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Glucose Oxidase (GOD)-Coupled Amperometric Microsensor with Integrated Electrochemical Actuation System

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Abstract – Recent developments for biosensors have been mainly focused on miniaturization and exploratory use of new materials. It should be emphasized that the absence of a novel "in-situ self-calibration/diagnosis technique" that is not connected to an external apparatus is a key obstacle to the realization of a biosensor for continuous use with minimum attendance. In order to address this issue, a novel solid-state glucose oxidase-coupled amperometric biosensor with integrated electrochemical actuation system has been designed and validated. There are two key components of the proposed glucose biosensor: solid-state GOD-coupled thin-film amperometric sensing element and O_2 depleting/saturating built-in electrochemical actuator. The actuator can be used to accomplish in-situ 1-point self-calibration by depleting O_2 (i.e., by simulating glucose-free environment). Also, it can be used at the same time to extend the proposed sensor's linear detection range by increasing O_2 (i.e., by enhancing glucose sensitivity). A prototype sensor was fabricated and a series of lab experiments was conducted. Collected data assures that the proposed sensor effectively establishes the zero calibration point and significantly enhances its measurement sensitivity and con dence.

Keywords – Solid-state biosensor, thin-film amperometric sensor, GOD (Glucose oxidase), Actuator, Glucose measurement.

I. INTRODUCTION

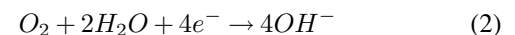
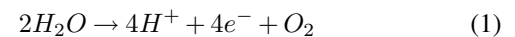
Although numerous techniques are being proposed in designing of accurate glucose biosensor, the number of major technical difficulties still should be addressed to implement a dependable solid-state glucose biosensor, such as unpredictable baseline drift and low measurement sensitivity during continuous use. Thus, a novel glucose microsensor array with a built-in electrochemical actuation system has been developed to achieve three novel functionalities which can overcome the major shortcomings mentioned above; one-point self-calibration (zero-point), extension of linear detection range, and increase in sensitivity. In the proposed sensor, there are two key components: an thin-film amperometric state glucose sensing element and an electrochemical actuator. On top of the sensing element, glucose-sensitive GOD (glucose oxidase) is coupled and its output current is used to measure the glucose concentration. Also, the electrochemical actuator can be used in three ways: to establish 1-point calibration point (zero point), to extend the sensor's linear detection range, and to enhance its sensitivity.

In the following two sections, the already proposed electrochemical actuator and GOD-coupled ISFET which is used as

foundation for proposed thin-film amperometric glucose sensor are discussed. Then, novel methods for in-situ 1-point self-calibration and linear detection range extension of thin-film amperometric glucose sensor are shown in section IV. Finally, data from the prototype sensors are presented in section V.

II. O_2 -CONTROLLING MICROACTUATOR

In order to create a controllable oxygen micro-environment, several electrochemical microactuators, based on water electrolysis have been reported with the use of micromachining techniques by other researchers. Gas pressure was electrochemically generated to be used to change the deflection of a micromechanical diaphragm [1] or to operate an active valve [2]. A micromachined electrochemically driven pump, capable of dosing precise nanoliter amounts of liquid, was introduced as well [3]. Recently, the same water electrolysis method has been adopted for a novel in situ self-diagnosis of oxygen microsensor [4]. Dissolved oxygen can be moderately generated or depleted at the generating electrode (AE) and counter-generating electrode (AE'), as shown in Figure (1) (a).



where reactions (1) and (2) happen at the anodic actuating electrode and at the cathodic actuating electrode, respectively.

Accumulation or depletion of dissolved oxygen near the AE, in turn, rapidly establishes a microenvironment of oxygen saturation or depletion. A microsensor, in close proximity to the surrounded AE, can be confined in a controlled local environment. The functionality of the sensor at a high and a low concentration can then be checked in the oxygen-saturated and in the oxygen-depleted phases, respectively. These transient perturbations of the microenvironment are expected to equilibrate rapidly with the surrounding medium.

