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Automated Oxidase-Coupled Amperometric Microsensor with Integrated Electrochemical Actuation System for Continuous Sensing of Saccharoids

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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. To address this deficiency, a novel needle-type biosensor system with fully automated operations is being developed, in which a novel oxidase-coupled amperometric sensor with oxygen depleting/generating actuator is interfaced with an electrochemical instrument and a perfusion system. Labview virtual instrument has been also developed to oversee the automatic control of the prototype sensor. Using the proposed system, a large amount of data can be rapidly collected for more effective sensor characterization and more advanced sensor designs. Autonomous and continuous sensing and self-calibration with minimal human intervention is also envisioned.*

Keywords – *Solid-state biosensor, Needle based amperometric sensor, GOD (Glucose Oxidase), LOD (Lactate Oxidase), Water Electrolysis, Actuator, Calibration, Continuous calibration/sensing, LabView, Automation.*

I. INTRODUCTION

Continuous monitoring of biochemical analytes such as glucose and lactate is very valuable for critically-ill patients to be used for therapeutic decisions and disease prognosis classification [1]. In order to achieve the goal of real-time monitoring of lactate and glucose, a reliable sampling and analysis system must be developed which meets the necessary clinical requirements such as size, response time, specificity, sensitivity, reliability and biocompatibility. In the last two decades, enzyme-based amperometric biosensors have played an increasing role in solving analytical and clinical problems. However, there are significant problems of most biosensors including the amperometric glucose sensors and lactate sensors:

1. Unpredictable baseline drift.
2. Sensitivity degradation during continuous use.
3. Dependency of enzyme activity on the background oxygen concentration in sample solutions.

Therefore the capability of on-demand *in situ* calibration and diagnosis of biochemical sensors is desired for reliable long-term monitoring with minimum attendance.

To address aforementioned issues, a novel biosensor with self-calibration capability and enhanced sensitivity was developed and some results and findings were reported recently in [2]. It is also desired to collect large amount of data to fully characterize the proposed sensor and to propose more advanced sensor designs. Thus, the proposed automated biosensor is interfaced with an electrochemical instrument for on-chip self-calibration and sensing and a perfusion system for accurate and continuous feeding of different analytes. The proposed automation also contributes to more effective solutions to the goal of long-term continuous monitoring with minimal intervention.

The sensor used for measurement is formed by depositing the glucose enzyme at the tip of the blunt-type needle. The surface area of the sensor is smaller compared to planar-type sensing element that was used in the previous prototype reported in [2]. Due to the enhanced responsiveness of the needle-type sensor, faster sensing operation is possible. The additional advantage is that reduced sensor size increases the applicability of the proposed sensor for *in vivo* applications.

II. PRELIMINARIES AND REVIEW

A. O_2-controlling microactuator

To create a controllable gaseous 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 [3] or to operate an active valve [4]. A micromachined electrochemically driven pump, capable of dosing precise nanoliter amounts of liquid, was introduced as well [5]. Recently, the same water electrolysis method has been adopted for a novel in situ self-diagnosis of oxygen microsensor [6], [7]. Dissolved oxygen can be moderately generated or depleted at the generating electrode (AE) and counter-generating electrode (AE').

