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Platinum Nanoparticles (PtNPs) - Laccase Assisted Biocathode Reduction of Oxygen for Biofuel Cells

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Reduction of dioxygen catalyzed by laccase was studied at platinum nanoparticles (PtNPs) modified electrodes. PtNPs with laccase are directly fabricated on glassy carbon electrode (GCE) and indium tin oxide electrode (ITO) by simple process. The PtNPs/laccase modified ITO electrode surface has been studied in detail using atomic force microscopy (AFM). This PtNPs/laccase modified GCE effectively exhibits the electro reduction signals for the detection of oxygen. PtNPs provided large surface area for laccase base on modified electrode. The PtNPs present on the electrode provided electrical connectivity between the electrode and the enzyme active sites. The cyclic voltammetry (CVs) has been used for the measurement of electroanalytical properties of analytes by means of modified electrodes. The stabilities of laccase, the reduction potentials and ratios of catalytic to background currents were compared. The power densities of biofuel cell was 84 $\mu W/cm^2$ at 0.62 V, respectively. The biofuel cell showed highly stable output in long-term performance. Efforts are underway to improve the interface transfer to achieve higher potential and current output.

Keywords:

1. INTRODUCTION

Nanomaterials have attracted much attention for designing novel biosensing systems and biofuel cell with enhanced performance of bioanalytical assay. Electrochemically functional nanoparticles (NPs) films have been of great interest for the past decade in electrocatalytic reactions because of their unique chemical and electrochemical properties. Gold (Au) and platinum (Pt) nanoparticles, in particular have been used extensively for the modifications of electrode [1-7].

Previous studies revealed that intensive research has been carried out on the fabrication of sensor devices using nano-Pt and nano-Au particles [8-9]. Between them, gold nanoparticles (AuNPs) have the ability to provide biocompatible surface for the immobilization of biomolecules, high surface area and good conductivity between electrode and biomolecules [10-12]. Platinum nanoparticles (PtNPs) too have properties similar to other noble metal nanoparticles and exhibit excellent electrocatalytic property for reduction towards O_2 [13-15]. Due to these reasons platinum nanoparticles modified electrodes are frequently used for the detection of target analyte O_2 which is a product of reduction of the substrates by reductase enzyme in the presence of oxygen. Suitable electrode materials and immobilization methods of enzymes onto the electrode surface are important for obtaining their direct electrochemical reaction and keeping their bioactivities [16-17]. On the one hand, numerous methods have been used for the modification and application of Pt in electroanalysis; an example is the monolayers of phosphododecatungstate, where Pt tends to activate them towards the efficient electrocatalytic reduction of oxygen in acidic medium [18].

The communication of this sitewith the electrode is possible either by mediator or direct electron transfer (DET). Mediated and DET coupling of enzymes to electrodes is important in realizing bioelectrocatalysis, which is often exploited as a basic principal of biosensors, biofuel cells, and other bio-based devices. To facilitate DET based bioelectrocatalysis nanoparticles were integrated into the structure of redox enzymes [19-20]. Modification of electrodes with nanoparticles was shown to enable DET of cytochrome *c* (Cyt *c*) [21-23], catalase (CAT) [24-26], cholesterol oxidase (ChOx) [27], myoglobin (MB) or hemoglobin (HB) [28-33], horseradish peroxidase (HRP) [34-36], superoxide dismutase (SOD) [37] and recently of bilirubin oxidase [38]. In this context, laccase is one of the interesting redox enzymes used in a variety of applications [39-41], including biofuel cells [42-46].

Laccases belong to the larger group of multi-copper enzymes. They are produced by plants, fungi, some bacteria and insects [47-48]. It contains four copper (II) atoms, denoted T1, T2 and T3 according to their spectroscopic properties. T1 and T2 are similar to planar Cu^{2+} complexes, whereas T3 is a binuclear $Cu^{2+/1+}Cu^{2+/1+}$ complex [49]. The copper center T1 can be reduced at high potential by phenolic compounds, redox mediators and direct electron transfer from electrodes. While substrates are oxidized at T1, further internal electron transfer leads to the reduction of molecular O_2 at the T2/T3 cluster [50-51]. The T2 and T3 sites form a trinuclear Cu cluster where O_2 is reduced to H_2O with four electrons transferred from the T1 site, which is the site of electron exchange with the redox donor molecules.

