

Nanomolar amperometric sensing of hydrogen peroxide using a graphite pencil electrode modified with palladium nanoparticles

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Abstract We report on a simple and rapid method for the preparation of a disposable palladium nanoparticle-modified graphite pencil electrode (PdNP-GPE) for sensing hydrogen peroxide (H_2O_2). The bare and PdNP-modified GPEs were characterized by cyclic voltammetry and SEM. The two electrodes displayed distinct electrocatalytic activities in response to the electrochemical reduction of H_2O_2 . The amperometric detection limits were 45 nM and 0.58 mM, respectively, for the PdNP-GPE and bare-GPE, at an S/N of 3. The electrodes can be prepared simply and at low cost, and represent a promising tool for sensing H_2O_2 .

Keywords Palladium nanoparticles · Graphite pencil electrode · Chemical preparation · Hydrogen peroxide · Amperometric sensor

Introduction

Hydrogen peroxide (H_2O_2) is an important analyte in the fields of food science, pharmaceutical development, chemical engineering, clinical practice, printing applications, and environmental sciences [1, 2]. H_2O_2 is a byproduct of a variety of enzymatic reactions, including the peroxidase enzymes: glucose oxidase and lactate oxidase [3, 4]. H_2O_2 is toxic to living organisms [5]; thus, the development of

simple, sensitive, inexpensive, disposable, and accurate H_2O_2 detection sensors is very important.

Electrochemical methods are attractive for their simplicity, sensitivity, and portability; however, conventional electrodes are relatively insensitive to the electrochemical reduction of H_2O_2 . More sensitive hydrogen peroxide enzyme-based sensors with high catalytic activities have been developed based on HRP-, myoglobin-, and cytochrome C-modified electrodes [6–8]. The complexities of enzyme immobilization procedures, instabilities, and high reagent (enzyme and substrate) have limited the use of enzyme-modified electrodes for the detection of H_2O_2 .

Nanomaterials with excellent electrocatalytic properties, a high surface-to-volume ratio, a high stability, and a low cost have been introduced as electrode surface modifications in an effort to develop more sensitive probes [9–13]. Some examples of modified electrodes that have been applied toward the electrochemical detection of H_2O_2 include: palladium nanoparticles (PdNPs) embedded in a layer of hierarchically porous TiO_2 hollow spheres immobilized on a glassy carbon electrode (GCE) [9], multi-walled carbon nanotube (MWCNT)-PdNPs immobilized on a GCE [10], CNT CuO nanoflowers immobilized on a GCE [11], composites of carbon nanotubes and silver NPs immobilized on a carbon ceramic electrode [12], and silver NPs immobilized in a carbon ion liquid electrode [13].

Platinum, gold, and glassy carbon conventional electrodes are expensive and display high background currents, unlike graphite pencil electrodes (GPEs), which have renewable surfaces, are low in cost, and provide a relatively low background current [14, 15]; however, GPEs are not very electrocatalytically active toward the electrochemical reactions of many electroactive molecules. The surfaces GPEs must, therefore, be modified with particular electrocatalysts for the fabrication of sensitive sensors [16, 17]. PdNP-modified electrodes exhibit good electrocatalytic properties toward a large number of electroactive molecules,

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including catecholamine neurotransmitters, methanol, and hydrogen peroxide [9, 18–20]. In view of these benefits, the present study sought to develop a novel, simple, rapid, and linker-free chemical method for preparing disposable PdNP-modified GPEs for the sensitive, stable, and reproducible electrochemical sensing of H_2O_2 .

Experimental

Reagents

Ammonium tetrachloropalladate(II) ($(\text{NH}_4)_2\text{PdCl}_4$), L-ascorbic acid (AA), uric acid (UA), 4-acetamidophenol (AAP), L-cysteine, and H_2O_2 were purchased from Sigma-Aldrich (<http://www.sigmaaldrich.com>). Disodium hydrogen phosphate and sodium dihydrogen phosphate were supplied by Fisher Scientific Company (<http://www.fishersci.com>). Hi-polymer graphite pencil HB black leads were obtained from Pentel Co. (Japan). All leads had a total length of 60 mm and a diameter of 0.5 mm, and were used as received. All solutions were prepared with deionized water with a resistivity of $18.6 \text{ M}\Omega\cdot\text{cm}^{-1}$, obtained directly from a PURELAB® Ultra Laboratory Water Purification System (<http://www.water.siemens.com>).

