

Preparation of Au/ polypyrrole composite nanoparticles and study of their electrocatalytical reduction to oxygen with (without) laccase

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Abstract

The colloids of Au/polypyrrole (AuPPy) composite nanoparticles were prepared by oxidizing pyrrole monomer with HAuCl_4 in a cetyltrimethylammonium bromide (CTAB) solution. Scanning electron microscopy (SEM) suggests the AuPPy nanoparticles in the form of regular spheres, approximately 200 nm in diameter. The resulting colloid of AuPPy composite nanoparticles strongly adheres to the surface of Au electrodes and exhibits better electrocatalytical reduction of oxygen than bare Au electrodes. It means that the complex procedures of centrifuge and wash are avoided. Also, no linker molecules are needed and the immobilization of nanoparticles is achieved easily in a single-step procedure. The experimental parameters were optimized with regard to the concentration of pyrrole and HAuCl_4 . The direct electron transfer of laccase is observed after it is immobilized on AuPPy modified electrodes by glutaraldehyde. With the help of mediator 2,2'-azino-bis-(3-ethylbenzothiazoline-sulfonic acid) (ABTS), laccase electrode gives an electrocatalytical reduction wave of oxygen at least at 0.8 V. This material is an excellent choice for the design of metal nanoparticle modified electrodes or biosensors

Keywords: Oxygen reduction, Laccase, Polypyrrole, Au nanoparticles

1 Introduction

Metal nanoparticles of gold and other noble metals, due to the large surface-to-volume ratio and the increased surface activity, have been studied extensively due to their unique physical and chemical characteristics, such as optical, electronic, magnetic, catalytic and electrochemical properties [Brito et al., 2007; Chen et al., 2007; Sonia et al., 2007; Zhou et al., 2008].

For electrocatalytical or electroanalytical applications, a variety of methods have been reported to immobilize metal nanoparticles onto the surface of electrodes [Calvo et al., 2007; Chu et al., 2007; Du et al., 2007; Lin et al., 2007]. Basically, almost all immobilization strategies have to draw support from the other materials. Nanoparticles are anchored onto the surface of electrodes by molecular self-assembly, covalent linking or being trapped into various gel or polymer films. The main limitation is that the immobilization procedure is complex. In addition, the centrifuge and wash of colloid make it more complex.

Conducting polymers have been used as the supporting matrix in different composites for intercalation of catalytically important nanoparticles so that the catalytic activity can be retained in the composite [Jin et al., 2007; Panda et al., 2007; Selvaraj et al., 2007]. In the past few years, there have been some reports on the synthesis of polypyrrole/gold nanocomposites, most of which were prepared in two steps. In a typical composite synthesis experiment, Au nanoparticles were synthesized firstly, and then polypyrrole was chemically oxidized to coat Au nanoparticles [Lu et al., 2006]. In fact, polypyrrole/gold colloids could also be prepared using a redox reaction between HAuCl_4 as an oxidant and pyrrole monomer as a reductant [Henry et al., 2001; Park et al., 2005].

Here, the stable colloids of AuPPy composite nanoparticles were prepared by oxidizing pyrrole monomer with HAuCl_4 in the presence of CTAB. The resulting colloid of AuPPy composite nanoparticles strongly adheres to the surface of Au electrodes. It is a facile and versatile route to immobilize AuPPy nanoparticles and the above-mentioned disadvantages of metal nanoparticles immobilization can be avoided. The study is focused on their electrocatalytical reduction of oxygen with (without) laccase.

2 Materials and methods

2.1 Reagents

HAuCl_4 and ABTS was obtained from Sigma. Laccase (Trametes Versicolor) was from Biochemika. CTAB, glutaraldehyde and pyrrole were purchased from SCRC (Shanghai, China). They were used as received. All other chemicals were of analytical grade and were used without further purification. All solutions were prepared by ultra-pure water from Milli-Q water. All experiments were carried out at room temperature.

2.2 Preparation of AuPPy composite nanoparticles

AuPPy composite nanoparticles were prepared by direct redox reaction between pyrrole monomers and HAuCl_4 . Typically, 0.01 μl pyrrole was dissolved in 2 ml 0.5 M H_2SO_4 solution with 10 mM CTAB. Then 10 μl 2 mM HAuCl_4 aqueous solution was added drop by drop. The solution turned transparent light yellow overnight, which resulted in a stable dispersion of AuPPy colloid.

