

## Glucose Sensors Using Lipoic Acid Self-Assembled Monolayers

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### Abstract

A novel approach to fabricating high-performance glucose sensors is reported, which is based on the process of self-assembled monolayers (SAMs). In this study, we have particularly used  $\alpha$ -lipoic acid (LA) SAMs for the glucose sensors. To our best knowledge, this study is the first one to use LA as SAMs for this purpose. *N*-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were deliberately attached at the same time on the LA SAM. Then, glucose oxidase (GO<sub>x</sub>) and horseradish peroxidase (HRP) were sequentially immobilized. Thus, the HRP/GO<sub>x</sub>/NHS-EDC/LA-SAM/Au/Cr/glass working electrode was developed. The glucose-sensing capability of the fabricated sensor was systematically measured by the use of cyclic voltammetry in the range of 1-30 mM glucose in phosphate-buffered saline. The result showed a good sensitivity, that is, as high as 27.5  $\mu\text{A}/(\text{mM}\cdot\text{cm}^2)$ . This result conspicuously demonstrates that LA can be one of promising substances for use as SAMs for accurately monitoring trace levels of glucose concentration in human blood.

**Key words:** Self-assembled monolayer, Glucose sensor, Thin film, Sol-gel

### 1. INTRODUCTION

Diabetes is a disease that is spreading worldwide with the increase in obesity. The number of diabetic patients is expected to increase to over 300 million people in the near future. Extreme care is required with respect to aspects such as eating habits and insulin injections in order to ensure the safety of diabetic patients. For this purpose, regular monitoring of the blood glucose level is essential. Therefore, there has been a strong demand for glucose sensors with high accuracy and sensitivity, as well as for miniaturization of sensors [1,2].

A literature survey shows that research is underway on the fabrication and characterization of glucose sensors by using nanowires [3,4], carbon nanotubes [5,6], self-assembled monolayers (SAMs) [7,8], nanopowders [9,10], and nano-patterned electrodes [11].

In particular, SAM-based glucose sensors can reliably detect

glucose in a low-cost process. The advantages of SAMs include (1) an easy and reliable synthesis process, (2) excellent adaptability to the human body, (3) easy manipulation of hydrophobicity or hydrophilicity, (4) possibility of immobilizing a very small amount of the enzyme, and (5) good long-term stability [12]. With regard to the type of thiol molecules, various types of SAMs can be created [13]. To expedite the implementation of SAM-based glucose sensors with superior sensing performance, a new SAM needs to be synthesized.

$\alpha$ -lipoic acid (LA) is known to prevent certain kinds of cell damage in the body, and also improve the function and conduction of neurons in diabetes [14]. In this sense, LA SAM can be applied to a platform of high performance glucose sensors. To the authors' knowledge, this study is the first to use LA in SAMs for detecting glucose with high sensitivity. The LA SAM-based sensor exhibited good properties for the detection of glucose with high reliability, repeatability, and sensitivity. These results strongly suggest that LA is a promising substance for use in SAMs in glucose sensors.

### 2. EXPERIMENTAL

The following steps were applied to fabricate LA SAM-based glucose sensors. The entire fabrication process is schematically shown in Fig. 1. All steps were performed in air at room temperature. In step 1, for the growth of an SAM, 90-nm-thick Au

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films were deposited on glass substrates by a sputtering technique. To enhance the adhesion between the Au film and the glass substrate, a 20-nm-thick Cr interlayer was inserted between them. Then, the Au/Cr/glass wafers were sequentially rinsed with alcohol and deionized water 3 times.

In step 2, an LA SAM was prepared on the Au/Cr/glass wafer. For this, the wafer was immersed in a 2% LA solution (in ethanol) for 24 h. The wafer was then rinsed with ethanol 3 times and dried under a stream of nitrogen gas.

In step 3, to promote the immobilization of glucose oxidase ( $\text{GO}_x$ ) to the SAM, an interlayer was synthesized. The wafer on which the SAM had been created was immersed in a mixed solution of 0.1 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) in phosphate-buffered saline (PBS, 7.2 pH) and 0.05 M *N*-hydroxysuccinimide (NHS) in PBS for 2 h. Then, the wafer was rinsed with PBS 3 times. In step 3, NHS ester molecules were immobilized on the SAM layer.

