Chiang Mai J. Sci. 2018; 45(3) : 1449-1459
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

Fabrication of a Home-made SPCE Modified with Thionine for Determination of Hydrogen Peroxide

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Received: 15 September 2016
Accepted: 31 May 2017

ABSTRACT

A disposable screen-printed carbon electrode (SPCE) was fabricated by a simple screen printing method which is widely used for the mass-production of the electrochemical sensor. These home-made SPCEs were modified to apply to the determination of hydrogen peroxide. The conditions of the SPCEs fabrication process were described including curing time, curing temperature, dilution ratio, and a number of repeated printing of carbon ink. The investigation of electrochemical surface pretreatment was then carried out in acidic solution regarding their influence on the electrochemical properties of SPCEs. In addition, the modification of thionine on SPCEs was carried out with electropolymerization to enhance the sensitivity of the electrode. The resulting thionine modified SPCEs was successfully applied to the determination of hydrogen peroxide in the real samples.

Keywords: screen-printed carbon electrode, SPCE, poly(thionine), hydrogen peroxide, voltammetry

1. INTRODUCTION

Hydrogen peroxide (H_2O_2) is a significant mediator in many fields such as food [1], industrial [2], pharmaceutical [3], environmental [4], and clinical [5] analyses. Additionally, H_2O_2 is also the by-product of many substances under the catalysis of their highly selective oxidases in the biological process [6]. Different detections were employed for H_2O_2 including titrimetry [7], chemiluminescence [8], spectrometry [4] and electrochemistry [10]. The first three techniques have obvious drawbacks due to time-consuming, expensive and suffer from various interferences. At present, the detection of H_2O_2 by electrochemistry is the attractive method because of its advantages, simplicity, rapid, accurate, reliable and reagentless [10-11].

H_2O_2 is measured electrochemically by direct oxidation at the electrode and it requires a high overpotential [12] which suffers from the electroactive interference by oxidizable

species, such as L-ascosbic acid (AA), uric acid (UA) and dopamine (DA), that present in the biological matrices. Therefore, the sensitivity and selectivity of electrochemical detection are in high demand for H_2O_2. To avoid these interferences, the H_2O_2 biosensor was developed by the immobilization of specific biomaterials on the electrode surface for improvement of selectivity. For example horseradish peroxidase (HRP) [13], myoglobin [14] and hemoglobin (Hb) [15], can catalyze the reduction of hydrogen peroxide. However, the enzyme modified electrodes have some weakness, e.g., the requirement of enzyme immobilization procedures, instability, and the high cost of the enzyme.

Conducting polymer was used to modify the electrode surface as useful for the sensitive detection of H_2O_2. Various conducting polymer including poly(aniline) [16], poly(pyrrole) [17], poly(3,4-ethylene-dioxythiophene) [18] and poly(thionine) [19] have been reported to use in the combination with nanomaterials. In recent years, the modification of poly(thionine) on the electrode has been a trend because thionine (Th) is a perfect electronic mediator and it has a good electrochemical reversibility, stability and a fast charge transfer characteristic. Xu *et al.* [20] reported the covalent immobilization of thionine on the potentiostatically activated glassy carbon electrode for the H_2O_2 biosensor. Baskar *et al.*[21] designed a flow injection analysis system based on a poly(thionine) modified ring disk electrode for the simultaneous detection of NADH and H_2O_2. These reports exhibit an electrocatalytic process toward H_2O_2 reduction on the Th-modified electrode.

Screen printed carbon electrode (SPCE) is a miniature system which consists of a three-electrode configuration (i.e., reference electrode (RE), working electrode (WE) and auxiliary electrode (AE)). Recently, the screen printing technique is widely used for large-scale fabrication of disposable electrochemical devices. The advantages of SPCE are its disposability, portability, simplicity, ease of operation, needless of electrode polishing, easy for chemical modification and low cost [22]. SPCE was designed in different configurations for various applications, and some parameters affecting on the electroactivity of SPCE were studied in order to enhance the performance of SPCE [23, 24].

In this work, we focus on the use of a home-made SPCE instead of the commercial electrode and the simple modification of poly(thionine) on the electrode for measuring H_2O_2. The effect of parameters for SPCE fabrication was investigated and the electrochemical response of electrode was studied by the cyclic voltammetric technique.

