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# Effect of self-assembled monolayers (SAMs) in binding glucose oxidase for electro-enzymatic glucose sensor with gold electrodes

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*Abstract*— **In this paper, a new approach to improve long term stability of electro-enzymatic glucose sensors is presented. Self-assembled monolayers (SAMs) are employed for surface treatment of the gold electrodes to improve adhesion. Three types of functional alkylthiols, namely 11-amino-1-undecanethiol hydrochloride, 1-hexadecanethiol (1-hexadecanthiol) and 1,9-nonanedithiol, and bovine serum albumin (BSA) are investigated in our study. Alkylhiols are used for surface treatment of gold electrodes and BSA is mixed in enzyme (glucose oxidase) solution to realize the proposed sensors. Furthermore, gold is investigated as an electrode material. Both reference and active electrodes are fabricated using only gold. The current response of the modified sensors showed long term stability. However, the unmodified sensors showed very short term stability because glucose oxidase does not adhere well to the electrodes. The proposed modified sensors also show lower drift than the unmodified sensors without surface treatment and BSA.**

## I. Introduction

Diabetes mellitus is a disease of major global importance, increasing in frequency at almost epidemic rates, such that the worldwide prevalence is predicted to at least double to about 300 million people over the next 10–15 years [1]. For a detailed account of the major aspects relating to diabetes the reader is referred to Pickup and Williams [2].

Diabetic patients must maintain a strict regime of either diet control or insulin injections in order to maintain blood glucose. This control can only be achieved by the regular monitoring of glucose level. The need for regular monitoring of glucose is highlighted in a study by the Diabetes Control and Complications Trial Research Group (1993) in the USA [3].

The development of reagent strips for detecting glucose, glucose meters, and finger prick systems, is now recognized as a major advance in the history of insulin therapy [4]. A variety of methods are currently used in analytical practice for glucose determination and concentration measurements [5], [6]. Most of these sensors are based on electro-enzymatic principles and employ enzymes as biological components for molecular recognition. Electro-enzymatic sensors are fabricated using platinum as an active electrode and silver/silver chloride (Ag/AgCl) as a reference electrode. Glucose oxidase (enzyme) is usually immobilized on an active (Pt) electrode using glutaradehyde.

The oldest electro-enzymatic principle [7] is still utilized because of its high selectivity to glucose. The stage of proof of concept for such a device has already been passed. Currently, efforts are made to improve the performance of the electro-enzymatic sensors by improving the unpredictability of the Ag/AgCl electrode fabrication and the potential drift. Furthermore, platinum is an expensive metal if large scale fabrication is desired, so other metals are of interest from a cost perspective. In addition, immobilized glucose oxidase loses its functionality over time which affects the sensor performance and life-time.

Towards finding solutions to these problems, we present an electro-enzymatic sensor using gold as both active and reference electrodes. The binding property of glucose oxidase to active gold electrodes is examined using different self-assembled monolayers (SAMs) and bovine serum albumin (BSA).

Gold is a very common metal for microfabrication and less expensive than Pt. Furthermore, gold electrodes can be easily fabricated without any special process steps like Ag/AgCl metal electrodes. Also, gold is more pliable, which can be of importance if flexible sensors are desired [7]. The sensors presented here are fabricated on glass substrates. Thiols are frequently used to increase adhesion of the gold surfaces in many bio-medical applications [9]. This increased adhesion of the gold surface helps to bind enzymes and proteins strongly to the gold electrodes resulting in long term stability of the sensor.

The micro-fabrication process of the electrodes is discussed in the fabrication process section, which also includes an explanation of the surface treatment of the active electrode using different SAMs and immobilization of enzyme (glucose oxidase). In the test results and discussion section, a brief explanation of the test setup is given followed by the detailed discussion of the test results obtained. Finally, the paper is concluded by extracting the main points from the experiments.

## II. Design

In this section, the design of the gold electrodes is explained followed by a description of surface modifications with SAMs and enzyme immobilization.

