Immobilization of glucose oxidase enzyme on NiAl-LDHs for application in microfluidic fuel cell and serotonin detection.

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Abstract. In this work, an enzymatic glucose oxidase (GOx) electrode immobilized on double layered hydroxides of NiAl (NiAl-LDHs) was developed for the detection of serotonin neurotransmitters that may be present in human blood samples and carry out the glucose oxidation present in this fluid for energy conversion, and can be applied as self-feeding electrodes in Lab-on-a-Chip devices. The self-feeding electrodes based on fuel cells have a very promising future in lab-on-a-chip devices because they do not occupy an external power source, their manufacturing processes are relatively simple and low cost, and can be developed for different applications such as the determination of biomolecules for diagnosis of various diseases.

1. Introduction

Lab-on-a-Chip technology focuses on the development of micro- or nano-scale devices with the integration of functions that are carried out at the laboratory level, which includes the manufacture and use of microelectromechanical systems with the use of improved the performance of the devices, offering a portable, extended, continuously renewed platform for diverse applications based on the idea of miniaturization [1]. Fuel cells are a promising technology for Lab-on-a-Chip systems, which are electrochemical devices that perform the conversion of chemical energy to electricity from different fuels where the development of electrodes is very important for performance improvement [2]. Due to its high specificity, high catalytic activity and the low number of wastes produced in the process, enzymes have attracted the attention for use in a great variety of applications. Although the advantages of the use of enzymes are numerous, there are some drawbacks, due to their solubility in water, is difficult to separate them from the products and they cannot be reused, not are very resistant to changes in the medium, for this reason, different methods have been sought to improve its stability without losing specificity or reduce the activity. This can be achieved by enzyme immobilizing, that is, physically confining in a physical support retaining its activity; this makes it insoluble and facilitates the separation of the enzyme from the products and can be reused, allowing to reduce costs.

The immobilization process can causes a loss of enzymatic activity; this loss often depends on the method of immobilization. Glucose oxidase enzyme has been used for applications in the food,
fermentation and textile industry but is mainly used in biosensors, due to its high specificity to catalyze the oxidation of the D-glucose. This enzyme is a dimeric flavoprotein with a molecular mass of 80 kDa / monomer [3]. Its maximum activity is between 20-40 °C with a pH between 3-6.5 and is rapidly inactivated at temperatures above 60 °C. The main sources from which glucose oxidase is obtained are the fungi of the families Aspergillus, Penicillium, and Saccharomyces [4]. Glucose oxidase catalyzes the oxidation of glucose with water and oxygen in gluconic acid and hydrogen peroxide.

Nickel has been shown to have a very great interaction with the amino acid groups of glucose oxidase [5]; together with aluminum in the double layered hydroxides (NiAl-LDHs) have promoted a better adsorption of the enzyme surface [6]. The NiAl-LDHs are excellent supports for immobilizing enzymes for several reasons, their high water content provides a biocompatible environment for the enzymes, presents high mobility of the analyte and the product, and they are non-toxic and have high chemical and hydrolytic stability [7]. These biosensors show good results in terms of sensitivity, response time and long-term stability and for application in Lab-on-a-Chip devices. For this reason, NiAl-LDHs were used for the development of a bioanode with tetrabutylammonium bromide Nafion and glucose GOx on Toray carbon paper to be applied in a microfluidic fuel cell using glucose as fuel.

2. Experimental

2.1 NiAl-LDHs nanoparticles synthesis

NiAl-LDHs nanoparticles were synthesized following a method reported by Abdolmohammad-Zadeh et al., [8]. In 300 ml of deionized water, add 0.375 g of AlN\textsubscript{3}O\textsubscript{9} \cdot 9H\textsubscript{2}O and 0.581 g of N\textsubscript{2}NiO\textsubscript{6} \cdot 6H\textsubscript{2}O under constant stirring and control the pH = 9.6 by the addition of a 0.05 M NaOH solution. The obtained solution was subjected to hydrothermal treatment at a constant temperature of 90 °C for about 24 h. The resulting product was separated by centrifugation at 6000 rpm for 10 minutes and washed three times with deionized water. Finally was dried at 60 °C for 6 hours.

2.2 Bioanode construction.

NiAl-LDHs were used for development a bioanode with Nafion tetrabutylammonium bromide and glucose oxidase enzyme onto Toray porous paper electrode (EC-TP1-060T) to be applied in a microfluidic fuel cell using glucose as fuel. A catalytic ink was prepared using 1mg of glucose oxidase (EC 1.1.3.4 Type XS, lyophilized powder, 100,000-250,000 units / g solid Aspergillus niger), 7 μL of Nafion solution (Sigma Aldrich), Vulcan carbon, 1 mL of 0.1M buffer phosphate pH= 7.5, and 3 mg of tetrabutylammonium bromide (Sigma Aldrich) and 1mg of NiAl-LDHs.

2.3 Microfluidic fuel cell evaluation.

The microfluidic fuel cell uses porous electrodes and has a “V” shape geometry design that generates a homogeneous distribution of fluids (Figure 1) [9] The poly-(methyl methacrylate) (PMMA) top plate support had two inlets for the reagents and one outlet to release the reaction products in the PMMA base plate, a home-made silicone elastomer film (Silastic®, Dow Corning, prepared using an Elcometer® Film Applicator with a final thickness of 200 μm) used as both the gasket and cell channel structure. The air intakes are disposed in both plates in order to improve oxygen concentration. The device was evaluated at different glucose concentration in 0.1M buffer phosphate pH 7.4 (4 U.P.D. Praxair) as fuel and oxygen taken from the air as the oxidant, respectively at 50 μL min⁻¹ flow rate for both streams.
3. Results and discussion

The electrode was used for the detection of serotonin in buffer solutions pH 7.5 at different concentrations using differential pulse voltammetry (Figure 2) technique. The purpose of this test was to provide versatility to the electrode for a future application in the detection of serotonin in human blood, and from the same concentration glucose contained in the blood to be able to power energetically an electronic biomedical device.

The evaluation of the microfluidic cell was carried out using different concentrations of glucose in buffered phosphate solution and 0.3M KOH saturated with O2 to cathodic compartment resulting in 0.677mWcm\(^{-2}\) of maximum power density in the presence of glucose (Figure 3a).
Figure 3. a) Polarisation and power density curves obtained from microfluidic fuel cell evaluated in absence and presence of 5Mm glucose in 0.1M buffer phosphates pH 7.4 at 10 mV s⁻¹ as fuel and b) Polarisation and power density curves obtained from microfluidic fuel cell evaluated at different glucose concentration in 0.1M buffer phosphates pH 7.4 at 10 mV s⁻¹ as fuel.

Figure 3b shows the evaluation of the microfluidic cell by increasing the glucose concentration in the solution of the anodic compartment.

4. Conclusions

These preliminary results showed that it possible to construct an electrode that performs the serotonin detection and also by means of the glucose oxidation by glucose oxidase enzyme could be evaluated in a microfluidic device. The detection of neurotransmitters present in blood samples has a great interest due to the connection with various neurological disorders, and to have a device that makes the detection from blood samples and that can be energetically fed from the oxidation of the glucose contained in the same sample could have a good impact.

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