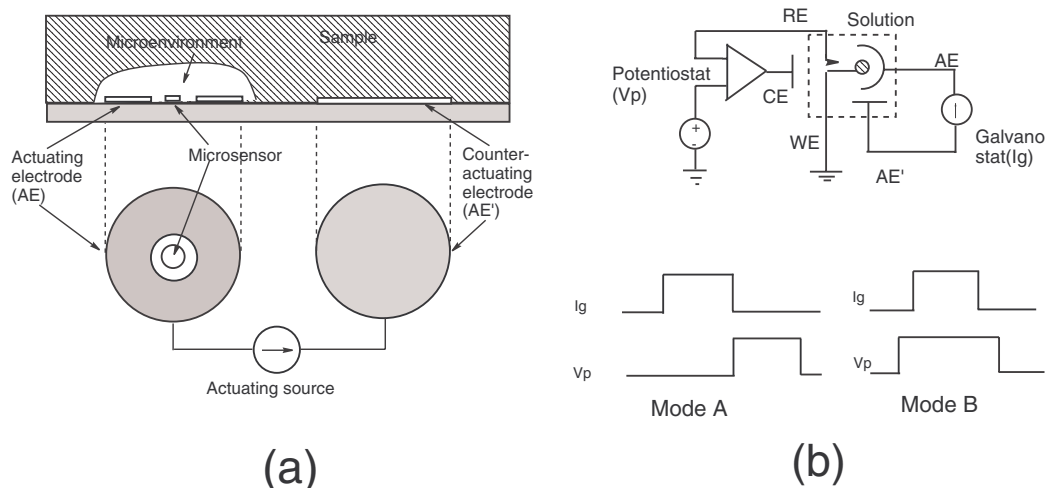


Fig. 1. (a) Concept for a novel oxygen sensor with in situ self-diagnosis capability. The microenvironment is generated by an actuating electrode (AE) which surrounds the microsensor. Oxygen-saturated or oxygen-depleted phases can be established by water electrolysis depending on the polarity. (b) A potentiostat and a galvanostat are employed for biasing the 3-electrode oxygen microsensor and for generating microenvironments, respectively, (A) Sequential generating mode for transient response, (B) Simultaneous generating mode for quasi steady-state response.

III. PERFORMANCE IMPROVEMENT OF GLUCOSE-SENSITIVE ISFET

Kim et al., reported extended upper detection limit and increased sensitivity of potentiometric glucose biosensors by using integrated electrochemical actuators [5], [6], [7]. These sensors are based on pH ion-sensitive field-effect transistor (pH-ISFET, also known as the chemically-sensitive field-effect transistor, CHEMFET). Operation of the glucose-sensitive ISFET is shown in Figure (2). An immobilized GOD membrane on top of the pH-sensitive gate layer serves as the recognition component and is selective to glucose molecules, only. Enzymatic reaction causes a pH change inside the GOD membrane that is proportional to the glucose concentration, thereby enabling a potentiometric determination of glucose by pH-ISFET.

The conventional glucose-ISFET measures the pH variation caused by the dissociation of gluconic acid, which provides low sensitivity due to the low dissociation constant of gluconic acid. An "amperometric stimulation technique," using the electrochemical actuator, was proposed to overcome this problem. Two additional hydrogen ions are produced by the electrolysis of the hydrogen peroxide with the integrated platinum microelectrode, as shown in Figure (2). With this new mechanism, which can provide two additional hydrogen ions per glucose molecules, the sensitivity and the detection range have been dramatically improved when compared to the conventional sensing mechanism. The generation of oxygen during the decomposition of hydrogen peroxide also significantly contributed to expedite the enzymatic reaction. These results obtained with the ISFET strongly suggest that both the detec-

tion range and sensitivity of amperometric biosensors for glucose and other saccharoids can also be improved with the aid of oxygen-generating electrochemical actuators.