The devices employing DET between laccase and the electrode have been favoured recently, because of the simplicity and the lack of thermodynamic losses due to mismatch between enzyme and mediator redox potentials. With these subtle properties, the bioelectrocatalytic reduction of oxygen by laccase has aroused extensive interest. However, the direct electron transfer of laccase is generally difficult due to the complex structures of its redox centers and the unfavorable orientations of laccase at electrodes. Thus, in recent years the electron transfer between laccase and the electrode was shuttled in most cases by redox mediators, such as 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS). In nature these electrons are supplied by several phenols, amines and lignins, as well as inorganic ions [52]. Laccase is the first enzyme for which DET was discovered on carbon electrodes [53], however, no efficient DET has been realized at metal electrodes [54-55]. Here we demonstrate a relatively rapid

DET between a laccase and PtNPs modified electrodes enabling efficient bioelectrocatalytic oxygen reduction. Laccase electrodes have aroused a considerable attention as biocathode for the development of biofuel cells [56-62].

In this work, we have exploited unique properties of PtNPs for the fabrication of a laccase electrode as a cathode for biofuel cells based on electrode surface. The proposed method is simple and would be applicable to enhance the power output of miniaturized biofuel cell. The immobilization of laccases for efficient electron exchange between enzyme and electrode surface is important for the development of new types of fuel cells, battery systems, and in vivo power supplies.

2. EXPERIMENTAL

2.1. Materials and Apparatus

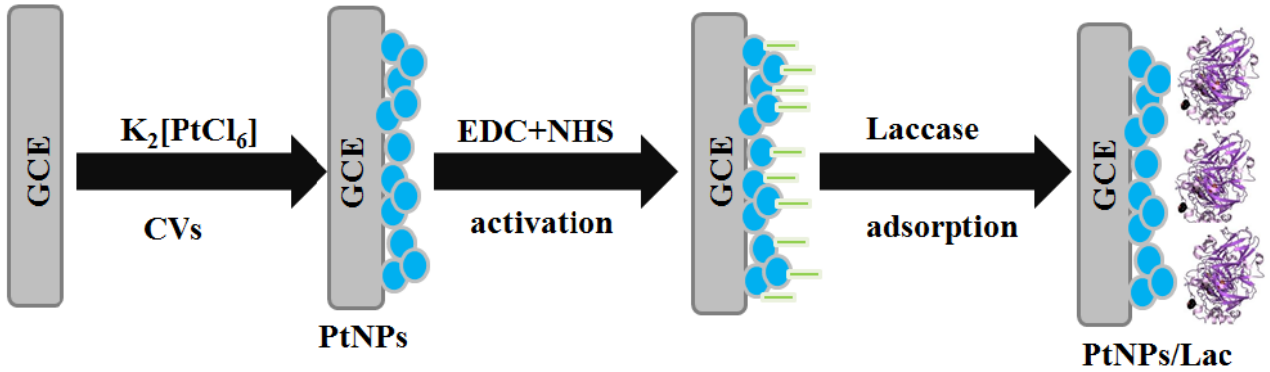
Laccase (EC 1.10.3.2), N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) and Hexachlorooplatinate ($K_2[PtCl_6]$) were purchased from Sigma–Aldrich (St. Louis, U.S.A.). All other chemicals used were of analytical grade and used without further purification 0.1 M pH 7.0 phosphate buffer solutions (PBS) and pH 1.0 H_2SO_4 solutions were used as supporting electrolyte. Aqueous solutions were prepared using doubly distilled deionized water. Nafion 117 was used as membrane.

Cyclic voltammetry (CVs) was performed in an analytical system model CHI-1205A potentiostat. A conventional three-electrode cell assembly consisting of an Ag/AgCl reference electrode and a Pt wire counter electrode were used for the electrochemical measurements. The working electrode was glassy carbon electrode (GCE; area 0.07 cm^2). In these experiments, all the potentials have been reported versus the Ag/AgCl reference electrode. The morphological characterizations of the films were examined by means of atomic force microscopy (AFM) (Being Nano-Instruments CSPM 5000). The power output measurements system by KEITHLEY 2400. All the experiments were carried out at room temperature (≈ 25°C).

2.2. Preparation of PtNPs/laccase modified electrodes

Prior to modification, glassy carbon electrode was polished with 0.05 μm alumina on Buehler felt pads and then ultrasonically cleaned for about a minute in water. Finally, the electrode was washed thoroughly with double distilled water and dried at room temperature. After that electrode was immersed in 0.1 M H_2SO_4 solution containing 1 mM $K_2[PtCl_6]$ from -0.4 V to 0.4 V, scan rate 50 mVs^{-1}. The PtNPs modified electrodes were gently washed with distilled water and dried. Then, it was dipped with 2 μL of the mixing solution of 0.1 M PBS containing 0.4 M EDC and 0.1 M NHS at room temperature for 2 hours. The laccase was immobilized onto the PtNPs modified electrode surface by adsorption attachment. 2μL laccase solution (5 mg/ml in pH 7.0 PBS) was pipetted onto the electrode and the surface was dried for approximately 1 h at room temperature. The resulted modified electrode was stored in a refrigerator at 4 ◦C for use. The film formed on the electrode surface can be expressed

as the Scheme 1.



