Nanometal-Decorated Exfoliated Graphite Nanoplatelet Based Glucose Biosensors with High Sensitivity and Fast Response

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ABSTRACT We report the novel fabrication of a highly sensitive, selective, fast responding, and affordable amperometric glucose biosensor using exfoliated graphite nanoplatelets (xGnPs) decorated with Pt and Pd nanoparticles. Nafion was used to solublize metal-decorated graphite nanoplatelets, and a simple cast method with high content organic solvent (85 wt %) was used to prepare the biosensors. The addition of precious metal nanoparticles such as platinum (Pt) and palladium (Pd) to xGnP increased the electroactive area of the electrode and substantially decreased the overpotential in the detection of hydrogen peroxide. The Pt−xGnP glucose biosensor had a sensitivity of 61.5 ± 0.6 μA/(mM · cm^2) and gave a linear response up to 20 mM. The response time and detection limit (S/N = 3) were determined to be 2 s and 1 μM, respectively. Therefore, this novel glucose biosensor based on the Pt nanoparticle coated xGnP is among the best reported to date in both sensing performance and production cost. In addition, the effects of metal nanoparticle loading and the particle size on the biosensor performance were systematically investigated.

KEYWORDS: metal nanoparticles · exfoliated graphite nanoplatelets · Nafion · glucose biosensor

Nanoparticles have been extensively used in electroanalysis due to their unique capabilities to enhance mass transport, facilitate catalysis, increase surface area, and control an electrode's microenvironment.[1] Conductive graphene nanoparticles are especially versatile because they are available in a variety of geometries, including spherical fullerenes, cylindrical nanotubes, and planar graphite nanoplatelets.[2,3] Carbon nanotubes have been widely used in biosensor applications,[4−6] largely due to their fast electron transfer and excellent electrocatalytic activity,[7] which promotes the electron-transfer reaction of hydrogen peroxide (H_2O_2),[8] NADH,[9,10] cytochrome *c*,[11] ascorbic acid,[12] *etc*. In our previous work, we demonstrated for the first time that exfoliated graphite nanoplatelets (xGnPs), which are 1−10 nm in thickness and from 100 to 1000 nm in diameter,[13] can be an inexpensive alternative (∼$5/lb) to carbon nanotubes (range from 20 to hundreds of dollars per gram) for use in biosensors.[14] Using xGnP, we observed a substantial decrease in overpotential for detection of H_2O_2 compared to the traditional carbon paste electrode prepared with micron-sized graphite particles. In addition, the resulting biosensors exhibited excellent sensitivity, low interference by ascorbic and uric acid, and relatively good long-term stability.

The integration of carbon nanoparticles with transition metal nanoparticles is relatively new in biosensor applications.[15−19] Precious metal nanoparticles make excellent catalysts, due to their high ratio of surface atoms with free valences to the cluster of total atoms.[16] For example, platinum (Pt) and palladium (Pd) nanoparticles are especially effective at H_2O_2 oxidation and reduction.[20] In addition, metal nanoparticles could even provide electrochemical reversibility for redox reactions, which is not possible on the bulk metal electrode.[21] Hrapovic *et al.* simply mixed a Pt nanoparticle suspension with single-walled carbon nanotubes (SWCNTs) Nafion suspension to create a glucose biosensor.[15] This study demonstrated that SWCNT provided an excellent support for the Pt nanoparticles for efficient electrical transfer; however, this method resulted in Pt nanoparticle agglomeration. The catalytic and electrochemical activities of metal nanoparticles depend heavily on their stability, particle size, and distribution.[19] To minimize the agglomeration of metal nanoparticles, a sol−gel technique,[19] Nafion solubilization,[16] and direct deposition of metal on carbon nanotubes[18] have been used. The synthesis of carbon nanotubes and nanofiber-supported metal

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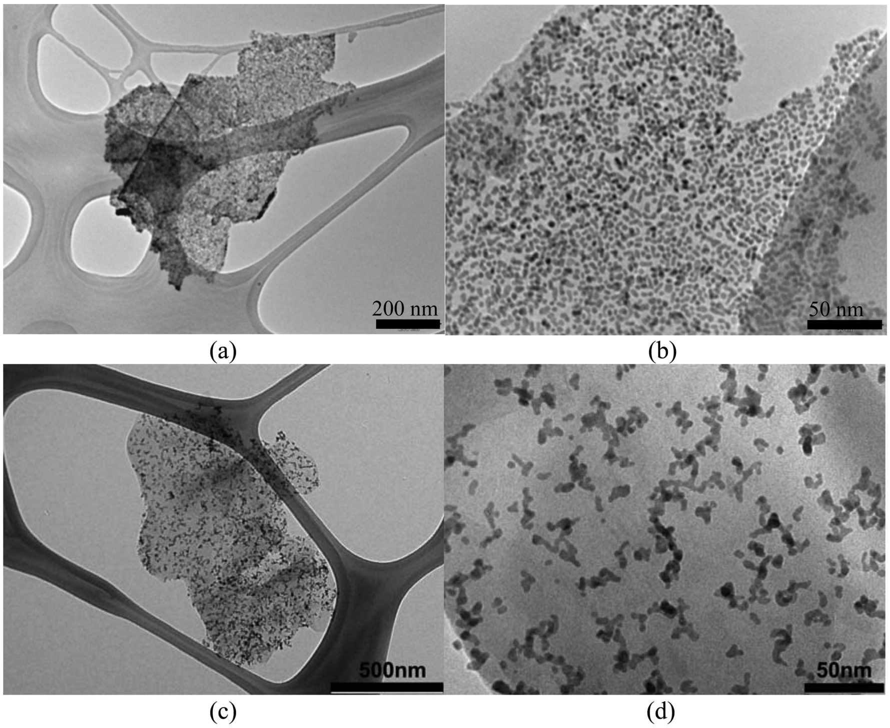