$$2H_2O \rightarrow 4H^+ + 4e^- + O_2 \quad (1)$$

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \quad (2)$$

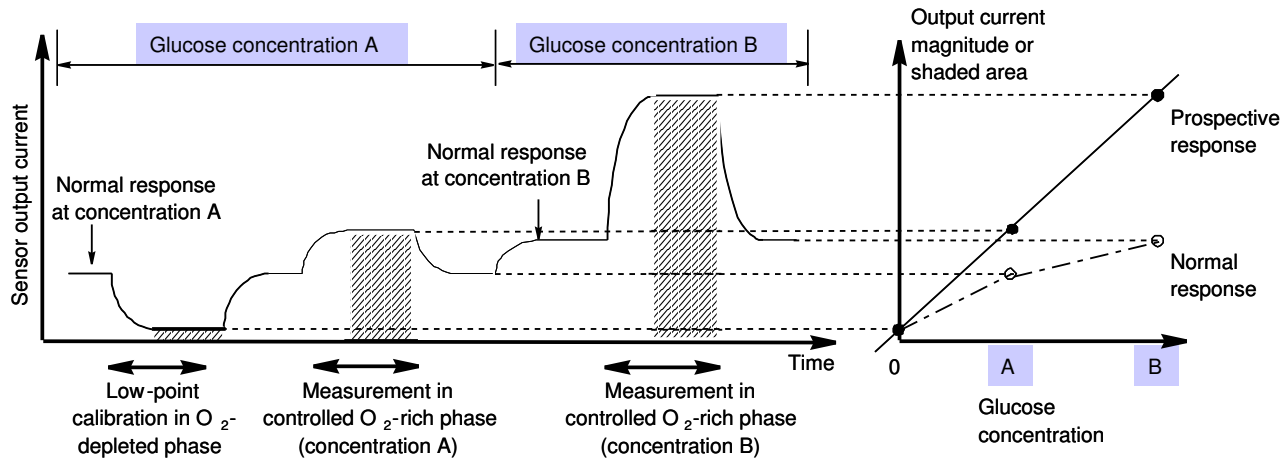

Fig. 1. One-point self-calibration and linear detection range extension using O_2-controlling microactuator

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.

B. One-point self-calibration and linear detection range extension of amperometric glucose/lactate sensor

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 as shown in Figure 1. 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 can be used in parallel as a cross-reference.

C. Glucose and Lactate Oxidase Enzyme Reactions and sensor calibration

The proposed glucose and lactate sensors are based on amperometric detection of hydrogen peroxide generated by the glucose (or lactate) oxidase-catalyzed oxidation of β-D-glucose (or L-lactate). These enzymes, GOD (Glucose Oxidase) and LOD (Lactate Oxidase), catalyze the following reactions:

$$\beta\text{-D-Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2 \quad (3)$$

$$\text{L-Lactate} + O_2 \xrightarrow{\text{LOD}} \text{Pyruvate} + H_2O_2 \quad (4)$$

For each case, the generated hydrogen peroxide is amperometrically detected by the working electrode that has positive bias (0.85V) with respect to the reference electrode.

A pair of oxygen and hydrogen bubbles can be reproducibly generated by the water electrolysis with a pair of calibration electrodes operating in a constant current mode. The electrolysis reactions occurring at the anodic and cathodic electrodes are described in Equations (1) and (2), respectively.

The zero-point sensor calibration can be performed by manipulating the hydrogen gas bubble which is generated at the cathodic electrode. The enzyme reaction within the membrane needs oxygen as shown in Equations (3) and (4). Once the hydrogen gas bubble is built up on the calibration electrode, carefully driven movement of solution can place the generated hydrogen gas bubble under the location of the sensing electrode. When the sensor is surrounded by the hydrogen bubble, this oxygen-free environment prevents the enzyme reaction. This technique results in a glucose (or lactate)-free microenvironment regardless of the actual presence of substrates in sample solutions. On the contrary, the oxygen gas bubble which is generated from anodic calibration electrode can be used for the measurement sensitivity enhancement. This artificial constant oxygen environment allows the enough oxygen for enzyme reaction. The enzyme reaction is not limited by insufficient oxygen tension in the sample solution anymore.

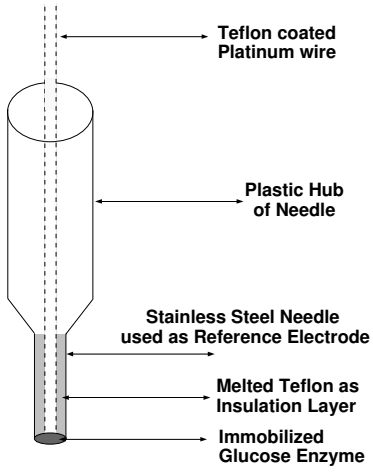

Fig. 2. The structure of the prototype needle based sensor

III. SENSOR PREPARATION

The proposed sensor has the following four major components:

1. *Solution Inlet:* Glucose (or lactate) solution can be applied from the perfusion system to the fluidic channel. Different solution channels can be selected for continuous measurements.
2. *Fluidic Channel:* Bidirectional movement of the solution, O_2 and H_2 bubbles is performed in the fluidic channel.
3. *Calibration Actuator:* Both O_2 and H_2 bubbles are generated on demand.
4. *Sensing Needle:* Needle-type sensing element measures amperometric responses. Its structure is shown in Figure 2.

An illustration of the proposed sensor system is shown in Figure 3.