2. MATERIALS AND METHODS

2.1 Reagent and Chemicals

Ag/AgCl ink was purchased from GWENT GROUP (England). Carbon ink (ACHESON, Singapore) was diluted in diethylene glycol monobutyl ether (MERCK, Germany). Commercial grade acetone was used as a cleaning solvent for both Ag/AgCl and carbon ink. Electrolyte solution was prepared by dissolving $K_3Fe(CN)_6$ (Ridel-De Haen, Germany) in 1 M KNO_3 (Ajax Finechem, Australia) solution. H_2SO_4 (LAB-SCAN, Poland), HNO_3 (MERCK, Germany) were used for acid pretreatment of SPCE. H_2O_2 (Ajax Finechem, Australia) solution was freshly prepared.

Thionine stock solution (200 μM) was prepared by dissolving 0.0014 g of thionine acetate salt (Sigma, USA) in 25 mL DI water. Real samples of disinfectant and hair-color solutions were purchased from a convenient store in Chiang Mai. The samples were

prepared by diluting in phosphate buffer (PB) solution of pH 7.4.

2.2 Apparatus

A μAutolab type II potentiostat (Eco Chemie, Utrecht, Netherlands) was used for carrying out an electrochemical measurement. The applied potential was scanned from -1.0 to +1.0 V (*vs.* Ag/AgCl) at a scan rate of 100 mVs^{-1} for $K_3Fe(CN)_6$ and H_2O_2 solution. Voltammogram was recorded by GPES program.

2.3 Fabrication of SPCE Electrode

Three-electrode configuration SPCE was designed and fabricated to the 3 screen meshes as shown in Figure 1. A polyvinyl chloride (PVC) sheet (0.5 mm thickness) was used as a substrate for sequential deposition of a Ag/AgCl ink, carbon ink and an insulator, respectively, to create the SPCE. Before screening the electrodes, the PVC substrate was cleaned by ethanol. Then, a layer of Ag/AgCl ink was deposited on the PVC sheet by screening through the first screen mesh to act as the conductive pads and the reference electrode (RE). After that it was cured by baking at 60°C for 30 min. Next, a layer of carbon ink was deposited through the second screen to form a working electrode (WE) and an auxiliary electrode (AE). It was then cured by baking in an oven at 150°C for 30 min. Finally, the insulating layer was deposited through the third screen to prevent the conducting pads from solution.

The electrochemical pretreatment of SPCE was carried out in acid solution by cyclic voltammetric scanning potential in the range of -1.2 to +1.5 V (*vs* Ag/AgCl) for 20 cycles [25]. These SPCEs were then rinsed with DI water prior to use.

2.4 Preparation of the Poly(thionine) (PTH) Modified SPCE

SPCE was equilibrated in 0.01 M PB pH 7.4 by cycling the potential between 0.0 and +1.3 V at a scan rate of 100 mVs^{-1} for 10 cycles. A pre-anodized SPCE (SPCE*) was prepared by applying potential of +2.0 V for 300 s in 0.01 M PB pH 7.4. The SPCE or SPCE* was immersed in PB solution of pH 7.4 containing 100 µM thionine and potential was scanned in the range between -0.5 and +1.1 V at 50 mVs^{-1} for 40 cycles, resulted in PTH film covered onto the SPCE or SPCE* [27].

3. RESULTS AND DISCUSSION

3.1 Fabrication of the Home-made SPCE

The SPCE was designed to be a combined three electrode system as depicted in Figure 1 in order to prevent the current passing through the RE so that a constant potential of the RE could be maintained. The entire electrodes fit on a 1×2.2 cm piece of PVC substrate. WE and RE were designed to be as close as possible to reduce the resistive effect between WE and RE. The geometric area of AE of about 29 mm^2 was larger than that of WE (12.57 mm^2) and RE (4.5 mm^2) to allow unlimited current flow at the AE.

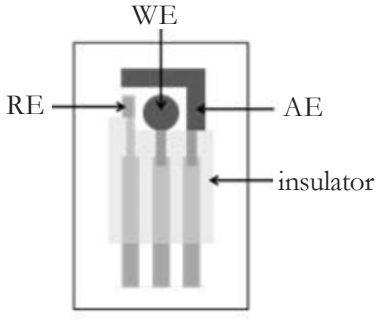

Figure 1. Configuration of a home-made SPCE; (RE)-reference electrode, (WE)-working electrode, (AE)-auxiliary electrode.