Apparatus and procedures

UV experiments were performed using a Cintra 303 spectrophotometer (<http://www.gbcsscientific.com>). A Jedo mechanical pencil (Korea) was used as a holder for both the bare and PdNP-modified graphite pencil leads. Electrical contact with the lead was achieved by soldering a copper wire to the metallic part of the mechanical pencil that held the lead in place. The pencil was fixed vertically such that 15 mm of the pencil lead was extruded outside of the pencil and 10 mm of the lead was immersed in the solution. This length corresponded to a geometric electrode area of 15.90 mm^2 . The pencil electrode is described in detail elsewhere [21]. CHI 660C (<http://www.chinstruments.com>) was used throughout the electrochemical experiments. The electrochemical cell contained bare- or PdNP-modified GPEs as the working electrodes, a Pt wire counter electrode, and an Ag/AgCl (Sat. KCl) reference electrode. The FE-SEM images were recorded using a TESCAN LYRA 3 (<http://www.tescan-usa.com>) at the Center of Research Excellence in Nanotechnology, King Fahd University of Petroleum and Minerals, Kingdom of Saudi Arabia.

Preparation of palladium nanoparticles-modified graphite pencil electrode

Equal volumes of 10 mL aqueous solutions of 1.1 mM AA and 1.0 mM ammonium tetrachloropalladate(II) were mixed

under magnetic stirring conditions at room temperature for 15 min. After forming the PdNPs, a bare GPE was immersed into a 3.0 mL test tube containing the synthesized PdNP solution. The PdNP-modified GPE was obtained by placing the test tube in a water bath preheated to $75 \text{ }^\circ\text{C}$ and incubated for 15 min. The PdNP-modified GPE was removed and washed by gently dipping twice in deionized water, followed by drying at $60 \text{ }^\circ\text{C}$ for 5 min prior to use.

Results and discussion

NP-modified electrodes are commonly prepared according to any of three methods: (i) ion capture on the electrode surface by linker molecules, followed by reduction [22], (ii) attachment of pre-synthesized NPs to the electrode via linker molecules [6, 23], and (iii) electrodeposition [13, 19]. The first two methods are complex and expensive because they require functionalization of the electrode surface with expensive linkers prior to immobilization of the NPs. Moreover, the linker molecules negatively affected the electron transfer reaction. Electrodeposition-based modification methods require a skilled technician and sophisticated instruments, and it is difficult to obtain reproducible surfaces at a given deposition rate. The development of a simple and linker-free chemical method for preparing PdNP-modified GPEs would constitute a significant advance in the field.

Aziz et al. and Compton et al. have prepared metal nanoparticles on ITO electrodes and glassy carbon spheres by immersing the ITO or glassy carbon spheres in an aqueous solution containing the appropriate metal precursor, successively adding ascorbic acid (AA), and then heating at 75 or $70 \text{ }^\circ\text{C}$ [24, 25]. In the present study, we took advantage of the good reducing properties of AA to prepare metal nanoparticles at room temperature without the need for stabilizers [26]. An aqueous solution of PdNPs was prepared simply by adding AA (aq.) to $(\text{NH}_4)_2\text{PdCl}_4$ (aq.) with stirring at room temperature. Upon addition of AA (aq.) to $(\text{NH}_4)_2\text{PdCl}_4$ (aq.), the color of the solution changed from pale yellow to a light dark brown and finally to a dark brown after 15 min of stirring. The color changes were characteristic of PdNP formation [27]. The UV–vis spectrum of the prepared PdNP solution was recorded (Fig. 1, curve c). For comparison, the UV–vis spectra of 0.5 mM $(\text{NH}_4)_2\text{PdCl}_4$ (aq.) (Fig. 1, curve a) and 0.55 mM AA solutions (aq.) (Fig. 1, curve b) were recorded. The UV–vis spectrum of AA displayed almost no absorbance bands over the spectral range tested. $(\text{NH}_4)_2\text{PdCl}_4$ showed a slightly higher absorbance with a λ_{max} at 403 nm. The UV spectrum of the aqueous solution containing AA and $(\text{NH}_4)_2\text{PdCl}_4$ stirred for 15 min (Fig. 1, curve c) displayed characteristics typical of the PdNP solution [28, 29]. The UV experiments confirmed the formation of PdNPs in an