Scheme 1. Construction of the PtNPs/laccase modified electrodes.

3. RESULTS AND DISCUSSIONS

3.1. Morphological characterization of PtNPs/laccase film

The surface morphology of electrodeposited Pt nanoparticles has been examined using AFM. Here the AFM studies could furnish the comprehensive information about the surface morphology of nano-Pt on the ITO surface. In prior to modification, ITO surfaces were cleaned and ultrasonicated in acetone–water mixture for 15 min and then dried. The AFM parameters have been evaluated for 1500×1500 nm and 3000×3000 nm surface area. Further, three different films; PtNPs, only laccase and PtNPs/laccase modified electrodes have been prepared on ITO electrode were characterized using AFM.

From Fig. 1, it is significant that there are morphological differences between both the films. The top views of nano structures Fig. 1 (A) on the ITO electrode surface shows uniformly deposited homogeneously dispersed PtNPs on this electrode. We can see the existence of Pt nanoparticles in obvious manner with the average size range of 25.6 nm. The other amplitude parameters such like roughness average (sa) for nano-Pt film (1500×1500 nm) was found as 6.15 nm. The root mean square roughness was found as 7.43 nm. The PtNPs/laccase film in Fig. 1 (C) reveals that the laccase had covered the entire PtNPs. Comparison of only laccase (B) and PtNPs/laccase (C) reveals, these results in could be explained as the increase in deposition of laccase presence of PtNPs. We can clearly see that the immersed PtNPs/laccase have been gathered together.

3.2. Electroanalytical response of oxygen at PtNPs/laccase film

In order to confer a higher activity to redox oxygen of laccase, the innovative combination of PtNPs was attempted. These three kinds of configurations were composed of only laccase, only PtNPs and PtNPs/laccase modified electrodes.

Cyclic voltammetry was used to characterize the bioelectrocatalytic activity of laccase electrodes toward the reduction of oxygen. For instance, Fig. 2 (A) shows the voltammograms

recorded at a laccase modified electrode in 0.1M PBS (pH 7.0 containing 1 mM ABTS) saturated with nitrogen (curve a), ambient air (curve b) and oxygen (curve c).

