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Comparison of amperometric biosensors fabricated by palladium sputtering, palladium electrodeposition and Nafion/carbon nanotube casting on screen-printed carbon electrodes

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Abstract

Different strategies, including palladium electrodeposition (Pd_{CV}), Pd sputtering (Pd_S) and Nafion-solubilized carbon nanotube casting (Nafion/CNT), were used to modify screen-printed carbon electrodes (SPCEs) for the fabrication of amperometric enzyme biosensors. The electrochemical properties of the bare and modified SPCEs and the optimal conditions for surface modification were determined. The electrochemical response of the bare SPCE to H_2O_2 under the potential of 0.3 V could be improved about 100-fold by Pd modification by electrodeposition or sputtering. By contrast, the electrochemical response of the bare SPCE was enhanced by only about 11-fold by Nafion/CNT casting. Moreover, the Pd_{CV}-SPCEs exhibited better reproducibility of electrochemical response (a relative standard deviation (R.S.D.) < 6.0%) than freshly prepared Pd_S-SPCEs (R.S.D. > 10%). The glucose biosensor fabricated from Pd-modified electrodes could be stored for up to 108 days without loosing significant activity. The Pd_{CV}-SPCE also showed very reliable signal characteristics upon 50 consecutively repeated measurements of ascorbic acid. The electrocatalytic detection of the Pd-SPCE was combined with additional advantages of resistance to surface fouling and hence good stability. In conclusion, this study demonstrated that deposition of Pd thin film on SPCEs by electrodeposition or sputtering provided superior enhancement of electrochemical properties compared to Nafion/CNT-SPCEs. Despite their high electrochemical response, Pd_S-SPCEs required an activation process to improve stability and Pd_{CV}-SPCEs suffered from poor between electrode reproducibility.

Keywords: Screen-printed carbon electrodes; Electrodeposition; Sputtering; Carbon nanotube; Palladium

1. Introduction

Recent trends in research and development of biosensors have increasingly emphasized the manufacture of portable biosensing systems (Thévenot et al., 2001; Malhotra and Chaubey, 2003; Zhang et al., 2000). Screen-printed carbon electrodes (SPCEs) are frequently used for the construction of simple portable devices for fast screening purposes and in-field/on-site monitoring because of their low cost and easy integration into mass-production processes (Albareda-Sirvent et al., 2000; Castillo et al., 2004; Yoon and Kim, 1996; Gao et al., 2003). Electroactive species generated during enzymatic reactions can be measured amperometrically by these sensors. However, such amperometric biosensors often suffer from electrochemical interference by oxidizable species, such as L-ascorbic acid, uric acid, and acetaminophen (Mizutani et al., 1998a,b). Like hydrogen peroxide, these interferants can be oxidized on the electrode resulting in a large current response. To avoid the interference from these oxidizable species, attempts have been made to modify electrodes with suitable electrocatalytic metals, such as platinum, rhodium, ruthenium, palladium as well as carbon nanotube, to reduce the overpotential for the oxidation of hydrogen peroxide (Xu et al., 2002; Yamato et al., 1997; Sarkar et al., 1999;

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Wang and Musameh, 2003; Wang et al., 2004; Wang, 2005; White et al., 1994; Newman et al., 1995; Chi and Dong, 1993; Johnston et al., 1995). Among available transition metals, palladium is often chosen to modify the electrode because it is difficult to oxidize, electrocatalytically active and relatively inexpensive (Xu et al., 2002; Yamato et al., 1997; O'Connell et al., 1998; Chang et al., 2003). Interestingly, carbon nanotube (CNT) can also be used for the modification of carbon electrodes, because it is capable of accelerating electron transfer during electrochemical reactions (Wang et al., 2004; Wang, 2005).

Carbon paste electrodes can be modified by dispersing palladium particle and/or CNT powder in carbon (graphite) paste prior to fabrication of the electrode (Wang et al., 1992, 1995; Rubianes and Rivas, 2003; Wang and Chen, 1994; Cai et al., 1995; Yamato et al., 1997; Sarkar et al., 1999). Pd- and CNT-dispersed carbon electrodes exhibited high electrocatalytic activity and increased response to hydrogen peroxide (Wang et al., 1992; Rubianes and Rivas, 2003; Wang and Chen, 1994; Cai et al., 1995; Yamato et al., 1997; Sarkar et al., 1999). However, their response times were slow and several minutes were required for the reaction to reach a steady-state. Moreover, the surface characteristics of the Pd- and CNT-dispersed electrodes were altered as the reaction proceeded, resulting in poor reproducibility of performance. Other modifications, such as preanodization and electrochemical cycling (Wang et al., 1996; Cui et al., 2001; Jae et al., 2001), were required for the activation of the electrodes, thereby enhancing their electrochemical activity and improving their reproducibility. Alternatively, carbon electrodes can be modified by the deposition of palladium or CNT (O'Connell et al., 1998; Nowall and Kuhr, 1995). The electrochemical properties of glassy carbon and metal electrodes could be improved by the electrodeposition of palladium (Zhang et al., 2000). Although the electrochemical activity of the electrodes was improved, the palladium on the surface of glassy carbon or metal electrodes was mechanically unstable (O'Connell et al., 1998; Sakslund and Wang, 1994), presumably because metal particles were not incorporated during electrodeposition. However, this approach has not been used in the improvement of screen-printed carbon electrode. The disadvantages exhibited in glassy carbon and metal electrodes may not occur in SPCE because of its rough and porous structure (Lubert et al., 2001a,b).