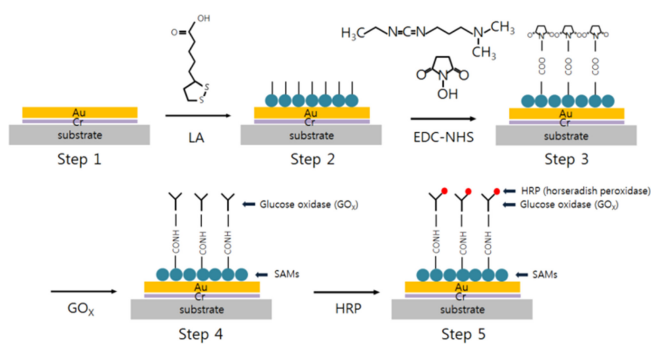
In step 4,  $\text{GO}_x$  was immobilized by forming peptide bonds with the NHS ester molecules. For that purpose, after performing the above 3 steps, the wafer was kept in a solution of 2 mg/mL  $\text{GO}_x$  in PBS for 2 h and then rinsed with PBS 3 times.

In step 5, the  $\text{GO}_x$ -immobilized wafer was immersed in a solution of 60 ng/mL horseradish peroxidase (HRP) in PBS for 2 h at 4°C. Then, the wafer was rinsed with PBS 3 times. By using this multiple-step process, HRP/ $\text{GO}_x$ /NHS-EDC/LA-SAM/Au/Cr/glass electrodes were prepared.

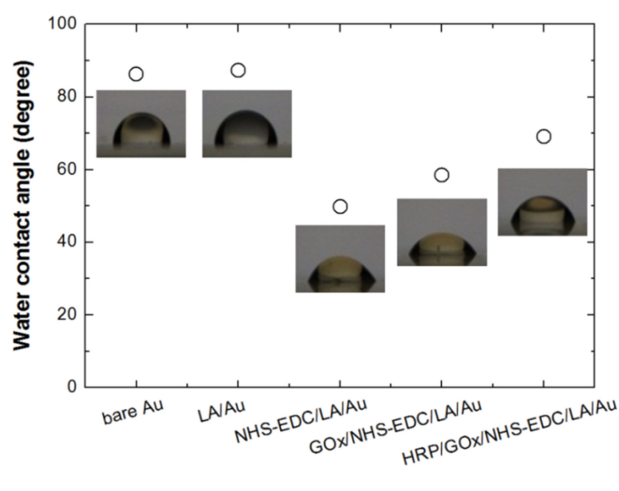
Cyclic voltammetry measurements were performed to investigate the electrochemical behavior of the fabricated electrode system to glucose. The measurements were based on a 3-electrode system consisting of an Ag/AgCl (3.0 M KCl) reference electrode, the fabricated HRP/ $\text{GO}_x$ /NHS-EDC/LA-SAM/Au/Cr/glass electrode that served as a working electrode, and a platinum wire that was used as a counter electrode. To estimate the glucose-sensing capability of the fabricated electrode system, I-V curves were obtained while the electrodes were dipped in PBS (pH = 7.2) containing various concentrations of glucose. The scan rate was set to 0.01 V/s. Water contact angles (WCAs) were measured to indirectly confirm the formation of SAMs at each step of the process.

### 3. RESULTS AND DISCUSSION

First, we have indirectly checked the chemical reactions at each step described in Fig. 1 by measuring the WCAs created on the substrates. It was expected that if the chemical reactions were



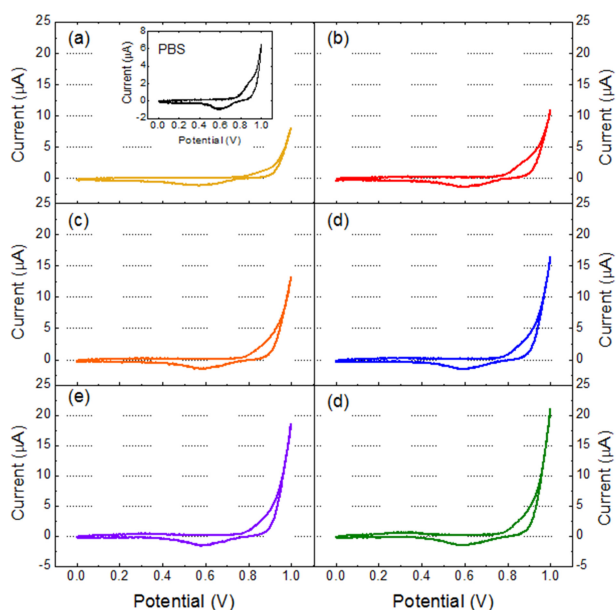
**Fig. 1.** Processing steps used for synthesizing the HRP/ $\text{GO}_x$ /NHS-EDC/LA-SAM/Au/Cr/glass electrode in this study.