The SPCEs were fabricated as described in experimental section. Effect of various parameters involved in the fabrication of SPCE on the sensitivity of the electrode was investigated. The evaluation of SPCE was carried out by cyclic voltammetric analysis of potassium ferricyanide as a model reaction as shown in the below reaction. (1).

$$Fe^{III}(CN)_6^{3-} + e^- \rightarrow Fe^{II}(CN)_6^{4-} \quad (1)$$

3.2 Effect of Curing Time of Carbon Ink

Carbon ink contains graphite particles, polymeric binder, and solvent. After the ink was screen printed on the PVC substrate, it was cured in order to firmly attach onto the substrate. Carbon ink was cured in the oven. The curing with heat is the polymerization by cross-linking of polymer chains and then promoted ink adhesion to the PVC substrate. The curing time of carbon ink was studied. In a preliminary step, carbon ink was cured at 100°C for 15 minutes. It was found that the absence of cyclic voltammogram of 10 mM $K_3Fe(CN)_6$ was observed due to its high resistance. Then curing time was increased to 30 and 60 minutes, it showed that the anodic peak current of 30 minutes was higher than 60 minutes (Figure 2A) and the resistance of SPCE at 30 minutes curing time was similar to that of SPCE at 60 minutes curing time. At 100°C curing temperature, it is insufficient to remove solvent resistance or solder resistance of the ink. Thus, 30 minutes curing time of carbon ink was chosen for further experiments. These resistances were considered to be high level as shown in Table 1. Therefore, the curing temperature and the dilution of carbon ink were studied later.

3.3 Effect of Curing Temperature of Carbon Ink

The curing temperature could cause the evaporation of solvent and decomposition of the polymeric binder, thus provided a greater appearance of graphite on the electrode surface. This parameter was varied in the range of 100-180°C, with curing time of 30 minutes. The temperature above 180°C was not studied because PVC substrate would be melted. It was found that 150°C gave a good result as its anodic peak currents were better than the others (Figure 2B).

3.4 Effect of Dilution Ratio of Carbon Ink

Diethylene glycol monobutyl ether was used to dilute carbon ink that composed of graphite and a polymeric binder in order to improve viscosity to be suitable for the screen printing process. The high viscosity provided generally the high density of graphite and a polymeric binder (high solder resistance), then the effective graphite surface area was decreased due to the aggregation of graphite particles. Carbon ink was diluted in two ratios, i.e., 1:10 (1 g ink: 10 drops solvent) and 1:5. The ratio over 1:10 fails because carbon ink is too dilute to be screen printed. It was found that 1:10 ratio gave higher anodic peak current than 1:5 ratio (Figure 2C), although the resistance of the 1:5 ratio was slightly lower.

3.5 Effect of Number of Repeated Printing of Carbon Ink

The number of repeated printing of carbon ink in the screen printing process was studied with the ink dilution ratio of 1:10 and under the curing condition at 150°C for

30 minutes. It was found that two times of repeated printing gave higher anodic peak current than either one time or three times printing as shown in Figure 2D and the resistance of carbon film obtained from two times repeated printing was lower as shown in Table 1 because of its homogeneous surface.