A. Preparation of Electrodes

The sensor electrode was prepared using a Teflon coated platinum wire (A-M Systems No.773000). The diameter of the wire measured was 0.005 inch with bare platinum and 0.008 inch with coating. This wire was inserted into a blunt type stainless steel needle with a plastic hub. The stainless steel part of the needle was heated so that the Teflon coating melts and fuses the platinum wire to the inside wall of the needle as shown in the Figure. 2. The Teflon acts as an insulator which separates the needle and the platinum wire and the needle can be used as a reference electrode. The immobilized glucose enzyme (i.e., preparation explained in the following subsection) was deposited on the tip of the needle and in contact with the platinum electrode and needle reference electrode for current measurement. Then, the needle was tested for any air or fluid leakage. Similar needle-based glucose sensors were reported in [9] and [10].

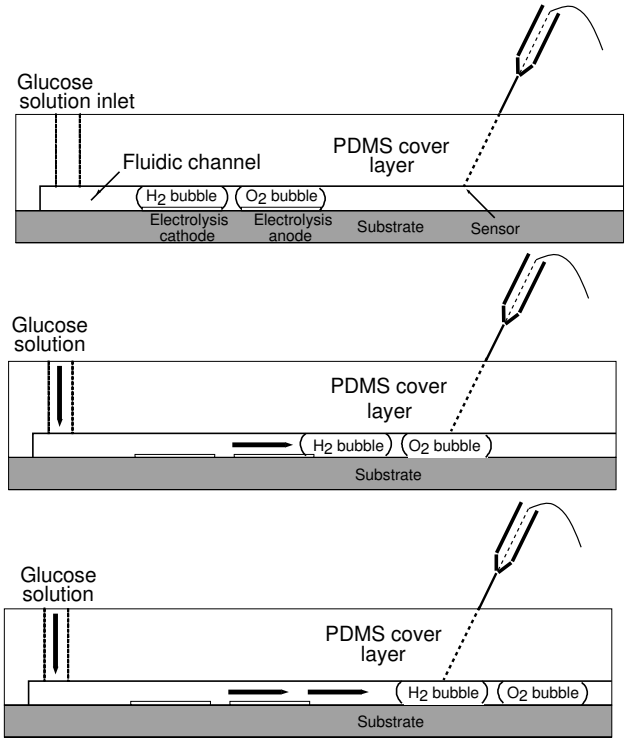

Fig. 3. The proposed fluidic chip with needle based sensor for built-in 1-point *in-situ* calibration of glucose sensor using water electrolysis. An electrochemically generated bubble (i.e., 0% oxygen) provides microenvironment for the zero-point calibration procedure.

B. Enzyme Preparation

The proposed amperometric sensors use glutaraldehyde cocrosslinking of glucose oxidase or lactate oxidase with bovine serum albumin. β-D(+) glucose (EC 207-756-2), L(+) lactate acid (EC 201-196-2), glucose oxidase (EC 1.1.3.4, 15500 units/g), bovine serum albumin (BSA, EC 232-936-2), lactate oxidase (EC 232-841-6, 29 units/mg), and glutaraldehyde (EC 203-856-5) were obtained from Sigma (Sigma Chemical Co., St. Louis, MO). All other chemicals were of analytical-reagent grade. Deionized water was used throughout the experiments for the preparation for the samples, buffers, and other solution.

In general, thicker enzyme layers result in better linearity over a wide concentration range, but the response time is longer. Smaller glutaraldehyde ratio may lead to inefficient immobilization, while higher ratio may lead to excessive crosslinking and blocking of some active sites of the enzyme [8]. The enzyme solution was prepared by mixing 1.0 mg of BSA in a 10 μl of 10 mM phosphate bubbler solution. To promote adhesion between the enzyme membrane and the electrode surface, small amount of 1 wt. % 3-aminopropyltriethoxysilane (3-APTES) was applied and cured for 30 minutes at 80 $^\circ C$ for surface silanization. Next, 10 μl of the enzyme layer was cast on the silanized area by dipping the needle in the enzyme solution. Then 10 μl of 5 wt. % GA was applied to

