Bimetallic PdCu/SPCE Non-Enzymatic Hydrogen Peroxide Sensors

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Abstract

Screen printed carbon electrodes (SPCE) were decorated with PdCu bimetallic alloys via a facile co-electrodeposition method to develop disposable non-enzymatic H₂O₂ sensors. The electrochemical performance of the biosensors was evaluated in terms of selectivity, sensitivity, and stability with a goal of demonstrating that employing a bimetallic PdCu non-enzymatic system can push forward the state of the art in hydrogen peroxide sensing. The physical characterization of the biosensors was conducted using Scanning Electron Microscopy (SEM), Energy-Dispersive X-ray Spectroscopy (EDS), and X-ray Diffraction (XRD) techniques. Sensors consisting of PdCu bimetals showed higher sensitivity than Pd/SPCE and Cu/SPCE electrodes towards H₂O₂. The fabricated PdCu/SPCE sensors showed a sensitivity of 396.7 $\mu\text{A mM}^{-1} \text{cm}^{-2}$, a linear range from 0.5 mM to 11 mM, and a low limit of detection (0.7 μM) at the applied potential of -0.3 V. The use of relatively low working potential eliminated the interference effect of the common electroactive species (ascorbic acid, uric acid, and glucose) present in a real sample, which are usually a concern for non-enzymatic sensing systems. In addition, the high reproducibility (RSD = 2.5 %), and excellent long term stability render PdCu/SPCEs as attractive materials for the construction of disposable enzyme-free H₂O₂ sensors.

Keywords: Palladium, copper, electrodeposition, hydrogen peroxide sensors, non-enzymatic, disposable sensors.

1. Introduction

Electrochemical sensing of H₂O₂ has become crucial in industrial, clinical, food, and environmental settings, creating a constant need for simpler and more effective H₂O₂ sensors, as well as methods for fabricating these sensors [1-4]. Also, H₂O₂ is produced in

many enzymatic reactions, which enables the detection of the chemical species involved in them [5-7]. Although there are various analytical techniques used for the detection of H_2O_2 including photometry, chemiluminescence, chromatography, titrimetry, colorimetry, an alternative, simpler, detection method is still to be designed [8]. Electrochemical methods are the most promising among all current hydrogen peroxide detection methods due to their high sensitivity, selectivity, and simplicity [8-10]. Peroxidase and heme proteins have been used to construct highly sensitive and selective electrochemical H_2O_2 sensors [1, 11-13]. However, enzyme based biosensors experience instability and enzyme denaturation over time in various environments [14, 15]. The better reproducibility and stability of non-enzymatic sensors increased their attractiveness as compared to enzymatic sensors [16].

Palladium (Pd) has gained much attention recently because of its favorable catalytic activity and low cost compared to other noble metals [17-21]. Therefore, Pd has found many applications as a catalyst material in the fields of hydrogen production, low temperature fuel cells and chemical sensors [12, 22-26]. A great deal of effort has been devoted to enhance the catalytic activity and the utilization of the noble metals, which in turn reduce the cost of the catalyst layers in electrochemical systems [27]. It was shown that the catalytic activity of noble metals can be enhanced by the introduction of dissimilar metal ions into the lattice [23, 28, 29]. In addition, bimetallic nanoparticles have become a promising candidate for the construction of electrochemical biosensors due to enhanced selectivity and sensitivity compared to pristine metal nanoparticles [29, 30]. The enhancement in the performance of the biosensors is attributed to the change in shape, surface structure, particle size, and chemical properties of the bimetallic catalysts induced by the interaction between the dissimilar metal atoms [31]. In the last couple of decades, various binary and ternary metallic systems were studied to develop a catalysts material with high catalytic activity and low cost [19]. Previously published works showed that Pd and

its alloys are promising catalyst materials for the electrochemical detection of H_2O_2 . For instance, Qiu et al. [24] prepared nanoporous PdCu alloys by a dealloying process and reported an remarkable enhancement in the activity of the biosensor toward H_2O_2 . Bimetallic catalyst systems can be prepared via a variety of methods including sputtering, chemical vapor deposition, pulse laser, colloidal deposition, melting, and electrochemical techniques [32-37].