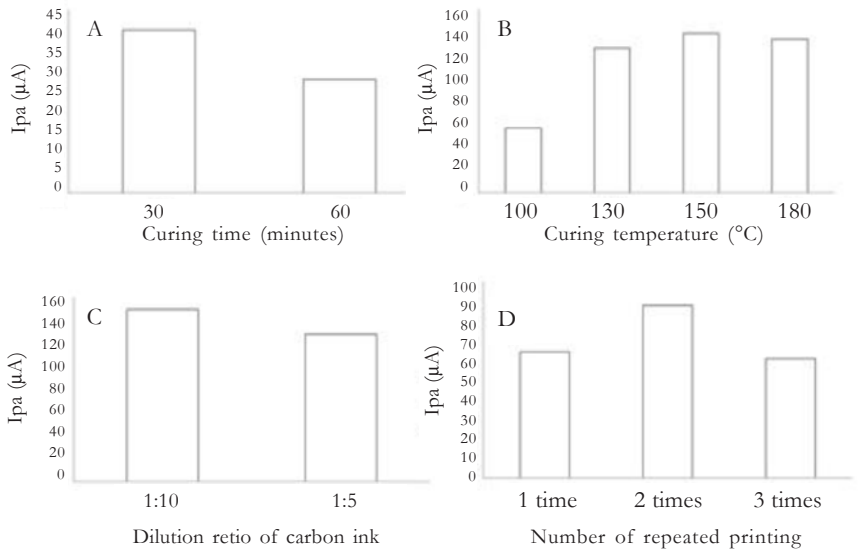

Figure 2. Optimization of conditions used for the fabrication of the home-made SPCE; the curing times (A), the curing temperature (B), the dilution ratio of carbon ink (C), the number of repeated printing (D).

Table 1. Summarized condition of the home-made SPCE fabrication.

Conditions	Optimization	Resistance (Ω)	Selected condition
Number of repeated printing of Ag/AgCl (times)	1	20	2
	2	8	
Curing time of carbon ink (minutes)	30	1300	30
	60	1400	
Curing temperature of carbon ink (°C)	100	170	150
	130	65	
	150	65	
	180	60	
Dilution ratio of carbon ink	1:10	90	1:10
	1:5	75	
Number of repeated printing of carbon ink (times)	1	226	2
	2	85	
	3	55	

3.6 Effect of Electrochemical Pretreatment of SPCE

In the screen-printed process, carbon ink was printed on a PVC substrate with the random alignment of graphite particles. The polymeric binder would also cover the active surface of graphite particles. The recent study of electrochemical behavior of graphite sheet revealed that the electrode kinetics at the edge plane is faster than at the basal plane [26]. Therefore, the electrochemical pretreatment of SPCE in acidic solution was carried out by applying oxidative potential to the electrode to remove residual polymeric binder in order to increase the active site and surface roughness of the electrode. Figure 3 shows the effect of pretreatment in HNO_3 and H_2SO_4 solutions. It was found that the anodic and cathodic peak potentials of pretreated SPCE were shifted negatively and positively, respectively, which indicated a good electrochemical response of the electrode.

In Figure 3A, various concentrations of HNO_3 were studied in the range of 0.1-1.0 M. The peak currents of pretreated SPCE were higher than those of the untreated SPCE due to the enhancement of active surface of the electrode by electrochemical acid pretreatment while the background current of treated SPCE was also significantly increased because of its capacitance. The capacitance of the electrode increased with the increase of its surface area, leading to the high capacitive current in the voltammogram.

In the treatment of SPCE with HNO_3, the back ground current was dramatically increased with increasing concentration of HNO_3. Since HNO_3 is a strong oxidizing acid it can oxidize polymeric binder to increase the surface area of SPCE, implied that increase both of basal plane and edge plane of graphite, causing a high capacitive current. Thus, HNO_3 is not suitable for pretreatment of SPCE in this work. In the other hand, it was used in the improvement of electrochemical performance of electrochemical double layer capacitors [27].

Effect of H_2SO_4 is shown in Figure 3B. It was found that the peak current of treated SPCE was higher than that of untreated SPCE, while the background current was lower. H_2SO_4 may increase more edge plane graphite that is dominant sites for fast electron transfer. The redox peak currents obtained from SPCE treated in HNO_3 and H_2SO_4 were not significantly different. Therefore, 0.5 M H_2SO_4 was selected for pretreatment of SPCE because the steady current of the electrode was achieved rapidly at this concentration.

