWNT/GC electrode and (b) SWNT/GC electrode in phosphate buffer (0.05 M, pH 7.5) containing 0.05 M KCl.

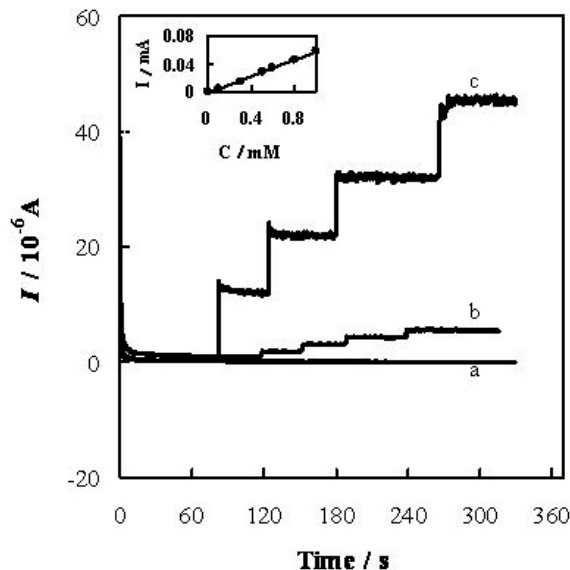
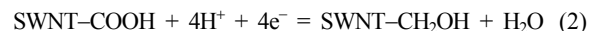


Figure 6. Amperometric response for CySH (2×10^{-4} M) at (a) GC (applied potential, 0.42 V), (b) SWNT/GC (applied potential, 0.42 V), and (c) PQQ/SWNT/GC (applied potential, 0.27 V) electrodes. Inset shows a calibration curve for CySH at PQQ/SWNT/GC electrode.

expected for the following four-electron, four-proton reaction (3):



The midpoint potential of PQQ at the PQQ/SWNT/GC electrode also showed a pH dependency with a slope of -0.057 V/pH (see figure 4d) indicating two-electron and two-proton reaction. Consequently, the oxidation of CySH at these two different electrodes may be responsible for the observed low slopes of -0.023 V/pH (at SWNT/GC) and -0.041 V/pH (at PQQ/SWNT/GC), and further studies are needed for the interpretation of the observed pH dependency on the oxidation of CySH at these electrodes. Similar results were observed for other thiols.

As the detection potential of CySH is moved less positive, the effect of the interferences is expected to decrease. It has also been found that the PQQ/SWNT/GC electrode is more sensitive to thiols at higher pH, which is consistent with that of PQQ/PPy-modified electrode (32). However, the electrochemical behavior of these two modified electrodes deteriorated at pH values higher than ~ 9 , and thiols can also be easily oxidized by atmospheric oxygen in basic solution. Hence, pH 7.5 (0.5 M phosphate buffer) was used for the following amperometric determination of thiols. Another reason to use pH 7.5 phosphate buffer for the determination of thiols is that it is closer to the pH of biological samples.

Figure 5 shows the effect of scan rates on the I_{pa} values of CySH at both SWNT/GC and PQQ/SWNT/GC electrodes. The oxidation peak currents of CySH increased linearly with the square root of the scan rate in the range of 10–400 mV/s with correlation coefficients of 0.998 and 0.995 at both PQQ/SWNT/GC and SWNT/GC electrodes, respectively. This suggests that the current is limited by semi-infinite linear diffusion of CySH in the interfacial reaction zones of both SWNT/GC and PQQ/SWNT/GC electrodes. Other thiols showed a similar behavior at both SWNT/GC and PQQ/SWNT/GC electrodes.