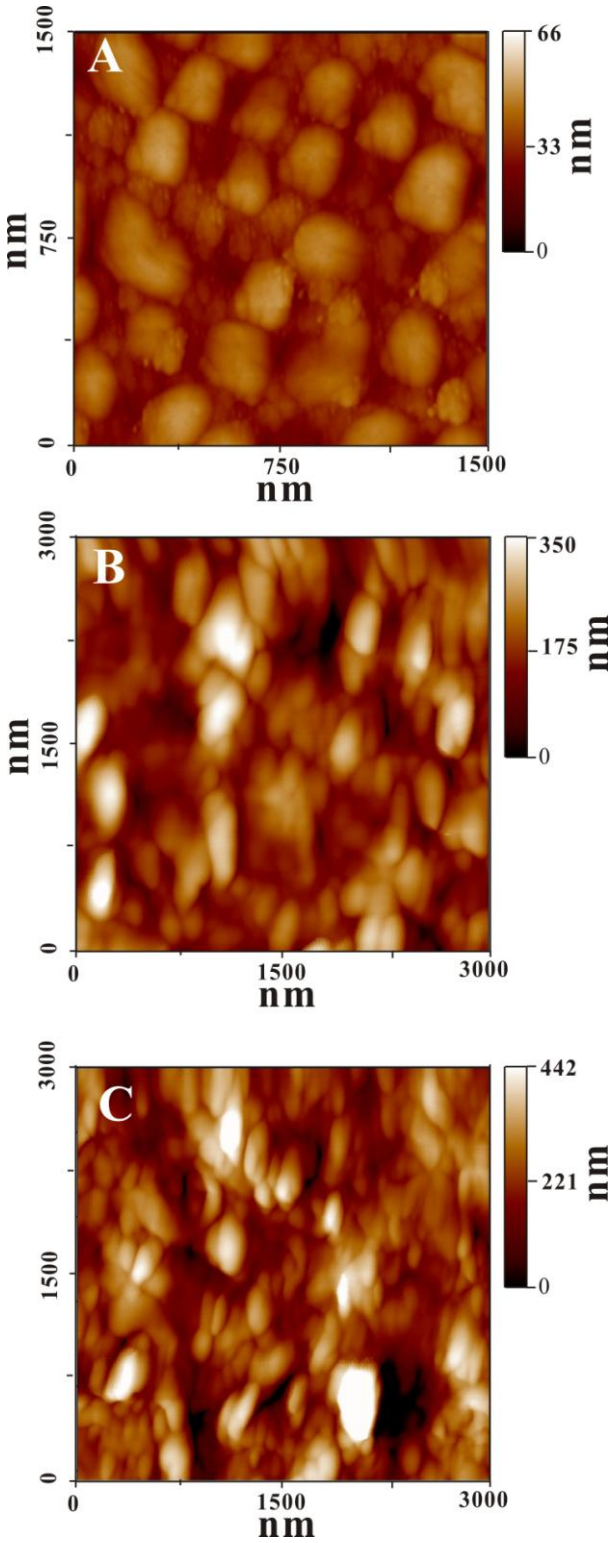


Figure 1. AFM images of (A) PtNPs; (B) only laccase; (D) PtNPs /laccase on ITO electrode.

The corresponding cyclic voltammograms have been obtained at 100 mVs^{-1} scan rate in the potential range of 0.6 to 0 V. Under nitrogen atmosphere, as can be seen, there is no obvious peak in the potential region studied. Curve b shows at ambient air (pH 7.0 PBS), we can obtain slight current at 120 mV. At saturation oxygen conditions, we obtained increased current dramatic from laccase reduction reaction.

