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In situ chemical reductive growth of platinum nanoparticles on glass slide for the mass fabrication of biosensors

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Abstract

Platinum nanoparticles (PtNPs) attached to glass slide surface was successfully prepared by using a simple in situ chemical reduction method. In this method, a ~10 nm gold layer was first sputtered uniformly onto the glass slide surface, PtNPs could be grown directly on the gold layer by immersing the glass slide into the grown solution containing H_2PtCl_4 and ascorbic acid. The gold layer sputtered uniformly serves as "seed" for the following growth of PtNPs and high dense of PtNP modified film can be prepared. Control experiment without the gold layer found no obvious formation of PtNPs indicating the importance of the "seed". The electrocatalytic effect of the PtNP film was investigated with the detection of hydrogen peroxide and for the fabrication of biosensors. Glucose oxidase was selected and directly electrodeposited onto the PtNPs modified surface. The resulting biosensor has a fast response time (<10 s) with wide linear range (5×10^{-6} to 2×10^{-2}). The fabrication method is simple, convenient and can be used for the mass fabrication of biosensors. The present preparation method of PtNPs modified film could be used for the preparation of other metal nanoparticle and find electrochemical applications as well as for optical uses.
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1. Introduction

The interesting optical, electronic, magnetic, and catalytic properties of metal nanoparticles have gained growing interest in recent years and metal nanoparticle modified surfaces are known to be effective for applications in catalysis, electrochemistry, and metal-enhanced spectroscopies [1–7]. Various methods have been explored to attach metal nanoparticles onto different surfaces, including physical, and chemical methods, such as electrochemical process, metal evaporation, and layer-by-layer self-assembly [8–10]. Compared with these strategies, wet chemical approach provides an easier control over nanoparticle size, and density by altering the conditions for chemical preparation [11]. For example, Chang et al. systematically investigated attachment of different metal nanoparticles on indium–tin oxide surface using seed-mediated wet chemical reductive method [12–14].

Although many papers have been devoted to the modification of metal nanoparticles on conductive surfaces such as gold, platinum, and indium–tin oxide, reports of the attachment of nanoparticles on non-conductive surfaces like glass slide, and glass coverslip are rather scarce. If we could attach metal nanoparticles on glass slide or glass coverslip in a simple way, in particular with keeping a moderate dispersion in a monolayer, then it would greatly facilitate the application of metal nanoparticle modified surfaces, for example for electrochemistry, metal-enhanced spectroscopy, and single molecular spectroscopy study.

Metal platinum is one of the mostly researched noble metals as it has been widely used for the fabrication of biosensors, and in fuel cell technology as effective catalyst [15–18]. The PtNPs modified substrates are used increasingly in many electrochemical applications owing to their extraordinary catalytic properties over bulk metal and also found effective for surface-enhanced Raman and IR spectroscopy [19–22]. Gomez et al. deposited platinum nanoparticle ensembles on electrodes and investigated in situ surface-enhanced Raman spectroscopy [21].

In this paper, we reported a simple method to attach platinum nanoparticles onto glass slide surface. First, a ~10 nm gold layer was sputtered uniformly onto the glass slide surface, PtNPs could be grown directly on the gold layer by immersing

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the glass slide into the grown solution containing H_2PtCl_4 and ascorbic acid. The attachment and structure growth of PtNPs were characterized in detail with scanning electron microscopy (SEM). The ~10 nm gold layer was found indispensable for the growth of nanoparticles as control experiment of bare glass slide without gold layer found very few nanoparticles. The electrocatalytic property of the PtNPs modified surface was investigated with the detection of hydrogen peroxide and for the fabrication of biosensor. For the fabrication of biosensors, glucose oxidase was selected and directly electrodeposited onto the PtNPs attached surface [23,24]. The resulting biosensor displays high sensitivity and wide linear range. The biosensor preparation method can be used for the mass fabrication of biosensors and the method for the attachment of PtNPs may be applicable to other metal nanoparticles and may find wide applications.

2. Experimental

2.1. Apparatus and reagents

Glucose oxidase (GOx, from *Aspergillus niger*; EC 1.1.3.4, type VIII-S; 196,000 unit g^{-1}) was purchased from Sigma. A 1/15 M phosphate buffer (PB, pH 6.98) solution was prepared using Na_2HPO_4 and KH_2PO_4. All other reagents were of analytical grade, and double distilled water was used throughout.

