



# Immobilization of glucose oxidase on reagentless ferrocene-containing polythiophene derivative and its glucose sensing application

M. Fatih Abasıyanık<sup>a,c</sup>, Mehmet Şenel<sup>b,c,\*</sup>

<sup>a</sup> Department of Genetics and Bioengineering, Faculty of Engineering, Fatih University, B. Cekmece, Istanbul 34500, Turkey

<sup>b</sup> Department of Chemistry, Faculty of Arts and Sciences, Fatih University, B. Cekmece, Istanbul 34500, Turkey

<sup>c</sup> Biotechnology Research Laboratory, Bionanotechnology Research Center, Fatih University, B. Cekmece, Istanbul 34500, Turkey

## ARTICLE INFO

### Article history:

Received 15 September 2009

Received in revised form 29 October 2009

Accepted 2 November 2009

### Keywords:

Redox polymer

Mediator

Ferrocene

Thiophene

Glucose oxidase

Biosensor

## ABSTRACT

A novel polymer electrode in the form of a thin film was prepared by electrochemical copolymerization of thiophene, thiophene-3-acetic acid, and dicyclopentadienyl iron-1,4-dienylmethyl-2-(thiophen-3-yl)acetate, for fabricating GO<sub>x</sub>-immobilized electrodes for amperometric sensing of glucose. Glucose oxidase was immobilized by covalent binding to the carboxyl groups on the electrode. The catalytic electrochemistry of the enzyme electrode with the copolymer was investigated. The enzyme electrodes act as a mediator between the redox center of glucose oxidase (GO<sub>x</sub>) and the electrode.

© 2009 Elsevier B.V. All rights reserved.

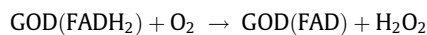
## 1. Introduction

Development of biosensor devices calls for creating an efficient interface between biological molecules and the transducer of the device. Conducting polymer interfaces are particularly suitable for localizing biomolecules onto micro-sized surfaces [1]. Besides, conducting polymers offer the facility to modulate their electronic properties via molecular interactions [2]. Many researchers have suggested that covalent functionalization of conducting polymers could be achieved by synthesis of functionalized monomers bearing a prosthetic group, which are subsequently polymerized [3,4].

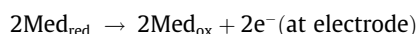
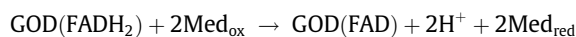
Development of polymeric mediators for applications in sensors and biosensors is essential because polymers allow incorporation of reagents to produce devices for reagentless sensing. Ferrocene derivatives are widely used as mediators in the construction of mediated amperometric biosensors. Direct attachment of the ferrocene-based mediators onto polymeric films prevents the mediator from leaching. Some examples of redox copolymers where covalent attachment of ferrocene has been attempted are poly(vinylferrocene-co-hydroxyethyl methacrylate) [5], poly(N-acryloylpyrrolidine-co-vinylferrocene) [6], acryl amide copolymers

[7], ferrocene-based polyamides [8], and poly(glycidyl methacrylate-co-vinylferrocene) [9].

The determination of glucose is important in the food- and fermentation industries and in clinical chemistry, and there have been many reports on this subject. The most frequently employed glucose biosensors are based on glucose oxidase (GO<sub>x</sub>). Most of the earliest glucose biosensors were based on the amperometric determination of oxygen consumed or hydrogen peroxide produced in the enzymatic oxidation of glucose by GO<sub>x</sub> [10]:



However, there were sensitivity problems in O<sub>2</sub> concentration and significant interference signals generated by ascorbate, urate, and paracetamol [11]. These problems were later overcome by introducing mediators to replace oxygen as the means of electron transfer [12–14]. Mediators (Med<sub>ox/red</sub>) are small molecules with lower redox potential, which can shuttle electrons between the embedded redox center of the enzyme and the electrode surface. The scheme of the sensing mechanism is as follows:



\* Corresponding author. Address: Department of Chemistry, Faculty of Arts and Sciences, Fatih University, B. Cekmece, Istanbul 34500, Turkey. Tel.: +90 2128663300.

E-mail address: msenel@fatih.edu.tr (M. Şenel).

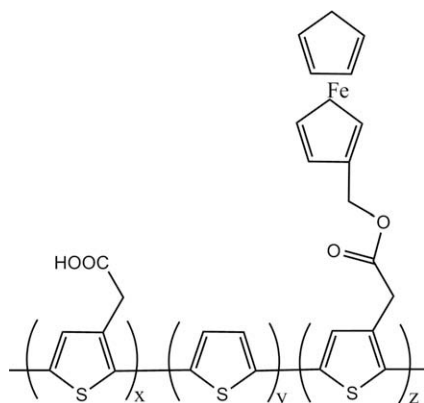


Fig. 1. Structure of Th/Th-COOH/Th-Fc.

Recently, because of fundamental interest evinced in the electron-transfer reaction between  $GO_x$  and electrodes and from the point of view of long-term stability of glucose sensors, several redox-active polymers have been prepared and utilized as polymeric mediators [15–20].

Since it has been established that glucose oxidase could be successfully immobilized in polythiophene derivatives [21,22] and that an electron acceptor to the enzyme, ferrocene, could be easily modified by chemical substitution [23–25], it was hoped that thiophene-ferrocene conjugates could be synthesized and used in the construction of reagentless mediated biosensors.