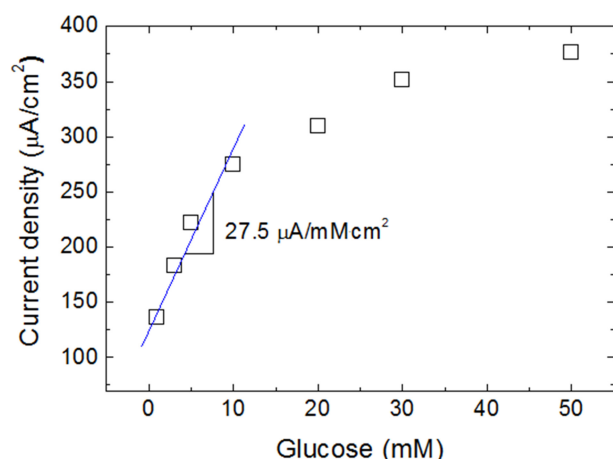
**Fig. 2.** Change in the water contact angle of the samples at each step.

performed properly and the layers were formed accordingly, the WCA at each step would show different values. The results are summarized in Fig. 2. As shown there, the sample at step 1 of the unmodified Au layer shows a WCA of 85°. The value remains almost the same for the sample at step 2, i.e., the LA-coated Au layer. This indicates that the LA formation little affects the WCA. However, for the sample at step 3, i.e., the NHS-EDC/LA/Au layer, the WCA greatly decreased to 50°. However, the samples at later steps show again increased WCAs. The changes in the WCA evidently indicate the successful preparation of the required layers at each step to fabricate the HRP/ $\text{GO}_x$ /NHS-EDC/LA-SAM/Au/Cr/glass electrode. A careful chemical analysis needs to be performed in the future in order to confirm the formation of each layer.

The electrochemical behavior of the HRP/ $\text{GO}_x$ /NHS-EDC/LA-SAM/Au/Cr/glass electrode system with various glucose concentrations in PBS was investigated by cyclic voltammetry. The results are shown in Fig. 3. As is clearly shown, the current increases gradually with increasing glucose concentrations. At the

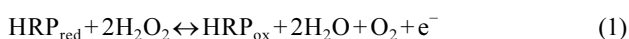
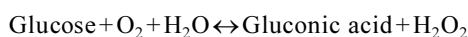


**Fig. 3.** I-V curves of the fabricated HRP/GO<sub>x</sub>/NHS-EDC/LA-SAM/Au/Cr/glass electrode, obtained by cyclic voltammetry; (a) 1, (b) 3, (c) 5, (d) 10, (e) 20, and (f) 30 mM glucose in PBS. The inset in (a) is the I-V curve obtained from pure PBS.



**Fig. 4.** Curve of current density versus glucose concentration in PBS. The slope indicates the sensitivity of the fabricated sensor.

working electrode, the following electrochemical reactions are known to occur [15]:



At the platinum counter electrode, the following reaction occurs:



In Fig. 4, the obtained current densities are plotted as a function

of the glucose concentration to evaluate the sensitivity of the fabricated sensor in detecting glucose. At low glucose concentrations ranging from 0 to 10 mM, the current density is proportional to the glucose concentration, exhibiting a sensitivity of 27.5  $\mu\text{A}/(\text{mM}\cdot\text{cm}^2)$ . The sensitivity is much higher than that of mercaptopropionic acid-SAM-based glucose sensors (0.41  $\mu\text{A}/[\text{mM}\cdot\text{cm}^2]$ ) [16]. However, it should be noted that the current density tends to saturate when the glucose concentration exceeds 20 mM, indicating that the LA SAM-based sensor can be used for the detection of very low concentration of glucose.

#### 4. CONCLUSIONS

In this work, LA SAM was used to fabricate glucose sensors. The final working electrode with the HRP/GO<sub>x</sub>/NHS-EDC/LA-SAM/Au/Cr/glass structure showed a good sensitivity that was as high as 27.5  $\mu\text{A}/(\text{mM}\cdot\text{cm}^2)$  in the range of glucose concentrations of 0–10 mM in PBS. This finding suggests that the use of LA as a base layer for the next stages of oxidase immobilization is a promising approach for obtaining sensitive glucose sensors.

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