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Synthetic Metals

A facile and simple polyaniline-poly(ethylene oxide) based glucose biosensor

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A B S T R A C T

In this work, the fabrication and performance of a simple and efficient glucose biosensor based on chemically synthesized polyaniline-poly(ethylene oxide) nanocomposite (PAni-PEO) have been demonstrated. The nanocomposite was synthesized through rapid-mixing polymerization and characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), and Fourier transform infrared spectroscopy (FT-IR). The nanocomposite and glucose oxidase (GOx) solutions were placed on a platinum electrode by drop-casting, to produce a glucose biosensor that was characterized by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and chronoamperometry. Under optimal conditions, the biosensor showed a linear range from 1 to 10 mM ($R^2 = 0.990$) with a high sensitivity of 16.04 μA mM^{-1} cm^{-2} and a detection limit of 0.82 mM. The interference from ascorbic acid (AA) and uric acid (UA) was also investigated. The results indicated that the biosensor exhibited a satisfactory linear range, sensibility, and selectivity for use as a glucose biosensor, considering the normal range of approximately 4–6 mM of glucose concentration in human blood.

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1. Introduction

Amperometric glucose biosensors have a high practical importance in medical diagnosis. For this purpose, electrodes have been fabricated by immobilizing glucose oxidase (GOx) on conducting polymers films [1]. Among the conducting polymers, polyaniline (PAni) stands out as one of the most studied polymers for use in sensors and biosensors, which have been extensively studied in recent years [2]. Initially, glucose biosensors were simple and were produced by electrochemical synthesis of PAni, entrapping the enzyme in one step [3,4], or were produced by the deposition of PAni chemically synthesized on an electrode, with subsequent adsorption of the enzyme [5].

Using the interfacial and rapid-mixing polymerizations [6–8], which produce highly pure PAni nanofibers in large quantity without the need for templates, the application of this polymer in glucose biosensors has become more promising. It is characterized by a simple and effective platform for the integration of enzymes in the electrodes [9]. Over the years, biosensors using PAni nano-structures have become more complex, involving different materials in order to improve the sensitivity, such as graphene [10], gold nanoparticles [11,12], TiO$_2$ nanoparticles [13,14], or a combination of these materials [15,16], resulting in biosensors that are complex, expensive, and hardly reproducible by industry. Furthermore, most of these biosensors are produced via electro-chemical deposition; however chemical deposition is more suitable for producing PAni on large scale for commercial use [17,18].

Although it has high electrical conductivity, good environmental stability, and ease of synthesis at nanoscale [19], PAni itself does not have good mechanical properties and, when dispersed in a medium, easily precipitates as a green insoluble powder, which hinders its application in biosensors, as it does not adhere on the electrode surface. To overcome these limitations, the production of nanocomposites with conventional polymers has been a good solution [20].

Among the conventional polymers, poly(ethylene oxide) (PEO) stands out because it allows the formation of polyaniline composites in a homogeneous viscous medium, combining its electrical conductivity with the mechanical properties of this classical polymer [21]. As far as we know, these PAni-PEO blends have been used in electrospinning processes and to produce polymer electrolytes, aiming the development of electrical devices such as transistors and batteries [22–26]. Applications in glucose biosensors have not been reported yet.

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For the construction of an amperometric glucose biosensor using conducting polymers, some conditions that directly influence the biosensor efficiency should be evaluated. The pH of the buffer solution used as electrolyte affects the electrochemical behavior of the mediator (when it is present) and the bioactivity of GOx when immobilized [27]. According to Dhand et al. [28], the ability of amino acids present at the active sites of the enzyme to interact with the substrate depends on their electrostatic state, which in turn depends on the pH of the solution.

As stated by Xia and coworkers [29], the sensitivity and response time of conducting polymer biosensors are highly dependent on the film thickness, where thinner films present a larger specific area for interaction with the target analyte and, consequently, a higher sensitivity and faster response time. The polymer is also responsible for attaching the enzyme to the electrode and causing electrical signal transduction [30]. Therefore the amount of polymer to be deposited on the electrode is a relevant parameter to be analyzed.

The performance of the biosensor can also be improved by the presence of mediators, whose influence is associated with the transport of electrons. The mediator shuttles the electrons between the redox center of the enzyme and the electrode, with a low oxidation potential. The most commonly used mediators are ferrocene and its derivatives, such as ferricyanide, which is used in home blood-glucose monitoring strips [31,32].

Another condition that has influence in the biosensor construction is the immobilized enzyme concentration [27]. The optimal concentration of GOx immobilized in the structure of the biosensor, together with the other conditions previously analyzed, is the concentration that shows the maximum electrical response, which can be observed from the signals generated from the redox reaction of the system [33].