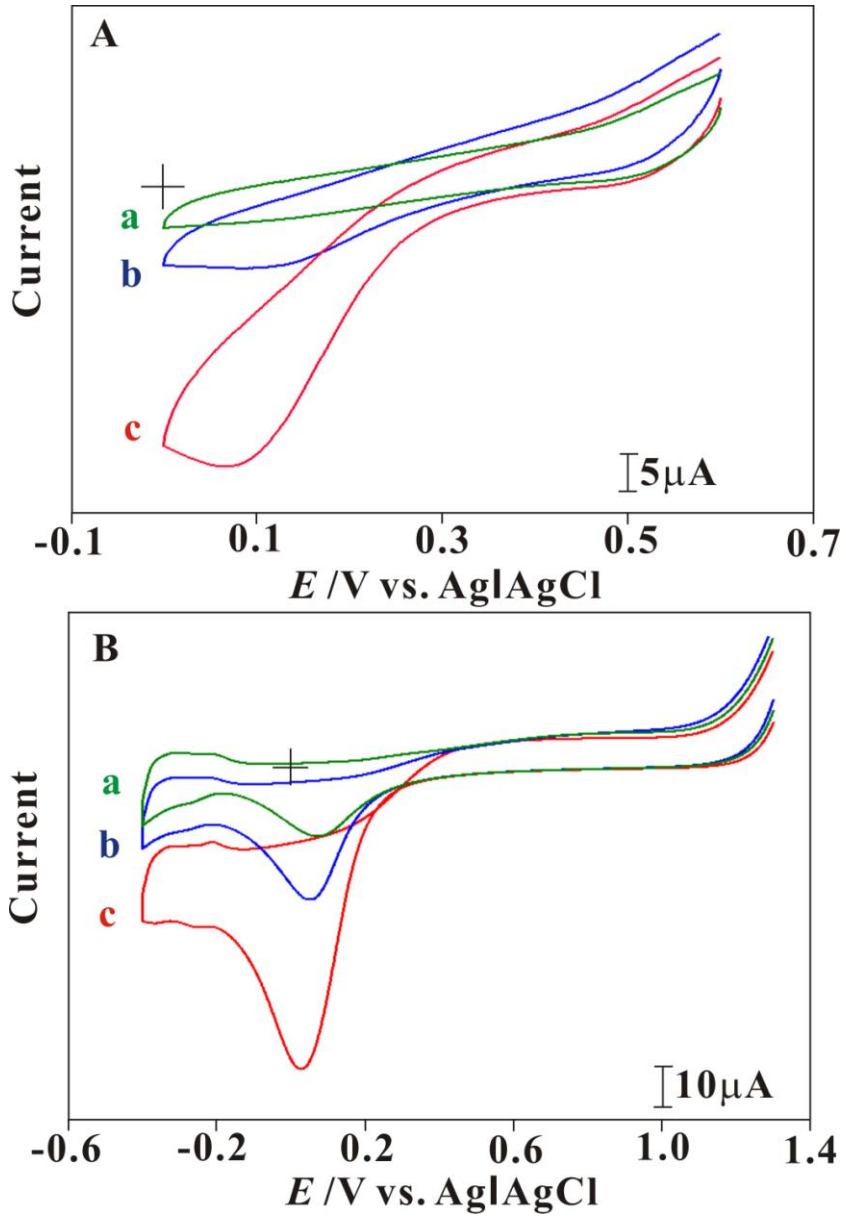


Figure 2. (A) CVs recorded at a laccase modified electrode in 0.1M PBS (pH 7.0 containing 1 mM ABTS) saturated with nitrogen (curve a), ambient air (curve b) and oxygen (curve c). Scan rate 100 mVs^{-1} in the potential range of 0.6 to 0 V. (B) CVs recorded at a PtNPs modified electrode in 0.1M PBS (pH 7.0 containing 1 mM ABTS) saturated with nitrogen (curve a), ambient air (curve b) and oxygen (curve c). Scan rate 100 mVs^{-1} in the potential range of 1.3 to -0.4 V.

Fig. 2 (B) shows the only PtNPs modified electrode at similar conditions. The corresponding

cyclic voltammograms have been obtained at 100 mVs^{-1} scan rate in the potential range of 1.3 to -0.4 V. Under nitrogen atmosphere (curve a), without oxygen added to the solution, the peak at potential 75 mV was obtained from Pt characteristic. Curve b and curve c showed that the reduction peak currents have increased with the increase of oxygen ratio. Bioelectrocatalytic of the peak potential 47 mV and 23 mV were obtained. PtNPs can electrocatalysis excellently of oxygen.

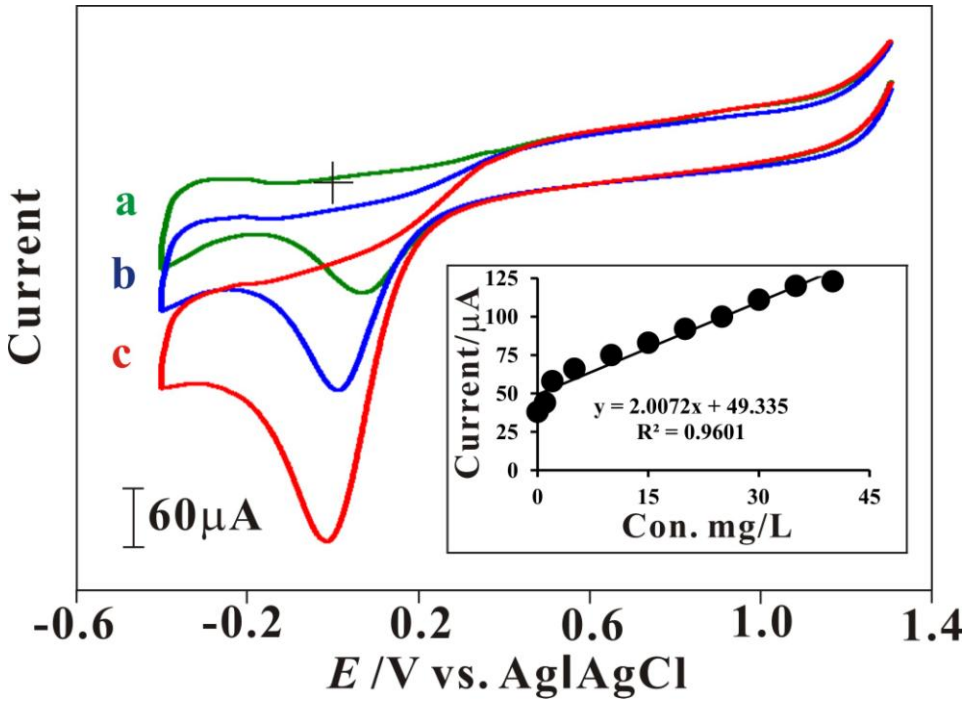


Figure 3. CVs recorded at a PtNPs/laccase modified electrode in 0.1M PBS (pH 7.0 containing 1 mM ABTS) saturated with nitrogen (curve a), ambient air (curve b) and oxygen (curve c). Scan rate 100 mVs^{-1} in the potential range of 1.3 to -0.4 V. Inset shows a current vs. concentration plot of oxygen at PtNPs/laccase electrode.