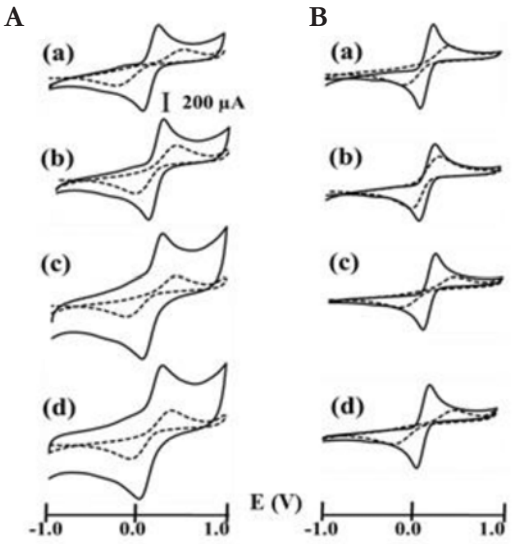

Figure 3. CVs of 10 mM $K_3Fe(CN)_6$ on the untreated (----), treated SPCE (——) with the different concentrations of HNO_3 (A) and H_2SO_4 (B): 0.1 M (a), 0.2 M (b), 0.5 M (c), 1.0 M (d). Scan rate, 100 mVs^{-1}.

3.7 Determination of Hydrogen Peroxide with SPCE

Repeatability of SPCE was checked by repeatedly performing cyclic voltammetric measurement of 10 mM $K_3Fe(CN)_6$ on the same electrode. It was found that %RSD (n=11) are in the range of 2.3.

After that, these home-made SPCEs were evaluated with the electrochemical reduction reaction of H_2O_2 as shown in reaction (2). The cathodic peak current at -0.60 V of H_2O_2 was recorded and used to construct a calibration graph in the range of 0.5-10 mM H_2O_2 (Figure 4). It was found that when the concentration of H_2O_2 increased, the current also increased, yielding a linear regression equation, $y = 7.741x + 29.02$ with $r^2 = 0.9940$. However, the sensitivity of the method has to be improved further.

$$H_2O_2 + 2e^- + 2H^+ \rightarrow 2H_2O \qquad (2)$$

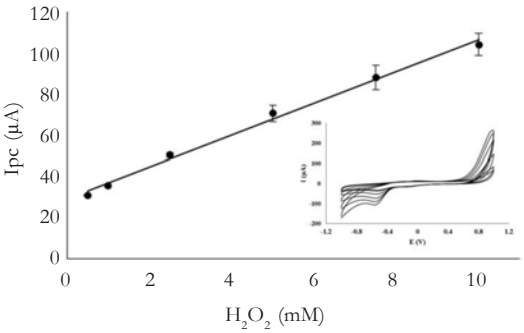

Figure 4. Calibration graph of 0.5-10 mM H_2O_2 on the treated SPCE.

3.8 Modification of Poly(thionine) (PTH) on SPCE

SPCE was first pre-anodized for increasing of the edge plane sites and carbonyl functional group on the surface of the electrode, in order to promote facile electron transfer in the electrochemical reaction. The oxygen functionalities on the graphite edge planes, that are negative charge, can interact with positively charged thionine. And the pre-anodization process also gives the increasing of background current due to the oxidation and reduction of the carbonyl functional group. Therefore, the current obtained from pre-anodized SPCE modified with PTH (PTH/SPCE*) was higher than that of PTH modified SPCE without pre-anodizing (PTH/SPCE).

The mechanism of thionine electropolymerization has been proposed and confirmed [28]. The initial adsorption of thionine monomer occurred on the electrode surface and the electropolymerization reaction started and proceeded at a fairly high potential at which reactive cation-radical species formed. The monomer units in PTH linked through -NH-bridges in the aromatic position α or β (both *ortho* with respect to -NH).

Cyclic voltammogram in Figure 5 shows the growth of the PTH film under consecutive scan. The redox peaks with cathodic and anodic potentials increased gradually and tended to become stable with increasing number of polymerization cycles. After that, cyclic voltammogram (Figure 5 inset) of PTH/SPCE* in PBS pH 7.4 confirmed the presence of surface-attached electroactive material.

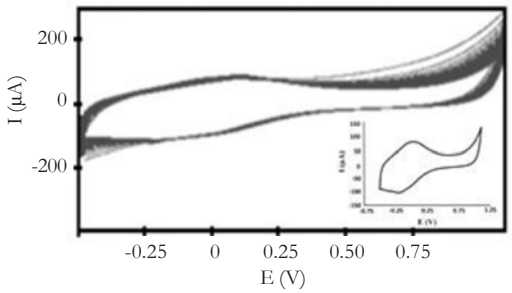

Figure 5. Cyclic voltammogram for during electropolymerization of PHT film on SPCE* with potential -0.5 and 1.1 V at scan rate 50 mVs^{-1} for 40 cycles in 100 μM thionine in PB pH 7.4. Inset: cyclic voltammogram for final electropolymerization of PHT film on SPCE*.