(a) (b) (c) (d)

Figure 1. TEM images of 20 wt % Pt−xGnP at (a) low magnification, (b) high magnification, and 20 wt % Pd−xGnP at (c) low magnification and (d) high magnification.

nanoparticles has drawn great attention since 1990, but the approach has been mostly limited to fuel cell electrocatalysts.[22] However, Xie *et al.* recently reported a glucose biosensor with high sensitivity using platinum-decorated multiwalled carbon nanotubes (MWCNTs).[18] The use of CNT-supported Pt catalysts reduces Pt loading by increasing the catalyst utilization and improving the catalyst activity/performance.

In this paper, we report for the first time use of xGnPs decorated with Pt and Pd nanoparticles as a transducer for a glucose biosensor. The previous work demonstrated that xGnP, which has the highest thermal oxidation resistance and the highest degree of graphitization, can be an inexpensive alternative to carbon nanotubes and carbon black as support for fuel cell applications.[23] The xGnPs and metal-decorated xGnPs were suspended and solubilized in Nafion. Nafion, a sulfonate tetrafluorethylene copolymer, is known to be a good solubilizing agent for carbon nanotubes, and it has good biocompatibility, thermal stability, mechanical strength, and antifouling properties.[8] A simple organic solvent casting method reported in a previous work[14] was used to develop a highly sensitive and fast responding glucose biosensor by the combination of metal-decorated xGnP, glucose oxidase (GOx), and Nafion. The performance of glucose biosensors based on two different metal-decorated xGnPs was compared, and the effect of the size and loading of Pt nanoparticles on the biosensor was also studied. This approach can provide extremely high surface area-to-volume ratio for the expensive metal, lowering the cost of the electrode.

RESULTS AND DISCUSSION

Dispersion of Precious Metal Nanoparticles on xGnP. Figure 1 shows the TEM images of 20 wt % Pt−xGnP and Pd−xGnP. Even though the xGnP are very thin, a stack of layers is clearly visible. Graphite nanoplatelets with a thickness of 10 nm would contain approximately 30 graphene sheets, considering an interlayer spacing of 0.335 nm.[24] Figure 1a,b indicates a homogeneous deposition of Pt nanoparticles with a surface coverage of approximately 60%. The nanoparticles appear spherical in shape, with a diameter range of 1−4 nm. An average particle size of 2.65 nm was calculated from the XRD broadening Pt (220) reflection peaks using the Scheller equation.[25] The surface area per unit mass (S) of these Pt nanoparticles was calculated to be 106 m^2/g based on the equation $S = 6000/(\rho \times d)$, where d is the mean diameter, and ρ is the density of Pt (21.41 g/cm^3). As shown in Figure 1c,d, the Pd particles are more polydisperse than the Pt particles and appear as worm-shaped aggregates. Also, the surface coverage for the Pd particles is lower (approximately 30% determined by the TGA measurement) and less homogeneous than for the Pt−xGnP, even though the total metal content is the same in both cases. For metal nanoparticle decorated carbon nanotubes, it is generally believed that the surface groups of carbon, such as carboxyl, hydroxyl, and carbonyl groups, are responsible for nucleating metal ions, which are reduced to nanosized particles.[26,27] This mechanism can be applied to the edges of graphite nanoplatelets, which have sufficient functional groups. However, for the inert basal plane, the surface basic sites are of Lewis type and are associated with π-electron rich regions (C_π sites),[28] the protonation of the basic carbon sites would lead to electron donor−acceptor complexes, which are responsible for the strong interactions between metal particles and graphite nanoplatelets.[23,28,29] Thus, further agglomeration of metal nanoparticles can be prevented during the biosensor preparation.