In this study, a novel copolymer of thiophene (Th), thiophene-3-acetic acid (Th-COOH), and dicyclopentadienyl iron-1,4-dienylmethyl-2-(thiophen-3-yl)acetate (Th-Fc), whose structure is shown in Fig. 1, was employed as a novel electron mediating support material for fabricating  $GO_x$ -immobilized electrodes. The copolymer was synthesized electrochemically in the form of thin films, and  $GO_x$  was immobilized covalently on the surfaces of the copolymer films through amide linkages formed by the condensation reaction with COOH groups on these surfaces. In this manner, the  $GO_x$ -immobilized electrodes were prepared with the copolymers of various compositions and applied to amperometric sensing of glucose. Their sensing ability was investigated in relation to the effect of copolymer composition on the conductivity of the film and the immobilization of  $GO_x$ . We demonstrate that these redox copolymers function as an enzyme binding support and a polymeric mediator for enzymatic glucose sensors.

## 2. Experimental

### 2.1. Materials

Thiophene-3-acetic acid, thiophene, ferrocene aldehyde and Fluka were used without further purification. Glucose oxidase ( $GO_x$ ) (EC 1.1.3.4) was obtained from Sigma. 1-Cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate (CMC) from Aldrich Chemical Co. was used as a condensing agent. Other chemicals and solvents were of guaranteed reagent- or analytical grade and were used without further purification.

### 2.2. Apparatus

The FTIR-ATR spectra ( $4000\text{--}400\text{ cm}^{-1}$ ) were recorded with a Bruker spectrometer. NMR spectra were recorded in  $CDCl_3$  using a Bruker 400 MHz spectrometer.

Electrochemical polymerizations and measurements were performed using a CHI Model 842B electrochemical analyzer. A small glassy carbon working electrode (2 mm diameter), a platinum

working electrode (for polymerization), a platinum wire counter electrode (0.2 mm diameter), an Ag/AgCl-saturated KCl reference electrode, and a conventional three-electrode electrochemical cell used were purchased from CH Instruments.

### 2.3. Synthesis of ferrocene alcohol

To a solution of ferrocene aldehyde in THF and methanol (1:2 v/v), a suspension of sodium borohydride in THF was added slowly with stirring. Once the addition was complete, the reaction was continued for 1 h at room temperature. After the solvent was removed, the crude product was extracted with ether and recrystallized from *n*-hexane. The solid was then dried at  $50\text{ }^\circ\text{C}$  under reduced pressure and characterized using FTIR-ATR,  $^1\text{H}$ , and  $^{13}\text{C}$  NMR spectroscopy (not shown).

### 2.4. Synthesis of dicyclopentadienyl iron-1,4-dienylmethyl-2-(thiophen-3-yl)acetate (Th-Fc) monomer

Th-Fc monomer was synthesized by coupling thiophene-3-acetic acid to ferrocene alcohol using EDCI and DMAP in dry chloroform (Fig. 2). The crude product was extracted with 5%  $\text{NaHCO}_3$ , 5% HCl, and brine solution five times and dried using  $\text{MgSO}_4$ . After the solvent was removed, the crude product was extracted with ether and recrystallized from *n*-hexane. The solid was then dried at room temperature under reduced pressure.

### 2.5. Electrochemical polymerization

Electrochemical polymerization was carried out in a solution of monomers of different concentrations (Th, Th-COOH, and Th-Fc, Table 1.) and tetraethylammonium perchlorate (0.10 M) in acetonitrile (20 ml). Before polymerization, nitrogen gas was bubbled through the solution for 20 min to remove oxygen. A potential of 2.2 V vs. Ag/AgCl was then applied to the Pt working electrode until the amount of charge passed reached  $0.80\text{ C/cm}^2$  (except for the special requirements). The copolymer Th/Th-COOH/Th-Fc was then obtained on the working electrode deposited in the form of a thin film. The composition of the copolymer was determined by means of FTIR-ATR spectroscopy.

### 2.6. Immobilization of $GO_x$ on Th/Th-COOH/Th-Fc copolymer film

The copolymer film-coated electrode was immersed in 3.0 ml distilled water containing 25 mg  $GO_x$  and 120 mg CMC and allowed to remain for 24 h at  $4\text{ }^\circ\text{C}$ . The copolymer films treated thus, i.e.  $GO_x$ -electrodes, were then rinsed with distilled water and stored in 0.1 M phosphate buffer (pH 7.4) at  $4\text{ }^\circ\text{C}$ .

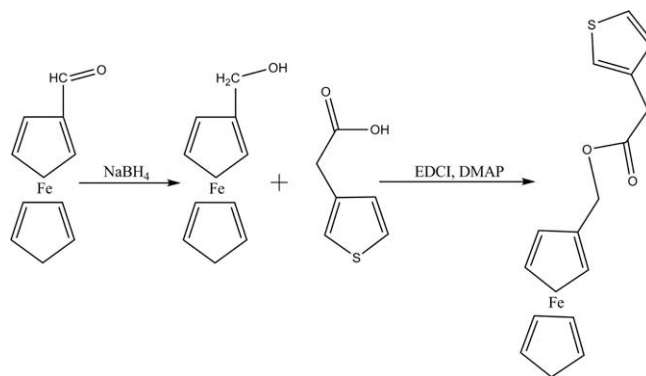


Fig. 2. Synthesis of thiophene-3-ethane ferrocene monomer.

Download English Version:

<https://daneshyari.com/en/article/220018>

Download Persian Version:

<https://daneshyari.com/article/220018>

[Daneshyari.com](https://daneshyari.com)