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Fabrication of a promising immobilization platform based on electrochemical synthesis of a conjugated polymer

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ABSTRACT

Since conjugated polymers are an important class of materials with remarkable properties in biosensor applications, in this study, a novel glucose biosensor based on a conjugated polymer was fabricated via the electropolymerization of the monomer 10,13-bis(4-hexylthiophen-2-yl)dipyridol[3,2-a:2′,3′-c]phenazine onto a graphite electrode surface. Glucose oxidase (GOx) was used as the model biological recognition element. As a result of the enzymatic reaction between GOx and glucose, the glucose amount was determined by monitoring the change in the oxygen level associated with substrate concentration via the amperometric detection technique. The proposed system possessed superior properties with K_M^{app} value of 0.262 mM, 2.88×10^{-3} mM limit of detection and 105.12 μA mM^{-1} cm^{-2} sensitivity. These results show that conjugated polymer film provides an effective and stable immobilization matrix for the enzyme. Finally, the biosensor was applied successfully to several commercially available beverage samples for glucose determination proving an inexpensive and highly sensitive system applicable for real time analyses.

1. Introduction

Development of analytical devices for the estimation and monitoring of biological samples have become the major interest in biotechnology and have been accelerated since it has been realized that biological systems can offer a commercial potential [1]. The biggest challenge in this area is the convertion of the biological data to a measurable signal since it is a complex issue to connect an electronic system to a biological environment. For this purpose, electrochemical biosensors are attractive devices to analze biological samples providing direct conversion of a biological event into an electrical signal [2]. *Diabetes mellitus* is the most common endocrine disorder related with the carbohydrate metabolism and it is characterized by abnormal level of blood glucose concentrations [3]. Moreover glucose is one of the most critical biological compounds for life among all the others found in nature since it is responsible for energy generation for growth and reproduction processes [4]. As a result, it is important to determine the glucose level quantitatively and continuously. Although there are a number of different methods for glucose detection such as liquid chromatography [5] and spectrometric techniques [6], these methods require skilled personnel, complicated sample preparation and have the limitation to be miniaturized. Considering these disadvantages, intense attraction has been shifted to the development of biosensors since they possess extensive sensitivity and specificity and have simple, easy-to-use formats [7,8]. Among the different types of biosensors, electrochemical methods, especially amperometric one, has become the most widely used technique in glucose sensing [9]. The principle of working of amperometric biosensors is the measurement of the current produced due to the reduction or oxidation of the electroactive species in the medium upon application of a constant potential [10–12].

In order to provide a suitable matrix for biomolecules where they can sustain their activity, it is essential to design a proper surface for the biosensor. Conjugated polymers (CPs) are one of

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the widely used materials in biosensor applications due to their unusual electronic properties mainly due to their π-conjugated system consisting of alternating single and double bonds providing charge mobility [13,14]. As a result, CPs have electrical conductivity, high electron affinity and low ionization potential making them an important class of materials for optoelectronic and biotechnological applications [15,16]. Moreover, they have the properties of processability, low cost, ease of preparation and they provide enhanced stability, sensitivity and fast response for the biosensors [17]. One of the biggest advantages of conjugated polymers in biosensors is their biocompatibility. They provide a matrix for the biomolecules which preserves their activity for a long time and are also compatible with many of the materials found in nature facilitating the interaction with biological molecules [18]. Moreover, they can act as transducers since they have the ability to transfer the electric charge generated by the biochemical reaction to the electric circuit. Also direct deposition of the polymer film on the electrode surface can be achieved by electrochemical synthesis providing the control of the conducting polymer film [19]. In addition, CPs have flexible chemical structures that can be modified to provide the desired electronic and mechanic properties [20–22].

In literature, several studies have utilized CPs in glucose biosensor applications. Ramanavicius et al. proposed a biosensor design based on the gold nanoparticles and conducting polymer-polyaniline nanocomposites. They used gold nanoparticles to facilitate electron transfer and improve the signal [23]. In another work, single walled carbon nanotubes and a conjugated redox polymer multilayer was combined in an amperometric glucose biosensor by layer-by-layer technique on a screen-printed carbon electrode [24]. Moreover, Yu et al. designed hydrogel heterostructures with platinum nanoparticles and polyaniline for electrochemical glucose detection [25]. Musameh et al. reported amperometric biosensors prepared by the co-immobilization of carbon-nanotube dopants and glucose oxidase within polypyrrole film which was electropolymerized via cyclic voltammetry [26]. In these studies, good biosensor parameters were obtained when conjugated polymers were combined with some other supporting materials. However, in this study, promising characteristics were obtained with the utilization of a single conjugated polymer.