IV. ONE-POINT SELF-CALIBRATION AND LINEAR DETECTION RANGE EXTENSION OF AMPEROMETRIC GLUCOSE SENSOR

Figure (3) shows a conceptual test cycle for the proposed microsensor using the O_2 -controlling microactuator. The O_2 -depletion phase will be performed first to determine the background current (zero-point calibration), followed by several measurements during the O_2 -generating phases with incremental glucose concentration to complete a response curve. Without the use of AE, microsensors with various microstructures and membrane thicknesses are first evaluated in terms of their sensitivities, linear ranges, response times, and residual currents. Then the feasibility of the proposed concept is demonstrated with various actuating signals, to achieve the novel functionalities of self-calibration/diagnosis, extended linear range, and increased sensitivity. Most important parameters of the actuation signal will be the duration and magnitude. During the entire characterization, a commercial "Gold Standard" instrument will be used in parallel as a cross-reference.

V. THE PROPOSED MICROSENSOR WITH INTEGRATED ELECTROCHEMICAL ACTUATION SYSTEM

A. Sensor Preparation

The chip consists of a cover layer, a substrate, and sensor electrodes. Figure 4 shows a layout of the assembled system: both

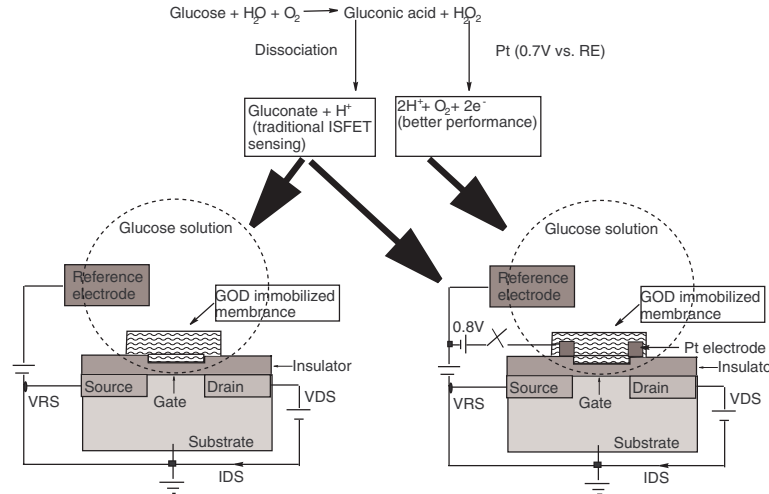


Fig. 2. Operational principle of the glucose-sensitive ISFET. Enzymatic reaction of glucose molecules inside the glucose oxidase (GOD) membrane causes pH change proportional to the glucose concentration. The pH-ISFET detects this local pH change to determine glucose concentration (left). By incorporating a Gate-surrounding platinum actuator, the hydrogen peroxide (byproduct) provides two more hydrogen ions per glucose molecule. The generated oxygen contributes to expedite the GOD reaction.