### *A. Electrode design*

For ease of testing, the sensors are designed with contact pads and connecting wires between contact pads and sensor area. All the sensors are designed using gold as a metal for

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all segments (sensor, connecting wire and contact pad). The active electrode can be identified only after the enzyme is immobilized onto it. Three different patterns have been fabricated for the design of the sensors (Fig. 1).

The area of the active sensor electrode in all three designs is same (see Fig. 1). The total area (enclosed in dashed lines) of each sensor is: 2 mm x 3 mm for the sensor in Fig. 1(a) and 2 mm x 2.5 mm for the sensors in Fig. 1 (b) and (c). Hence, the separation between active and reference electrodes is 1 mm (Fig. 1(a)) or 0.5 mm (Fig. 1(b), (c)). The area of the active and reference electrode is 1 mm x 2 mm in all three designs. The different widths of the connecting wires, 1 mm for Fig. 1(a) and 0.5 mm for Fig. 1(b)-(c), between sensor and contact pad are designed to examine the effect on the sensor current. The contact pads in all the sensors (Fig. 1) are 2 mm x 2mm to connect wires using silver epoxy for electrical tests.

### *B. SAM and enzyme immobilization scheme*

Different alkylthiols as self-assembled monolayers are used for the surface treatment of the gold electrodes so that the glucose oxidase can bind more reliably to the electrodes. The various surface modifications that we pursued are summarized in Table I. Prior to testing these modified sensor designs, we attempted to immobilize the enzyme without any surface treatment or BSA (group E in Table I).

As can be seen in Table I, five groups of sensors have been fabricated utilizing each option. These sensor sets, labeled A, B, C, D and E, are distinguished based on their surface treatment or enzyme solution preparation.

## III. Fabrication Process

Fabrication of the electro-enzymatic sensors is divided in two parts. The first part discusses the fabrication process for the gold electrodes on glass substrates and the second part discusses the enzyme immobilization process along with surface treatment of the gold electrodes.

### *A. Fabrication of the electrodes*

The fabrication process for gold electrodes on the glass substrate is shown in Fig. 2.

The fabrication process for gold electrodes on glass

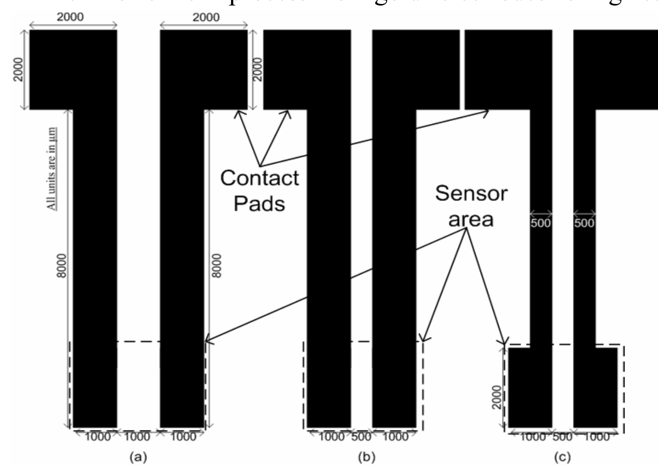

Fig. 1. Schematics of the glucose sensors

TABLE I
SELF-ASSEMBLED MONOLAYERS AND OTHER MODIFICATIONS TESTED ON VARIOUS SENSORS

| Sensor set | Scheme utilized |
|---|---|
| A | 11-amino-1-undecanethiol hydrochloride |
| B | 1-hexadecanethiol |
| C | 1,9-nonanedithiol |
| D | Bovine serum albumin (BSA) only |
| E | None |

