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Hybrid Bioelectrocatalytic Reduction of Oxygen at Anthracene-modified Multi-walled Carbon Nanotubes Decorated with $Ni_{90}Pd_{10}$ Nanoparticles

Sidney Aquino Neto[a], Rodrigo G. Da Silva[a], Ross D. Milton[b], Shelley D. Minteer[b], Adalgisa R. De Andrade[a,*]

[a] Departamento de Química, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, 14040-901, Ribeirão Preto, SP, Brazil
[b] Departments of Chemistry and Materials Science and Engineering, University of Utah, Salt Lake City, Utah 84112, United States

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ABSTRACT

Considering the importance of the oxygen reduction reaction (ORR) for many energy conversion technologies, the design of cost-effective and active electrocatalysts is quite important for the future energy economy. In this investigation, we report the preparation of hybrid biocathodes containing multi-walled carbon nanotubes (MWCNTs) decorated with both enzymes and $Ni_{90}Pd_{10}$ metallic nanoparticles, targeting the bioelectrocatalytic reduction of oxygen in buffered solutions. The electrochemical characterization showed that the presence of the hydrophobic moieties did not affect the metal nanoparticle synthesis protocol, attesting to the possibility of obtaining a bifunctional material containing both the metallic nanoparticles and the aromatic moieties employed for enzyme orientation. Based on the electrochemical data obtained with the hybrid biocathodes, enhanced direct bioelectrocatalytic reduction of oxygen could be clearly observed at both laccase and bilirubin-based biocathodes, with a larger improvement for the hybrid bioelectrode prepared with laccase. In the laccase bioelectrode, the targeted reaction required a lower overpotential and provided about 50% higher current density, thus, demonstrating the benefit of incorporating small amounts of $Ni_{90}Pd_{10}$ metallic nanoparticles into enzymatic cathodes for the oxygen reduction reaction.

1. Introduction

The oxygen reduction reaction is at the core of several renewable energy conversion technologies, such as fuel cells, metal-air batteries, electrolyzers, among others [1]. The reduction potential of oxygen is 1.23 V vs SHE (standard hydrogen electrode) under standard conditions; however, there is always overpotential associated with this reaction, which is highly dependent on the electrode material [2]. Particularly in fuel cells, this reaction is of major interest, because it has limited the power density of many of these systems with either metal-based catalysts or biocatalysts [3,4].

Considering traditional metal-based fuel cell systems, platinum is considered as the best electrocatalyst for the oxygen reduction reaction, since its first demonstration in 1823 [5]. Alloys and bimetallic catalysts also play an important role on the electrocatalytic reduction of oxygen and different nanomaterials and synthetic protocols had been extensively investigated focusing on the achievement of more efficient electrocatalysts [6–10]. In recent years, palladium-based electrocatalysts have also been exploited for the oxygen reduction reaction, mainly because of its higher abundance in nature when compared to platinum materials [11–14]. The activity of palladium itself is slightly lower compared to platinum; however, in the presence of another metal (such as Sn, Co, Fe, Ni, among others), the performance can be even higher than platinum-based materials [14,15]. Non-precious metal catalysts such as iron, nickel, and cobalt-based materials have also been recently explored in the literature for decreasing the cost in high-power density fuel cell applications [16–18]. In this context, Ni-based materials have shown quite good performance when compared with pure precious-based catalysts. An example is the possibility of improving the electrocatalytic reduction of oxygen using Pd/Ni alloys supported on carbon, which display lower onset potentials and higher oxygen reduction activities over Pd/C catalyst in alkaline media [19,20]. Moreover, metal-free materials

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[*] Corresponding author.
E-mail address: ardandra@ffclrp.usp.br (A.R. De Andrade).

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have been under investigation in order to obtain commercially viable materials [21].

Apart from the traditional fuel cell electrocatalysts, biological materials are also employed in energy conversion devices, such as fuel cells. These biological fuel cells employing enzymes, microorganisms, or organelles display interesting features when compared to the noble metal-based system, especially considering that they can operate at lower temperatures and under physiological conditions [22]. Enzymatic systems used at the cathodic side have shown high efficiency towards the oxygen reduction reaction [4,23]. The multicopper oxidase enzymes laccase and bilirubin oxidase catalyze the four electron reduction of oxygen to water, with concomitant oxidation of aromatic organic substrates such aromatic amines and phenols [24]. They are considered robust and environmentally friendly catalysts for many technological purposes [25]. In biological fuel cells, there is a possibility of preparing enzymatic biocathodes using a direct electron transfer architecture, where the enzymes can be orientated to transfer electrons directly with electrode surfaces without the assistance of any mediator molecules [26,27]. Among different orientating methodologies, anthracene-modified MWCNTs can orientate the enzymes for direct bioelectrocatalytic oxygen reduction through the type-1 copper site, affording efficient electrocatalysis [28,29]. In previous investigations, we have shown that this methodology can orientate laccase and bilirubin oxidase enzymes onto carbon paper surfaces for efficient oxygen reduction in both methanol/O_2 and ethanol/O_2 biofuel cells [30,31].

Considering the importance of the oxygen reduction reaction for many energy conversion technologies, the design of cost-effective and active electrocatalysts is quite important for the future energy economy. It is in this context that many research groups have focused on the development of inorganic, organic, and biological catalysts [14]. Based on the advantages presented by each material, literature has shown that a good alternative for increasing the catalyst efficiency is to explore the synergy of hybrid materials [32,33]. In the case of enzymatic-based materials, the integration of enzymes and metal catalysts can provide not only lower overpotentials, but also higher current densities for the desired electrode reaction [34].