This study presented the analysis of the effect of palladium modification of the SPCE surface by electrodeposition and sputtering (Os et al., 1996; Kuwabata and Martin, 1994). Electrochemical properties, such as electrochemical response, response time, and cyclic voltametric behavior, and mechanical stability were carefully investigated on the electrodeposited and sputtered Pd-SPCEs. These properties were compared with those of a CNT-modified electrode. An amperometric glucose biosensor was fabricated using the Pd-modified electrodes, and its characteristics and sensitivity were studied. The easy availability of the electrode modification processes presented in this study suggests the potential for future development of SPCE-based biosensors with high sensitivity and reproducibility on a commercial scale.

2. Experimental

2.1. Reagents

Bovine serum albumin (BSA), glucose and palladium chloride ($PdCl_2$) were purchased from Sigma Chemical Co. (St. Louis, USA). PVA-SbQ was obtained from Toyo Gosei Kogyo Chemical (Tokyo, Japan) (Chang et al., 2003). The L-Glucose oxidase (GOx, EC 1.1.3.4), hydrogen peroxide were purchased from Fluka. The palladium strip electrode was obtained from Boehringer–Mannheim (Yuan et al., 2005). The screen-printing carbon strip was obtained from ApexBichem (Hsinchu, Taiwan). Multiple wall carbon nano tubes (CNT) with an average external diameter of 20–40 nm and length of 5–15 μm were obtained from Conyuan Biochemical Technology Co. (Taipei, Taiwan). The buffer for assay is 100 mM phosphate buffer saline (PBS). One hundred millimolar phosphate buffer were prepared by mixing stock standard solution of K_2HPO_4 and KH_2PO_4 and adjust the pH with NaOH. The common chemicals used for preparation of buffers, etc., were of analytical reagent grade. All of the solutions were prepared with deionized distilled (D–D) water.

2.2. Electrodeposition of palladium on the SPCE

Oxygen plasma treatment was performed using an UV/O_3 cleaner (Nippon laser and electronics, Japan) and UV light irradiation in a pure oxygen atmosphere. All measurements were performed at room temperature. The electrodes were then immersed in a solution containing 5 mM palladium chloride. The surface concentration of the palladium films could be controlled by the appropriate choice of the number of cyclic scans. Electrodeposition on the strip was performed by cyclic potential scanning between −0.4 and 1.0 V versus Ag/AgCl at 50 mV/s. Unless otherwise stated, Pd-modified electrodes were developed using cyclic scans for 15 cycles.

2.3. Sputtering of palladium on the SPCE

SPCEs with a working area of 4.8 mm^2 were sputtered using a S200C radio frequency sputter coater designed by Branchy Technology Co. Ltd. (Tao-Yuan, Taiwan) using 99.999% pure palladium. A sputtering time of 2–4 min at a pressure of 5 kPa and a current of 70 mA were used to yield a 50–150 nm thick sputtered-Pd layer.

2.4. Casting CNT on the SPCE

Multiple wall CNT was solubilized in 0.5% Nafion solution to give a concentration of 1 mg/mL via mild sonication and subsequently film-cast onto the SPCE surface (Lim et al., 2005).

2.5. Enzymes immobilization

The enzymes were immobilized by a combination of PVA-SbQ photo cross-linking and glutaraldehyde exposure. A mixture of 50 mg PVA-SbQ and 150 mg L-GOx (500 U/mL) was prepared and 1 μL of this mixture was deposited onto the active

area of the electrode, the sensor was then placed in a dark sealed box containing glutaraldehyde vapor. The box was kept at 4 °C for 8 h, followed by exposure under UV light for 25 min.

2.6. Amperometric measurements procedure

Amperometric measurements were performed using a home-made potentiostat (Chang et al., 2003). Input and output signals from the potentiostat were coupled to a PC (Pentium 600 MHz) using a peripheral interface card (AT-MIO-16E, National Instruments, Austin, TX, USA). The interface card consisted of a 16-channel analog-to-digital (A/D) converter (12 bit) and a 2-channel digital-to-analog (D/A) converter (12 bit). Voltage output, data display and recording were programmed using the LabVIEW 6.1 Software package (National Instruments). All measurements were taken with a three-electrode system under a fixed working potential of 0.5 V versus Ag/AgCl using a modified commercial palladium strip with an active area of 4.8 mm^2 as the working and counter electrodes. The working solution was 9.9 mL PBS buffer (pH 7.0) in a cylindrical cell, with temperature controlled using a thermostat. All experiments were carried out at 37 °C. For the measurement of glucose, 0.1 mL glucose stark solution was injected into the test solution using a micro syringe when a steady-state of the testing-system had been obtained. Following the injection of glucose, the response current was displayed and simultaneously recorded by the computer until a steady-state was achieved. Magnetic stirring during the operation was used to ensure the homogeneity of the solution. The difference between the baseline and the steady-state current was used to calculate the concentration of glucose.

3. Results and discussion

3.1. Modification of the SPCE with Pd

To reduce the overpotential for the oxidation of hydrogen peroxide the electrocatalytic metal, Pd, was deposited on the surface of the SPCE by either electrodeposition (Pd_{CV}-SPCE) or sputtering (Pd_S-SPCE). The electrodeposition of Pd was performed using cyclic voltammetry in the 100 mM PBS buffer (pH 7.0) containing 5 mM palladium chloride with a scanning range of −0.4 to 1.0 V and a scan rate of 50 mV/s. The SPCE was also modified with palladium using a sputter coater. A thin sputtered Pd layer (50–150 nm) was obtained using sputtering times ranging from 2 to 4 min at a pressure of 5 kPa and a current of 70 mA. As shown in Fig. 1, the bare and 50 nm Pd layer-coated (Pd_{S50}-SPCE) electrodes displayed similar cyclic voltammograms, while a substantial increase in the current response was observed on the 100 nm (Pd_{S100}-SPCE) and 150 nm thick Pd layer (Pd_{S150}-SPCE) coated electrodes. Among the Pd-coated SPCE electrodes, the Pd_{S100}-SPCE achieved the highest response, whereas the response of Pd_{S150}-SPCE showed only moderate amplification. A surface area differences among three Pd-coated SPCEs may account for their differential electrochemical responses to H_2O_2. The surface characteristic of SPCE and Pd_S-SPCEs was further studied by scanning electron micrograph (SEM) (Fig. 2). As illustrated in Fig. 2A, the surface of