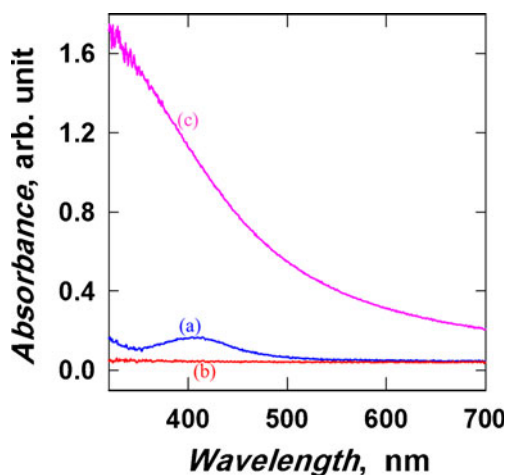


Fig. 1 UV-visible spectra of aqueous solutions containing: (a) 0.5 mM $(\text{NH}_4)_2\text{PdCl}_4$, (b) 0.55 mM ascorbic acid, (c) PdNPs synthesized by mixing equal volumes of 1.0 mM $(\text{NH}_4)_2\text{PdCl}_4$ and 1.1 mM ascorbic acid, with stirring for 15 min at RT

aqueous solution containing AA and $(\text{NH}_4)_2\text{PdCl}_4$, stirred for 15 min. The bulk PdNP solution was used to modify a large number of GPEs.

A GPE was immersed in the synthesized PdNP solution and incubated for 15 min at room temperature. After washing and drying, the cyclic voltammogram (CV) of the treated electrode was obtained in 0.1 M NaOH (Fig. 2, curve b). The anodic current in the CV (Fig. 2, curve b) of the treated GPE was slightly higher than the current obtained from the bare GPE (Fig. 2, curve a). PdNP-modified carbon electrodes previously yielded a higher anodic current due to the formation of hydroxide or oxide on the Pd surface [30]. The cathodic currents of the treated GPE and the bare GPE were indistinguishable. These experiments indicated that the PdNPs were not extensively immobilized on the

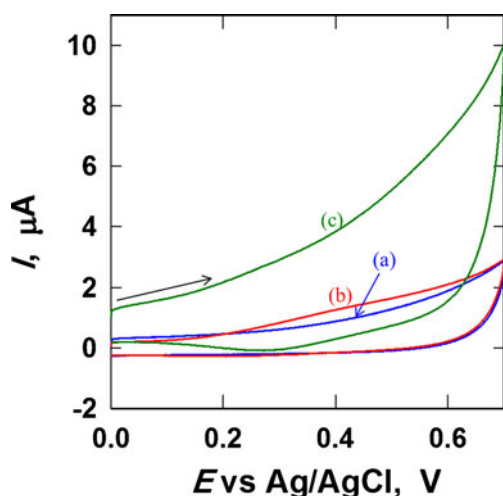


Fig. 2 CVs collected in a 0.1 M NaOH aqueous solution at GPE before (a), and after immersing in the PdNP solution at room temperature (b) or at 75 °C (c) for 15 min. The scan rate was $100 \text{ mV} \cdot \text{s}^{-1}$

GPE surfaces from the PdNP solution at room temperature. The efficient immobilization of the PdNPs on the GPE surfaces was achieved by increasing the temperature from room temperature to 75 °C. To increase the temperature, the PdNP solution and the immersed GPE were placed in a preheated water bath for 15 min. The CV (Fig. 2, curve c) of the GPE treated at 75 °C displayed a high anodic current in 0.1 M NaOH. The cathodic scan of the GPE treated at 75 °C displayed a hump-type peak at +0.263 V (Fig. 2, curve c). The reaction associated with the cathodic peak has not yet been identified, although similar peaks have been reported previously in the presence of PdNP-modified carbon electrodes [30]. The CV experiments suggested that heat treatment played an important role in immobilizing the PdNPs on the GPE. The bare GPE and PdNP-modified GPE were characterized by FE-SEM imaging at two different magnifications (Fig. 3A and B). Figure 3(a)–(c) show FE-SEM images of the bare GPE and the PdNP-modified GPE at room temperature and at 75 °C, respectively. Figure 3 clearly shows that the PdNPs were only immobilized on the GPE after immersion in the aqueous PdNP solution at 75 °C. The low-magnification view of the PdNP-GPE (Fig. 3A(c)) confirmed the presence of a homogeneous distribution of PdNPs on the surface of the PdNP-GPE. The PdNP size on GPE surface ranged from 20 to