Cyclic voltammetric and amperometric measurements were carried out on CHI 760B electrochemical workstation (Shanghai, China). Scanning electron microscopy (SEM) analysis was performed using a JSM-5600LV microscope (JEOL Ltd., Japan). A three-electrode cell (10 mL) with the modified glass slide as the working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum foil electrode as counter electrode was used. All potentials were measured and reported versus the SCE and all experiments were carried out at room temperature.

2.2. Attachment of PtNPs on glass slide surface

Typical procedures to attach PtNPs on glass slide are as follows: a piece of glass slide (1 cm^2) was used after washing in acetone, ethanol, and pure water with sonication, and drying with nitrogen gas. First, a ~10 nm gold layer was sputtered onto the slide surface, then, the slide was immersed into solution containing 0.05 mM H_2PtCl_4 and 5 mM ascorbic acid for a constant time at room temperature. After a given time, the slide was removed from the solution, and rinsed with pure water several times, then dried with nitrogen gas.

2.3. Fabrication of biosensors

For the preparation of electrode, an insulated copper wire was connected to the corner of the slide by applying a drop of conductive silver epoxy followed by insulating epoxy. For the fabrication of biosensor, GOx was immobilized onto the PtNPs modified glass slide surface by an electrodeposition method. Electrodeposition was performed using chronoamperometry in PB containing 20 mg/mL GOx and 0.8 mM Triton 100 at a potential of 1.3 V for 30 min.

3. Results and discussion

3.1. Morphological characterization of the PtNP attached surface

The central theme of the present study is to modify the glass slide surface with PtNPs using a simple and reproducible method. A ~10 nm gold layer was uniformly sputtered onto the slide surface and serves as "seed" for the following growth of nanoparticles. Former literature reported using small nanoparticles (size less than 5 nm) as seed and adsorbing the seed onto substrate followed by chemical reduction [12–14]. We assume the sputtered gold layer would be more uniform and stable, and the method would be quite simple. First, the function of the gold layer was investigated. As a control experiment, a bare glass slide was immersed into the growth solution containing H_2PtCl_4 and ascorbic acid. During the chemical reductive process, the colour of the glass slide changed from transparent to darkish brown. It turned out that the PtNPs were just simply falls onto the surface, not grown on the glass slide, as after 12 h of reaction, taking out the slide and rinsing with water for several times, the PtNPs were easily rinsed away. After rinsing, the slide was characterized with SEM as shown in Fig. 1A. It can be seen that very little PtNPs with small size, which were recognized as bright dots, were left on the slide, indicating that without the gold layer, the interaction between glass slide and PtNPs is very weak, because there is no nucleation sites for the growth of nanoparticles. On the contrary, when the slide was sputtered with gold before immersion into the growth solution, the PtNPs are strongly attached onto the slide surface. After washing with water and even sonication, no distinct removal of PtNPs was observed. We expect that this firm binding comes from the initial stable gold layer, and the following PtNPs were grown on this layer.

Fig. 1B shows SEM images recorded after treatment in the growth solution for 2 h. It can be seen PtNPs grew on the glass slide surface with a moderate dispersion. The shape of PtNPs was almost spherical, and the size dispersion was relatively regular, with the diameters of the PtNPs around 50 nm. But there are also small nanoparticles around 10 nm, indicating the particles were grown through a progressive nucleation process, that is, the big nanoparticles were grown from the smaller ones.

The effect of reduction time on the morphology of the nanoparticle film was investigated in detail. Fig. 2 shows SEM images of the glass sample with reduction time of 4 h. It can be seen, increasing the reduction time, the size of the nanoparticles increased, reaching around 80 nm. However, small nanoparticles could be still observed, which shows that during the reduction process, smaller nanoparticles grown into larger ones and at the same time, small nanoparticles are continuously formed. From the lower magnification image (Fig. 2B), one can see that although there are some voids exist, the entire film is relatively intact, dense and has an ordered structure, suitable for further applications. The morphology of the film was further changed