2.3 Anchoring AuPPy nanoparticles onto the Au electrodes and fabrication of laccase electrodes

The Au disk electrodes (diameter 2 mm) were mechanically polished successively with 1 and 0.05 μm alumina slurries to a mirror finish. Then they were cleaned with piranha solution (H_2O_2 : H_2SO_4 = 1:3) for 20 min followed by a thorough rinse with pure water.

20 μL of AuPPy colloid solution was cast on the surface of Au electrodes. After 12 h, the nanoparticle-modified electrodes were rinsed extremely with pure water and named as Au-AuPPy electrodes.

For the preparation of laccase electrodes, 2 μl 10 mg/ml laccase solution was added onto the AuPPy-modified

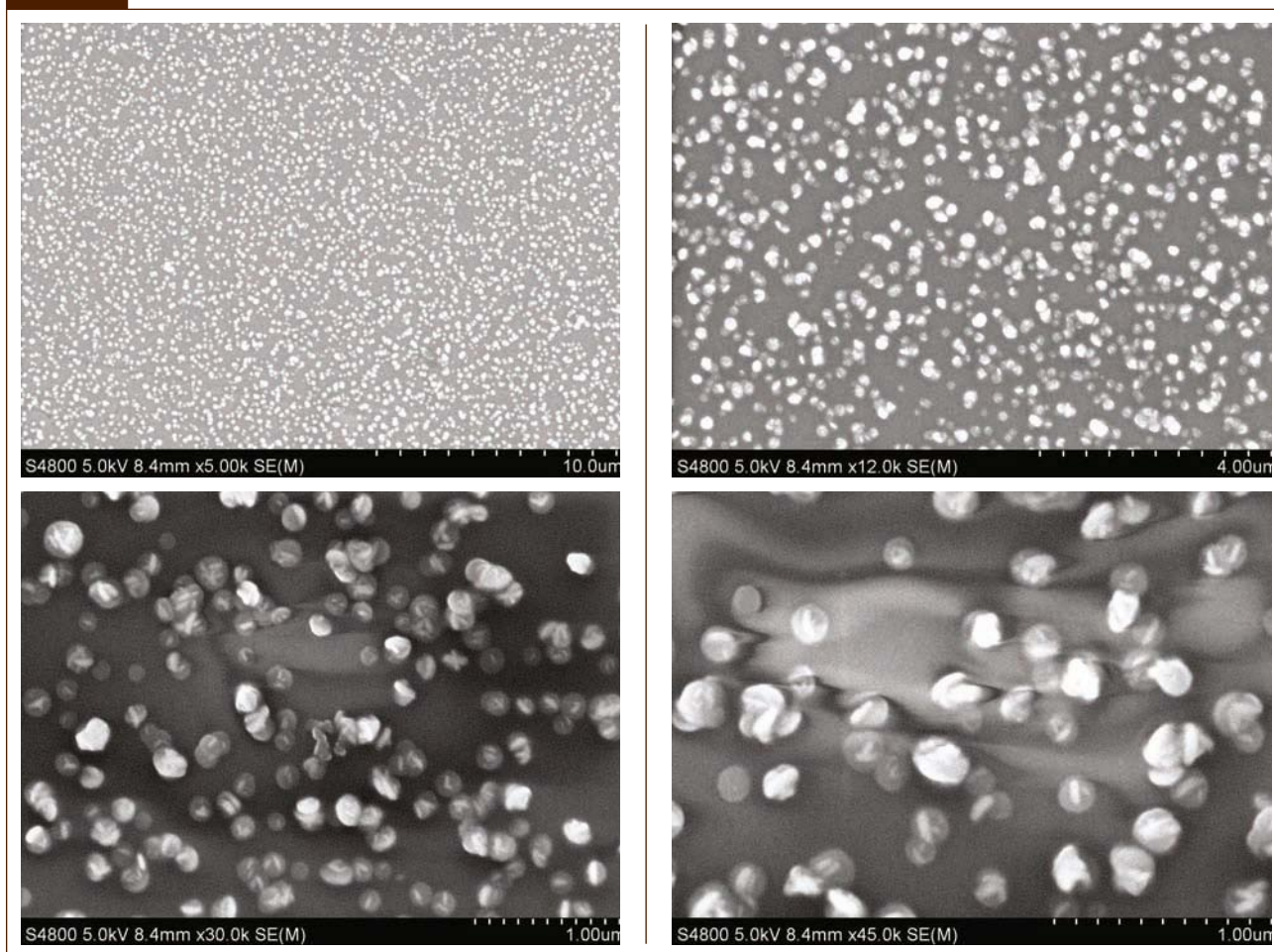
electrodes and dried, then 1 μl 0.1 % glutaraldehyde was dropped onto the electrode surface and the electrodes were allowed to dry at room temperature. The resulting electrodes are named as Au-AuPPy-laccase electrodes. As contrast, 2 μl 10 mg/ml laccase solution was added onto the bare Au electrode and dried, then 1 μl 0.1 % glutaraldehyde was dropped onto the electrode surface and the electrodes were allowed to dry at room temperature. The resulting electrodes are named as Au-laccase electrodes.