Morphology of Biosensor Composite Films. Nafion is an effective solublizing agent for suspending metal-decorated graphite nanoplatelets. A water−2-propanol Nafion mixture (15 wt % water and 85 wt % 2-propanol) was found to be an effective medium in which to suspend metal-decorated graphite nanoplatelets. This result was consistent with previous work, which showed

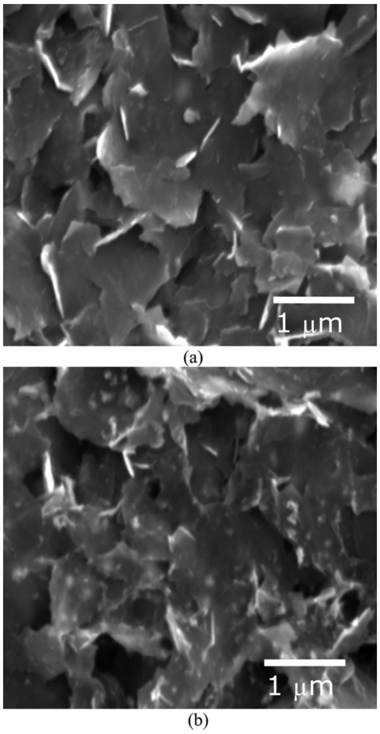

Figure 2. SEM images of Pt−xGnP−Nafion nanocomposite (a) and GOx−Pt−xGnP−Nafion biocomposite films.

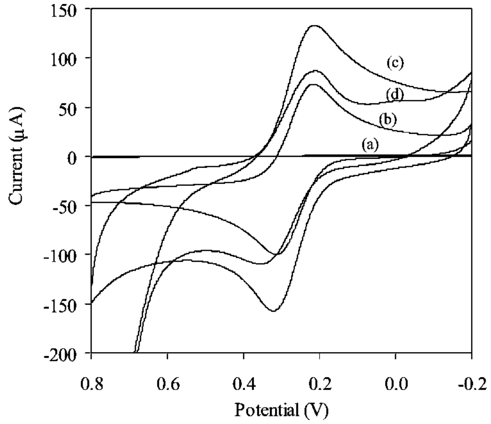

Figure 3. Cyclic voltammograms (second run recorded) for Nafion (a), xGnP (b), 20 wt % Pt−xGnP (c), and 20 wt % Pd−xGnP (d) modified glassy carbon electrodes in 5 mM $Fe(CN)_6^{4-}$ and 1 M KCl at 20 mV · s^{-1} *vs* Ag/AgCl reference electrode.

that Nafion 2-propanol solution had a better solubility for xGnP than aqueous solution.[14] Furthermore, deposition of the enzyme−Nafion layers from water−organic solutions with a high concentrated organic solvent showed better sensitivity and stability because Nafion was deposited from a solution where it was truly dissolved.[30]

Figure 2 shows the SEM images of Pt−xGnP−Nafion and GOx−Pt−xGnP−Nafion composite films. As shown in Figure 2a, graphite platelets are densely packed but do not lay flat, resulting in a surface exhibiting high microscale roughness. At this magnification, the size of the xGnP can be seen to vary significantly, but the Pt nanoparticles are not visible. The GOx−Pt−xGnP−Nafion composite film shows a very similar morphology, except the GOx-containing Nafion deposits appear as white dots with a particle size of approximately 100 nm, on the graphite nanoplatelets. At the high content of 2-propanol (85 wt %) used in this study, GOx does not fully dissolve but forms a colloidal suspension[14] that can stabilize the GOx, so that up to 100% of its activity is preserved.[30] The colloidal enzyme nanoparticles are aggregates of enzyme molecules, each of which is approximately 4−5 nm in size.[17]

Biocatalytic Activity of Metal−xGnP−Nafion Composite Film. Cyclic voltammetry is a useful tool to evaluate the performance of metal−xGnP−Nafion composite films as transducers. Figure 3 shows the cyclic voltammograms of Pt−xGnP (20 wt % Pt, 80 wt % xGnP), Pd−xGnP (20 wt % Pd, 80 wt % xGnP), and xGnP−Nafion-modified electrodes recorded in 5 mM ferrocyanide (in 1 M KCl) at a 20 mV/s scan rate. The Nafion-modified carbon electrode shows no redox peaks because Nafion film acts as a barrier for the electron transfer. Well-defined oxidation and reduction peaks due to the Fe^{3+}/Fe^{2+} redox couple were observed at +0.309 and +0.220 V *vs* Ag/AgCl reference electrode for the xGnP and Pt−xGnP−Nafion-modified electrodes in both the forward and reverse scans. The peak separation is 89 mV, which is slightly higher than the peak separation of the glassy carbon electrode (72 mV), indicating a reversible redox process. For the Pt−xGnP-modified electrode, the peaks are more significant than the ones for the xGnP-modified electrode. The electroactive surface area (*A*) for the composite films was estimated based on the Randles−Sevcik equation, which assumes mass transport only by diffusion process:[31]