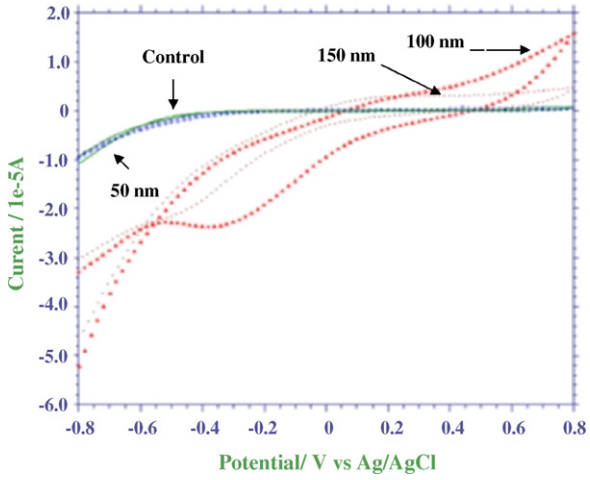

Fig. 1. Cyclic voltammograms of electrodes modified by Pd sputtering of different thicknesses.

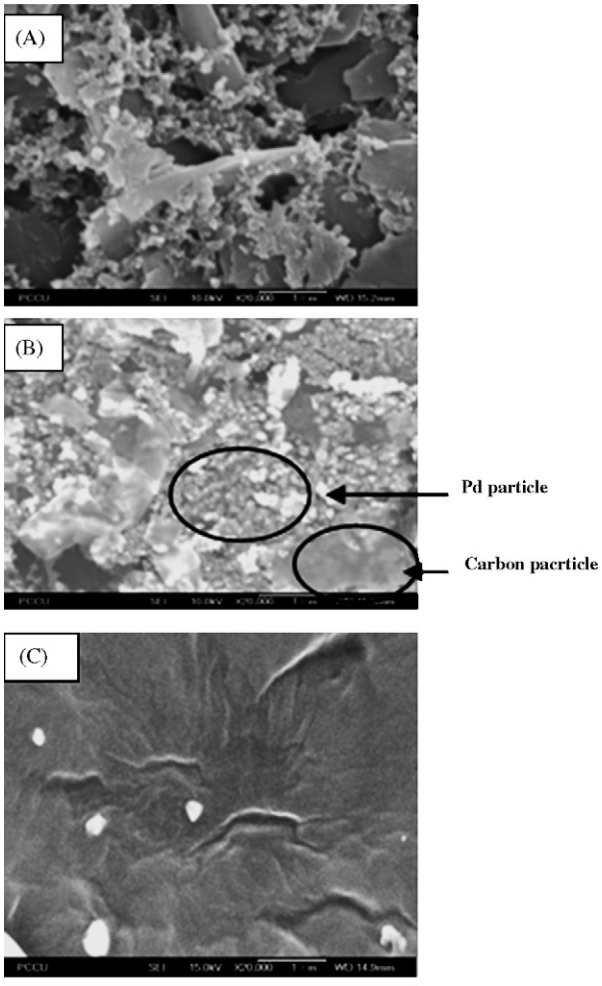

Fig. 2. Typical scanning electron micrograph of (A) SPCE electrode, (B) Pd sputtering SPCE electrode and (C) commercial Pd strip electrode (magnification 20,000-fold). This microscopic images show a rough and jagged structure with randomly distributed carbon particles and binder on the surface of SPCE electrode (A and B).

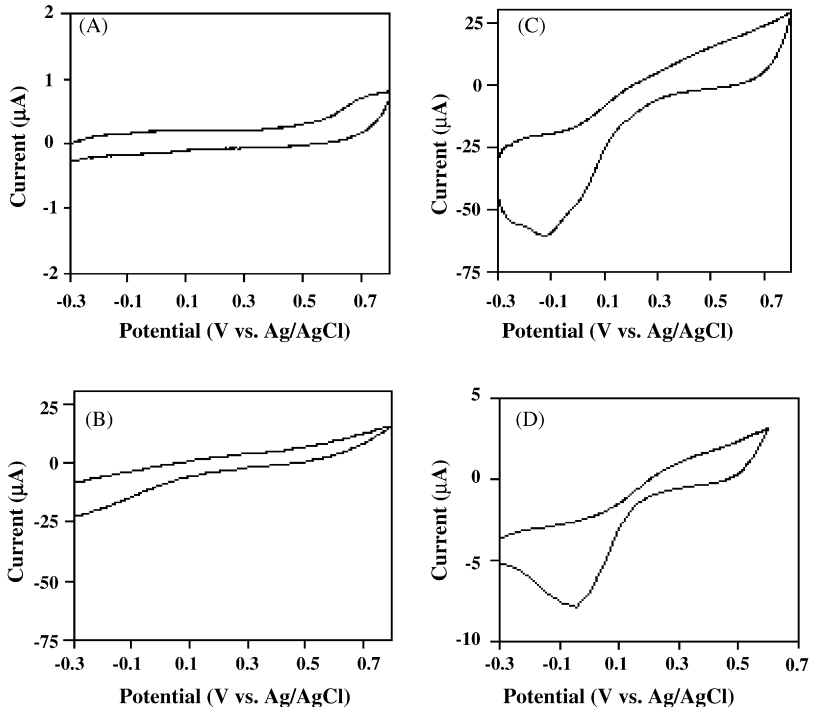

Fig. 3. Cyclic voltammograms of the bare SPCE (A), Pd_{S100}-SPCE (B), Pd_{CV15}-SPCE (C) and commercial Pd strip electrode (D). Scan rate was 50 mV/s.