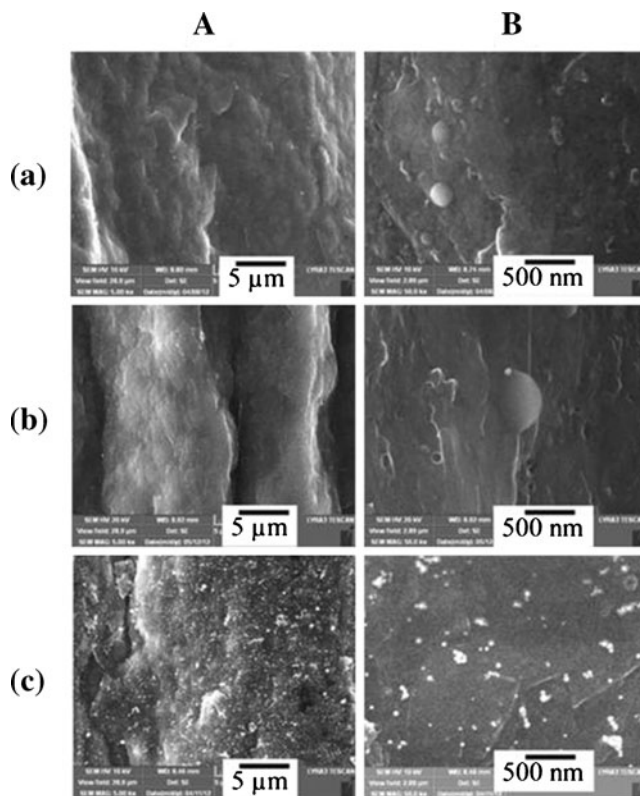


Fig. 3 FE-SEM images at two different magnifications: 5 μm (A) and 500 nm (B), for the bare (a) and PdNP-modified GPE prepared by immersing the bare GPE in the PdNP solution at RT (b) or at 75 °C (c) for 15 min

65 nm (Fig. 3B(c)). The effect of temperature on the immobilization reaction was examined by preparing PdNP-modified GPEs at 50 °C (Figure S-1a) and 90 °C (Figure S-1b), and FE-SEM images of the resulting electrode surfaces were recorded. The SEM images (Fig. 3 and Figure S-1) clearly revealed that the PdNP-modified GPE prepared at 75 °C presented the highest density of PdNPs. The electrodes with the second and third highest density PdNP-modified surfaces were obtained from the PdNP-modified GPEs prepared at 90 °C and 50 °C, respectively. The PdNP-modified GPE, which was modified at 75 °C, is denoted PdNP-GPE in this manuscript.

The effects of the GPE PdNP surface modifications on the electrocatalytic properties were examined by collecting the CVs in phosphate buffer (PB) (0.1 M, pH 7) in the absence (Fig. 4, curves a and b) or presence of 1.0 mM H₂O₂ (Fig. 4, curves a' and b') on the bare- (Fig. 4, curves a and a') and PdNP-GPE (Fig. 4, curves b and b'). The CV data shown in Fig. 4, curves a and a' confirmed that the bare GPE displayed poor electrocatalytic properties with respect to both the electro-oxidation and the reduction (redox) reactions of H₂O₂. On the other hand, the background current of the PdNP-GPE (Fig. 4 b) was higher than that of the bare GPE (Fig. 4, curve a). The background current of PdNP-GPE was not high, whereas the PdNP-GPE (Fig. 4, curve b') showed a much higher electrooxidation current in the presence of H₂O₂ compared to that observed for the bare GPE (Fig. 4, curve a'). Both the reduction and oxidation of H₂O₂ at the PdNP-GPE could be attributed to the excellent electrocatalytic properties of the PdNPs. The PdNP-GPE showed a significantly lower overvoltage for the redox reaction of H₂O₂ compared to the bare GPE. Therefore, the PdNPs acted as suitable mediators to shuttle electrons

between H₂O₂ and GPE, and they facilitated the electrochemical generation following electron exchange with hydrogen peroxide.