The PtNPs/laccase film was synthesized on GCE at similar conditions as described in Materials and Methods. Then the PtNPs/laccase modified electrode was washed carefully in deionized water and transferred to pH 7.0 PBS (containing 1 mM ABTS) for the electrocatalysis of oxygen (Fig. 3). Under nitrogen atmosphere (curve a), there is small peak in the potential 70 mV. Curve b shows at ambient air (pH 7.0 PBS), current of reduction oxygen was obtain at 13.6 mV. At saturation oxygen conditions (curve c), we obtained highest increased current from laccase reduction oxygen then others at the potential 0.2 mV. Inset shows a current vs. concentration plot of oxygen at PtNPs/laccase electrode. Upon addition of oxygen a new growth in the reduction peak of respective analytes have appeared at the current values. An increase in concentration of oxygen, simultaneously produced a linear increase in the reduction peak currents of the oxygen. Reduction of oxygen by the biocatalyst yields the responsed laccase redox site. The ABTS mediates redox of laccase. $ABTS^{2-}$ as one-electron donor for the laccase catalyzed reduction of dioxygen (Eq1).

$$O_2 + 4\ H^+ + 4\ ABTS^{2-} \rightarrow 2\ H_2O + 4\ ABTS^- \quad (1)$$

The observations at bare electrode (not shown) clearly indicate that the fouling effect of the electrode surface with the reduction obtaining the no single peak for oxygen. From all these above results it is clear that PtNPs/laccase film is more efficient and exhibits enhanced functional properties comparing to that of bare alone. This seems to indicate that the presence of PtNPs affect the potential value and laccase can increase reduction currents. The potential value for the oxygen reduction was close to the redox potential of the laccase used. This work have investigated the mechanism of electron transfer properties of laccase at different kinds of electrodes and concluded that at PtNPs modified electrode observed voltammetric response could be attributed to the redox process of T2 copper ion, while those at the carbon-based electrodes could be ascribed to the redox process of T1 copper ion in laccase.

3.3. Stability of PtNPs/laccase modified electrodes

Fig. 4 shows the stability plot of PtNPs/laccase modified electrodes stored in PBS at 4 °C for 10 consecutive days.