2.4 Characterization and measurement

The physical characteristics of the AuPPy nanoparticles were studied by SEM (S-4800 UHR FE-SEM).

Electrochemical experiments were conducted with an Autolab PGSTAT 30 System (Ecochemie, The Netherlands) in a three electrode system. Nanoparticle-modified Au electrode with the diameter of 2 mm was used as working electrode, a platinum wire as auxiliary electrode, and an Ag/AgCl electrode as reference electrode. The supporting electrolyte is 10 mM phosphate buffer solution (PBS, pH 5.5) containing Na_2HPO_4 and KH_2PO_4 in 0.1 M KCl. All experiments were carried out at the room temperature.

Figure 1



SEM image of AuPPy nanoparticles. AuPPy colloid was prepared by adding 14 μl pyrrole and 100 μl 2mM HAuCl_4 in turn into 2 ml 0.5 M H_2SO_4 solution with 10 mM CTAB

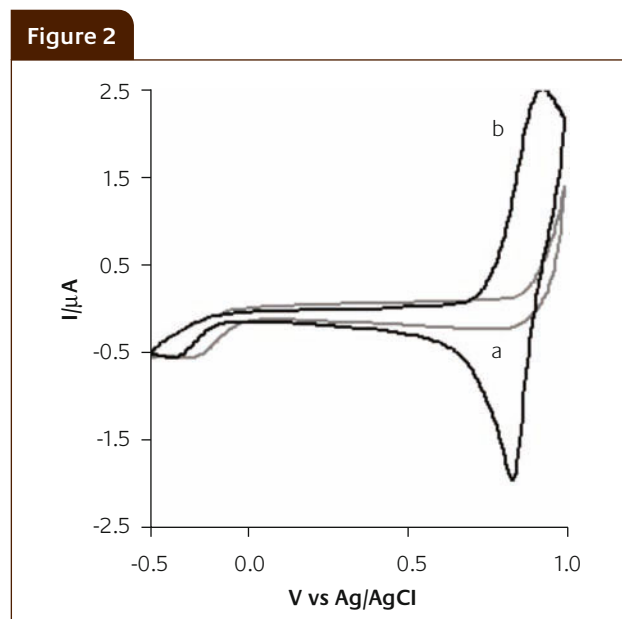
3 Results and discussion

After the addition of HAuCl_4 into the pyrrole solution with CTAB as dispersant, the color of solution was slowly changed to light yellow, red, violet or black, which depends on the concentration and rate of HAuCl_4 and pyrrole. The resulting solution is clear and transparent, which means that well dispersed colloid is formed with the help of surfactant CTAB. Actually, the polymerization of pyrrole into PPy and the reduction of HAuCl_4 to elemental Au occur instantaneously and simultaneously. The mixture was stirred overnight at room temperature for complete reaction.

The morphology and size of AuPPy were investigated with SEM (Fig.1). With the help of CTAB, the prepared AuPPy nanoparticles are dispersed well and have a uniform spherical structure. It can be considered that the protective cation surfactant CTAB might disperse pyrrole well in water solution. SEM revealed that the average diameter of AuPPy is about 200 nm. The results of SEM suggest that the dispersions prepared by one-step synthesis are not mixture of the Au and PPy nanoparticles but composed of the AuPPy composite clusters.

20 μL of AuPPy colloid solution was cast on the surface of Au electrode. After 12 h, the nanoparticles-modified electrode was rinsed extremely with pure water for electrochemical characterization. Fig.2 shows the CVs of the bare and AuPPy modified Au electrode in a pH 5.5 PBS.