$$I_p = 2.69 \times 10^5 A D^{1/2} n^{3/2} \gamma^{1/2} C \qquad (1)$$

where *n* is the number of electrons participating in the redox reaction, *D* is the diffusion coefficient of the molecule (equal to $(6.70 \pm 0.02) \times 10^{-6}$ cm^2/s), *C* is the concentration of the probe molecule in the solution (mol/cm^3), and γ is the scan rate ($V \cdot s^{-1}$). The calculated *A* value for the xGnP−Nafion composite film was 0.18 ± 0.01 cm^2 and that for the Pt−xGnP film was 0.27 ± 0.01 cm^2. Thus, the addition of Pt nanoparticles increased the effective electrode surface area by roughly 45%. The Pd−xGnP electrode showed pseudoreversible redox peaks, presumably due to the larger particle size and non-uniformity of Pd nanoparticles. The electroactive surface area for the Pd−xGnP electrode was 0.19 cm^2, which is only slightly higher than the xGnP electrode.

Figure 4 shows the effect of Pt loading on the cyclic voltammetry curves. Increasing Pt loading increased the redox peak currents and the peak separation. These effects are attributed to increased surface area and surface roughness. Surprisingly,

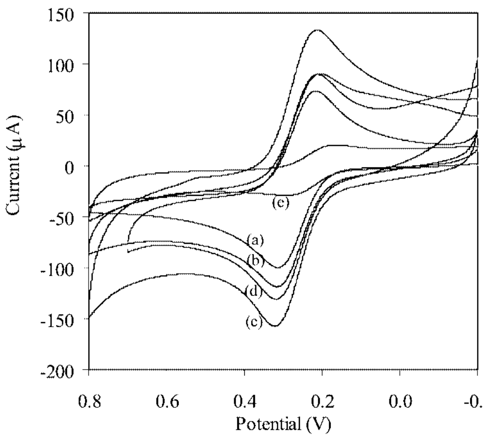

Figure 4. Cyclic voltammograms for Pt−xGnP-modified glassy carbon electrodes with varying Pt loadings in 5 mM $Fe(CN)_6^{4-}$ and 1 M KCl at 20 mV s^{-1} *vs* Ag/AgCl reference electrode: (a) 100 wt % xGnP, (b) 10 wt % Pt, (c) 20 wt % Pt, (d) 50 wt % Pt, and (e) 100 wt % Pt.

the peak currents were lower for 50 wt % Pt loading than for 20 wt % loading, perhaps due to an increase in the particle size. At high Pt loading, controlling particle size became more difficult. The TEM data image indicated an average particle size for 50 wt % Pt loading of approximately 5 nm, as shown in Figure 5. The surface coverage of 50 wt % Pt loading is even lower than 20 wt % Pt loading, when compared to Figure 1b, which resulted in lower electroactive area. One hundred percent Pt−Nafion composites exhibited well-defined redox peaks, but peak currents were much lower. This result is attributed to Pt nanoparticles agglomerating in Nafion solution when they are not firmly anchored on the substrate, resulting in a significant decrease of electroactive area. Therefore, it is important to control the relative rates of Pt nanoparticle nucleation and growth on the xGnP surface *versus* in solution. In the following experiments, 20 wt % Pt- and Pd-decorated xGnP were used, unless otherwise indicated.

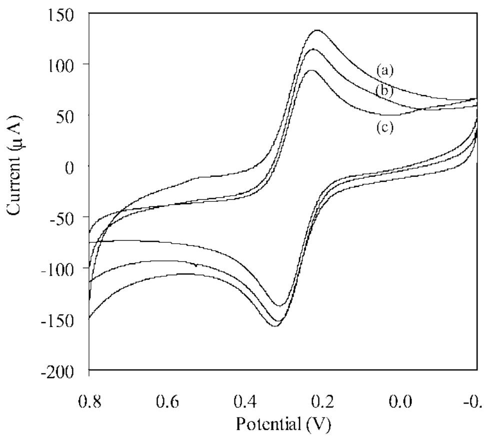

Figure 6. Cyclic voltammograms for 20 wt % Pt−xGnP-modified glassy carbon electrode with different size of Pt in 5 mM $Fe(CN)_6^{4-}$ and 1 M KCl at 20 mV s^{-1} *vs* Ag/AgCl reference electrode. (a) Particle size <2 nm, (b) 2.65 nm, and (c) >3 nm.