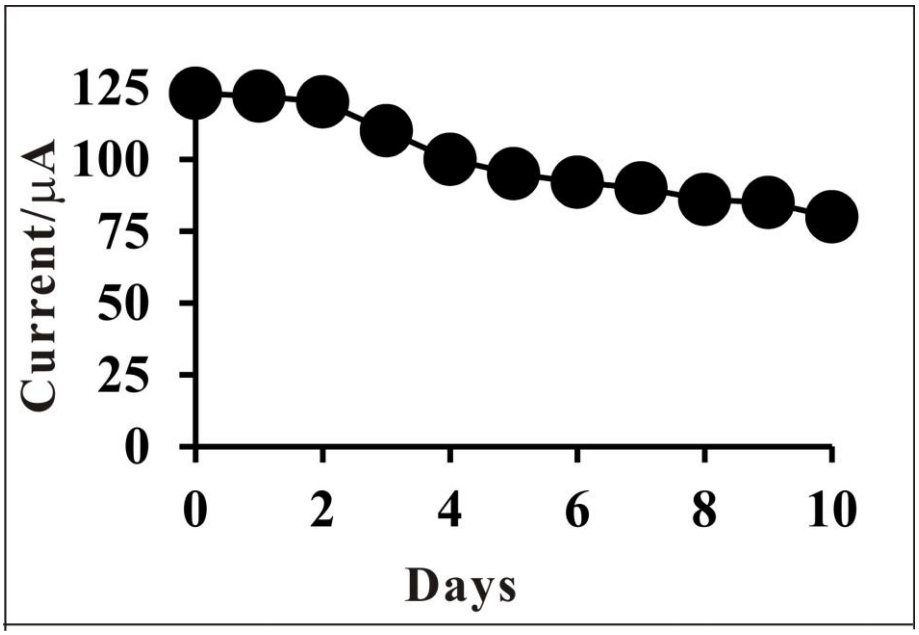


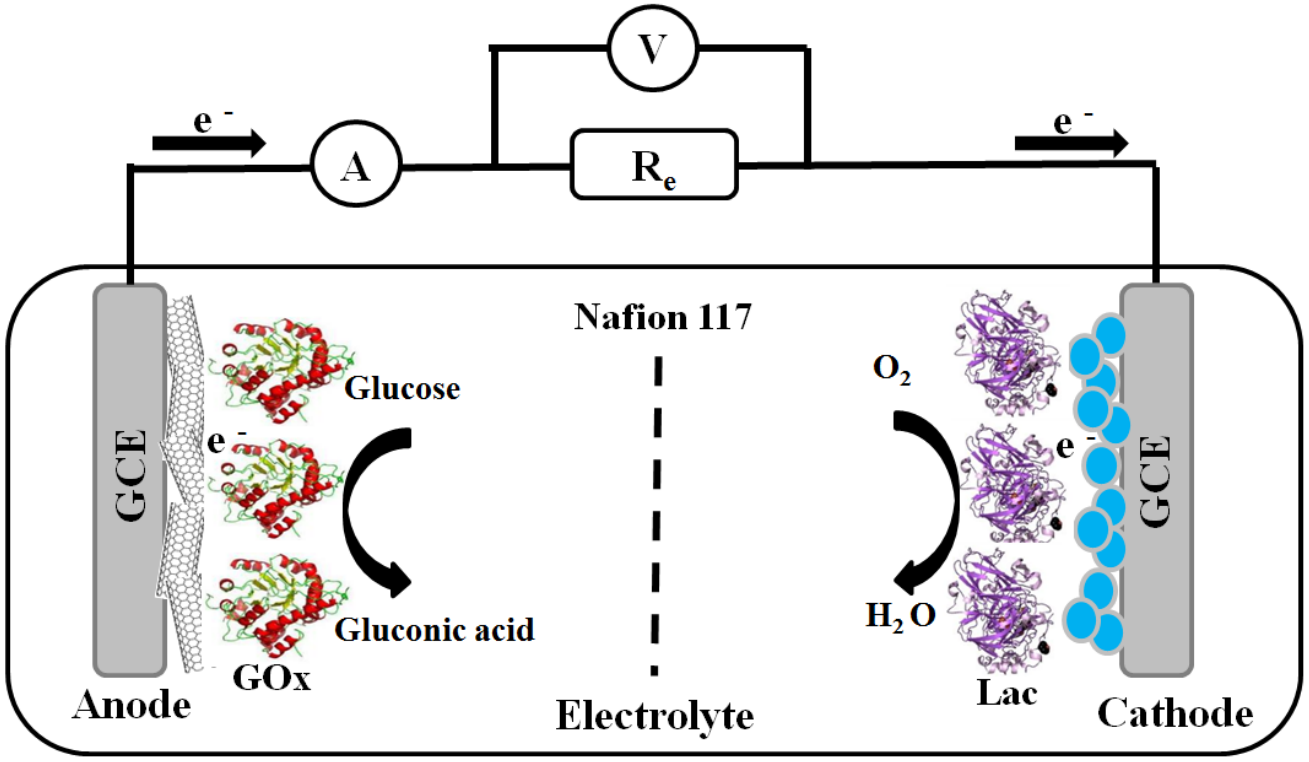
Figure 4. Reduction oxygen peak current of PtNPs/laccase modified electrodes stored in pH 7.0 PBS at 4 °C for 10 consecutive days.

The PtNPs/laccase modified electrodes maintains the redox peaks at same position and it retains 97.56 % of reduction peak current after 3 days. About 81.3 % peak current is retained at the modified electrodes even after 5 days storage in PBS. However, the peak current drops significantly in the consecutive days. This result reveals that the modified electrodes exhibits a reasonable stability for 10 days. Here, the good stability of PtNPs/laccase modified electrodes could be attributed to the

stability of PtNPs and the high affinity between laccase.

3.4. Biofuel cell performance of PtNPs/laccase modified cathode

As model for biofuel cell working under physiological conditions. This biofuel cell was built by association of a composite MWCNTs/GOx as bioanode and the optimized PtNPs/laccase electrode as biocathode. The configuration biofuel cell with bioanode and biocathode can be expressed as the Scheme 2.



Scheme 2. The configuration biofuel cell with bioanode and biocathode.