4.3. Amperometric determination of thiols at SWNT/GC and PQQ/SWNT/GC electrodes

Figure 6 shows amperometric response curves upon subsequent additions of a 0.2 mM CySH solution in phosphate buffer (0.05 M, pH 7.5) at (a) bare GC, (b) SWNT/GC, and (c) PQQ/SWNT/GC electrodes, respectively. Both SWNT/GC and PQQ/SWNT/GC electrodes responded rapidly to the change in the level of CySH, producing steady-state signals. The response time (time required to reach 90% of the maximal response) with 5×10^{-5} M CySH were 3 s and 2 s at SWNT/GC and PQQ/SWNT/GC electrodes, respectively. Inset shows a calibration curve based on the amperometric response with CySH at a PQQ/SWNT/GC electrode. A linear relationship between the peak current and the thiol concentration up to 1 mM was obtained. Even though PQQ catalyzes the oxidation of thiols (see figure 1), an amperometric response current recorded at a PQQ/GC electrode (without SWNTs) was significantly lower than that observed at the PQQ/SWNT/GC electrode

Table 3. Analytical data for the amperometric detection of thiols using SWNT/GC and PQQ/SWNT/GC electrodes

Thiol	Linear range (10^{-6} M)	Sensitivity (10^{-6} A / 10^{-6} M) ¹	Detection limit (10^{-6} M) ¹
L-cysteine	10 – 2000 ²	0.013	5.2 ± 0.4
	1 – 2000 ³	0.059	0.7 ± 0.2
L-homocysteine	30 – 3000	0.006	8.1 ± 0.5
	5 – 5000	0.026	2.2 ± 0.3
N-acetyl-L-cysteine	50 – 5000	0.005	6.5 ± 0.4
	5 – 5000	0.019	3.2 ± 0.5
L-penicillamine	50 – 5000 ²	0.008	5.6 ± 0.3
	3 – 3000 ³	0.031	1.1 ± 0.4
Glutathione	50 – 5000 ²	0.003	15.2 ± 0.8
	30 – 3000 ³	0.011	6.2 ± 0.5

¹ Data given as mean ± SD (n = 3) (S / N = 3), ² SWNT/GC, ³ PQQ/SWNT/GC.

Table 4. Stability of SWNT/GC and PQQ/SWNT/GC electrodes¹

Sensor	SWNT/GC		PQQ/SWNT/GC	
Storage	Dry	PBS ²	Dry	PBS
	% of Initial Sensor Response to CySH			
3 days	98 ± 1	93 ± 2	97 ± 1	90 ± 1
7 days	95 ± 2	88 ± 2	94 ± 2	87 ± 1
3 weeks	93 ± 1	80 ± 1	91 ± 1	75 ± 2

¹ Data given as mean ± SD, n = 5 for dry storage in refrigerator at 4 °C or in PBS at 4 °C. ² PBS, phosphate buffer solution (0.05 M, pH 7.0).

(approximately 1/2000) with the same concentration of CySH. In addition, amperometric curves observed at the PQQ/GC electrode showed an irregular pattern upon successive additions of a CySH solution due to the quasireversible electron transfer of PQQ at the GC electrode (43). Similar results were obtained for L-homocysteine, N-acetyl-L-cysteine, glutathione, and L-penicillamine. The results are summarized in table 3.

In order to test the stability of both SWNT/GC and PQQ/SWNT/GC electrodes, amperometric experiments with CySH were carried out. The results are summarized in table 4. When these two types of electrodes were stored at 4°C in dry condition for three weeks, the response of these electrodes with CySH dropped only by several percent indicating a good stability of these sensors. However, the electrodes kept in phosphate buffer showed a 20-25% decrease of response after three weeks. Nevertheless, both sensors were chemically and electrochemically stable, particularly those stored in dry condition at 4°C.

4.4. Effects of potential interfering agents

The electroactive species such as ascorbic acid and uric acid may be main interferents for the detection of thiols in biological media. In order to overcome this problem, both SWNT/GC and PQQ/SWNT/GC electrodes were prepared using SWNTs dissolved in a solution containing both DMF and Nafion (see 3. MATERIALS AND METHODS). Nafion not only helps to solubilize SWNTs but also minimizes the effects of potential interferents such as ascorbic acid and uric acid. Meanwhile, the well-distributed Nafion/SWNT film is a better conductor than Nafion film itself, because SWNTs improve the electron transport of the Nafion membrane (53). The levels of endogenous ascorbic acid and uric acid

are approximately 0.125 and 0.33 mM in blood samples, respectively (53). The optimum Nafion concentration in SWNT solution for eliminating the interference of such concentrations of ascorbic acid and uric acid was found to be 0.5% (v/v). Both SWNT/GC and PQQ/SWNT/GC electrodes prepared with Nafion exhibited no response current with these species of such concentration levels. Under the optimum experimental conditions, other species including glucose, amines and amino acids in biological media, with fifty multiple relative to CySH concentration, did not disturb the determination of CySH. Therefore, the method provides the best selectivity for thiol determination in the presence of these compounds.