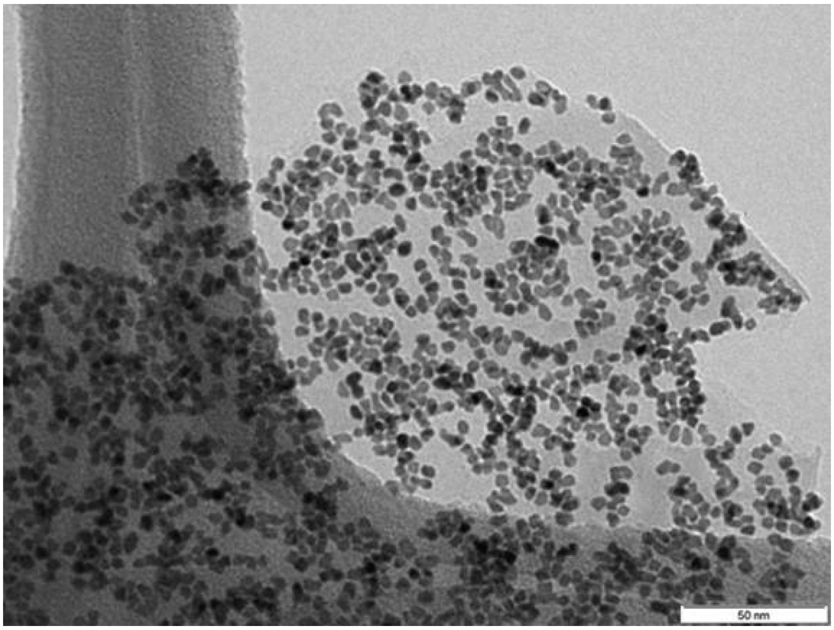

Figure 5. TEM image of 50 wt % Pt−xGnP.

Figure 6 shows the effect of Pt particle size on the cyclic voltammetric response on the glassy carbon electrode. As expected, the redox peak currents increased with decreasing particle size, which is due to the increased surface coverage of Pt nanoparticle.

Electrocatalytic Effect toward H_2O_2. The combined electrocatalytic effect of metal nanoparticles and carbon nanotubes toward H_2O_2 is well-documented in the literature.[15,17−19] Figure 7 shows the catalytic activity of metal-coated xGnP toward 5 mM H_2O_2 in 50 mM phosphate buffer solution evaluated with cyclic voltammetry. As shown in Figure 7b (inset, left), the xGnP−Nafion-modified electrode exhibits strong oxidation and reduction currents starting at around 0.4 and −0.15 V. This substantially lowered the detection potential for H_2O_2 compared to the plain glassy carbon electrode, which shows an oxidation of H_2O_2 starting at +0.8 V.[32] Thus, graphite nanoplatelets have similar catalytic activity for H_2O_2 as carbon nanotubes.[33−35] Both Pt−xGnP and Pd−xGnP−Nafion composite films show more pronounced currents than the xGnPs do without metal nanoparticles. In both cases, H_2O_2 can be detected over the whole range of potential, as a result of the excellent catalytic effect of metal nanoparticles.[15,17,18,20]

Amperometric Measurement. The combination of metal nanoparticles and xGnP not only increased the electroactive area of the electrode but also facilitated amperometric detection of H_2O_2 at milder potentials and provided higher detection sensi-

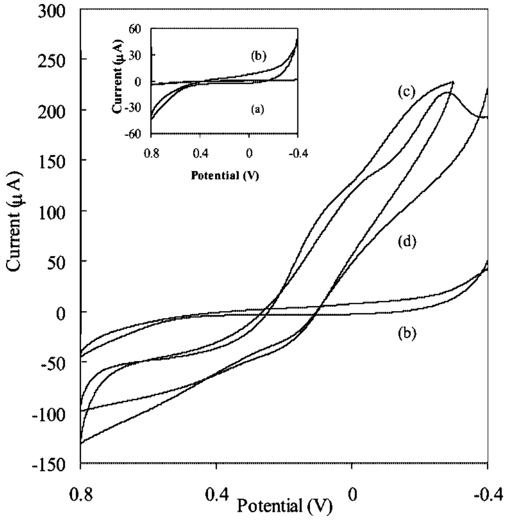

Figure 7. Cyclic voltammograms obtained with functionalized glassy carbon electrodes immersed in 5 mM H_2O_2 in 50 mM phosphate buffer solution (pH = 7.4) at (a) Nafion, (b) xGnP−Nafion, (c) Pt−xGnP−Nafion, and (d) Pd−xGnP−Nafion composites; scan rate = 20 mV/s.

tivity. Thus, these composite materials have attractive features for the fabrication of oxidase-based biosensors.

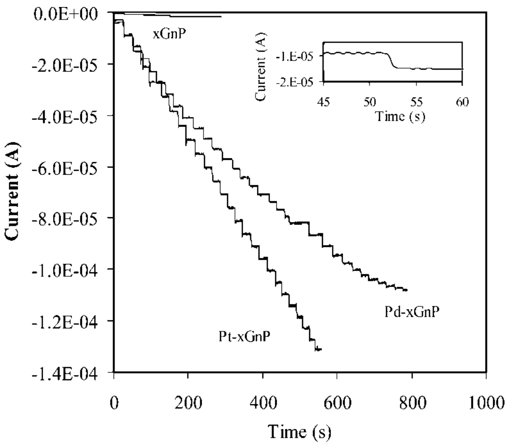

Figure 8. Amperometric responses for xGnP, Pt−xGnP, and Pd−xGnP glucose biosensors upon subsequent addition of 0.5 mM glucose solution (10 times) and then 1.0 mM glucose solution in 50 mM phosphate buffer solution at 700 mV. Inset shows the fast response of Pt−xGnP glucose biosensor upon the addition of 0.5 mM glucose solution.

