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Zero-Length Cross-Linkers Modify Direct Electron Transfer of Glucose Oxidase on Pre-Anodized Paper/Carbon Electrodes for Glucose Biosensors

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Abstract

Simple covalent immobilization of Glucose Oxidase (GOX) on a carbon electrode surface with zero-length cross-linkers resulted in the development of a disposable glucose biosensor with Direct Electron Transfer (DET) of GOX. This glucose biosensor displayed a high electron move rate (ks,3.363 s^{-1}) as well as great proclivity (km, 0.03 mM) for GOX while keeping intrinsic enzymatic exercises. Moreover, the DET-based glucose discovery was achieved by utilizing both square wave voltammetry and chronoamperometric procedures, and it accomplished a glucose location range from 5.4 mg/dL to 900 mg/dL, which is more extensive than most monetarily accessible glucometers. Using the negative operating potential, this low-cost DET glucose biosensor avoided interference from other common electroactive compounds and displayed remarkable selectivity. Especially for self-monitoring of blood glucose, it has great potential to monitor various stages of diabetes, from hypoglycemic to hyperglycemic states.

Keywords: Direct electron transfer • Glucose biosensors • Glucose oxidase immobilization

Introduction

The casual plasma glucose concentration of more than 200 mg/dL is considered to be diabetes, and its incidence and prevalence have been rising worldwide. Diabetes affected 30.3 million adults and children in the United States in 2015, representing 9.4 percent of the population. A total of 23.1 million people were estimated to be undiagnosed, or 2.2% of the US population, and 7.2% of those had been diagnosed with diabetes. Starting around 2015 in the US, around 5% of individuals determined to have diabetes are type 1 diabetics, and the rest are type 2 diabetics. When their biomarker glycosylated haemoglobin ($HgbA_{1c}$) is greater than 5.5% but less than 6.4%, 33.9%, or 84.1 million people, are considered to be pre-diabetics [1]. A person has pre-diabetes if their Fasting Plasma Glucose (FPG) is between 100 mg/dL and 125 mg/dL (5.6–6.9 mmol/L) or their hemoglobin A_{1c} is between 5.7% and 6.5% (39–47 mmol/mol). They also have impaired glucose tolerance if their plasma glucose levels are higher than normal but not high enough to be considered diabetes. Additionally, gestational diabetes affects 3–10 percent of pregnancies and causes high blood glucose levels in pregnant women.

The rules of the American Diabetes Affiliation (ADA) and the International Diabetes Federation (IDF) recommend that Self-Monitoring of Blood Glucose (SMBG) conventions be individualized to meet the particular requirements of every patient (T1D and T2D patients) and be performed habitually during pregnancy in diabetic patients. As a result, a crucial parameter for the diagnosis and treatment of diabetes is the speed with which glucose levels in body fluids can be determined at the point of care [2]. For instance, if a patient's glucose level is greater than 600 mg/DL, it will result in diabetic hyperosmolar syndrome, which is a condition that can be fatal and necessitates immediate hospitalization. Due to their portability, low operational costs, and ease of fabrication, GOX-based glucose biosensors have received a lot of attention. The commercially available GOX, Glucose Dehydrogenase(GDH), Flavin-Adenine-Dinucleotide(FAD), and GDH (glucose dehydrogenase)- PQQ (pyrroloquinoline quinone)-based glucometers, on the other hand, only cover the range of 20 to 600 mg/dL and are unable to address all diabetes scenarios. Just two of them meet the ISO (International Organization for Standardization) 2013 measures for the worldwide principles for blood glucose observing frameworks [3,4].

Description

A composition of Highly Concentrated Graphene Oxide (HC-GO) with a sheet resistance of 50/sq. (20% oxygen and 79% carbon) at a thickness of 25 millimeters) was obtained from Graphene Laboratories in Calverton, New York, USA. Conductive compounds provided Ag/AgCl ink with a surface resistivity of less than 75 m/square mil and a viscosity of 5570–14,600 CPS as well as carbon ink with a 79% C-220 carbon resistive ink. Fisher Scientific supplied the potassium ferricyanide [K3Fe(CN)6] (CAS 13746-66-2) that was required. SU-8 negative photoresist detailing 50 was bought from Miniature Chem (Newton, Mama, USA). In all of the studies, Phosphate Buffer Solution (PBS) with a pH of 7.4 was used. A Millipore purification system (Millipore, Burlington, MA, USA) produced deionized water (>18 MΩcm) [5].

Conclusion

Using a Spectrum 100 FT-IR Spectrometer, the FT-IR spectra of various electrodes were recorded to confirm the electrode modification. A Class II/2 Helium-Neon (HeNe) laser with a 633 nm wavelength provides continuous radiation in the optical module. The powerful technique known as X-Ray Photoelectron Spectroscopy (XPS) provides useful information regarding the glucose biosensor's chemical bonding and molecular composition. A PHI 5600 spectrometer with a hemispherical energy analyzer and a 1487 eV aluminium (Kα) source at 100 Watts was used for the XPS analysis. During XPS analysis, the pressure in the analysis chamber remained within the low range of 10^{-9} Torr. The examined area was approximately 1 mm^2. With a 1.0 eV step, all spectra were recorded at a 54°C take-off angle. A carbon signal (C1s) at 284.5 eV was used to further correct the spectra, and Casa-XPS software version 2.3.18 was used to analyze them. The background that was required for the curve fitting was extracted using the Shirley method.

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We identified the C=O stretching vibrations of the peptide linkages in the GOX backbone at 1600 cm1 to 1700 cm^{-1} and 1650 cm^{-1} to 1750 cm^{-1} using FT-IR spectroscopy which revealed the presence of the amide I band (an overlapping spectrum of α-helices, β-sheets, turns, and random coils, which form the basic structure of the protein. Additionally, the N-H in-plane bending and C-N stretching linkages for the peptide groups combined to produce the 1462 cm^{-1} amide band II peak. Another small band, amide band III, was present at 1239 cm^{-1}, and its correlation with the Delfino data from 2013 is excellent. In addition, we discovered two large bands in the range of 3400 to 2900 cm^{-1}, which correspond to the amide A and bond linkages to $-CH_3$ stretching at 2958 cm^{-1}, respectively. As a result, the FT-IR spectra show that GOX was successfully immobilized on the PA-PPE surface.

Acknowledgement

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Conflict of Interest

There are no conflicts of interest by author.

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