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**Glucose Biosensor Based on the Hexacyanoferrate 11-Mercaptoundecyl-N',N'',N''''-trimethylammonium/6-(Ferrocenyl)hexanethiol**

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**Abstract**

An amperometric glucose biosensor based on hexacyanoferrate 11-mercaptopoundecyl-N',N'',N'''-trimethylammonium (HMA)/6-(ferrocenyl)hexanethiol (FT)/GOx modified gold electrode was fabricated and evaluated. The GOx/HMA/FT/Au electrode was characterized using cyclic voltammetry (CV) and used for glucose concentration estimation in standard solutions using CV and amperometric techniques.

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Keywords: self-assembled monolayer; biosensor; glucose oxidase; amperometric sensor

**1. Introduction**

Since Clark and Lyons proposed the first glucose enzyme electrode in 1962 [1] there has been a lot of attention paid to developing novel biosensors for the fast, stable, reproducible, sensitive and selective quantification of glucose. Most glucose measurements are based on immobilization of *glucose oxidase* (GOx) for detecting H₂O₂ concentration which is produced from the GOx enzymatic reaction. The self-assembled monolayer (SAM) of alkanethiol on gold electrode has been receiving considerable interest because of its easy fabrication and ordered interfacial structure. In addition, SAM provides a means of tuning the chemical nature of the electrode-solution

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interface and serves as a promising interface for constructing fast responding amperometric biosensors with low over-potential.

This work described the characterization of a glucose biosensor based on hexacyanoferrate 11-mercaptopoundecyl-N',N''',N'''-trimethylammonium (HMA)/ 6-(ferrocenyl)hexanethiol (FT)/GOx modified gold electrode.

2. Experimental

2.1. Reagents

Glucose oxidase (Aspergillum niger, 162,000 units/g), glucose, bromide 11-mercaptopoundecyl-N',N''',N'''-trimethylammonium (BrMA) and 6-(ferrocenyl)hexanethiol (FT) were obtained from Sigma–Aldrich and used as received. All solutions were prepared using deionized water.

2.2. Apparatus

Cyclic voltammetry (CV) was conducted with a μ-Autolab type III (Eco Chimie) connected to a microcomputer and controlled by GPES software. All amperometric measurements were carried out in a 25 mL thermostatic glass cell containing three-electrodes: gold electrode coated with hexacyanoferrate 11-mercaptopoundecyl-N',N''',N'''-trimethylammonium/6-(ferrocenyl)hexanethiol/Glucose Oxidase as biosensor, a saturated calomel (SCE) as the reference, and a platinum auxiliary electrode.

2.3. Sensor preparation

The gold square (1.0 cm²) electrodes were polished to a mirror-like surface with 0.01 μm aluminate powder, sonicated in distilled water, acetone and ethanol, respectively. After, the electrodes were treated in the piranha solution for 15 min, and then thoroughly rinsed with water. Prior to use, the electrode was voltammetrically cycled in sulfuric acid (0.5 mol L⁻¹) until a stable cyclic voltammograms was obtained. This procedure enabled the electrode to yield a fresh surface for the efficient formation of self-assembly monolayer. To fabricate the SAM, the fresh gold electrode was immersed in 10 mmol L⁻¹ aqueous bromide 11-mercaptopoundecyl-N',N''',N'''-trimethylammonium (BrMA) solution / 20 mmol L⁻¹ 6-(ferrocenyl)hexanethiol/Glucose Oxidase as biosensor, a saturated calomel (SCE) as the reference, and a platinum auxiliary electrode.