consists of only three simple and cost effective fabrication steps. First of all, the glass slides were cleaned using the RCA 1 clean process (not shown in Fig. 2). A 50-nm thick chromium adhesion layer and a 50-nm thick gold layer were sequentially sputter-deposited in a Corona sputtering system (Fig. 2(1)). After metal sputtering, the gold electrodes were patterned using photolithography and wet etching. Shipley S1813 positive photoresist was spun, soft baked and patterned using the electrode mask (Fig. 2(2)). The photoresist was hard baked after developing in MF-319 developer. The gold was etched using a KI based gold etchant (from Transene) followed by chromium etching using chrome etchant from Transene (Fig. 2(3)). After a DI water rinse and drying, the glass substrate was ready with gold electrodes. Each individual sensor was then separated using a dicing saw. Finally, wires for electrical test were connected to the contact pads using conducting silver epoxy.

The sensors were then marked with each sensor group identification letter (A, B, C, D or E) and surface treatment with enzyme immobilization was done as outlined in the next sub-section.

### *B. Enzyme immobilization and surface treatment process*

The process used for surface treatment of the gold

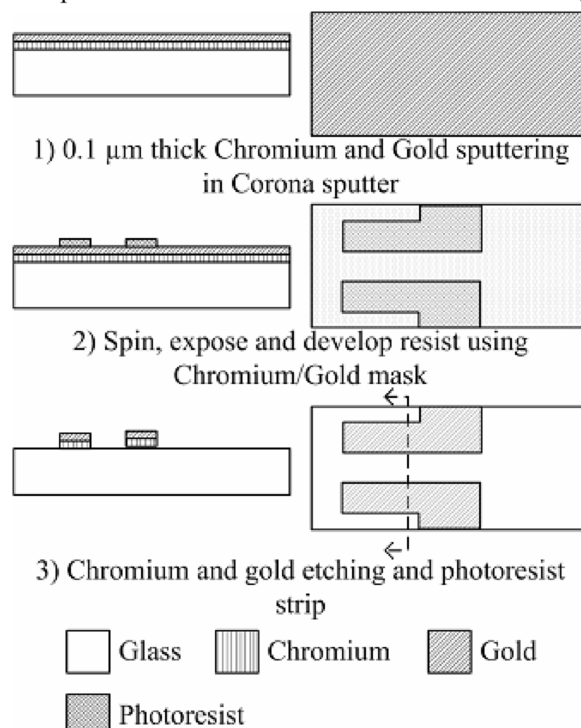

Fig. 2. Fabrication process for gold electrodes on glass substrate

electrodes on sensor group A, B and C (shown in Table I) is described and followed with an explanation for the procedure to immobilize the enzyme.

The sensors were cleaned in air plasma for 30 seconds. Each type of alkylthiol was then diluted in ethanol in individual vials. All the sensors of group A were immersed into a solution of 11-amino-1-undecanethiol hydrochloride. Similarly, sensors of group B and C were submerged in vials with 1-hexadecanethiol and 1,9-nonanedithiol respectively. All the vials were sealed with parafilm to prevent solvent evaporation. The sensor groups A, B and C were immersed overnight in each respective solution of alkylthiol.

Before immobilizing glucose oxidase onto the electrodes, a thin polydimethylesiloxane (PDMS) mask with rectangular access holes was created to confine the enzymes to the active area of the gold electrode. The process steps for fabricating the PDMS mask is explained in following steps:

1. PDMS elastomer was mixed with a curing agent (10% of the elastomer).
2. The mixture of elastomer and curing agent was degassed in vacuum for 30 min.
3. The mixture was poured on glass slide followed by the curing process of the PDMS.
4. After the PDMS was cured, rectangular holes were made in the PDMS mask using a sharp knife.

To immobilize glucose oxidase:

1. Glucose oxidase was mixed into a 7.4 pH phosphate buffer.
2. Upon the addition of glutaraldehyde and BSA the solution was thoroughly mixed and stored in a 4 ºC fridge until needed.
3. Meanwhile, all the sensors were dried under a filtered stream of nitrogen gas.
4. The pre-fabricated PDMS mask was aligned and placed over the gold electrode for selective deposition of enzyme.
5. Holes in the PDMS mask were filled with the chilled solution of enzyme and left at room temperature for 30 min to immobilize the enzyme.
6. Sensors with immobilized enzyme were stored at 4 ºC until needed for further processing or analysis.