SPCE is mostly rough and pitted. Although the surface of SPCE coated with Pd by sputtering still exhibited a rough texture, most of pits on the surface of Pd_{S100}-SPCE disappeared (Fig. 2B). This result suggests that a layer of Pd film thicker than 100 nm may further smooth the surface of SPCE leading to smaller surface area. This postulation can partially explain the result that the electrochemical response of Pd_{S150}-SPCE is lower than that of Pd_{S100}-SPCE (Fig. 1). This postulation was further confirmed by the observation that a commercial Pd strip with a smooth surface (Fig. 2C) exhibited a low electrochemical response to H_2O_2 (Fig. 3D). On the other hand, 50 nm Pd film might not be thick enough to provide enough H_2O_2 binding and catalytic sites on the SPCE surface (Hall et al., 1998a,b). Thus, the sputtering time should be carefully controlled to achieve sufficient thickness of the Pd layer to cover the entire surface of SPCE. In addition, a critical thickness of Pd is likely to be required for the electrodes to allow optimal electrochemical responses to occur (Johnston et al., 1995).

3.2. Cyclic voltammetric studies of the bare and Pd-coated SPCEs

Fig. 4 illustrates the cyclic voltammograms of the bare SPCE, Pd_{S100}-SPCE, Pd_{CV15}-SPCE (a Pd-modified SPCE by 15 cycles of CV) and a commercial Pd strip electrode. A significant charging current of the bare SPCE was observed in the potential range of 0.5–0.7 V without a cathodic arm (Fig. 3A), while the Pd_{CV15}-SPCE (Fig. 3C) and commercial Pd strip electrode (Fig. 3D) showed similar cyclic voltammograms, exhibiting a potential window in the range of 0.2–0.6 V and a pronounced cathodic arm associated with the reduction of oxides on the surface of these electrodes. These results indicate that the Pd_{CV}-SPCE exhibited electrochemical properties and cyclic voltametric behavior which were similar to those of the commercial Pd strip electrode (O'Neill et al., 2004).

Despite its similar cyclic voltammetric behavior, the Pd_{CV15}-SPCE exhibited a 10-fold higher electrochemical response to hydrogen peroxide than the commercial Pd strip electrode (Fig. 3C and D). It is possible that electrodeposition on the rough and jagged surface resulted in the generation of a larger reaction area on the electrode (Fig. 3B and C). The cyclic voltammogram of the Pd_{S100}-SPCE (Fig. 3B) showed a moderate charging current in the potential range of 0.5–0.7 V. Moreover, both the Pd sputtering-coated and Pd-electrodeposited SPCEs (Pd_{CV15}-SPCE) exhibited higher background current at 0.1 V in the cyclic voltammograms than that of the bare SPCE (Fig. 3). This result

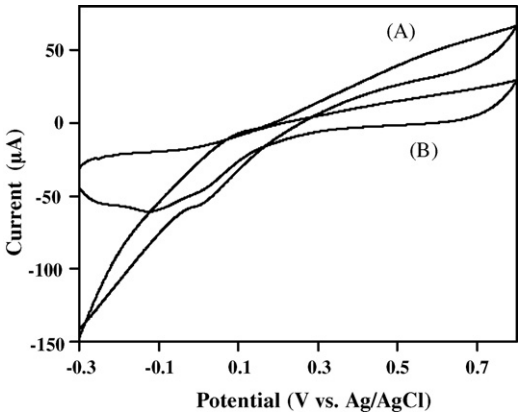

Fig. 4. Cylcic voltammograms of the Pd-SPCE determined in 100 mM phosphate buffer solution containing 1 mM H_2O_2 (A) and in 100 mM phosphate buffer solution (B).

Table 1
The H_2O_2 concentration-dependent responses of the SPCE and Pd-SPCE

Concentration (mM)	Response of the SPCE (nA)[a]	Response of the Pd_{CV}-SPCE (nA)[a]	Folds[b]
0.2	1.94 (11.16)[c]	323.85 (2.30)	167
0.4	3.83 (6.05)	637.49 (2.81)	167
0.6	6.45 (7.00)	927.65 (0.76)	144
0.8	9.63 (4.96)	1202.69 (0.46)	125
1.0	11.67 (3.94)	1440.71 (0.43)	123

[a] Each value is the mean of three measurements. Measurements were carried out at 37 °C in 100 mM sodium phosphate buffer (pH 7.0) under stirring. The polarizing potential applied to the sensor was 0.4 V vs. Ag/AgCl.
[b] Response of the Pd_{CV}-SPCE/response of the SPCE.
[c] Relative standard deviation.

could be partially explained by the enlargement of the surface area of the SPCE due to Pd modification by electrodeposition or sputtering.