In this work, PAni-PEO nanocomposite was chemically synthesized through rapid-mixing polymerization to yield a homogeneous viscous polymer that was easily applied over an electrode, together with glucose oxidase (GOx), to produce a simple, efficient, and easily reproducible glucose biosensor. All parameters discussed above for the construction of the biosensor were studied to optimize the linear detection range, detection limit, response time, and stability.

2. Materials and methods

2.1. Materials

Poly(ethylene oxide) (PEO, 600.000 g/mol), glucose oxidase (GOx, from *Aspergillus niger* Type VII, >100 U/mg), D-(+)-glucose, potassium ferricyanide ($K_3Fe(CN)_6$), ascorbic acid (AA), and uric acid (UA) were purchased from Sigma and used as received. Aniline (Synth) was purified by distillation prior to use. Ammonium peroxydisulfate (APS) was purchased from Synth and used as received. Purification of the PAni-PEO nanocomposite was performed through dialysis using a molecular porous tubular membrane (molecular weight cut-off, MWCO, in the range of 12–14 kDa) purchased from Fisher Scientific. Phosphate buffer solution (0.1 M, pH 6 and 7) was prepared from NaH_2PO_4 (Neon) and Na_2HPO_4 (Neon). The glucose stock solution was allowed to mutarotate at room temperature overnight before use. All aqueous solutions were prepared with deionized water.

2.2. Synthesis of PAni-PEO nanocomposite

Rapid-mixing polymerization was used to produce polyaniline nanofibers in the presence of PEO. The synthesis was based in the method proposed by Li and Jing [34] with some modifications. Initially a predetermined amount of PEO was dissolved in 100 mL

of 1 M HCl and stirred well to obtain a transparent solution. Then, APS was added to the medium. In another glass vial, aniline was dissolved in 50 mL of 1 M HCl and then mixed with 50 mL of PEO in 1 M HCl solution. After that, oxidant and aniline solutions containing PEO were mixed for a few minutes and the reaction was left to proceed for four hours without agitation. The molar ratio between monomer and oxidant was kept at 1:1 and equal to 0.5 M, while the concentration of PEO was 5 g/L. Impurities, such as unreacted APS, oligomers of PAni, and excess HCl, were removed from the solution using dialysis against distilled water for 24 h. The resultant polymer solution was 3.7 wt% in concentration and the polymer was identified as PAni-PEO.

2.3. Preparation of Pt/PAni-PEO/GOx modified electrodes

Prior to modification, platinum (Pt) electrodes were carefully cleaned by immersion in sulfochromic concentrated solution and then rinsed with distilled water. The biosensor was prepared by pipetting reagents over the electrode surface. First, 50, 75 or 100 μL of PAni-PEO solution have been pipetted, spread over the electrode and dried in an oven at 60 °C. After that, 20 μL of different GOx solutions (5, 25, 50 or 75 mg/mL) was pipetted over the polymer and dried at 35 °C. The modified electrodes were identified as Pt/PAni-PEO/GOx.

2.4. Characterization

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images were obtained using JEOL JSM 6060 and JEM 1200 microscopes, respectively. The Fourier transform infrared (FT-IR) spectra were recorded on a Perkin Elmer Spectrum 1000 FT-IR spectrophotometer in a wave-number range of 4000–500 cm^{-1} under ambient conditions. The conductivity of the polymer was measured through the four-point probe method using Cascade Microtech CS 4-64 device connected to a Keithley 6430 sourcemeter at room temperature in disks (diameter: 1.2 cm; thickness: 1.65 mm) prepared with a press.

2.5. Electrochemical measurements

All electrochemical measurements were performed using a PGSTAT 302N/Autolab potentiostat (EcoChemie, Netherlands), with Nova 1.10 software, in a standard three-electrode cell. A platinum wire was used as a counter electrode and Ag/AgCl/ saturated KCl as a reference electrode. All reported potentials were measured versus this reference electrode. The working electrode was a 1.5 cm^2 platinum plate. Cyclic voltammograms (CVs) were obtained with a potential range of −0.5–+0.8 V at a scan rate of 30 mV/s. Electrochemical impedance spectroscopy (EIS) measurements were performed at the open circuit potential with an amplitude of 5 mV and frequencies ranging from 10 kHz to 0.05 Hz. CVs and EIS were performed in triplicate. Chronoamperometric measurements were carried out at an applied potential of +0.2 V, requiring the transient background to decrease to a steady-state value. All measurements were conducted in phosphate buffer (0.1 M, pH 6 or 7) in the presence of 1, 5 or 10 mM $K_3Fe(CN)_6$ in an unstirred cell at room temperature (∼25 °C).

3. Results and discussion

3.1. Characterization of PAni-PEO nanocomposite

The main purpose of this work was to chemically synthesize a PAni-PEO nanocomposite with suitable properties for application to an electrode surface, producing a glucose biosensor that is efficient without the need for complex and expensive materials