4.5. Applications

The PQQ/SWNT/GC electrode was used to determine the amount of both L-cysteine and N-acetyl-L-cysteine in a dietary supplement (see 3. MATERIALS AND METHODS). Figure 7 shows an amperometric response curve for CySH at the PQQ/SWNT/GC electrode in pH 7.5 phosphate buffer at an applied potential of 0.27 V. The amounts of both L-cysteine and N-acetyl-L-cysteine in the tablets (each tablet contains 500 mg each of those thiols) were determined to be 481±12 mg (n=3) and 485±10 mg (n=3) indicating the recovery of 96±2% and 97±2%, respectively. Recovery experiments were also carried out by adding known amounts of cysteine to the tablet solution with satisfactory recovery (95 ± 3%).

This type of thiol sensors may have a potential application in other biological samples. Although thiol levels in biological media are normally quite low [e.g. cysteine levels in blood and in urine are $4\text{--}240 \times 10^{-6}$ M and $20\text{--}80 \times 10^{-6}$ M, respectively, for healthy individuals (52)], they are within the detection range of the PQQ/SWNT/GC electrode.

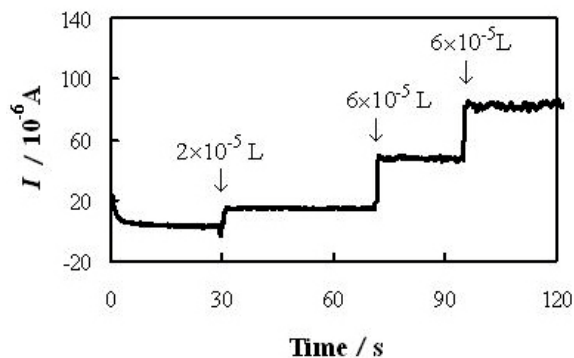


Figure 7. Amperometric response for the determination of CySH in pharmaceutical products at a PQQ/SWNT/GC electrode. 2×10^{-5} L of a 2.0 mg tablet/3 mL was added followed by two successive addition of 6×10^{-5} L of a 2.0 mg tablet/3 mL in phosphate buffer (pH 7.5). Applied potential: 0.27 V (vs. Ag/AgCl).

5. SUMMARY

The SWNT film modified GC (SWNT/GC) electrode exhibited a fast electron transfer for the catalytic oxidation of thiols at the electrode, which enabled the direct electrochemical detection of thiols at much less positive potentials than those at Boron-doped diamond (BDD) and other carbon electrodes. A thin film of coenzyme pyrroloquinoline quinone (PQQ) coated on the SWNT/GC electrode further improved the electrocatalytic behavior of the SWNT/GC electrode for the detection of thiols. Cyclic voltammetry showed the reversible redox currents of PQQ at the SWNT/GC electrode because of the fast electron transfer at the SWNT/GC electrode. The amperometric determination of thiols was carried out by applying much less positive potentials at both SWNT/GC (0.42 V) and PQQ SWNT/GC (0.27 V) electrodes which helped to improve the sensitivity and to minimize the effects of interfering agents for the detection of thiols. Both SWNT/GC and PQQ/SWNT/GC electrodes showed a fast response time (2-3 s) and storage stabilities over three weeks. Nafion used for solubilizing SWNTs for the preparation of the SWNT/GC electrode eliminated the effects of interfering species such as ascorbic acid and uric acid for the determination of thiols. These two types of thiol sensors are easy to prepare and are sensitive enough to determine thiols in biological media.

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