Another set of enzyme solutions was also prepared without mixing BSA in glucose oxidase, phosphate buffer, and glutaraldehyde. One separate group of sensors was also prepared utilizing this enzyme solution.

## IV. Test set-up, Results and discussion

Test results are discussed after a brief explanation of our experimental test setup.

### A. *Test set-up*

Test solutions with glucose concentration of 50 mg/dl, 200 mg/dl, 400 mg/dl and 600 mg/dl were prepared in a phosphate buffer. Concentrations were calibrated using a commercially available digital blood glucose meter (LifeScan OneTouch® Ultra blood glucose monitoring system). The calibrated solutions were used for electrical testing of all sensors.

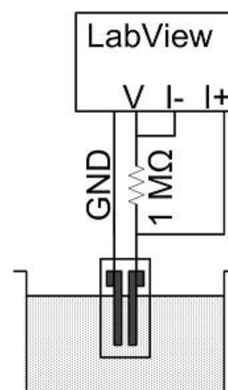


Fig. 3. Test setup using LabView®

Current response of all sensors was measured using Labview® provided by National Instruments. The test setup using Labview® is shown in Fig. 3 along with a photograph of the actual test setup (Fig. 4). A 1 MΩ resistance was connected to convert the current output generated by the sensor to a voltage read-out.

### B. *Results and discussion*

After successful fabrication and isolation of each sensors, the sensors were each examined by optical microscopy for defects due to the fabrication process. The photograph of a typical sensor is shown in Fig. 5.

For this sensor, no fabrication defects were observed in the gold electrodes. The current generated at different glucose concentration for the sensor without surface treatment and BSA (group E) is shown in Fig. 6.

From Fig. 6, current measured at 50 mg/dl was higher than the current measured at 200 mg/dl because the sensor was first submerged in 50 mg/dl solution followed by 200 mg/dl test solution. This response clearly indicates lack of adhesion between electrode and enzyme. The enzyme (glucose oxidase) seems to be dissolved in the test glucose solution. Optical microscopy also indicated the absence of enzyme after the electrical test.

Unexpectedly, all group C sensor wires came off from the contact pads which made them unfit for further test. Hence, all other groups of sensors except group C and E had been tested over a 45 min period (Fig. 7).

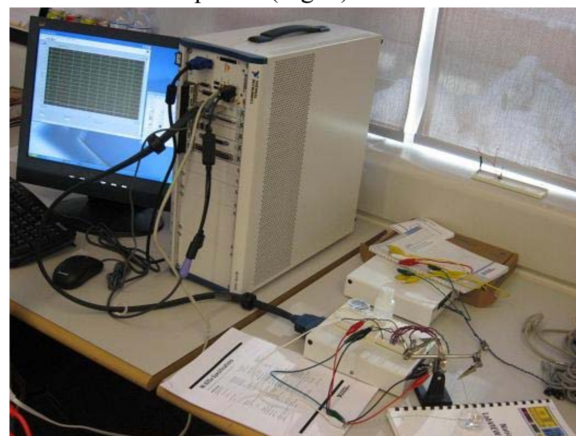

Fig. 4. Picture of the actual test setup

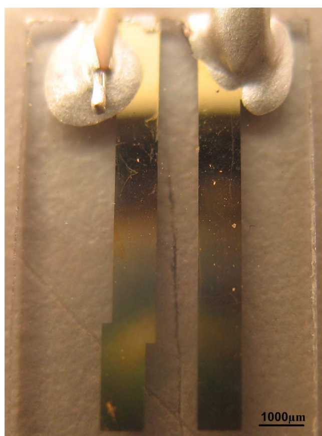

Fig. 5. Photograph of a single sensor

We can see from Fig. 7 that the sensor group A response changed from 0.87 nA to 2.13 nA over the 45 minute period with 600 mg/dl glucose concentration. Initially, the current decreased for 5 min and then continuously increased becoming steady over time. The current generated using sensor group B changed from 1.3 nA to 2.45 nA. The current response of this set of sensors fluctuated significantly (see Fig. 7). The current generated using sensor set D varied from 196 nA to 328 nA which was significantly larger than the other sensor groups A and B. Furthermore, the current for sensor D decreased initially but then increased and became almost steady.