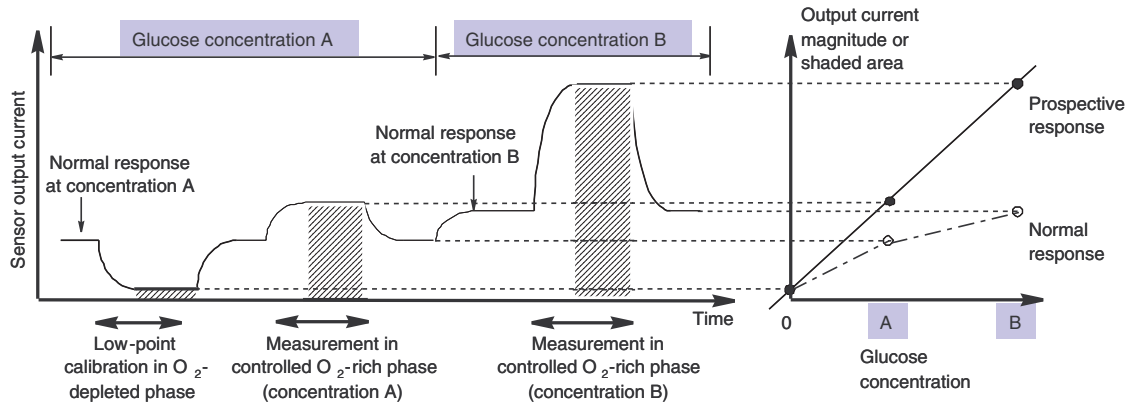


Fig. 3. Conceptual in situ one-point self-calibration/self-diagnosis (zero-point for background current) and extension of linear detection range with enhanced sensitivity using the proposed methodology. Both will be performed in controlled microenvironments of oxygen-depleted or oxygen-rich phases during the electrochemical actuation period. All measurement is done in physiological phosphate buffer solutions with known glucose concentrations. A commercial glucose monitoring system is used in parallel to serve as the reference.

cross-section and top view. A thick photoresist (MicroChem, SU-8) was used to prepare a template for the molding process of a polydimethylsiloxane (PDMS) cover layer (Corning, Sylgard 184) to include the channel structure. The thick photoresist was patterned on a boro-silicate glass substrate to have a thickness of 100 μm . The PDMS was cast onto the glass substrate and cured for 24 hours at room temperature in a vacuum desiccator. The thickness of the PDMS cover layer was about 0.75 cm. The substrate was a silicon wafer with a silicon nitride layer coating. A platinum/titanium thin film (100 nm/20 nm) was deposited by e-beam evaporation and patterned by lift-off technique to define the actuation electrode.

The PDMS cover layer was attached to the substrate by simply pressing against the substrate to seal the interface between the PDMS and the silicon nitride layer. The large patterns of the channel and the actuator electrodes allowed manual alignment of the cover layer with the substrate. The glucose oxidase (GOD, 10,000U), bovine serum albumin (BSA, 50 mg), 3-aminopropyltriethoxysilane (3-APTES, 100 ml), and 25 wt. % aqueous solution of glutaraldehyde (GA) were obtained. All reagents were of pure analytical grade. Deionized water was used throughout the experiments for the preparation of the samples, buffers, and other solutions. The enzymatic solution consisted of 0.5 mg GOD and 0.5 mg BSA in a 10 μl of 10 mM

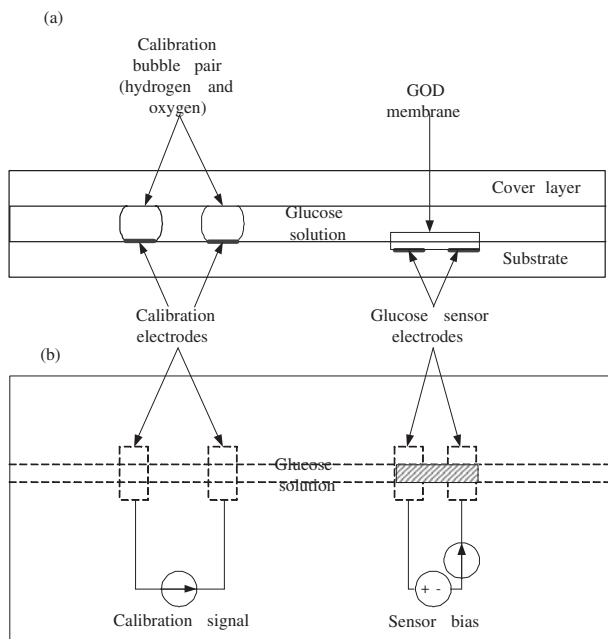


Fig. 4. The proposed fluidic chip for built-in 1-point *in-situ* calibration of glucose sensor using water electrolysis. An electrochemically generated bubble (i.e., 0% oxygen) provides microenvironment for the zero-point calibration procedure. (a) Simplified cross-section and (b) Layout of the system.

phosphate buffer solution. To increase the adhesion between the enzyme-immobilized membrane and the sensor, electrode were coated with 1 wt. % 3-APTES and then cured for 30 mi at 80°C. Next, 10 μ l of the enzymatic solution was dropped o the sensor electrodes. Then 10 μ l of 5 wt. % GA was droppe for a GOD immobilization. A photograph of fully-assemble microsensor is shown in Figure 5.