The Pd-based catalytic scheme for measuring H_2O_2 has been described elsewhere (Hall et al., 1998a,b; Johnston et al., 1995). Under a sufficient anodic potential, Pd(0) is first oxidized to Pd(II) and then to $Pd(OH)_2$. The presence of $H_2O{\cdot}Pd(OH)_2$ offers bonding sites for H_2O_2 to allow subsequent reactions to occur (Hall et al., 1998a,b). As illustrated in Fig. 4, on a Pd-modified SPCE, the electrochemical response to H_2O_2 could be easily induced starting from 0.2 V (line A). This was presumably due to the drastic increase of $Pd(OH)_2{\cdot}H_2O_2$ on the electrode (Hall et al., 1998a,b). Under such circumstances, the conversion of $Pd(OH)_2{\cdot}H_2O_2$ to Pd(0) becomes the rate-limiting step so that the conversion from Pd(0) to Pd(II) can occur as soon as Pd(0) is generated. These results thus suggest that the palladinized electrode had substantially decreased applied potential and amplified oxidative current resulting from the oxidation of H_2O_2. The advantageous of Pd deposited on the SPCE in the catalytic activity of H_2O_2 was further demonstrated by electrochemical responses of SPCE and Pd_{CV}-SPCE to various concentrations of H_2O_2 (Table 1). The electrochemical responses of Pd_{CV}-SPCE to H_2O_2 showed 123–167-folds higher than that of unmodified SPCE.

3.3. Comparison of Pd-electrodeposited SPCE with Nafion/CNT-modified electrodes

An optimal working potential for Pd-modified SPCE to H_2O_2 could be estimated from the steady-state hydrodynamic voltammograms. The net steady-state anodic currents of H_2O_2 of bare, Nafion/carbon nanotube (CNT)-deposited and Pd-electrodeposited SPCEs were obtained within potentials ranging from 0 to 0.8 V (Fig. 5). Compared with the bare SPCE the electrochemical responses of the Pd-electrodeposited electrode to 1 mM H_2O_2 exhibited a sharp increase in response to applied potentials higher than 0.2 V. At 0.3 V, the steady-state anodic current of the Pd_{CV15}-SPCE (i_a = 5.533 μA) was about 100-fold higher than that of the bare SPCE (i_a = 0.055 μA), and about 70-fold (i_a = 22.181 μA) higher than that of the bare SPCE at 0.5 V. By contrast, the steady-state anodic currents to H_2O_2 of the bare SPCE remained unchanged within the potential range tested. Apparently, the improved sensitivity of the SPCE was associated with the electrocatalytic activity of the palladium surface coating to H_2O_2. Furthermore, the electrochemical responses of the Pd_{CV15}-SPCE to H_2O_2 were highly reproducible with a relative standard deviation (R.S.D.) lower than 6.0% when determining electrochemical responses of three electrodes to 1 mM H_2O_2 at three different potentials 0.4, 0.6 and 0.8 V. This observation indicates that the Pd-electrodeposited SPCE has good operational stability for multiple usage or continuous analysis. By contrast, the reproducibility between batches of the Pd_{CV}-SPCE was poor, with R.S.D.s of 18.8, 16.2 and 9.3% when determining electrochemical responses of three electrodes to 1 mM H_2O_2 at three different potentials.

The sensitivity and electrochemical characteristics of palladium-modified electrodes were also compared with those of Nafion/CNT-deposited electrodes. Interestingly, the Nafion/CNT-deposited SPCE (CNT-SPCE) exhibited a sigmoid steady-state anodic current curve with maximum current at 0.7 V (Fig. 5). Although the maximum steady-state anodic current of the Pd_{CV}-SPCE and CNT-SPCE was about the same at 0.7 V, the steady-state anodic currents of the CNT-SPCE were only about 12% (i_a = 0.647 μA) and 22% (i_a = 5.895 μA) of those of the Pd_{CV}-SPCE at 0.3 V (i_a = 5.533 μA) and 0.5 V (i_a = 22.181 μA),

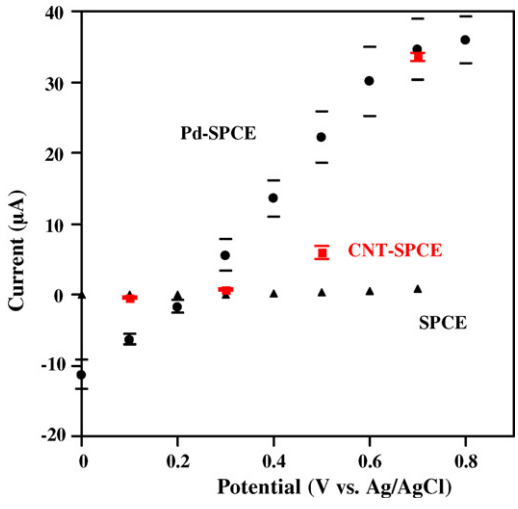

Fig. 5. Hydrodynamic voltammograms of (●) Pd_{CV15}-SPCE, (■) Nafion/CNT-deposited SPCE and (▲) bare SPCE to 1 mM H_2O_2. Measurements were carried out at 37 °C in 100 mM sodium phosphate buffer (pH 7.0) under stirring. The net steady-state anodic current was calculated by subtracting the background current from the H_2O_2 oxidative current at the designated working potential. The data are presented as mean ± S.D. of three independent experiments.

respectively (Fig. 5). This result indicates that deposition of Pd on the SPCE allows the modified electrode to detect H_2O_2 with a high sensitive even under a low applied potential, e.g. 0.4–0.5 V, which may not be feasible for the Nafion/CNT-deposited SPCE (Fig. 5).

Hydrogen peroxide could be effectively oxidized on the bare SPCE above a working potential of 0.6 V (Fig. 4, line B), a result consistent with the reported oxidative potential of hydrogen peroxide. However, under such a high working potential, interference from electroactive species, such as ascorbic acid, uric acid and acetaminophen, could also be induced. In the present study, the palladium-modified SPCE exhibited a very sensitive response to H_2O_2, even at a relatively low working potential (e.g. +0.3 to +0.5 V). Thus, in order to minimize the interference from electroactive species, an operation potential of +0.5 V was selected for the following experiments.

