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Development of a novel biosensor based on a conducting polymer

Saniye Soylemez [a], Fulya Ekiz Kanik [b], Merve Ileri [a], Serife O. Hacioglu [a], Levent Toppare [a,b,c,d,*]

[a] Department of Chemistry, Middle East Technical University, 06800 Ankara, Turkey
[b] Department of Biotechnology, Middle East Technical University, 06800 Ankara, Turkey
[c] Department of Polymer Science and Technology, Middle East Technical University, 06800 Ankara, Turkey
[d] The Center for Solar Energy Research and Application (GUNAM), Middle East Technical University, 06800 Ankara, Turkey

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ABSTRACT

A new type of amperometric cholesterol biosensor was fabricated to improve the biosensor characteristics such as sensitivity and reliability. For this purpose, a novel immobilization matrix 2-(4-fluorophenyl)-4,7-di(thiophene-2-yl)-1*H*-benzo[*d*]imidazole (BIPF) was electrochemically deposited on a graphite electrode and used as a matrix for the immobilization of cholesterol oxidase (ChOx). Due to strong π–π stacking of aromatic groups in the structures of polymer backbone and enzyme molecule, one can easily achieve a sensitive and reliable biosensor without using any membrane or covalent bond formation between the enzyme molecules and polymer surface. Moreover, through pendant fluorine group of the polymer, H-bond formation between with enzyme molecules and polymer was generated. Cholesterol was used as the substrate and amperometric response was measured in correlation with cholesterol amount, at −0.7 V vs. Ag/AgCl in phosphate buffer (pH 7.0). Consequently, optimum conditions for this constructed biosensor were determined. $K_M$app, I_{max}, LOD and sensitivity values were investigated and calculated as 4.0 nM, 2.27 μA, 0.404 μM and 1.47 mA/mM cm^2, respectively. A novel and accurate cholesterol biosensor was developed for the determination of total cholesterol in food samples.

1. Introduction

Biosensors have attracted great attention due to their easy recognition of various important analytes in biological systems [1]. A biosensor represents two main parts: (1) a recognition part in which biological component is immobilized on a solid surface and (2) a transducer. Combination of these parts allows measuring a target analyte without using reagents. The aim of the biosensor is to convert a biological event into an electrical signal [2]. Solid support is used to anchoring a sensing molecule on transducer. It should be adaptable to different environments and resistant to a wide range of physiological conditions.

Conducting polymers (CPs) have attracted great interest since they were used for construction of the electrochromic devices [3,4] and as suitable matrices for enzyme molecules due to enhanced sensitivity, versatility of biosensors for the detection of desired substances [5]. In fabrication of efficient cholesterol biosensors conducting polymers are the most studied materials for matrix preparation [6–10]. Due to their good electrochemical and physical properties, they can be used as immobilization matrices in the preparation of biosensors [11,12]. Besides, CPs afford increased surface area, thickness control, produce a long-life biosensor, as well as enhanced electron transfer during the electrochemical reactions on the electrode surface [13].

There are several problems as regards to loss of enzyme on a support surface and maintenance of enzyme stability and shelf life of the biosensors. In order to overcome these problems, several enzyme immobilization methods such as adsorption, covalent bonding, entrapment and cross-linking have been used to fabricate desired biosensors [14]. The procedure of biomolecule immobilization on conductive surfaces remains as a fundamental step for the production of mechanically durable and stable biosensors. To improve the performance of the biosensor, it is preferable to find a managable immobilization method and a stable material that can maintain the biocatalytic activity of the biomolecules. Physical adsorption is favored by many researchers due to the simple adsorption of the enzyme molecules onto the number of CPs where it is fixed on the surface by hydrogen bonding and Van der Waals forces [15,16]. By the help of these bonding forces, entrapment of enzymes in conducting polymers provides the localization of biologically active molecules on electrodes of any size or three-dimensional geometry to fabricate amperometric biosensors [17]. This method has been used for the preparation of many biosensors [18–20].

* Corresponding author at: Middle East Technical University Department of Chemistry 06800 Ankara Turkey. Tel.: +90 3122103251; fax: +90 3122103200.
E-mail addresses: saniyesoylemez@gmail.com (S. Soylemez), fulyaekiz@gmail.com (F. Ekiz Kanik), ileri.merve@gmail.com (M. Ileri), serifeirem@gmail.com (S.O. Hacioglu), toppare@metu.edu.tr (L. Toppare).

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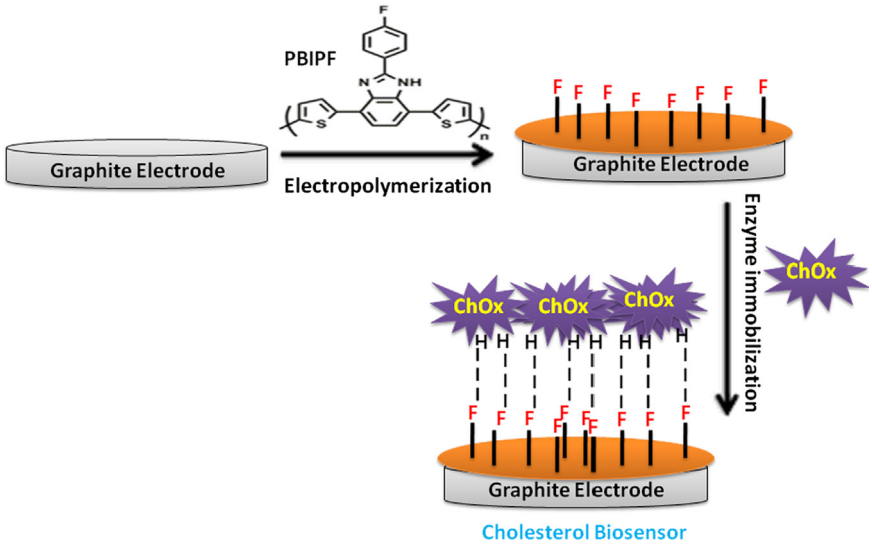

Scheme 1. A schematic representation of the proposed biosensor.