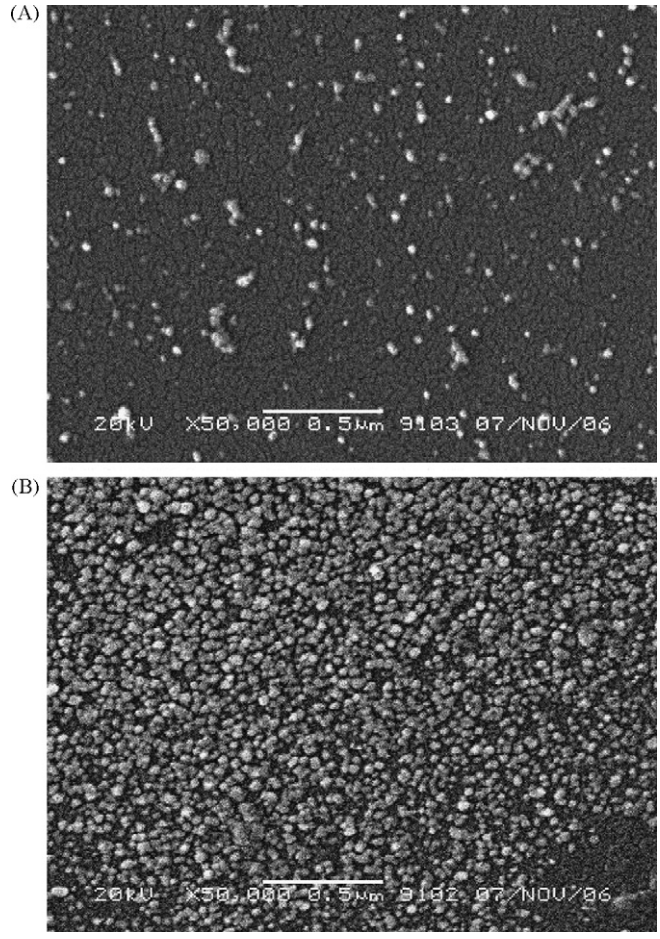

Fig. 1. (A) SEM image of the bare glass slide after immersion into growth solution for 12 h. (B) SEM image of the glass slide sputtered with 10 nm gold and immersion into growth solution for 2 h.

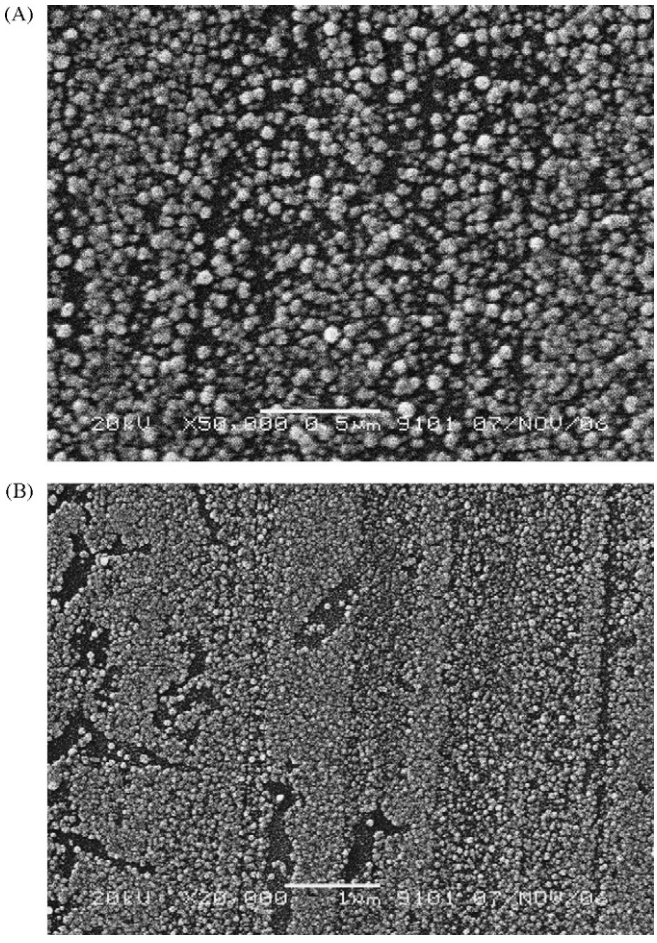

Fig. 2. SEM images of the glass slide sputtered with 10 nm gold and immersed into growth solution for 4 h.

after 12 h of reduction as shown in Fig. 3. At this time, the diameter of the nanoparticles increased to 300 nm, and particles contacted to each other, forming a layered film.