In previous studies, Pd_{CV}-SPCEs have been shown to exhibit higher electrochemical responses to hydrogen peroxide than Nafion/CNT-dispersed SPCEs (Fig. 5). In this study, the resistance to surface fouling of the Pd_{CV}-SPCE and Nafion/CNT-dispersion electrode was further investigated and compared. The Pd-modified electrode exhibited a consistent signal readout during 50 consecutive measurements of ascorbic acid (decrease in response to ascorbic acid of only 7%). This observation is in agreement with the findings of Matos et al. (2000). The Nafion/CNT-based electrode, however, showed a decrease in response to analyte over all of the 40 detection runs (up to 14–16%) (Wang and Musameh, 2003; Wang et al., 2004; Wang, 2005). The electrocatalytic detection of the Pd-SPCE is coupled to its resistance to surface fouling, which provides good stability.

3.4. Fabrication and characterization of PVA-SbQ/GOx/Pd-SPCE electrodes

The properties of the Pd-modified electrodes were further characterized by immobilizing glucose oxidase with PVA-SbQ on the modified SPCE and subjecting it to electrochemical analysis. The results showed that the glucose biosensor fabricated by using a Pd-electrodeposited SPCE (PVA-SbQ/GOx/Pd_{CV}-SPCE), exhibited a dramatic increase in electrochemical response to 1 mM glucose. The electrochemical responses of PVA-SbQ/GOx/Pd_{CV}-SPCE to glucose seemed to be affected by the amount of Pd deposited on the SPCE (Table 2). The PVA-SbQ/GOx/Pd_{CV10}-SPCE exhibited a steady-state anodic current of 3658 ± 248 nA; whereas the glucose biosensors fabricated from Pd_{CV}-SPCEs after 15 and 20 CV cycles, i.e. Pd_{CV15}-SPCE and Pd_{CV20}-SPCE, showed steady-state anodic currents of 5366 ± 259 and 4130 ± 232 nA, respectively. Compared with the glucose biosensor fabricated from an unmodified SPCE (14.5 nA) the electrochemical responses to 1 mM glucose increased 250–370-fold. However, the electrochemical response of glucose sensor made from PVA-SbQ/GOx/CNT-SPCE, to 1 mM glucose was 139 ± 10.6 nA. Notably, the glucose biosensors developed with Pd_{CV10}-, Pd_{CV15}- and Pd_{CV20}-SPCEs showed high reproducibility with R.S.D.s of 6.77, 4.82 and 5.63%, respectively. The electrochemical responses of bear SPCE or Pd-modified SPCE without enzyme was also examined. The result showed that their exhibited no electrochemical response to glucose (data not shown). This observation suggests that Pd_{CV}-SPCEs have a commercial potential for fabrication of biosensors despite moderately poor reproducibility between batches of prepared electrodeposited Pd-SPCEs. This may be due to the uneven deposition of Pd(0) on the SPCE surface, resulting from the uneven distribution of palladium chloride near the SPCE surface. Apparently, the ragged and porous SPCE surface could be the main cause of the heterogeneous reduction reaction of Pd(II) on the SPCE surface and hence the uneven distribution of the palladium chloride solution during electrodeposition.

Although the glucose biosensor made using a sputtering-deposited Pd_{S100}-SPCE exhibited a high electrochemical response (4865 ± 515 nA) to 1 mM glucose, it had poor reproducibility with an R.S.D. of around 10.6% (Table 2). This might be attributable to loosely bound Pd particles in the outer layer of the electrode-during sputtering, which could easily be stripped during the first several runs of electrochemical measurements. Hence, the electrochemical pre-treatment of the Pd-modified electrodes with cyclic voltammetry (ranging from 0 to 1 V versus Ag/AgCl) in phosphate buffer, pH 7.4 was performed to try to improve the reproducibility of SPCEs developed using Pd sputtering (Zhang and Rechnitz, 1994; Yamato et al., 1997). The result shows that the reproducibility of glucose biosensor fabricated from electrochemically pre-treated Pd_S-SPCE increased from the R.S.D. of 10.6% to the R.S.D. of 5.13% (data not shown), which is similar to that for biosensors made from electrodeposited Pd_{CV15}-SPCE (Table 2).

3.5. Measurement of glucose concentration and reproducibility of the glucose sensor

The cyclic voltammetry pre-treated PVA-SbQ/GOx/Pd_{S100}-SPCE biosensor exhibited a linear calibration range for glucose concentrations from 0.5 to 1000 μM (data not shown), with a slope of 4.1 nA/μM and a correlation coefficient of 0.993 ($n = 11$). The detection limit of glucose was 0.5 μM (S/N = 3). The pre-treated PVA-SbQ/GOx/Pd_{S100}-SPCE biosensor exhibited a good reproducibility with a R.S.D. of <5.0% when the glucose concentration was 50 μM or below. This result indicated that PVA-SbQ/GOx/Pd_{S100}-SPCE exhibited a good operational

Table 2
Comparison of the response and reproducibility of PVA-SbQ/GOx/Pd-SPCEs developed using carbon paste electrodes modified by Pd via CV and sputtering

Modification	Response (nA)[a]	S.D. (nA)	R.S.D. (%)
SPEC	14.5	–	–
Pd_{CV10}	3658	248	6.8
Pd_{CV15}	5366	259	4.8
Pd_{CV20}	4130	232	5.6
Pd_{S100}	4865	515	10.6
CNT-SPCE	139	10.6	6.9

[a] Each value is the mean value of three independent measurements. Measurements were carried out in 1 mM glucose at 37 °C in 100 mM sodium phosphate buffer (pH 7.0) under stirring. The working potential applied to the sensor was 0.5 V vs. Ag/AgCl.