From these results, we conclude that the sensors immobilized with BSA and the electrodes with a non-treated surface give the best results for the glucose sensor. Long term stability as well as the current magnitude obtained for sensor group D is best among all other groups. The surface treated sensors (group A and B) also give similar responses to sensor group D but the current magnitude is lower by two orders of magnitude than the sensor group D. Hence, it can be concluded that the electron transfer from the enzyme to the gold surface is strongly blocked by the self-assembled monolayers. However, the surface treated sensor binds glucose oxidase (enzyme) very well to the electrode for long term stability of the sensor.

## V. Conclusion

A new approach using self-assembled monolayers as a binding agent between an enzyme and gold electrodes for bio-sensor applications is demonstrated with detailed results. Glucose oxidase strongly adheres to gold electrodes resulting in an electro-enzymatic glucose sensor. Three thiols (11-amino-1-undecanethiol hydrochloride, 1-hexadecanethiol and 1,9-nonanedithiol) with different terminal groups are successfully tested. Bovine serum albumin (BSA) is also examined for its property of binding glucose oxidase on active sensor electrodes.

Furthermore, glucose sensors using only gold electrodes as both active and reference electrodes are also fabricated. The current generated using surface treated and non-treated sensors show that the surface treated sensors as well as

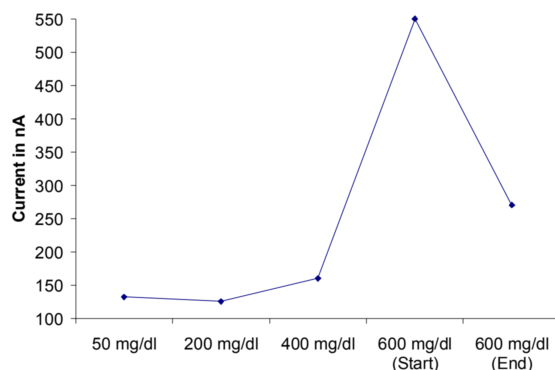

Fig. 6. Current generated at 0.8 V with different glucose concentrations for sensor without surface treatment and without BSA in enzyme solution

sensors with BSA showed better long term stability than the one without any surface treatment and without BSA. These sensors also showed lower drift than the sensors without surface treatment and without BSA. For the sensors without surface treatment and without BSA, the glucose oxidase (enzyme) was dissolved in the test glucose solutions during electrical testing. Hence, the sensors without BSA or SAMs show very short term stability.

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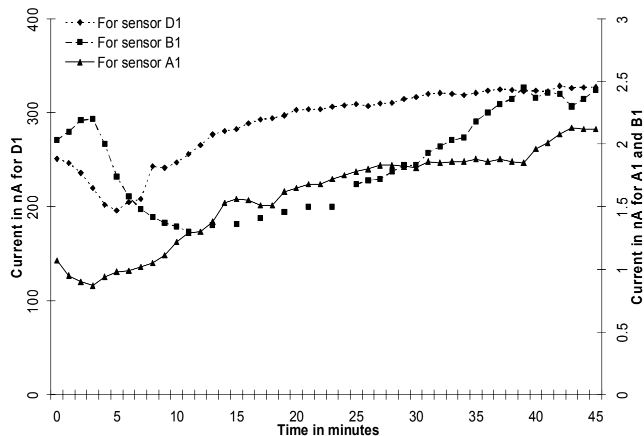

Fig. 7. Electrical test results for sensor sets A, B and D