3.9 Effect of Amount of Thionine

Changing thionine solution concentration from 100 µM to 200 µM was studied in this work. It was found that the current of H_2O_2 on 200 µM PTH/SPCE* was higher than that of 100 µM while the sensitivity was not different as shown in Figure 6.

Xu *et al.* [29] have explained that the effect of pre-anodization was critical which created thionine cation radical. When the electrode was pre-anodized, large amount of positive charges were accumulated on the electrode surface to create thionine cation radical for polymerization. This resulted in the formation of a layer of poly(thionine) film on the electrode. In this work, 100 µM of thionine solution was selected.

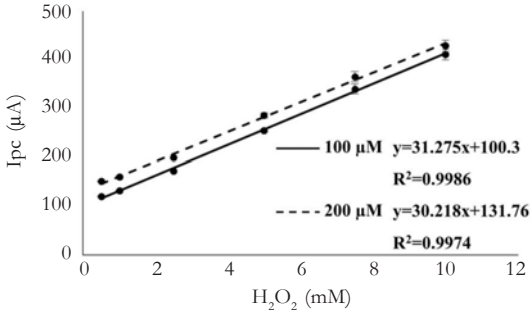

Figure 6. Calibration graph of H_2O_2 in the range of 0-10 mM with the different PTH/SPCE*, the current at -0.6 V.

3.10 Effect of Scan Rate

The effect of scan rate on the electrochemical response of PTH on SPCE* was investigated. CVs of PTH/SPCE* in the buffer at different scan rate is shown in Figure 7. Both the cathodic and anodic currents were increased with increasing of scan rates, indicating a surface-controlled electrode according to the following Eq. (1);

$$i_p = \frac{n^2F^2A\Gamma\upsilon}{4RT} \quad (1)$$

Where Ip is the peak current, n is the number of electrons, F is Faraday constant, A is the electrode surface area, Γ is the surface coverage concentration and ν is the scan rate [30].

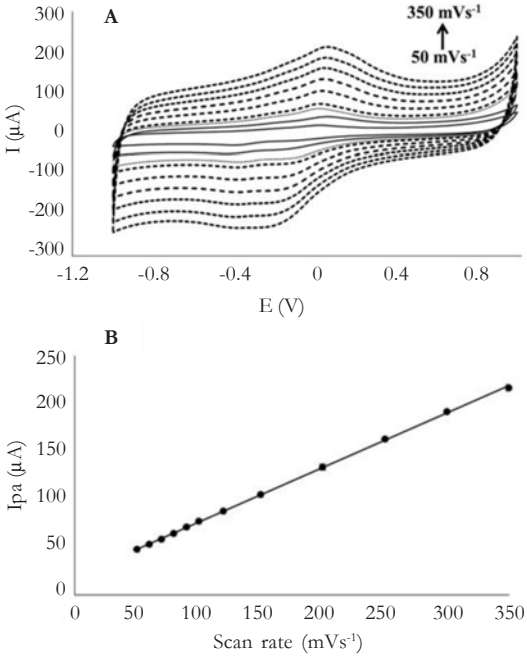

Figure 7. Cyclic voltammograms for the different scan rates on PTH/SPCE* in PB pH 7.4 (A), the plot of the relationship between scan rate *vs* current (B).

3.11 Determination of Hydrogen Peroxide with PTH/SPCE*

CVs of H_2O_2 on PTH/SPCE* showed high current level but it was without reduction peak of H_2O_2 (Figure 8). This may be due to the reaction involved adsorption of H_2O_2 on the PTH/SPCE* surface, therefore, less mass transfer by diffusion of species from the bulk solution to the electrode surface. So, the poly(thionine) film was used to increase the adsorption of H_2O_2 on the electrode surface to enhance sensitivity.