3.2. Electrochemical characterization of the PtNP attached glass slide

From the above SEM characterization, it can be seen that the present method could be used to form PtNP film with different structures, from monolayer to multilayer, and the size of the PtNP can also be altered. At the reduction time of 4 h, the glass slide surface was almost covered with nanoparticles, so this slide could be used as electrode and it would be interesting to investigate the electrochemical performance of the modified glass slide. The glass slide after growth of 4 h was chosen and the following experiments were done using this slide as electrode. First, the conductivity of the electrode was determined and Fig. 4 shows the cyclic voltammograms (CVs) of the glass slide in 20 mM $K_3Fe(CN)_6$ and 0.2 M KCl with different scan rates. The well-defined anodic and cathodic peaks due to the Fe^{3+}/Fe^{2+} redox couple were observed and at 50 mV s^{-1} the peak potentials were 330 and 164 mV with peak separation of 166 mV, which were similar to those observed with the bulk electrode [25,26]. In addition, both of the peak current clearly increase with increasing potential scan rate and are linearly related with the square root of the scan rate in a range from 10 to 200 mV s^{-1}, suggesting a diffusion controlled process. Thus, the conductivity of the electrode is good, indicating the PtNP film is efficient in transferring

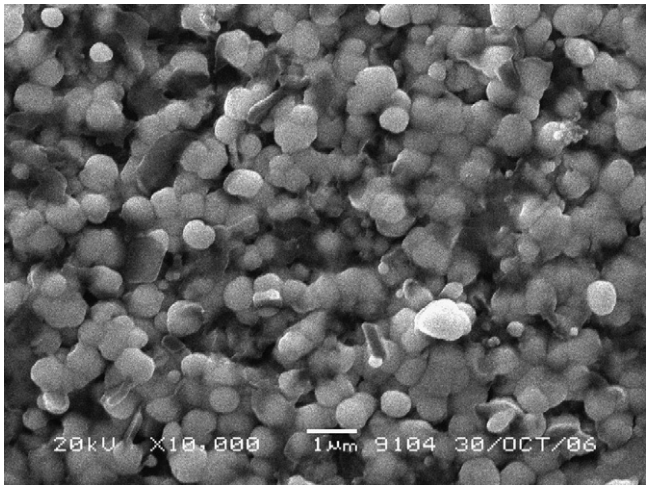

Fig. 3. SEM image of the glass slide sputtered with 10 nm gold and immersed into growth solution for 12 h.

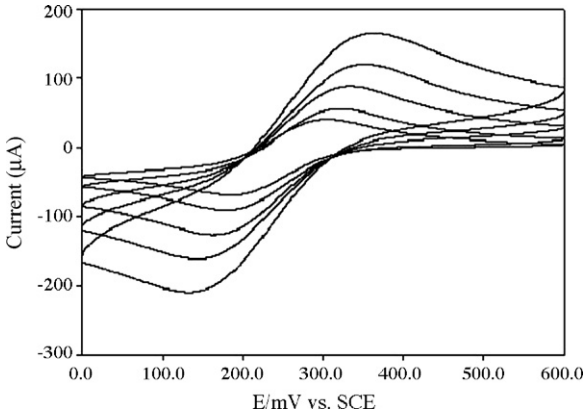

Fig. 4. Cyclic voltammograms (CVs) of the PtNP-modified glass slide in 20 mM $K_3Fe(CN)_6$ containing 0.2 M KCl with different scan rates. From inner to outside: 10, 20,50, 100, and 200 mV s^{-1}.

electrons and forming a diffusion layer. This good conductivity made it possible for further application.

The electrochemical response of the PtNP film toward H_2O_2 was also investigated with potential design of a biosensor. Fig. 5A shows cyclic voltammograms of the film in phosphate buffer in the absence (a) and in presence of 5 mM H_2O_2 (b) between −0.6 and 0.6 V at the scan rate of 100 mV s^{-1}. When H_2O_2 was added to the buffer, an obvious increase of the cathodic and anodic current was observed, indicating the film exhibited excellent electrocatalytic activity toward H_2O_2. Fig. 5B depicts the typical current–time curve at the film modified slide for successive addition of 10 μM H_2O_2 at 0 V. Fast and sensitive response can be observed at the electrode with steady-state current reached within 5 s, showing the reduction of H_2O_2 at the PtNP film is facilely easy.