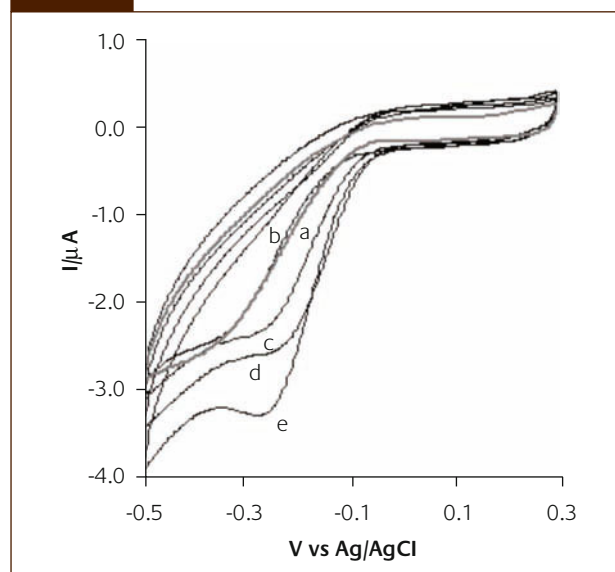
AuPPy modified Au electrode shows typical anodic peak (Au oxide, ca. 0.92 V) and cathodic peak (reduction of Au oxide, ca. 0.83 V) as shown in Fig. 2b. The potentials of both anodic and cathodic peak of AuPPy-modified Au electrode shift negatively as compared to that of bare Au electrode



Cyclic voltammograms (CVs) of bare Au (a), and Au-AuPPy electrode in pH 5.5 PBS. Scan rate: 100mV/s. AuPPy colloid solution was prepared by adding 14 μL pyrrole and 100 μL 2mM HAuCl_4 in turn into 2 ml 0.5 M H_2SO_4 solution with 10 mM CTAB

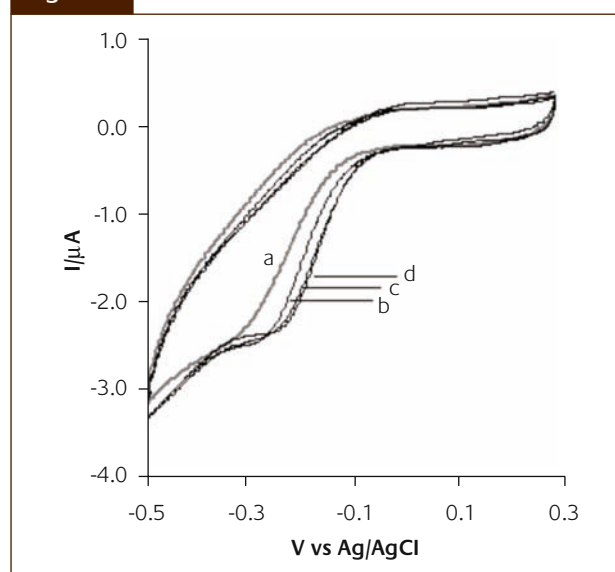
(Fig. 2a). This enhanced electrochemical activity is attributed to the presence of nano-sized Au in AuPPy nanoparticles. The CVs keep unchanged by continuous cycling, which means that AuPPy nanoparticles strongly adhere to the Au electrode and exhibit their inherent electrochemical activity. The oxidation or reduction peak of polypyrrole hasn't been observed. It is possible that the amount of polypyrrole is far less than that of Au within AuPPy composite nanoparticles and the electrochemical response of former far smaller than that of the latter. The peak potential of oxygen reduction for

Figure 3



CVs of bare Au (a), and Au-AuPPy electrodes in a pH 5.5 PBS. Scan rate: 100mV/s. AuPPy colloid solution was prepared by adding 0.8 (b), 0.4 (c), 0.2 (d), 0.1 (e) μL pyrrole and 20 μL 2 mM HAuCl_4 in turn into 2ml 0.5 M H_2SO_4 solution with 10 mM CTAB