The effects of the PdNP-modified GPE preparation temperature on the electrocatalytic properties of the electrode in the presence of the electrochemical redox reactions of H₂O₂ were determined by recording the CVs in 0.1 M PBS (pH 7.0) containing 1 mM H₂O₂ in the presence of the PdNP-modified GPEs prepared at different temperatures (Figure S-2 A). The PdNP-GPE (Fig. 4, curve b', and Figure S-2 A, curve c) displayed the smallest starting potential (i.e., the lowest overpotential) for the electrochemical redox reactions of H₂O₂. The redox current levels generated by H₂O₂ in the presence of the PdNP-modified GPEs prepared at different temperatures are compared in the bar chart show in Figure S-2 B. Figure S-2 B, curve c was constructed using reduction at -0.25 V and oxidation at +0.8 V. The bar chart shows that the PdNP-GPE (Figure S-2 B, curve c) showed the highest H₂O₂ reduction or oxidation signal. The best electrocatalytic properties of the PdNP-GPE arose from the presence of a high amount of PdNP among the prepared PdNP-modified GPEs. The PdNP-GPE was, therefore, selected for further experiments.

The reproducibility of the PdNP-GPE preparation method was tested by measuring the CVs of seven different modified electrodes in PBS containing 1.0 mM H₂O₂ (data not shown). The recorded CVs were quite similar and, hence, the prepared PdNP-GPEs were reproducible and were considered good candidates for the electroanalysis of H₂O₂.

The H₂O₂ concentration-dependent signal and detection limits at the bare GPE and at the PdNP-GPE were measured using the amperometric method. Figure 5 shows typical

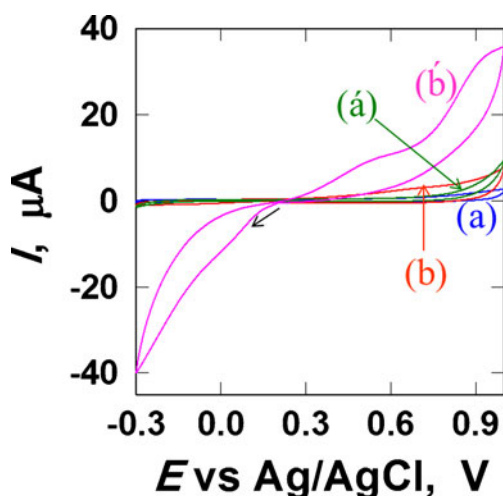


Fig. 4 CVs collected in PBS (0.1 M, pH 7) in the absence (a, b) or presence (a', b') of 1 mM H₂O₂ at the bare-GPE (a, a') or PdNP-GPE (b, b'). The scan rate was 100 mV·s⁻¹. CVs were recorded after purging with argon for 20 min. Other preparation conditions were as described in Fig. 3c

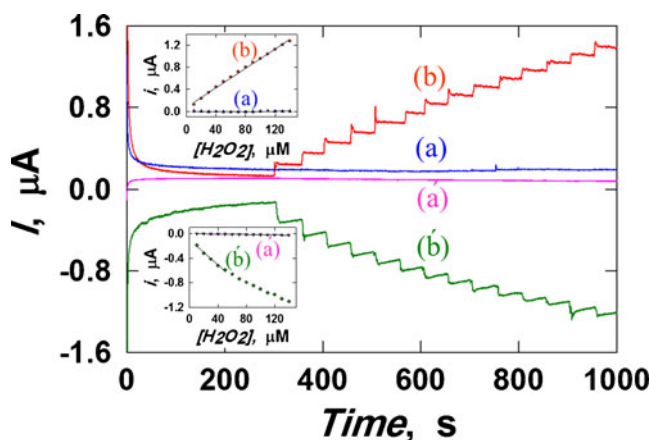


Fig. 5 Amperograms of (a, a') bare or (b, b') PdNP-modified graphite pencil electrodes in 10 mL PBS (0.1 M, pH 7) at +0.80 V (a, b), during the successive addition of 10 μM of H₂O₂ and at -0.25 V (a', b'), during the successive addition of 50 μM H₂O₂. Amperograms (a, b) and (a', b') were recorded before and after purging with argon for 20 min, respectively. The insets show the corresponding calibration plots. Other preparation conditions were as described in Fig. 3c