B. Sensor Operation

Each time of operation, the proposed microsensor measures output responses under three different microenvironments: air-saturated, O_2 -saturated (in an O_2 -bubble) and O_2 -depleted (in a H_2 -bubble). The microsensor operation consists of the following steps:

1. The micro-fluidic channel is initially filled with glucose solution under measurement from the glucose solution inlet.
2. The embedded electrodes generate both H_2 and O_2 bubbles in the micro-fluidic channel using water electrolysis.
3. The first output response is initially measured in the air-saturated microenvironment in the channel.
4. The glucose solution in the syringe is pressurized so that the O_2 -bubble is placed over the sensor. Then, the second output response is measured in the O_2 -saturated microenvironment in the channel.

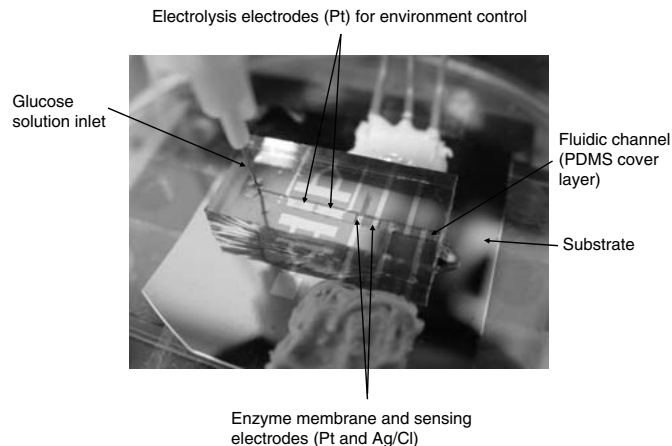


Fig. 5. Photograph of the fully assembled fluidic chip.

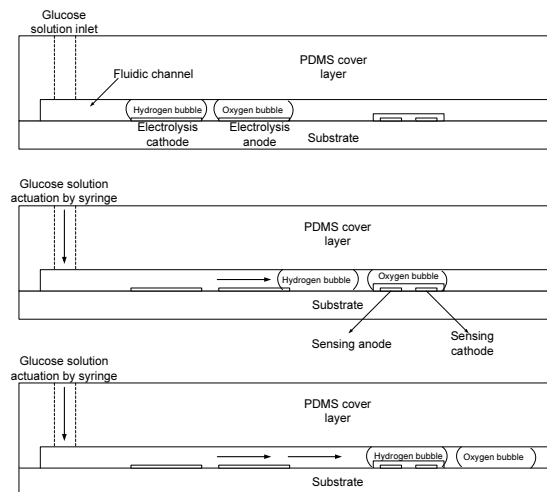


Fig. 6. **Top:** Generation of H_2 and O_2 bubbles using water electrolysis. **Middle:** Sensor output measurement under O_2 -saturated microenvironment for enhanced sensitivity. **Bottom:** Sensor output measurement under O_2 -depleted microenvironment for *in situ* 0-point self-calibration.

5. The glucose solution in the syringe is pressurized once again so that the H_2 -bubble is placed over the sensor. Then, the second output response is measured in the O_2 -depleted microenvironment in the channel.

The overall sensor operation is depicted in Figure 6.

C. Measurements

An electrochemical instrument (Gamry Instruments, FAS1) was used to provide the chronoamperometric operation for the glucose sensor (i.e. constant voltage mode) and the galvanostatic operation for the water electrolysis actuation (i.e. constant current mode). The output current of the sensor was measured with respect to the concentration of glucose. During the experiment, a microscope with a CCD camera was used to check the

images of calibrant bubble generation and bidirectional motion in the channel. Figure 7 shows the calibration/measurement curves obtained with the prepared sensor. The lower curve was obtained when the sensor was enclosed in a hydrogen bubble (i.e., 0% oxygen environment). Since the oxygen is not available within the hydrogen bubble the enzyme (glucose oxidase) reaction can not be completed, which means the sensor output is zero regardless of the actual glucose concentration in the sample solution. Therefore the in-situ one-point calibration (i.e., zero-point) of glucose sensor is possible in a hydrogen bubble. Also as shown in the upper curve, the dynamic range and the sensitivity were improved when the sensor was enclosed in an oxygen bubble (i.e., 100% oxygen environment), compared to the middle curve obtained in a normal air-saturated sample solution (i.e., 21% oxygen environment). In an oxygen bubble, the enzyme reaction is not limited by the oxygen supply and the reaction is independent on background oxygen contents in the sample solution.