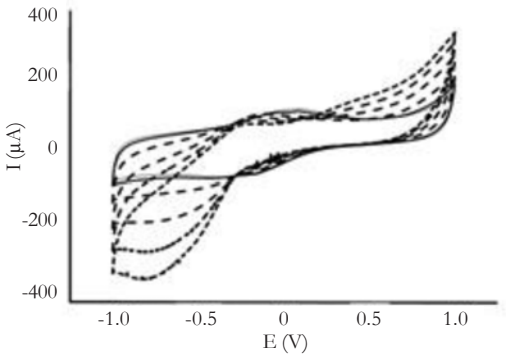

Figure 8. CVs of different concentrations of H_2O_2 in the range of 0.5-10 mM with TH/SPCE*.

The determination of H_2O_2 was carried out with cyclic voltammetry. The potential was scanned between -1.0 to +1.0 V (*vs.* Ag/AgCl) with scan rate 100 mVs^{-1}. It was found that the cathodic current in the negative potential range increased with the increasing of H_2O_2 concentration. The cathodic current at -0.6 V was used to construct the calibration graph in the range of 0.5 to 10 mM H_2O_2, the equations of linear regression on different electrodes are; y=27.238x + 91.718 (PTH/SPCE*), y=13.85x + 24.135 (PTH/SPCE), and y=7.741x + 29.024 (SPCE) as shown in Figure 9.

It was found that PTH/SPCE* provided the highest sensitivity. However, it is still inadequate for determination of the low concentration of H_2O_2. Therefore, this PTH/SPCE* was used to apply with samples containing high concentration of H_2O_2.

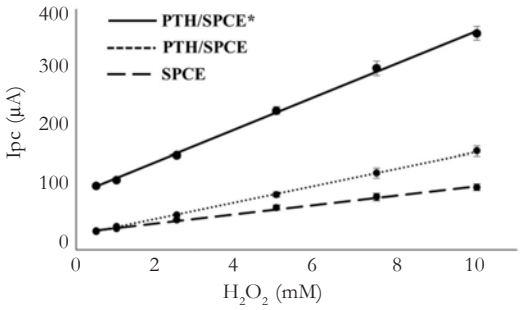

Figure 9. Calibration graph of 0.5-10 mM H_2O_2 on SPCE*.

3.12 Real Sample Analysis

The developed PTH/SPCE* were applied for cyclic voltammetric determination of H_2O_2 in disinfectant and hair-colorant solutions. The external standard method was performed for analysis of samples. A disinfectant and the hair-colorant sample were diluted 200-folds and 10-folds by PB of pH 7.4 before the analysis, respectively. The results obtained were compared with the label values as summarized in Table 2. In addition, $KMnO_4$ titration method was also employed for the determination of H_2O_2 in these samples as shown in this table.

Table 2. Determination of H_2O_2 in a disinfectant and hair-colorant samples by cyclic voltammetry (CV) using the PTH/SPCE* and $KMnO_4$ titration.

		H_2O_2 content (%)		
Sample	Label	Found by CV/ PTH/ SPCE*	Found by titration	% Different
Disinfectant (v/v)	1 3.0	3.1±0.1	3.27±0.05	-5.2
Hair-colorant (w/w)	2 9.0	9.2±0.4	8.9±0.4	3.4
	3 12.0	12.3±0.3	12.0±0.2	2.5

The reproducibility of PTH/SPCE* was investigated in the calibration graph of H_2O_2 in the range of 0-10 mM. It was found that RSD of 13 electrodes was 4.5% in the presence of high sensitivity. After that the reproducibility of PTH/SPCE* was investigated for analysis of the sample, it was found that the RSD (n=11) of one electrode in the same sample solution were 1.4% for disinfectant and 4.3% for hair-color samples.

4. CONCLUSION

Home-made SPCEs were successfully fabricated with a simple screen printing technique. The modification of poly(thionine) on SPCE can enhance the sensitivity of electrode and then the proposed modified SPCE can be applied to the H_2O_2 determination in real samples. The SPCE is low cost, disposable, and provides good sensitivity and precision. It can be used as a platform for developing of various sensors/biosensors.

ACKNOWLEDGEMENTS

The authors would like to thank Science Achievement Scholarship of Thailand (SAST) for providing scholarship to Ms. Preeyaporn Reanpang. We thank the National Research University Project under Thailand's Office of the Higher Education Commission, Center of Excellence for Innovation in Chemistry, the Graduate School and Department of Chemistry, Faculty of Science, Chiang Mai University for all supports.

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