2.4. Electrochemical measurements

The CV studies were carried out to determine the effect of scan rate, pH and to estimate glucose concentration. Glucose estimation studies for GOx/HMA/FT/Au electrode were conducted via CV and amperometric investigation. CV studies were carried out in 0.10 mol L⁻¹ potassium hexacyanoferrate(III) solution for 120 min. The immobilization of the glucose oxidase (GOx) on gold electrode modified by self-assembled monolayers of thiols (HMA/FT) was obtained by the droplet evaporation method placing on the electrode surface. The GOx solution (5 mg L⁻¹ in acetate buffer) was prepared previously to be deposited in the electrode surface. After 24 h immobilization, the GOx/HMA/FT modified Au electrode was ready, then rinsed and stored in acetate buffer solution at 4 °C when not in use.
3. Results and Discussion

3.1. Electrochemical studies of the GOx/HMA/FT/Au

The cyclic voltammogram (Figure 1) were recorded at potential sweep rate of 25 mV s\(^{-1}\) from range of -0.50 to 0.80 V vs. SCE in 0.10 mol L\(^{-1}\) acetate buffer solution (pH 4.6). The typical cyclic voltammogram showed two redox couple at 0.24 V (peak I) / 0.08 V (peak IV) and 0.50 V (peak II) / 0.28 V (peak III), which can be attributed to redox reactions of [Fe(CN)\(_6\)]\(^{4-}\) to [Fe(CN)\(_6\)]\(^{3-}\) and [Ferroceno] to [Ferrocenium]\(^{+}\), respectively.

![Cyclic voltammogram of the modified gold electrode in acetate buffer (pH = 4.6). Scan rate = 25 mV s\(^{-1}\).](image1.png)

The surface coverage of electrochemically active on gold was calculated from the area under the reduction wave and the electrode surface area. After cycling the electrode in 0.10 mol L\(^{-1}\) acetate buffer, the estimated surface concentration was found to equal \(8.68 \times 10^{-10}\) mol cm\(^{-2}\) for hexacynaoferate immobilized.

3.2. Performance of glucose biosensor

The activity of the biosensor when tested towards glucose exhibited a favorable response with electron mediator and redox center of enzyme (FAD) in the surface of working electrode (see Fig. 2).

![Voltammetric response of the proposed biosensor for different glucose concentration in acetate buffer (pH = 4.6) at 25 mV s\(^{-1}\).](image2.png)
The performance of the biosensor for glucose is based on the cycle of four subsequent with three chemical step (Eqs. 1 to 3) and followed by an electrochemical step (Eq. 4), resulting in a reduction current measured as the biosensor response:

\[
\text{Glucose} + \text{FAD}_{\text{enzyme}} \rightarrow \text{gluconic acid} + \text{FADH}_2(\text{enzyme}) \quad (1)
\]
\[
\text{FADH}_2(\text{enzyme}) + \text{O}_2 \rightarrow \text{FAD}_{\text{enzyme}} + \text{H}_2\text{O}_2 \quad (2)
\]
\[
2[\text{Fe(CN)}_6]^{4-}\text{(electrode)} + \text{H}_2\text{O}_2 + 2\text{H}^+ \rightarrow 2[\text{Fe(CN)}_6]^{3-}\text{(electrode)} + 2\text{H}_2\text{O} \quad (3)
\]
\[
[\text{Fe(CN)}_6]^{3-}\text{(electrode)} + \text{e}^- \rightarrow [\text{Fe(CN)}_6]^{4-}\text{(electrode)} \quad (4)
\]

The peaks currents varied linearly with the square root scan rate, suggesting that the redox process is controlled by diffusion.

In order to evaluate the performance of the biosensor as an amperometric sensor for glucose determination in acetate buffer (pH 4.6), experiments involving choroamperometry at different applied potentials (0.4 V to -0.4 V vs. SCE) with sensor was performed to determine the best potential for the amperometric oxygen determination. The highest amperometric response for glucose was obtained at 0.06 V vs. SCE. The response time for sensor was fast in each glucose addition. The range linear from 1.0 x 10^{-5} to 8.4 x 10^{-4} mol L^{-1} was obtained from biosensor, which is shown \( I (A) = 1.5 \times 10^{-7} + 6.2 \times 10^{-2} \text{[glucose (mol L}^{-1})\text{]} \) equation (n = 6; r = 0.990). The dynamic response of biosensor is shown in Fig. 3.

Fig. 3. Amperometric response of the biosensor in acetate buffer for different glucose concentrations.

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