The stability of the PtNP film in detection of H_2O_2 was investigated. For example, 1 mM H_2O_2 was measured continuously for 10 times, and a relative standard deviation (R.S.D.) of 4.9% was obtained. The satisfactory repeatability indicates the reliability of the film for the determination of H_2O_2 and made it feasible for the further biosensing application.

3.3. Performance of the glucose biosensor

The good performance of the PtNP film toward H_2O_2 and the simple, convenient method for the preparation of the PtNP film made it an ideal biosensing platform for the fabrication of oxidase-based biosensors. In this study, glucose oxidase was selected and directly electrodeposited onto the PtNP film surface. A uniform and biologically active enzyme film could be formed with electrochemically mediated deposition of enzyme on an electrode in the presence of nonionic detergent [23]. The electrodeposition method could produce a much more extensive and compact enzyme film than enzyme film through physical adsorption. After electrodeposition, the biosensor can be used for the detection of glucose. At 0 V, in phosphate buffer with careful magnetic stirring, adding standard glucose solution resulted in an instantaneous current response. Fig. 6 is the calibration curve of the biosensor toward glucose and the insert shows the current plotted versus time for successive addition of

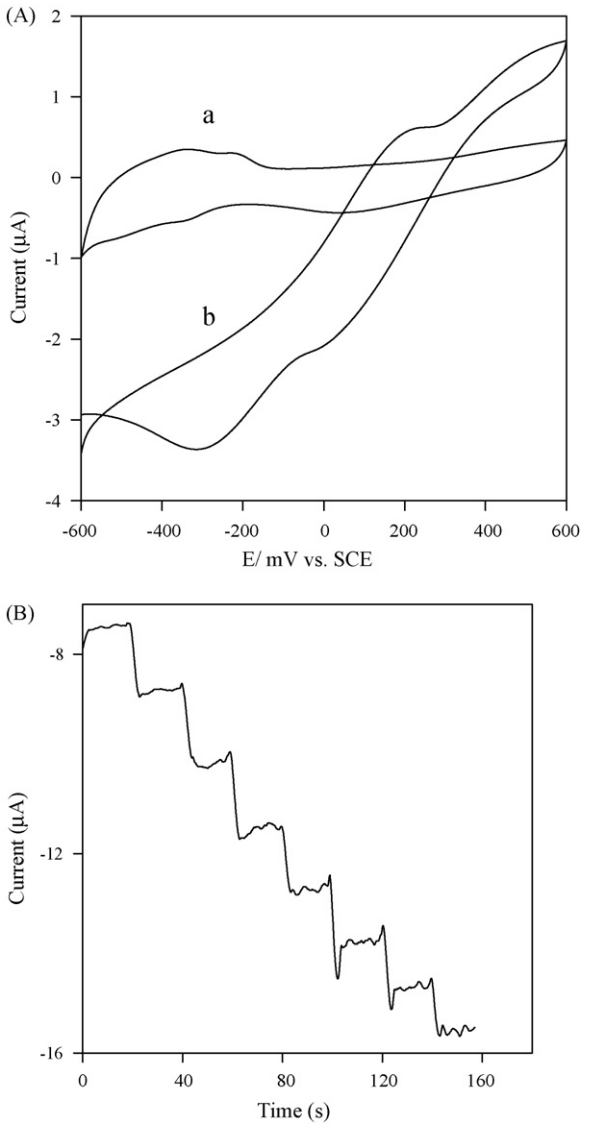

Fig. 5. (A) CVs of the PtNP film in the absence (a) and presence (b) of 5 mM H_2O_2. Scan rate: 100 mV s^{-1}. (B) Current–time curve of the PtNP film to the successive addition of 10 μM H_2O_2 at 0 V.