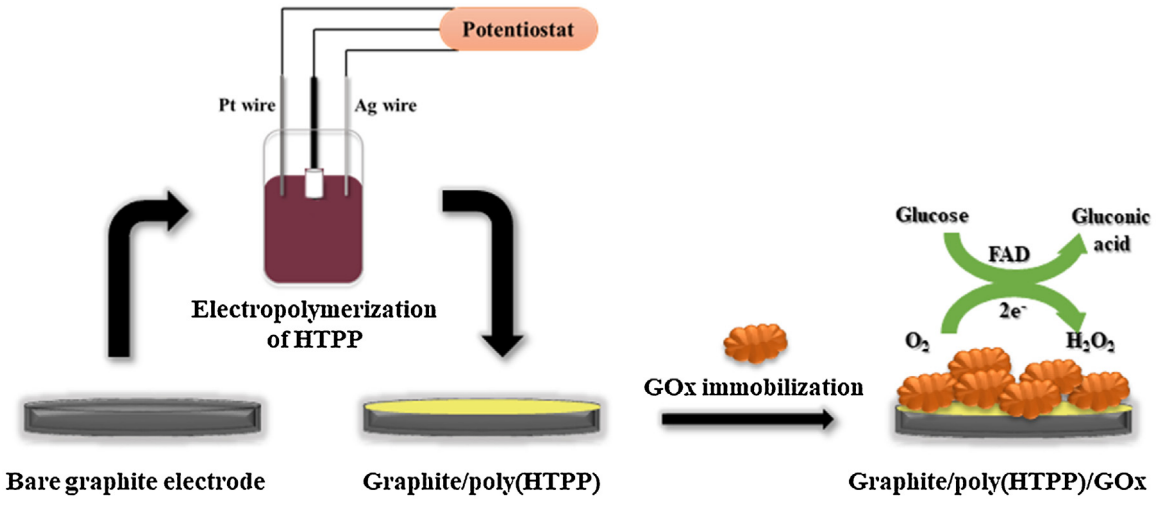
Herein, with these motivations we fabricated a glucose biosensor utilizing the electropolymerization of 10,13-bis(4-hexylthiophen-2-yl)dipyridol[3,2-a:2′,3′-c]phenazine. HTPP monomer was used for the first time in glucose biosensor application and showed enhanced biosensor properties. Due to the hydrophobic alkyl chains on the polymer backbone, proper attachment of the enzyme with both hydrophilic and hydrophobic parts can be achieved. In addition, conjugated polymer provides an improved surface for immobilization by enhancing the catalytic reactions on the electrode surface due to its electroactive nature. Moreover, as a result of the presence of organic residues of the polymer with π bonds, the non-covalent interaction known as π-π stacking provides a good adhesion of the enzyme on the polymer coated surface. After electropolymerization process, immobilization of glucose oxidase (GOx), which is the most used enzyme for glucose biosensor applications having a high glucose selectivity, was performed on the conjugated polymer coated electrode surface using glutaraldehyde (GA) as the cross linking reagent. The working principle of the glucose biosensor is based on the oxidation of β-D-glucose by molecular oxygen which is catalyzed by GOx where this results in the production of gluconic acid and hydrogen peroxide [27]. Electrochemical measurements were made by amperometric detection technique by monitoring the level of the oxygen consumption at −0.7 Ag wire reference electrode. Scheme 1 represents the construction procedure of the proposed amperometric glucose biosensor. The characterization studies of the biosensor were performed successfully.

2. Experimental

2.1. Materials and methods

Glucose oxidase (GOx, β-D-glucose: oxygen 1-oxidoreductase, EC 1.1.3.4, 17300 units/g solid) from *A. Niger*, D-glucose, glutaraldehyde (GA, Grade II 25% in H_2O), $NaClO_4$ and $LiClO_4$ were purchased from Sigma-Aldrich and were used without further purification. Dichloromethane (DCM), acetonitrile (ACN) were purchased from Merck (Darmstadt, Germany). Phosphate buffer solution (PBS) was prepared using 0.025 M Na_2HPO_4 (Fisher Scientific Company) and 0.025 M NaH_2PO_4 (Fisher Scientific Company). As the substrate, glucose solution was prepared by dissolving 0.18 g of glucose in 10 mL pH = 7.0 PBS. All chemicals were of analytical reagent grade.

PalmSens potentiostat (PalmSens, Houten, The Netherlands) was used for all the cyclic voltammetry studies and the amperometric measurements. Three electrode system containing a graphite electrode (Ringsdorff Werke GmbH, Bonn, Germany, type RW001, 3.05 mm diameter and 13% porosity) as the working electrode, a platinum electrode (Metrohm, Switzerland) as the counter electrode and Ag wire as the reference electrode was used for all the electrochemical experiments. The data were given as the average of three measurements and standard derivations were recorded as



Scheme 1. Schematic representation of the proposed biosensor.