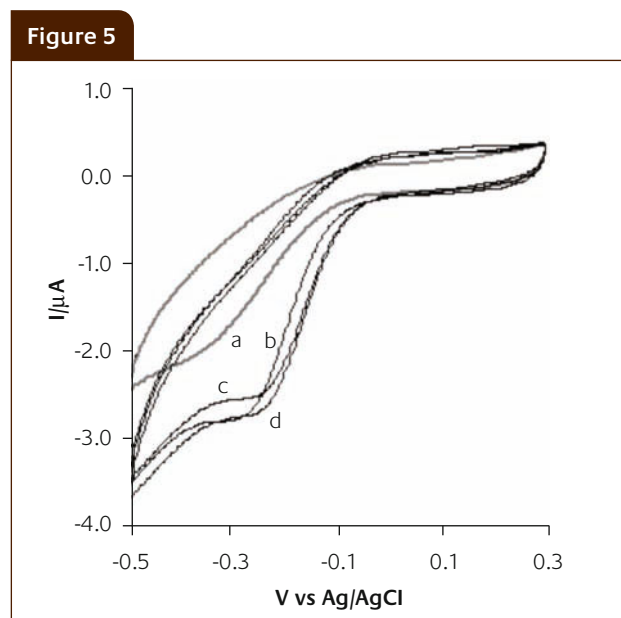
Figure 4



CVs of bare Au (a), and Au-AuPPy electrodes in a pH 5.5 PBS. Scan rate: 100mV/s. AuPPy colloid solution was prepared by adding 0.001 (b), 0.005 (c), 0.01 (d) μL pyrrole and 20 μL 2 mM HAuCl_4 in turn into 2 ml 0.5 M H_2SO_4 solution with 10 mM CTAB

AuPPy-modified electrode shift negatively as compared to that for bare Au electrode. Obviously, the presence of polypyrrole within AuPPy affects the electrocatalytical reduction of AuPPy toward oxygen.

To optimize the electrocatalytical activity of AuPPy toward oxygen reduction, the effect of pyrrole concentration in reaction solution was studied firstly. AuPPy colloid solution was prepared by adding 0.8, 0.4, 0.2, 0.1 μl pyrrole and 20 μl 2 mM HAuCl_4 in turn into 2 ml 0.5 M H_2SO_4 solution with 10 mM CTAB. As shown in Fig.3, compared with the bare Au electrode, the peak potential of the oxygen reduction shifts positively and the peak current increases largely with the decrease of pyrrole concentration. It seems that the presence of excess polypyrrole has a negative effect on the electrocatalytical reduction of oxygen. However, the electrocatalytical ability of oxygen reduction decreases when the concentration of pyrrole is reduced further (Fig.4). These results show suitable amount of pyrrole is necessary for the immobilization of AuPPy nanoparticles.

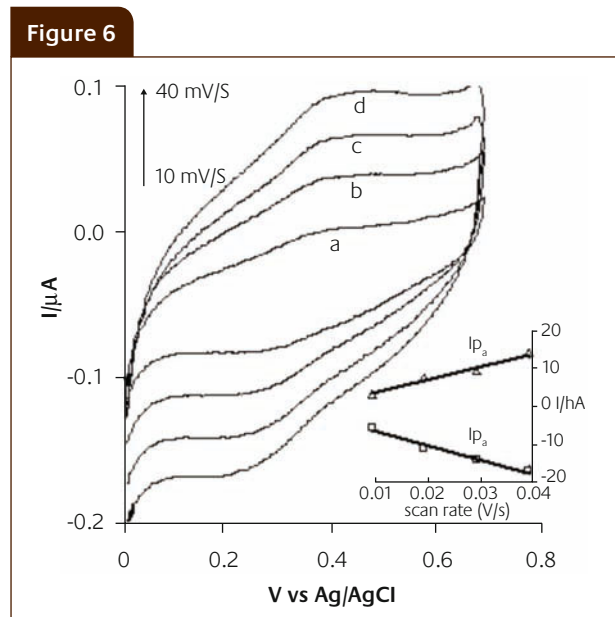


CVs of bare Au (a), and Au-AuPPy electrodes in a pH 5.5 PBS. Scan rate: 100mV/s. AuPPy colloid solution was prepared by adding 0.01 μl pyrrole and 5 (b), 10 (c), 20 (d) μl 2 mM HAuCl_4 in turn into 2 ml 0.5 M H_2SO_4 solution with 10 mM CTAB