Electrodeposition, also known as electroplating, is an electrochemical method which enables the formation of metal coating or particles on the surface of a conducting material [38]. Electrodeposition has three important advantages over other methods; (i) this technique is useful for both large and small scale production, (ii) no complicated procedure is required, (iii) preparation of PdCu alloys with electrodeposition is more economical compared to other methods [39]. Electrodeposition of metal nanoparticles and metal hexacyanoferrates has been used to prepare electrochemical sensors with enhanced performance for the detection of hydrogen peroxide and glucose [30, 40].

In this study, we report on the construction of novel disposable hydrogen peroxide sensors based on PdCu decorated screen printed carbon electrodes (SPCEs). The sensors were prepared in only 10 minutes using a facile co-electrodeposition method in the absence of any surfactants. To the best our knowledge, this approach of fabrication of disposable non-enzymatic hydrogen peroxide sensors has not been reported yet. The electrochemical performance of the biosensors was evaluated in terms of selectivity, sensitivity, and stability with a goal of demonstrating that employing a bimetallic PdCu non-enzymatic system can push forward the state of the art in hydrogen peroxide sensing.

2. Experimental

PdCl₂, CuCl₂, sodium-L ascorbate, uric acid, and D-(+)-glucose were obtained from Sigma. Hydrogen peroxide (H₂O₂) and perchloric acid (HClO₄) were purchased from Riede-de Haen and Mallinckrodt, respectively. Screen printed carbon electrodes (SPCE, 4x5 mm) was obtained from Pine Research Instrumentation.

The electrodeposition of metals was conducted using a 3 electrode cell and a potentiostat obtained from BASi (West Lafayette, IN) and Biologic, respectively. The deposition baths were prepared by dissolving required amount of PdCl₂ and CuCl₂ precursors in 0.1 M HClO₄ solution. Co-electrodeposition of PdCu on the screen printed carbon electrodes (SPCEs) (0.2 cm²) was performed in different electrodeposition baths containing certain amounts of Pd and Cu precursors using a constant-potential electrodeposition process at room temperature. While the concentrations of each of the metal ions were adjusted depending on the desired Pd/Cu ratio, the total metal ion concentration of the solution was fixed at 10 mM. In order to obtain Pd:Cu (1:3) molar ratio, for example, a homogeneous electrodeposition bath containing 7 mM Pd and 3 mM Cu was prepared. The co-electrodeposition process was conducted at -0.5 V vs. Ag/AgCl. Pt wire and Ag/AgCl were used as the counter and reference electrodes, respectively. Argon gas was purged through the electrodeposition bath to remove oxygen molecules from the solution.

PdCu particles deposited on SPCEs were observed by scanning electron microscopy (FEI Philips XL-40) coupled with an X-ray energy dispersive spectroscopy system (EDX), which was used to determine the chemical composition of the deposits. X-ray

diffraction of the samples were conducted using a Bruker D8 Focus X-ray diffractometer (XRD) with Cu K α ($\lambda=0.15406$ nm) radiation.

The same electrochemistry unit was implemented to carry out the electrochemical performance of the constructed biosensors towards H₂O₂. Phosphate buffer solution (PBS, 0.1 M, pH 7.4) was used as the electrolyte during the experiments. The electrochemical properties of the constructed sensors were evaluated at the working potential of -0.3 V.

3. Results and Discussion

3.1. Preparation of PdCu Decorated SPCEs

In order to determine the optimum PdCu loading on the surface of the SPCEs, the effect of electrodeposition time on the catalytic activity of the constructed biosensors towards H₂O₂ was studied. For this purpose, the surface of screen printed carbon electrodes were decorated with Pd for varying deposition times ranging from 5 to 30 minutes. The relationship between the electrodeposition time and activity of the sensors are shown in Figure 1a. It was observed that when the deposition time of Pd was extended from 5 min to 10 min, a significant rise in the response current of Pd/SPCE electrode was obtained. On the other hand, the further increase in the deposition time resulted in a drop in the activity of the electrodes. When the deposition time was longer than 10 min, the response current of the electrodes towards 5 mM H₂O₂ decreased, which may be attributed to the reduction in the total area of the catalytic sites due to the deposition of large amount of Pd metal on the electrode surface. The electrodes prepared with the deposition time of 30 minutes showed slightly higher activity than that of prepared with 20 minute electrodeposition time. This may be due to a slight

increase in the total metal surface area available for H_2O_2 reduction. Based on these results, the deposition time of 10 min was found to be the optimum value and selected as the deposition time for further experiments.