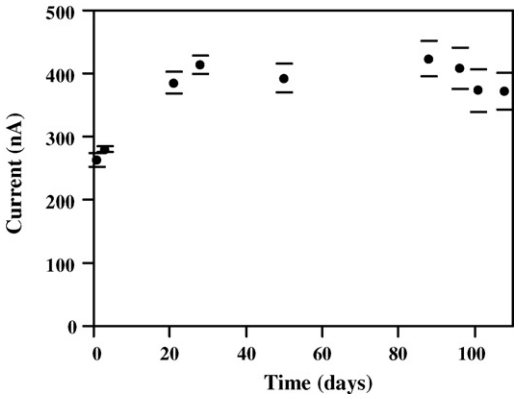

Fig. 6. Long-term stability of PVA-SbQ/GOx/Pd_{S100}-SPCE. The PVA-SbQ/GOx/Pd_{S100}-SPCE was stored under dark and dry conditions and was placed in phosphate buffer at 4 °C before testing. Measurements were performed with 100 μM glucose as substrate in 100 mM sodium phosphate buffer (pH 7.0) at 37 °C. Each value is the mean ± S.D. of three independent measurements. The working potential applied to the sensor was 0.5 V vs. Ag/AgCl.

stability for multiple-usage and continuous analysis. This study also revealed that the fabricated glucose biosensor was quite reliable when detecting low concentrations of substrate, but only moderately reliability when higher concentrations of substrate were detected.

3.6. Long-term stability of the glucose sensor

The stability of the glucose sensors made from various Pd-coated SPCEs stored dry at 4 °C was investigated. The electrochemical responses of stored glucose biosensors were tested periodically during storage. The PVA-SbQ/GOx/Pd_{CV15}-SPCE showed no apparent change in the electrochemical responses over a storage period of 5 months (data not shown). The long-term stability of PVA-SbQ/GOx/Pd_{S100}-SPCE prepared from cyclic voltammetric pre-treated Pd_{S100}-SPCE was examined by determining electrochemical responses to 100 μM glucose three times a month over a 108-day storage period. Prior to electrochemical analysis, the electrodes were pre-wetted by immersion in the phosphate buffer solution (pH 7.0) for 30 min. As shown in Fig. 6, after 1 day of storage, the electrochemical response of PVA-SbQ/GOx/Pd_{S100}-SPCE was increased about 30% compared with the initial response, and at 21 days of storage was increased about 50%. These changes may be attributable to the formation of an optimal matrix structure of PVA-SbQ polymer during storage that allowed oxygen and/or substrates to rapidly access the encapsulated enzyme. The changes in structure leading to the increased response might be due to buffer mediated matrix swelling or the loss of soluble PVA-SbQ from polymer matrix due to generation of a porous polymer matrix resulting from immersion in the buffer (Rouillon et al., 1994). About 10% soluble PVA-SbQ monomers leaked from the polymer matrix when the content of SbQ in the polymer was 1.1% (Rouillon et al., 1994). Although the response of the PVA-SbQ/GOx/Pd_{S100}-SPCE to 100 μM glucose gradually increased during storage from 1 to 21 days, the reproducibility of the prepared enzyme electrode remained good with R.S.D. lower than 5%. The electrochemical responses of the prepared glucose biosensor reached a plateau at day 21 of storage which was unchanged for the remainder of the storage period (Fig. 6). This result suggests that enzyme can be stably entrapped in the PVA-SbQ polymer matrix by cross-linking with glutaraldehyde. The high stability of entrapped enzymes can also be attributed to the presence of a great number of OH groups in the matrix; the enzymes are kept in a hydrophilic environment even in a dry state, thereby preventing enzyme denaturation (Jaffrezic-Renault et al., 1999).

4. Conclusion

SPCE modification by Pd electrodeposition or sputtering markedly enhanced electrochemical responses to hydrogen peroxide, resulting in higher electrochemical responses to the oxidation of hydrogen peroxide than Nafion/CNT-dispersed SPCE. The electrocatalytic detection with a Pd-SPCE also provides the additional benefit of resistance to surface fouling, which results in good stability. The glucose biosensor made from Pd_{CV}-SPCE showed 250–370-fold higher response to 1 mM glucose than that fabricated from the unmodified SPCE. The glucose biosensors based on Pd-modified SPCEs maintained stable response characteristics over a storage period of 108 days. The palladium modified electrodes developed in this study exhibited several advantages over transition metal particle-dispersed and/or CNT-dispersed electrodes, such as high electrochemical response, easy fabrication compared to particle dispersion electrodes and low cost. In addition to the fact that the CNT-modified electrodes suffer from problems of insolubility of CNT during doping (Wang and Musameh, 2002) and fragility of CNT during storage (Rubianes and Rivas, 2003), the CNT-modified electrode in this study was less stable than that made from Pd-modified SPCE. The electrode-based biosensor based on this construction lost about 40% of its initial response to analytes within 50 days (Lim et al., 2005).