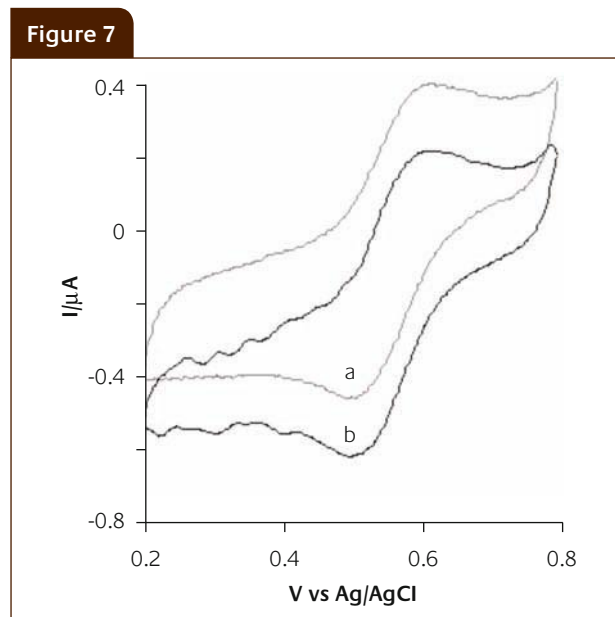
Fig.5 shows that the peak potential of AuPPy nanoparticles toward oxygen reduction shifts positively with the increase of HAuCl_4 . However, the peak current of AuPPy nanoparticles toward oxygen reduction decreases when the amount of HAuCl_4 is raised further to 20 μl . In fact, a compromise should be made to get optimised electrocatalytical results for AuPPy toward oxygen reduction.

Fig. 6 shows the CVs of Au-AuPPy-laccase electrodes in N_2 -saturated pH 5.5 PBS at different scan rates. A pair of redox peaks at 0.27 V and 0.40 V respectively are observed at 40 mV/s, and its formal potential is around 0.33 V. In contrast, Au-laccase electrode gives no obvious

electrochemical signal, which means that laccase hasn't been immobilized onto the bare Au surface. Therefore, the redox peaks are attributed to the redox reactions of laccase immobilized on AuPPy modified electrode, and AuPPy nanoparticles play an important role in improving the laccase immobilization and facilitating the direct electron transfer between laccase and Au electrode. The effect of scan rate on the redox response of laccase has been studied. Both redox peak currents and peak-to-peak separation increase with the increase of scan rate. The anodic and cathodic peak currents (I_{pa} and I_{pc}) for laccase are linearly proportional to the scan



CVs of Au-AuPPy-laccase electrodes at 10, 20, 30, 40 mV/s in N_2 -saturated pH 5.5 PBS. Inset: plots of peak currents vs scan rate



CVs of Au-AuPPy-laccase electrodes in pH 5.5 PBS containing 0.1 mM ABTS. Solution deoxygenated with N_2 (a) and saturated with O_2 (b). Scan rate: 100 mV/s

rate ranging from 10 to 40 mV/s, as expected to be a surface-confined electrode reaction (inset of Fig.6).

In order to increase the catalytic efficiency of the laccase, a common mediator ABTS has been added to the solution to make the electron transfer more efficient. Fig.7 shows CVs of Au-AuPPy-laccase electrodes in pH 5.5 PBS containing 0.1 mM ABTS in the absence and presence of O₂. The redox peaks of ABTS appear at 0.50 and 0.61 V respectively, in agreement with the literature data. The catalytic reaction starts immediately when enzyme, mediator and substrate are contacted, namely when the electrode is immersed in the electrolyte solution. The oxidization current of ABTS decreases and reduction current increases in the solution saturated with oxygen compared to that deoxygenated with nitrogen. The redox mediator ABTS is oxidized by laccase and the regeneration of the enzyme is achieved by the reduction of molecular oxygen to water. This resulted in an electrocatalytic reduction wave of oxygen at least at 0.8 V. The scan range hasn't extended over 0.8 V in order to avoid the oxidation current of nanosized Au within AuPPy nanoparticles.

4 Conclusions

In this paper, we describe a very simple and direct one step procedure to immobilize AuPPy composite nanoparticles. The colloids of AuPPy composite nanoparticles are directly added onto the electrode surface, and nanoparticles strongly adhere to the surface of Au electrodes. The AuPPy composite nanoparticle-modified electrodes exhibit good electrocatalytic reduction toward oxygen. In addition, the presence of gold in the AuPPy composite nanoparticles have the ability to facilitate direct and fast electron transfer between enzyme and electrodes. Here, the polypyrrole within AuPPy serves as a linker to immobilize AuPPy onto the electrode surface. This material is an excellent choice for the design of metal nanoparticle modified electrodes or biosensors.

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