After the optimization of the deposition time, the effect of the composition of the electrodeposition bath was investigated using deposition baths consisting of varying Pd/Cu molar ratios. While the total concentration of the electrodeposition bath was fixed to 10 mM, the molarity of each metal ion was altered to observe the impact of the co-electrodeposition of Pd and Cu metals on the surface of the electrodes. As shown in Figure 1b, the addition of Cu^{2+} ions up to the concentration of 3 mM into the electrodeposition bath enhanced the performance of the biosensors. In other words, the electrodeposition of Cu metal on the electrode surface enhanced the activity of Pd towards H_2O_2 . The effect of the introduction of Cu^{2+} into the deposition bath on the performance of the electrochemical H_2O_2 sensors was first observed by Guadagnini et al. [41]. The authors reported that Cu^{2+} -loaded copper hexacyanoferrate film electrodes prepared via an electrodeposition method showed a higher performance compared to its pristine counterpart [41]. It is noteworthy that since total concentration of the electrodeposition bath was fixed to 10 mM, the sensor prepared in the bath consisting of 7 mM Pd^{2+} and 3 mM Cu^{2+} ions had a smaller amount of Pd metal on its surface compared to those decorated in electrolytes with 10 mM Pd^{2+} . On the other hand, further increase in the molarity of Cu ions in the deposition bath led to a significant decrease in the catalytic activity of Pd. The decrease in the performance of the electrode is attributed to the presence of excess amount of Cu metal which has much lower catalytic property towards H_2O_2 compared to Pd.

The representative SEM images of electrodeposited PdCu bimetals on screen printed carbon electrodes are shown in Figure 2 a-b. It was observed that the prepared PdCu alloy had porous and dendrite-like structures exposing a large electroactive surface area for H₂O₂ reduction. The EDS results obtained from the deposits confirmed the coelectrodeposition of Pd and Cu on SPCEs (Figure c-d). The composition of the alloy revealed that the average atomic ratio of Pd to Cu was found to be 7.2:2.8, which is consistent with the composition of the electrodeposition bath. The atomic ratio of Pd to Cu revealed that, however, the deposition rate of Pd is slightly higher than Cu at the working potential of -0.5 V.

The XRD patterns of the prepared Pd/SPCE and PdCu/SPCE are shown in Figure 3. The characteristic XRD peaks of Pd are located at 40.1°, 46.7°, 68.1°, and 82.1° corresponding to (111), (200), (220), and (311) planes [19]. The XRD results obtained from the samples, however, showed only weak (111) peak located at around 40°. This is due to the fact that the contribution of screen printed carbon electrode was much higher than that of Pd and PdCu deposits to the XRD signal, which in turn obscured the signals obtained from Pd and PdCu deposits. When the XRD pattern obtained from the blank SPCE was subtracted from those obtained from Pd/SPCE and PdCu/SPCE, the peak located at ca. 40.1° became more visible (inset of Figure 4). The inset revealed that when the co-electrodeposition of Pd and Cu was carried out, the (111) peak shifted slightly towards higher 2θ values, confirming the formation of Pd-Cu solid solution. Although PdCu deposit includes approximately 30 % at. of Cu, no additional phase associated with Cu was observed.

3.2. Electrochemical Performance of the Constructed Electrodes

The influence of the scan rate on the CV results of the PdCu/SPCE electrodes was studied in the presence of 0.5 mM H₂O₂ in the PBS solution. The CV voltammograms of the electrode at different scan rates ranging from 10 to 400 mVs⁻¹ are shown in Figure 5. It was found that the reduction of H₂O₂ on PdCu/SPCE surface had diffusion controlled electron transfer process since the cathodic peak current showed a linear relation with the square roots of the scan rates. This relationship is shown as the inset of Figure 4. The CV results also revealed that the increase in the scan rate shifted the reduction potential to more negative values indicating an irreversible reduction process [42].

The optimum working potential for H₂O₂ reduction on PdCu/SPCE surface was determined by measuring the response current of the electrodes at various working potentials ranging from 0 to -0.5 V. The current vs. applied potential graph reached a plateau at -0.3 V, which is shown in Figure 5. Although H₂O₂ can also be reduced at 0.3 V, which is seen in Figure 5, our primarily results showed that the reduction current of H₂O₂ obtained at that voltage was lower than that obtained at -0.3 V (not shown in the manuscript). Therefore, to obtain the higher catalytic activity, a constant potential of -0.3 V was used for the electrochemical characterization of constructed sensors.