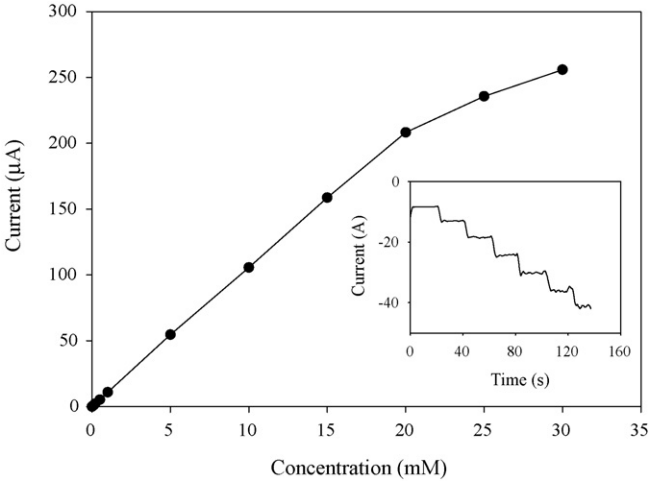

Fig. 6. Calibration curve of the glucose biosensor. Insert: current–time curve of the biosensor to the successive addition of 0.5 mM glucose at 0 V.

Table 1
Comparison of glucose concentration in serum samples determined with the biosensor and the hospital method (values were mean value of three determinations)

Sample	Concentration determined by the biosensor (mM)	Concentration determined by hospital method (mM)
1	7.89	8.01
2	8.65	8.94
3	10.32	10.88
4	11.23	11.78
5	12.67	13.13

0.5 mM glucose. The resulting biosensor has a fast response time (<10 s). The linear calibration range is extended from 5×10^{-6} to 2×10^{-2} M, and the detection limit of 1×10^{-6} M was obtained based on S/N = 3. The wide linear range indicates large amount of enzymes was immobilized onto the electrode surface through electrodeposition. The repeatability of the biosensor was also tested and found satisfactory. The glucose concentration of 0.5 mM was measured continuously for six times, and a R.S.D. of 5.1% was obtained. The low R.S.D. means that both of the enzyme and PtNP film are relatively stable.

For the mass fabrication of biosensors, the reproducibility of the biosensor is very important. To investigate the reproducibility, six different biosensors were prepared carefully, and the current response of the biosensors toward 0.5 mM glucose was detected with R.S.D. of 6.5%. The satisfactory repeatability and reproducibility means the biosensors can be used for practical application.

The selectivity of the biosensor was determined. In this study, the determination of glucose is practically free of interferences. At the low detection potential of 0 V, 0.1 mM ascorbic acid, 0.1 mM uric acid, and 0.1 mM acetaminophen gave slight response current, but compared to the response of 1 mM glucose, the current response are negligible, indicating high selectivity of the biosensor.

The long-term stability of the biosensor was determined. When not in use, the biosensor was stored at 4 °C. The response of the biosensor to 0.5 mM glucose was tested intermittently (every 3 days), and 1 month later, the biosensor still obtained 80% of the initial value, indicating good stability of the sensor.

3.4. Real sample analysis

The applicability of the biosensor was tested by the determination of serum sample glucose concentration. Results were compared with hospital method with glucose kit (Shanghai Kehua-Dongling Diagnostic Products Co. Ltd.). Five samples were analyzed with the results shown in Table 1. Glucose concentrations determined by the two methods agreed rather well, indicating the reliability of the biosensor.

4. Conclusions

In the present work, PtNP film can be grown on glass slide surface by the in situ chemical reduction method. The 10 nm gold layer uniformly sputtered on glass slide surface served as "seed" for the following growth of nanoparticles. The morphology of the PtNP film can be altered by altering the reduction time, that is with increasing reduction time, the size of the nanoparticle increased and the density of the PtNP changed from a monolayer to a multilayer film. The electrochemical application of the PtNP film modified glass slide as working electrode was investigated in detail. In $K_3Fe(CN)_6$ solution, well-defined anodic and cathodic peaks and diffusion controlled process were observed, indicating good conductivity of the film. The electrocatalytic activity of the PtNP film was discussed with hydrogen peroxide detection. Glucose oxidase was selected and directly electrodeposited on the PtNP film. The resulting biosensor has good performance toward glucose. The method for the attachment of PtNPs may be applicable to other metal nanoparticles and may find wide applications.

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