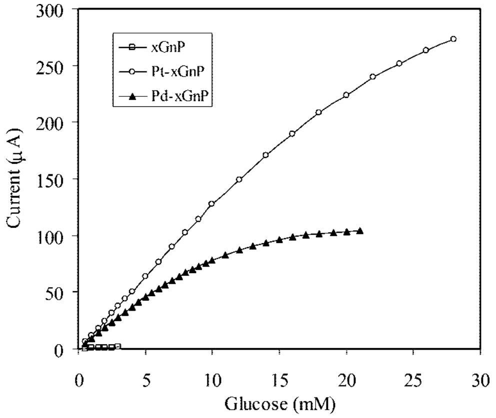

Figure 9. Calibration curves for xGnP, Pt−xGnP, and Pd−xGnP biosensors.

Figure 8 shows the typical amperometric responses of the metal-decorated xGnP-based compared to the xGnP-based biosensor for the successive additions of glucose at an applied potential of +0.7 V. Both metal-coated xGnP glucose biosensors exhibited rapid step increases. As shown in the inset, the time to achieve 95% steady state current for the Pt−xGnP biosensor was no more than 2 s, similar for a Pd−xGnP biosensor. The glucose detection limits were 1.0 and 4.0 μM (S/N = 3) for Pt−xGnP and Pd−xGnP biosensors, respectively. Figure 9 shows the calibration curves of Pt−xGnP and Pd−xGnP biosensors compared to xGnP biosensor. The linear detection ranges of the Pt−xGnP- and Pd−xGnP-based glucose biosensors were up to about 20 and 10 mM, respectively. The sensitivities of the Pt−xGnP and Pd−xGnP biosensors were 61.5 ± 0.6 and 47.9 ± 3.2 μA/ (mM · cm^2), respectively. On the basis of these results, the Pt−xGnP glucose biosensor exhibited slightly better performance than the Pd−xGnP biosensor. Pt has been reported to exhibit a slightly better catalytic effect than Pd toward H_2O_2, with similar performance at a potential of 700 mV,[36] and our results confirmed these findings (Figure 7). The enhanced performance of Pt−xGnP is mostly attributed to the smaller size and uniformity of Pt nanoparticles on the xGnP, which results in shorter H_2O_2 diffusion paths between the enzyme particles and the catalytic metal nanoparticles. The Pt−xGnP glucose biosensor exhibited a higher sensitivity than that reported for a glucose biosensor containing Pt-decorated MWCNT and a much quicker response than a Pt−MWCNT glucose biosensor prepared by the sol−gel technique (response time 15 s) or carbon paste preparation (response time 30 s).[18] This improved performance could be attributed to the strong adhesion of Pt nanoparticles to the graphene basal plane's C_π sites, which favors electron transfer.[23,29] The Pt−xGnP-based glucose biosensor is among the best glucose biosensors reported so far.

The effect of Pt nanoparticle loading on the performance of biosensors was investigated. As shown in Figure 10, the biosensor sensitivity was maximal at 20 wt % Pt. The biosensor sensitivity is expected to increase with increasing Pt loading. The decrease in sensitivity at high Pt loading is possibly attributed to the particle size increase, which could reduce the surface coverage and catalytic effect of Pt nanoparticles; in addition, it could also reduce the effective contact between Pt nanoparticles and the enzyme. As shown in Figure 10, the sensitivity of 100% Pt Nafion biosensor is much lower than Pt−xGnP biosensors. The agglomeration of Pt nanoparticles is visible on the carbon electrode by the naked eyes, which can dramatically decrease the catalytic effect. In addition, the Pt−Nafion composite film lacks a conductive support for the electrical contacting with the electrode, inhibiting the effective elec-

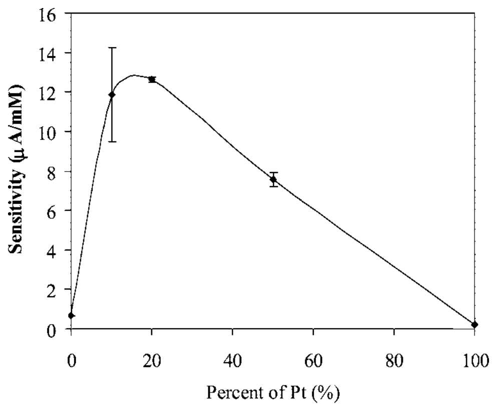

Figure 10. Effect of the Pt loading on the sensitivity of glucose biosensors.

tron transfer. Incorporation of xGnP increases the effective carbon electrode surface area and also allows an increased loading of catalytic Pt without facilitating Pt nanoparticle aggregation.