In Figure 7, linear trendlines (LTLs) of the sensor output curves under O_2 -saturated and air-saturated microenvironments are also shown (e.g., LTL of enhanced output and LTL of normal output, respectively). The LTL of enhanced output curve is $y = 15.571x + 580.81$ and $R^2 = 0.9082$, while the LTL of normal output curve is $y = 3.0468x + 160.57$ and $R^2 = 0.8946$. Notably, the measurement sensitivity of the sensor is significantly improved since the slope of the linear trendline of the normal curve is enhanced from 3.0468 to 15.571. The enhanced output curve obtained from the O_2 -saturated microenvironment make possible more accurate glucose level measurement. For even more confidence in sensor output reading, a multi-degree polynomial trendline can be modeled. For example, a 3-rd degree polynomial trendline (PTL), $y = 0.0003x^3 - 0.1827x^2 + 43.887x - 117.69$, is shown as well. It has a significantly improved R^2 value (e.g. the goodness-of-fit measure) of 0.9779.

VI. CONCLUSION

This paper has presented a novel glucose oxidase (GOD)-coupled amperometric microsensor with integrated electrochemical actuation system. The proposed glucose sensor system has three embedded components; micro-fluidic channel for bidirectional glucose movement and O_2 -depleting/ saturating built-in electrochemical actuator and solid-state GOD-coupled thin-film amperometric sensing element. Each measurement operation, three different output responses can be obtained: air-saturated, O_2 -saturated and O_2 -depleted. The O_2 -depleted output response can be used for *in situ* 1-point self-calibration/diagnosis. Also, the O_2 -saturated output response can be used to achieve significantly enhanced measurement sensitivity/ linearity over the normal air-saturated output response.

A series of lab experiments was conducted on the fabricated prototype sensor system and data was collected for different

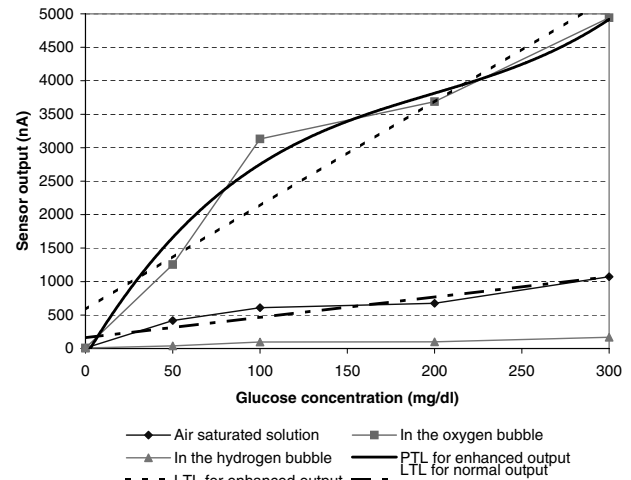


Fig. 7. Glucose sensor calibration/measurement curves. In the hydrogen bubble (i.e., 0% oxygen), the one-point (zero-point) calibration is feasible mimicking glucose-free solution regardless of the actual glucose concentrations in the sample solution. In the oxygen bubble (i.e., 100% oxygen), stable responses can be obtained due to the constant background oxygen microenvironment. A 3-rd degree polynomial trendline for the O_2 -enhanced curve is also shown.

glucose concentration levels. The collection of data verifies that the proposed sensor system successfully establishes the zero calibration point using the O_2 -depleted microenvironment and significantly improves its measurement sensitivity and confidence using the O_2 -saturated microenvironment. The proposed glucose sensor system can be used for continuous glucose level monitoring purposes with minimum attendance.

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