The amperometric response of the metal decorated SPCE sensors was investigated at the working potential of -0.3 V by successive addition of 0.5 mM H₂O₂ into PBS solution (Figure 6a). It was observed that the highest sensitivity value was obtained from PdCu decorated electrode showing that PdCu solid solution has higher catalytic activity towards H₂O₂ compared to its constituents. The sensitivity of the constructed sensors followed the order of PdCu/SPCE > Pd/SPCE > Cu/SPCE. It is known that the

performance of monometallic noble metal catalysts can be enhanced by the introduction of dissimilar noble metal or transition metal atoms into the system, which is fully compatible with the result obtained from this work. Although noble metal-noble metal alloys show higher catalytic properties than noble metal-nonnoble metal catalysts, nonnoble metal containing systems offer an economical way to achieve enhanced activity. The increase in the catalytic activity of Pd is attributed to the change in its electronic structure induced by alloy formation [43]. Another important factor affecting the catalytic properties of Pd is its dispersion on the electrode surface. Haller et al. [43] stated that the formation of PdCu alloy plays a role in maintaining and enhancing Pd dispersion. The current-concentration plots of the sensors shown in Figure 6b revealed that although the Pd containing electrodes had a good linear relationship between current and concentration in the range of 0.5 to 6.5 mM, the Cu/SPCE electrode displayed slight deviation from linearity ($R^2 = 0.9369$). When 0.5 mM H_2O_2 was added into PBS solution, Pd-based electrodes showed faster response compared to Cu/SPCE indicating that Pd based metals have higher catalytic activity towards H_2O_2 reduction compared to Cu, which was also evidenced by the sensitivity values of the electrodes.

Figure 8 shows the current-concentration graph of a PdCu/SPCE sensor obtained by the addition of H_2O_2 in PBS solution up to 12 mM at the working potential of -0.3 V. The sensor showed a good linearity up to 11 mM. The response of the sensor started to deviate from the linearity when more than 11 mM H_2O_2 was added into PBS. The sensitivity of the PdCu/SPCEs was found to be 79.34 $\mu A/mM$ (%RSD = 2.5, n=3). The low % RSD value indicates the good reproducibility of the sensors. The limit of detection (LOD) calculated using the equation 1 was 0.7 μM .

$$\text{LOD} = \text{std}_{\text{background}}/S \quad (\text{S: sensitivity, Std: standard deviation}) \quad \text{Eq. 1}$$

The effect of the presence of molecules, which are well known interferants to the detection of hydrogen peroxide, on the sensor performance, was investigated by the addition of 0.1 mM uric acid (UA), ascorbic acid (AA), and glucose into PBS solution at a working potential of -0.3 V. It is known that the presence of these species results in a significant change in the response current of the biosensors due to their highly electro-active nature, especially at relatively high working potentials [7]. Since no permselective polymer layer was deposited on the surface of PdCu/SPCEs, it is imperative to have minimum interference effect to detect H₂O₂ concentration correctly. The *i*-t curve obtained by the consecutive injection of H₂O₂ and potential interfering species is shown in Figure 8. It was found that the interfering effect of AA, UA, and glucose is completely negligible at the working conditions of the constructed biosensors; thus, the biosensors can be used to detect H₂O₂ in real samples. The use of negative working potential, -0.3 V, allowed us to construct interference free PdCu/SPCE biosensors without any permselective polymer film.

The sensitivity, LOD, linear response range, and working potential of the constructed biosensors were compared with previously published results (Table 1). The constructed PdCu/SPCEs showed a comparable sensitivity with nanoporous PtPd/C/GCE and PtPd/MWCTN/GCE electrodes [23, 29]. In our work, however, a relatively lower working potential was used during the amperometric detection of the analyte, which brought in the advantage of removing the importance of the interfering species present in a real sample. This is a significant characteristic, since the most important challenge of non-enzymatic biosensors is maintaining the specificity that is usually conferred by enzymatic reactions. In addition, a wider linear range was obtained from our sensors compared

to some of the previously published results (Table 1). As mentioned before, the high catalytic activity of PdCu/SPCE sensors towards H₂O₂ reduction may be attributed to the formation of Pd-Cu solid solution.. The stability of the sensors was examined using the response current to the addition of 5 mM H₂O₂ into PBS solution. The stability tests were carried out for one month and the electrode was kept at 4 °C when not in use. As shown in Figure 9, after 35 days, no drop in the response current of the sensor was observed, indicating excellent long term stability, thus overcoming the main disadvantage of the enzymatic biosensor counterparts.