Table 1 A comparison of the fabricated sensor with other reported metal particle-based sensors for H₂O₂ detection

Method	Sensing material	Sensing media/working potential (V vs. Ag/AgCl)	Analytical ranges (μM)	Detection limit (μM)	Ref.
Amperometry	PdNP-GPE	0.1 M PB, pH 7.0 +0.8 V −0.25 V	10–140 10–50	0.045 1.0	This Work
Amperometry	PdNPs-TiO ₂ Hollow Spheres- GCE	PBS, pH 7.0 / −0.2 V (vs. SCE)	10–860	3.81	[9]
Amperometry	Nafion-MWCNT-PdNPs- GCE	0.1 M PBS, pH 7.4 / −0.2 V	1–10000	0.30	[10]
Amperometry	CNT-wired CuO Nanoflower- GCE	0.1 M PB, pH 8.0 / −0.3 V (vs. SCE)	0.5–82	0.16	[11]
Amperometry	AgNPs-CNT-Carbon Ceramic Electrode	0.1 M PB, pH 7.0 / −0.2 V (vs. SCE)	10–8000	0.20	[12]
Amperometry	AgNPs-ZnO-GCE	0.1 M PB, pH 7.4 / −0.25 V	2000–5500	0.42	[31]
Amperometry	CuONPs-Ionic Liquid-Carbon Electrode	0.1 M NaOH / −0.2 V	1–2500	0.50	[32]
Amperometry	Co ₃ O ₄ Nanowalls-GCE	0.01 M PB, pH 7.4 +0.8 V −0.2 V	50–1400 50–5350	2.8 10.0	[33]
Amperometry	PdNPs-Gold Nanowire Array Electrode	0.01 M PBS, pH 7.4 / −0.2 V	0.1–2000	5.0	[34]
Amperometry	PtNPs-MWCNT/ polyaniline-GCE	0.1 M PB, pH 6.5 / −0.25 V (vs. SCE)	7–2500	2.0	[35]
Cyclic voltammetry	AgNPs-Carbon Ion Liquid Electrode	0.1 M PB, pH 7.0	2–200	0.7	[13]
Cyclic voltammetry	Cytochrome C- and ZrO ₂ NPs-GCE	0.1 M PB, pH 7.0	40–270	Not Given	[36]
Luminescence	CdSe/ZnS Quantum Dots	0.01 M HEPES, pH 7.2	0.1–10000	0.10	[37]
Phosphorescence	Nano TiO ₂ /SiO ₂ Composite	Water	7–70000	0.16	[38]

amperometric responses of the (a) bare GPE and (b) PdNP-GPE at +0.8 V upon successive additions of 10 μM H₂O₂. The PdNP-GPE (Fig. 5b) yielded a well-defined and sensitive signal for each addition of H₂O₂, whereas the bare GPE (Fig. 5a) gave a poor signal. The concentration-dependent signal (upper inset in Fig. 5) was linear over the entire H₂O₂ concentration range tested in the presence of the PdNP-GPE ($R^2=99.68$) or the bare GPE ($R^2=97.2$), after subtracting the mean of the corresponding zero hydrogen peroxide response. Both electrodes followed a linear trend that could be fit to the equation $y = ax + b$. The detection limits of H₂O₂ at an applied potential of +0.8 V for the PdNP-GPE and bare GPE were 45 nM and 0.58 mM, respectively. Although the detection limit achieved at +0.8 V for the PdNP-GPE was impressively low, numerous other substances were expected to be oxidized at such a potential, interfering with the measurement. These factors were expected to present a significant obstacle to the detection of low concentrations of H₂O₂ in the presence of relatively high concentrations of interferents. These factors can be eliminated or reduced by applying a lower potential. A potential of −0.25 V was applied to minimize the biases from the potential interferences for the amperometric detection of H₂O₂ at PdNP-GPE. Figure 5 shows typical amperometric responses of bare GPE (a') and PdNP-GPE (b') at −0.25 V upon successive additions of 50 μM H₂O₂. The PdNP-GPE (Fig. 5b') at −0.25 displayed well-defined and sensitive signals after each addition of H₂O₂, whereas the bare GPE (Fig. 5a') gave a poor signal. A linear relation (lower inset of Fig. 5) between the concentration and the