Cholesterol oxidase (ChOx) which catalyzes the oxidation of cholesterol by molecular oxygen to 4-cholesten-3-one and hydrogen peroxide is an industrially important enzyme molecule for clinical determination of cholesterol. Since several clinical disorders such as hypertension, arteriosclerosis and coronary artery disease increase the alarming level, determination of cholesterol in food samples is very important in an industrialized world [2]. Hence, the development of a new sensitive and durable biosensor is an important aspect of biosensor technology.

In this study immobilization of cholesterol oxidase (ChOx) was performed via the adsorption technique onto a conducting polymer of 2-(4-fluorophenyl)-4,7-di(thiophene-2-yl)-1*H*-benzo[*d*]imidazole (BIPF). Electrochemical polymerization of BIPF monomer was undertaken by cyclic voltammetry in acetonitrile (ACN)/dichloromethane (DCM) solution. The polymer was chosen as the immobilization matrix since it has strong π–π stacking of aromatic groups and fluorine group which is open to generate hydrogen-bonding. Additionally, use of conducting polymer as the host matrix for immobilization of ChOx enhanced the electron transfer during the enzymatic reactions. Conducting polymer was electrochemically deposited on a graphite electrode surface by cyclic voltammetry technique. Electropolymerization enables to control morphology and thickness of the polymer [6]. Hence, immobilization of biomolecules by electrochemically generated polymers can be achieved succesfully. ChOx was immobilized onto the polymer coated electrode to construct the amperometric cholesterol biosensor. Immobilization of biological molecule onto the solid support is a fundamental step in the development of a stable and robust biosensor. This immobilization was performed by physical adsorption process where the enzyme molecules were adsorbed in the polymer interface due to π–π stacking effect and the hydrogen bond formation between the functional groups of enzyme molecule and fluorine of conducting polymer. Since immobilization matrix has functional fluorine moiety in its structure, it is easy to achieve sensitive and reliable biosensor without using any membranes or covalent bond formation between the enzyme molecules and polymer surface. It was reported that attachment of the enzyme molecules to the functionalized conducting polymers can be obtained by affinity of the functional groups that can selectively interact with tags on the biomolecules [6]. Moreover, taking the advantage of conductivity and redox stability, conducting polymers can be modified for certain purposes. With this motivation, BIPF was designed and synthesized. To incorporate biomolecules and to improve the binding, novel monomer containing particular functional group and aromaticity were proposed. There are limited applications of adsorption technique for conducting polymer and enzyme based biosensors which display the novelty and value of the present work [21–25].

Hence, an efficient, highly sensitive and fast response biosensor was successfully fabricated in this work. A representative preparation for the proposed biosensor is depicted in Scheme 1. The obtained biosensor was characterized by scanning electron microscopy (SEM) technique. Optimization and characterization studies were done and practical application of modified electrode was tested via determining total cholesterol in food samples.

2. Material and methods

2.1. Materials

Cholesterol oxidase (E.C.1.1.3.6) (26.4 U/mg protein) from *Pseudomonas fluorescens*, cholesterol, Triton-X 100 and glutaraldehyde were purchased from Sigma-Aldrich and used with no further purification. A solution of cholesterol (0.005 M) was freshly prepared by dissolving cholesterol in 1% (v/v) Triton-X 100 in 2-propanol (Merck). This mixture provides solubility and stability of cholesterol in aqueous solutions at room temperature. To obtain a clear solution it was then diluted with 50 mM PBS (pH 7.0) consisting of 0.025 M Na_2HPO_4 (Fisher Scientific Company) and 0.025 M NaH_2PO_4 (Fisher Scientific Company) and distilled water. The chemicals used in the synthesis of the monomer were purchased from Sigma Aldrich. All other chemicals were analytical grade. Cholesterol oxidase colorimetric kit was obtained from Human GmbH-65205 (Wiesbaden-Germany).

2.2. Apparatus

All electrochemical measurements were performed using an Ivium CompactStat (The Netherlands) potentiostat in a reaction cell equipped with three electrodes consisting of a graphite electrode (Ringsdorff Werke GmbH, Bonn, Germany, type RW001, 3.05 mm diameter and 13% porosity) as the working electrode, platinum electrode as the counter electrode and Ag/AgCl electrode (3 M KCl filled) as the reference electrode. Electropolymerization was performed with a Voltalab 50 potentiostat. Measurement of amperometric analyses were calculated as an average of four measurements and standard derivations were given as ± SD. Scanning electron microscope (SEM) (JEOL JSM-6400 model) was used for investigation