Table 1. Comparison of the analytical performance of the constructed PdCu/SPCE with previously published studies.

Sensor	Sensitivity ($\mu\text{A mM}^{-1} \text{cm}^{-2}$)	Linear range (mM)	LOD (μM)	Applied Potential (V vs. Ag/AgCl)	Ref.
PdNPs/Nafion/GCE	766	0.0001-9	0.018	-0.2	[42]
PdNPs-GNs/GCE	NA	0.0001-10	0.05	-0.25	[44]
HRP-Pd/Gr	92.82	0.025-3.5	0.05	-0.05	[12]
Nanoporous PtPd/C/GCE	239.8	up to 3	114	+0.3	[29]
Nanoporous PtCu/C/GCE	69.4	up to 4	12.2	+0.3	[29]
AgNP/SnO ₂ /GCE	NA	0.01-35	5	-0.3	[8]
Nanoporous PdCu/GCE	NA	0.5-20	1	+0.6	[24]
Cu/PSi/CPE	NA	0.5-3.8	0.27	-0.2	[10]
PdNPs/PEDOT/GCE	215	0.0025-1	2.84	-0.4	[45]
PtPd/MWCTN/GCE	414.8	0.0025-0.125	1.2	+0.3	[23]

PdCu/SPCE	396.7	0.5-11	0.7	-0.3	This work
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4. Conclusion

PdCu/SPCEs were prepared using a constant-potential co-electrodeposition technique to construct highly selective non-enzymatic amperometric H₂O₂ sensors with a high sensitivity and selectivity. Since the optimum working potential of the sensor was -0.3 V, no interference effect of AA, AU, and glucose was observed indicating that the electrodes can be used to detect H₂O₂ in real samples and no permselective membrane is required. The highest sensitivity was obtained with the electrode prepared in an electrodeposition bath consisting of 7 mM Pd²⁺ and 3 mM Cu²⁺ at the deposition potential of -0.5 V for 10 min. A linear range from 0.5 mM to 11 mM and a low limit of detection (0.7 μM) were obtained from the constructed sensors. These sensors are promising candidates for the construction of commercial disposable H₂O₂ biosensors due to their high selectivity, sensitivity, excellent long term stability, ease of fabrication, and low cost.

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Figure 1a and 1b.

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 1. A) Effect of the electrodeposition time on the response current of Pd/SPCE sensors. B) Variation of the response current of the sensors with increasing Cu^{2+} concentration in the deposition bath.

Figure 2. (A-B) SEM images of the electrodeposited PdCu on SPCEs. (C-D) Representative EDS results of the deposits.

Figure 3. X-Ray diffraction patterns of SPCE, Pd/SPCE, and PdCu/SPCE. (inset: Pd (111) peak obtained from Pd and PdCu deposits.)

Figure 4. Cyclic voltammograms of PdCu/SPCE in the presence of 0.5 mM H_2O_2 in PBS solution at various scan rates. The inset shows the linear relationship between the peak current and square root of the scan rate.

Figure 5. Response current values of PdCu/SPCE to the addition of 5 mM H_2O_2 into PBS solution at different applied potentials.

Figure 6. (A) Current-time curves of Pd, Cu, and PdCu decorated SPCEs obtained by the successive addition of 0.5 mM H_2O_2 into PBS at the applied potential of -0.3 V. (B) Current-concentration curves of the sensors obtained by the successive addition of 0.5 mM H_2O_2 at the applied potential of -0.3 V.

Figure 7. (A) Current-concentration curve of a PdCu/SPCE obtained by the successive addition of 1 mM H_2O_2 into PBS at the applied potential of -0.3 V. The inset shows the response of the PdCu/SPCEs to the addition of 0.5 mM H_2O_2 into PBS (n=3).

Figure 8. Amperometric responses of PdCu/SPCE to the successive addition of 0.5 mM H_2O_2 and 0.1 mM potential interferences at the working potential of -0.3 V.

Figure 9. Long term stability of PdCu/SPCE in 35 day period (addition of 5 mM H_2O_2 into PBS solution).

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