response current was obtained after subtracting the mean of the corresponding zero hydrogen peroxide response for bare ($R^2=0.987$) and PdNP-GPE ($R^2=0.992$). The linearity of the relationship deviated at higher concentrations of H₂O₂ at −0.25 V for the PdNP-GPE, possibly due to kinetic limitation at the electrode. At −0.25 V, both electrodes yielded a linear relationship. The detection limits at an applied potential of −0.25 V were 1.0 and 59 μM for the PdNP-GPE and bare GPE, respectively. The sensor described here is compared with a variety of other H₂O₂ sensors in Table 1, for a variety of detection methods, sensing materials, analytical ranges, and detection limits. Table 1 shows that the performance of the sensor developed here was comparable to the performances of other H₂O₂ sensors.

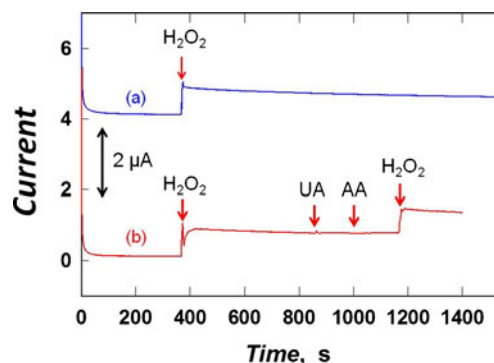


Fig. 6 Amperometric responses of the PdNP-GPE to a 100 μM H₂O₂ solution over (a) a long period of time, (b) successive additions of hydrogen peroxide, UA, and AA. Other preparation and working conditions were as described in Figs. 3c and 5

PdNP-GPE displayed a stable and strong catalytic activity and facilitated the amperometric measurement of hydrogen peroxide. Figure 6a shows the amperometric response to a 100 μM H_2O_2 solution at +0.8 V during a prolonged 20.0 min experiment. The response remained stable throughout the entire experiment, with a current decrease of less than 10 %, indicating that the H_2O_2 and/or its oxidation products did not inhibit catalysis at the PdNP-modified GPE.

Figure 6b presents the amperometric response to successive additions of H_2O_2 , UA, and AA, and again H_2O_2 , at +0.8 V for a given PdNP-GPE surface. A well-defined H_2O_2 response was observed in which subsequent injections of relevant physiological levels (1 μM) of UA and AA did not produce additional signals or even modify the obtained current response. Further additions of H_2O_2 yielded well-defined and reproducible sensor responses, reflecting a good H_2O_2 sensing selectivity and sensitivity in the fabricated PdNP-GPE. Interference in the electrocatalytic reduction of H_2O_2 by other analytes was examined under a -0.25 V potential. Successive additions of 50 μM H_2O_2 , 100 μM 4-acetamidophenol, 100 μM L-ascorbic acid, 10 μM UA, and 10 μM L-cysteine were introduced into the solution in which a PdNP-GPE was immersed (data is not shown). A well-defined and invariant H_2O_2 response was observed after each addition. With the exception of L-cysteine, none of the compounds induced a change in the response. The sensor developed here apparently could not tolerate thiol-containing molecules that blocked the catalytic surface of the metallic electrode [39].

Conclusions

We successfully fabricated a novel, low-cost, disposable, and easily fabricated H_2O_2 sensor based on the simplest method yet reported for preparing metal NPs-modified carbon substrates. The PdNP-GPE were prepared by immersing a bare GPE in a PdNP solution, followed by heating at 75 $^\circ\text{C}$ for 15 min. PdNP-GPE exhibited excellent electrocatalytic activity towards H_2O_2 and the fabrication method was highly reproducible. With its remarkable electrocatalytic activity, a very low detection limit could be achieved with greater analytical selectivity, sensitivity, and stability. The performance quality of the novel PdNP-GPE proved to be excellent, and the electrodes were found to be suitable for the analytical determination of H_2O_2 concentrations in a variety of applications.

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