The influence of the Pt particle size on the biosensor performance was also examined. It was found that the biosensor sensitivity increased with decreasing Pt particle size. For a given Pt loading, the smaller particle sizes increase electroactive area and enhance electron transfer. For a given nanoparticle surface area, decreasing particle size decreases the Pt loading required, controlling fabrication costs. Therefore, to optimize performance of biosensor interfaces in which catalytic nanoparticles are used to facilitate electron transfer, it is critical to control both the nanoparticles' size and loading.

Biosensor Interference and Storage Stability. Addition of catalytic Pt and Pd nanoparticles can increase the glucose biosensor's sensitivity; however, these noble metals have also been shown to catalyze redox reactions of common electroactive interfering substances, such as ascorbic acid (AA) and uric acid (UA).[20] In addition, Wang, *et al.* reported that the permselective properties of Nafion are not sufficient to fully eliminate anionic interferences, especially at high potential.[8] For these reasons, the interfering effects of 0.1 mM ascorbic acid and 0.2 mM uric acid compared to 4 mM glucose (the average physiological concentration of blood glucose[37]) were evaluated on the Pt−xGnP glucose biosensor at the potential of +0.7 mV. Addition of 0.1 mM AA or 0.2 mM UA to a 4 mM glucose solution resulted in a 8.0 or 18% increase in the biosensor's output, respectively. In comparison, the percent signal increase for a xGnP biosensor without Pt nanoparticles was considerably greater: 56.8% for AA and 125% for UA. Thus, the Pt nanoparticles increased the biosensor's selectivity for H_2O_2 compared to that for AA and UA under the conditions studied. In addition, the interference of both compounds could be eliminated by using a potential of −50 mV, although the biosensor's sensitivity to glucose is also significantly decreased, which is due to less catalytic activity of both Pt and xGnP toward H_2O_2 at this potential, as shown in Figure 7.

The storage stability of these new biosensors was studied by storing the biosensors dry at 4 °C and studying the response to 0.5 mM glucose intermittently. The response maximized after 1−2 days storage and kept stable for a week. After a week, the response slightly decreased due to the possible leakage of weakly bound enzyme to xGnP. The biosensor could still uphold 70% of the sensitivity after 1 month and 50% in 2 months.

CONCLUSIONS

In this paper, for the first time, metal-decorated xGnP has been used to construct oxidase-based amperometric biosensors. xGnP incorporated into the biosensor interface increased the effective electrode surface area and served as an excellent support for metal nanoparticles. Pt and Pd nanoparticles immobilized on the xGnP provided a large electroactive surface area that effectively catalyzed redox reactions involving H_2O_2. The Nafion provided a permselective matrix in which the metal-decorated xGnP could be immobilized on the glassy carbon electrode's surface. Glucose biosensors produced using this approach exhibited a sensitivity up to 61.5 ± 0.6 μA/(mM · cm^2) and a response time less than 2 s. These performance properties are among the best reported to date for glucose biosensors. Both the metal loading and the nanoparticle size had a significant effect on biosensor performance. Use of nanoparticles having an average diameter less than 2 nm provided large ratio of Pt surface area to mass for low fabrication costs. Furthermore, the xGnP provide high carbon electrode surface area and facilitate uniform distribution of Pt nanoparticles but have a projected cost much lower than carbon nanotubes. The combination of high performance and inexpensive nanomaterial components suggests that this approach may be suitable for commercial applications.

EXPERIMENTAL SECTION

Reagents. GOx (Type X-S, *Aspergillus niger* (EC 1.1.3.4), 179 units/mg), Nafion (5 wt % in lower aliphatic alcohol and water mixture), 2-propanol, glucose, ascorbic acid, uric acid, sodium phosphate monobasic, sodium phosphate dibasic, chloroplatinic acid hexahydrate ($H_2PtCl_6 \cdot 6H_2O$), palladium(II) nitrate hydrate ($Pd(NO_3)_2 \cdot xH_2O$), and room temperature ionic liquid (RTIL), 1-butyl-3-methylimidazolium acetate ($bmimCH_3CO_2$) were obtained from Sigma-Aldrich and used as received. Ethylene glycol (EG, bp = 197.3 °C) from J.T. Baker was used as an agent to reduce the Pt precursor. Exfoliated graphite nanoplatelets (xGnP) with an average diameter of 1 μm and a thickness of 10 nm were made in house by a microwave process, sonicated, and milled.[13] All aqueous solutions were prepared with deionized (DI) water (>18.1 mΩ) supplied by a Barnstead nanopure Diamond-UV purification unit equipped with a UV source and final 0.2 μm filter.