Considering the aforementioned characteristics of Pd/Ni alloys as well as the advantages of employing the multicopper oxidase enzymes such as laccase and bilirubin for the oxygen reduction reaction, this investigation reports on the preparation of hybrid biocathodes containing enzymes and metallic nanoparticles targeting the bioelectrocatalytic reduction of oxygen in buffered solutions. The goal was to immobilize either laccase or bilirubin oxidase onto anthracene-modified MWCNTs decorated with $Ni_{90}Pd_{10}$ metallic nanoparticles.

2. Material and Methods

2.1. Chemicals

Hydroxyl functionalized MWCNTs (OH-MWCNTs, OD < 8 nm, ID 2-5, OL 10–30 μm) were purchased from www.cheaptubes.com. Toray carbon paper electrodes were purchased from Fuel Cell Earth. Bilirubin oxidase from *Myrothecium verrucaria* lyophilized powder, 8.6 units mg^{-1}; laccase from *Trametes Versicolor*, lyophilized powder 23.1 units mg^{-1}; Nafion® 1100 EW 5% wt. suspension; potassium tetrachloropalladate(II) (K_2PdCl_4); nickel (II) chloride hexahydrate ($NiCl_2.6H_2O$); and oxalyl chloride were purchased from Sigma-Aldrich and used as received. 2-anthracene carboxylic acid was purchased from TCI (USA). All the other reagents were of analytical grade and used without further purification. All solutions were prepared with high-purity water from a Millipore Milli-Q system.

2.2. Synthesis of anthracene-modified MWCNTs decorated with $Ni_{90}Pd_{10}$ metallic nanoparticles

Anthracene-modified MWCNTs were prepared as previously described [28]. Briefly, anthracene-2-carbonyl chloride is first synthetized through the reaction between 2-anthracenecarboxylic acid (0.270 g) with oxalyl chloride (0.343 g) in dichloromethane. The product is then reacted with 1.096 g of hydroxylated MWCNTs in acetonitrile and washed with dichloromethane.

$Ni_{90}Pd_{10}$ metallic nanoparticles were synthetized as previously described, by a modified polyol method [35,36]. In this successive chemical reduction (in situ redox-transmetalation), the Ni metallic nanoparticles act as a sacrificial platform for Pd reduction. This redox reaction proceeds spontaneously at a favorable redox potential and therefore is an efficient route for selective formation of bimetallic structures [37]. Briefly, nickel(II) chloride hexahydrate was first dissolved in 100 mL ethylene glycol, followed by the addition of 1 mol L^{-1} sodium hydroxide (ethylene glycol solution) and 1 mol L^{-1} hydrazine (12-fold excess in an acetonitrile solution). The mixture was stirred for about 1 h at 60 °C. Nanoparticle formation can be observed by a gray metalized colloidal solution. The solution was cooled down to 25 °C and stirred overnight for complete volatilization of excess hydrazine. Appropriate amounts of potassium tetrachloropalladate(II) were dissolved in 100 mL ethylene glycol at pH 11, and then added to the previous nickel colloidal solution. This final solution was kept under magnetic stirring for about 12 hours until complete reduction of the palladium species (dark red-brown solution). After the reaction was completed, the anthracene-modified MWCNTs were added to the mixture and stirred overnight, keeping a 2.5 wt % metal loading ratio. The final suspension was filtered and exhaustively washed with water and 0.3 mol L^{-1} sodium nitrate, and the solid product was dried at 60 °C for 12 h under nitrogen atmosphere. Control electrodes were also prepared by supporting the $Ni_{90}Pd_{10}$ metallic nanoparticles onto hydroxyl functionalized MWCNTs keeping the same metal loading ratio, but without the anthracene moieties.

2.3. Hybrid biocathode preparation and Electrochemical measurements

The hybrid biocathodes containing enzymes and metallic nanoparticles were prepared by immobilizing the enzymes onto a 1 cm^2 carbon paper support using tetrabutylammonium bromide (TBAB)-modified Nafion and anthracene-modified MWCNTs decorated with $Ni_{90}Pd_{10}$ metallic nanoparticles (An-MWCNTs/$Ni_{90}Pd_{10}$). First, 1.5 mg of enzyme (laccase or bilirubin oxidase) was dissolved in 75 μL of citrate/phosphate buffer 0.1 mol L^{-1} (pH 5.0 for laccase and 7.0 for bilirubin oxidase). This solution was added to 7.5 mg of An-MWCNTs/$Ni_{90}Pd_{10}$, followed by a vortex-mixing and gentle sonication procedure. After mixing, 25 μL of TBAB-modified Nafion 5 wt % suspension in ethanol (prepared as described in [38]) was added to the mixture and vortex-mixed. Approximately 30 μL of the final mixture was painted onto the carbon paper electrodes, which was dried under a positive airflow.

Cyclic voltammetry experiments were conducted in a single compartment cell using a spiraled platinized platinum wire (15 cm) as the counter electrode. For the electrochemical characterization of the synthetized material (An-MWCNTs/$Ni_{90}Pd_{10}$), 5 μL of a 25 mg mL^{-1} 99% ethanol/1% Nafion solution were drop-cast onto a 0.071 cm^{-2} glassy carbon electrode. Cyclic voltammograms were performed in 0.1 mol L^{-1} NaOH at 20 mV s^{-1} from −0.6 to 0.8 V *vs.* a Hg/HgO reference electrode (0.905 V vs a reversible hydrogen electrode).

For evaluation of the bioelectrocatalytic reduction of oxygen by the hybrid biocathode, cyclic voltammetry experiments were