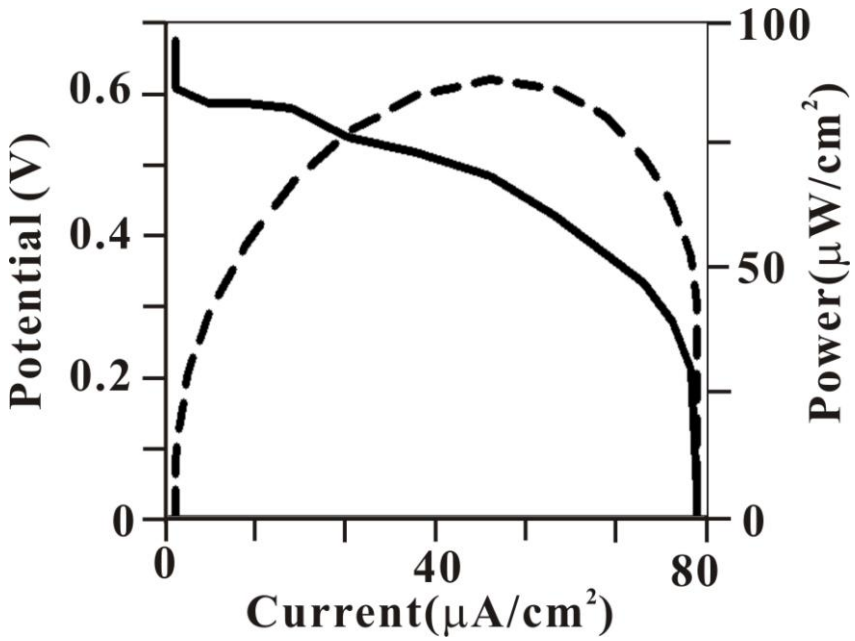


Figure 5. Polarization curve (solid line) and dependence of the power density on the operating voltage (dashed line) for a biofuel cell consisting of MWCNTs/GOx modified electrode as anode in combination with a PtNPs/laccase electrode as cathode. As fuel a 5 mM solution of glucose in PBS (pH 7.0) containing 1 mM ABTS was used.

Fig. 5 shows the influence of the fuel (glucose) and the oxidizer (O_2) on the open circuit voltage (OCV) of the resulting biofuel cell. Analysis has shown that the electrocatalytic oxidation current of glucose appears at 0.68 V with a current density of 0.02 $\mu A/cm^2$ and reaches 78 $\mu A/cm^2$ at 0 V vs. Ag/AgCl. Current density was calculated versus geometric electrode area, giving 0.07 cm^2. In air saturated 0.1 M pH 7.0 PBS (not shown), the biofuel cell displays an OCV of 0.48 V. Upon addition of glucose (5 mM), a stable OCV value of 0.51 V was reached after 10 min. According to the Nernst equation, this OCV increase reflects the negative shift of the half-wave potential of mediator due to the enzymatic oxidation of glucose, GOx consuming the oxidized form of mediator at the bioanode. Similarly, in nitrogen-saturated (not shown) containing glucose (5 mM), the OCV value was stabilized to 0.31 V after 30 min. Upon addition of O_2, the OCV sharply increased to 0.6 V. In presence of oxygen and laccase, the half-wave potential of ABTS shifts positively due to the enzymatic oxidation of ABTS. Fig. 5 displays the relationship between the power density and the cell voltage of the assembled glucose/O_2 biofuel cell in oxygen-saturated 0.1M PBS (pH 7.0) containing 5 mM glucose. The open circuit potential of the GOx modified electrode is close to the redox potential of the FAD/$FADH_2$ cofactor in the enzyme itself. As the current is produced, the cell voltage starts to decrease, the cell voltage drops faster and become 0 V at 78 $\mu A/cm^2$ of the short circuit current (SCC). From the measured I–V curves, maximum power densities are calculated to be 84 $\mu W/cm^2$ at 0.62 V. To further illustrate the efficient role of PtNPs in the biocathode functioning, the parameters of a biofuel cell composed of an identical bioanode and a laccase electrode without PtNPs as biocathode, were evaluated. This demonstrates the beneficial role of PtNPs for the electron transport.

4. CONCLUSIONS

We have demonstrated application of PtNPs/laccase modified electrode as cathode for biofuel cell. The laccase-based electrodes gave high catalytic currents of O_2 reduction in redox mediators with remarkable operational stability. High sensitivity and stability together with very easy preparation makes PtNPs/laccase electrode as promising candidate for constructing simple electrochemical sensor for oxygen. We suggest that these PtNPs with the laccase region that surrounds the T2 site, allowing fast electron transfer between this Cu site of the enzyme and the electrode. The AFM results have shown the difference between type films morphological data. The experimental methods of CVs with film biosensor integrated into the GCE which are presented in this paper, provide an opportunity for qualitative and quantitative characterization, even at physiologically relevant conditions. Finally, the functional properties of designed laccase-based electrodes are very suitable for their possible application as. It is expected that this easy fabrication of enzyme electrodes will paves the way for the

development of a new generation of biofuel cells and also will be useful for the development of bioreactors and biosensors.

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