Preparation of Precious Metal Nanoparticle Decorated xGnP. A modification of the microwave-heated polyol process[22,38,39] was used to deposit metal nanoparticles on xGnP with well-controlled particle size and metal loading as high as 50 wt %.[40] More detailed methods regarding the preparation of metal-coated xGnP can be found in a previous work.[23] To fabricate Pt−xGnP catalysts with 20 wt % Pt loading in the absence of RTIL, xGnPs were dispersed in 20 mL of EG for 30 min by sonication. A solution of Pt precursor dissolved in 5 mL of EG was added to the xGnP suspension, mechanically mixed in a 100 mL beaker for 60 min, transferred into a commercial microwave oven, and irradiated under air for 50 s to reduce the Pt precursor. After cooling, the resulting suspension was centrifuged, and the residual slurry was washed with acetone several times and dried in a vacuum oven at 373 K overnight. The weight ratio of Pt precursor and xGnP was controlled according to the targeted metal loading on the support. For Pt (20 wt %)/xGnP synthesized in the presence of the RTIL, various contents of the RTIL were added and dissolved in 20 mL of ethylene glycol prior to the dispersion of xGnP. The use of RTIL resulted in smaller size of Pt nanoparticles.

Characterization of Precious Metal Nanoparticle Coated xGnP. Pt nanoparticles dispersed on xGnP were characterized by recording their X-ray diffraction (XRD) patterns on a Regaku Rotaflex 200B X-ray diffractometer using Cu Kα radiation with a curved graphite monochromator in order to identify the phases present in the catalyst and to evaluate the lattice parameter and the particle size of the Pt crystallites dispersed on xGnP. The 2θ region between 10 and 100° was scanned at 45 keV and 100 mV at a scan rate of 5°/min. The average metal nanoparticle size was calculated using Scheller equation according to the XRD broadening Pt (220) reflections.[25] Transmission electron microscopy (TEM) was carried out with a JEOL 100CX and JEOL 2200FS operating at a voltage of 100 and 200 keV to determine the morphology of the dispersed Pt and Pd electrocatalysts and the average size of metal particles. Specimens were prepared by ultrasonically redispersing the sample powders in acetone for 15 min, applying the powder suspension onto lacey carbon-coated Cu grids, and drying them in air at ambient temperature. Thermogravimetric analysis (TGA 2950, TA instrument) was performed to determine the Pt and Pd loading. Samples weighing 3−4 mg were tested over temperatures ranging from room temperature to 800 °C (for supported Pt and Pd catalysts) or 1000 °C (carbons) at 20 °C/min under flowing air.

Preparation of Precious Metal Nanoparticle Coated xGnP−Nafion Composite Film. A 0.5 wt % Nafion stock solution was prepared by diluting the 5 wt % Nafion with 2-propanol. Ten milligrams of Pt/Pd−xGnP was dispersed in 1 mL of 0.5 wt % Nafion 2-propanol solution by ultrasonication for ~20 min. Prior to surface modification, glassy carbon electrodes (3 mm in diameter) were polished with grit 4000 polishing paper and then alumina powder (1, 0.3, and 0.05 μm) to obtain a mirror finish. The electrodes were then sonicated for 5 min, thoroughly rinsed with DI water and ethanol, and dried under a nitrogen stream. A 6 μL aliquot of metal-coated xGnP−Nafion solution was drop cast on the glassy carbon electrode and allowed to dry at room temperature.

Preparation of Glucose Biosensor. A GOx enzyme suspension in a water−2-propanol solution (85% alcohol) was prepared as follows. A 30 mg/mL GOx solution in 50 mM phosphate buffer (pH = 7.4) was added to the xGnP−Nafion−2-propanol suspension to obtain the desired final enzyme concentration. The suspension was sonicated for 5 min using a bath sonicator (longer sonication is not recommended because it may denature the enzyme). Six microliters of this suspension was drop cast onto the glassy carbon electrode and dried at room temperature for 1 h. The GOx-modified electrodes were then soaked in 50 mM phosphate buffer (pH 7.4) for 20 min, washed thoroughly with DI water, and stored dry at 4 °C.

Electrochemical Measurement. Cyclic voltammetry and amperometric experiments were performed using an electrochemical analyzer (CH instruments, Austin, TX, model 650 A) connected to a personal computer. All experiments were carried out using a conventional three-electrode system with the glassy carbon electrode as the working electrode, a platinum foil as the auxiliary electrode, and a saturated Ag/AgCl electrode as the reference. The cyclic voltammograms of the metal-coated xGnP−Nafion composite film were obtained in aqueous solution containing 5 mM $K_4Fe(CN)_6$ and 1 M KCl. In steady state amperometric experiments, the potential was set at 700 mV *vs* Ag/AgCl electrode with magnetic stirring. All measurements were carried out in 50 mM phosphate buffer solution at a pH of 7.4.

Surface Characterization of Biosensor Composite Film. The morphology of Pt−xGnP−Nafion composite and GOx−Pt−xGnP−Nafion biocomposite films was characterized using scanning electron microscopy (SEM, JEOL 6300F). The films were sputter-coated with gold using a Denton sputter coater for 30 s.

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