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References

Albareda-Sirvent, M., Merkoci, A., Alegret, S., 2000. Rev. Sens. Actuat. B 69, 153–163.
Cai, X., Kalcher, K., Kolbl, G., Neuhold, C., Diewald, W., Ogorevc, B., 1995. Electroanalysis 7, 340–347.
Castillo, J., Gáspár, S., Leth, S., Niculescu, M., Mortari, A., Bontidean, I., Soukharev, V., Dorneanu, S.A., Ryabov, A.D., Csöregi, E., 2004. Sens. Actuat. B 102, 179–194.
Chang, K.S., Hsu, W.L., Chen, H.Y., Chang, C.K., Chen, C.Y., 2003. Anal. Chim. Acta 481, 199–208.
Chi, Q., Dong, S., 1993. Anal. Chim. Acta 278, 17–23.
Cui, G., Yoo, J.H., Lee, J.S., Yoo, J., Uhm, J.H., Cha, G.S., Nam, H., 2001. Analyst 126, 1399–1430.

Gao, Q., Cui, X., Yang, F., Ma, Y., Yang, X., 2003. Biosens. Bioelectron. 19, 277–282.
Hall, S.B., Khudaish, E.A., Hart, A.L., 1998a. Electrochim. Acta 43, 579–588.
Hall, S.B., Khudaish, E.A., Hart, A.L., 1998b. Electrochim. Acta 43, 2015–2024.
Jae, G.C., Yoo, H., Lee, J.S., Yoo, J., Uhm, J.H., Cha, G.S., Nam, H., 2001. Analyst 126, 1399–1403.
Johnston, D.A., Cardosi, M.F., Vaughan, D.H., 1995. Electroanalysis 7 (6), 520–526.
Jaffrezic-Renault, N., Senillou, A., Martelet, C., Wan, K., Chovelon, J.M., 1999. Sens. Actuat. B 59, 154–164.
Kuwabata, S., Martin, Ch.R., 1994. Anal. Chem. 66, 2757–2762.
Lim, S.H., Wei, J., Lin, J., Li, Q., You, J.K., 2005. Biosens. Bioelectron. 20, 2341–2346.
Lubert, K.H., Guttmann, M., Beyer, L., Kalcher, K., 2001a. Collection Czechoslovak Chem. Commun. 66, 1457–1472.
Lubert, K.H., Guttmann, M., Beyer, L., Kalcher, K., 2001b. Electrochem. Commun. 3, 102–106.
Mizutani, F., Sato, Y., Sawaguchi, T., Yabuki, S., Iijima, S., 1998a. Sens. Actuat. B 52, 23–29.
Mizutani, F., Sato, Y., Hirata, Y., Sawaguchi, T., Yabuki, S., 1998b. Anal. Chim. Acta 364, 173–179.
Matos, R.C., Augelli, M.A., Lago, C.L., Angnes, L.L., 2000. Anal. Chim. Acta 404, 151–157.
Malhotra, B.D., Chaubey, A., 2003. Sens. Actuat. B 91, 117–127.
Nowall, W.B., Kuhr, W.G., 1995. Anal. Chem. 67, 3583–3588.
Newman, J.D., White, S.F., Tothill, I.E., Turner, A.P.F., 1995. Anal. Chem. 67, 4594–4599.
O'Connell, P.J., O'Sullivan, C.K., Guilbault, G.G., 1998. Anal. Chim. Acta 373, 261–270.
O'Neill, R.D., Chang, S.C., Lowry, J.P., McNeil, C.J., 2004. Biosens. Bioelectron. 19, 1521–1528.
Os, P.J.H.J., Bult, A., Koopal, C.G.J., Bennekom, W.P., 1996. Anal. Chim. Acta 335, 209–216.
Rouillon, R., Tocabens, M., Marty, J.L., 1994. Anal. Lett. 27, 2239–2248.
Rubianes, M.D., Rivas, G.A., 2003. Electrochem. Commun. 5, 689–694.
Sakslund, H., Wang, J., 1994. J. Electroanal. Chem. 374, 71–79.
Sarkar, P., Ibtisam, E.T., Steven, J.S., Turner, A.P.F., 1999. Analyst 124, 865–870.
Thévenot, D.R., Toth, K., Durst, R.A., Wilson, G.S., 2001. Biosens. Bioelectron. 16, 121–131.
Wang, J., Pedrero, M., Pamidi, P.V.A., Cai, X., 1995. Electroanalysis 7, 1032–1035.
Wang, J., Pedrero, Sakslund, H., Hammerich, O., Pingarron, J., 1996. Analyst 121, 345–350.
Wang, J., Chen, Q., 1994. Analyst 119, 1849–1851.
Wang, J., Musameh, M., 2002. Anal. Chem. 74, 1993–1997.
Wang, J., Musameh, M., 2003. Anal. Chem. 75, 2075–2079.
Wang, J., Naser, N., Angnes, L., Wu, H., Chen, Q., 1992. Anal. Chem. 64, 1285–1288.
Wang, J., Chen, Q., Chatrathi, M.P., Musameh, M., 2004. Anal. Chem. 76, 298–302.
Wang, J., 2005. Electroanalysis 17, 7–14.
White, S.F., Turner, A.P.F., Schmid, R.D., Bilitewski, U., Bradley, J., 1994. Electroanalysis 6, 625–632.
Xu, F., Wang, L., Gao, M.N., Jin, L.T., Jin, J.Y., 2002. Talanta 57, 365–373.
Yamato, H., Koshiba, T., Ohwa, M., Wernet, W., Matsumura, M., 1997. Synth. Met. 87, 231–236.
Yoon, H.C., Kim, H.S., 1996. Anal. Chim. Acta 336, 57–65.
Yuan, C.J., Hsu, C.L., Wang, S.C., Chang, K.S., 2005. Electroanalysis 17, 2239–2245.
Zhang, S., Wright, G., Yang, Y., 2000. Biosens. Bioelectron. 15, 273–282.
Zhang, X., Rechnitz, G.A., 1994. Electroanalysis 6, 361–367.