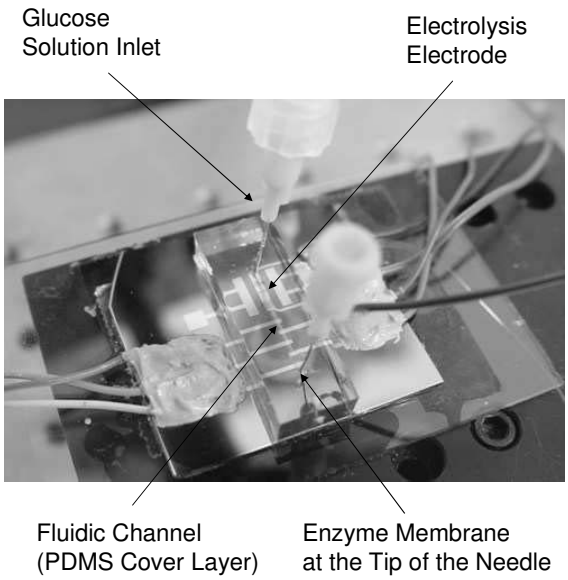

Fig. 4. Photograph of the fully assembled fluidic chip with needle type sensor.

initiate the chemical crosslinking reaction of BSA on the electrode surface in similar manner. For the glucose sensor, the enzymatic enzyme solution included 0.5 mg of GOD and 0.5 mg of BSA in a 10 μl of 10 mM phosphate buffer solution. 0.2 mg of LOD and 2.0 mg of BSA was added in a 10 μl of 10 mM phosphate buffer solution for lactate sensor.

Each glucose and lactate sensor chip consists of: (1) a cover layer, (2) a substrate, and (3) calibration electrodes. Figure 3 shows a layout of the assembled system and calibration procedure with bubble movement. 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 electrodes. 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. A photograph of fully-assembled microsensor is shown in Figure 4.

IV. AUTOMATION OF THE MEASUREMENT SETUP

The automation of the measurement setup involved interfacing a PC with LabView, an electrochemical instrument (Gamry Instruments PC750 and FAS-1), the proposed prototype needle based sensor and a perfusion system (Automate Scientific).

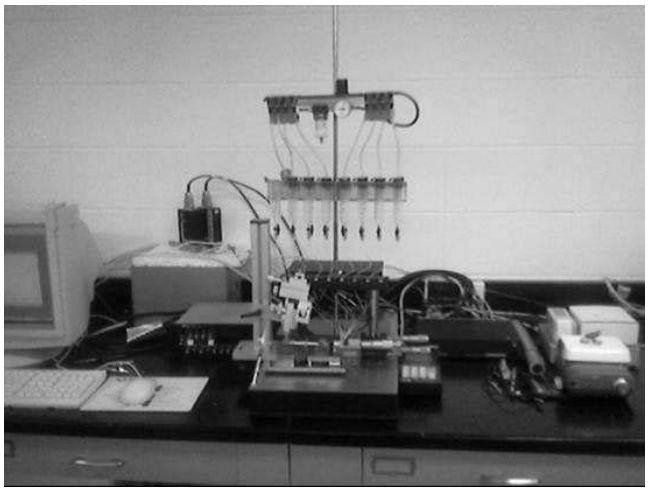

Fig. 5. The prototype system with Gamry instrument, perfusion system and control PC.

Figure 5 shows the workbench with Gamry instrument, perfusion system and control PC. The basic data and control flow is depicted in the Figure 6. The LabView Virtual Instruments (VI) running on the PC controls the perfusion system using parallel communication port (LPT). The perfusion system in turn delivers the required fluid to the sensor chip inlet. The Gamry Instrument is interfaced with the PC using PCI slot and its electrodes are connected to the sensor and actuator. The Gamry System continuously measures the current from the sensor and the data is fed back to the PC. The VI stores the acquired data on the disk. The basic drivers (LV 500)[11] to control electrochemical instruments were provided by Gamry Instruments. These virtual instruments were modified and customized to automate the entire setup.

A. Automated Calibration/Sensing and Fluidic Movement

In this section we describe the basic structure of the LabView VIs. The main control panel of VI is as shown in the Figure 7. The basically VI consists of 3 parts namely 1) Perfusion System Control 2) Sensor Current Measurement and 3) Bubble Generation.

Perfusion System Control

Automate Scientific perfusion system provides an option to control the perfusion channels through TTL (Transistor-Trnasistor Logic) signals. The ValveBank control unit has a standard female DB-25 connector through which each channel can be controlled independently and simultaneously. As shown in the Figure 7 every channel has a separate control button in the VI through which a channel valve can be opened or closed. The ValveBank was programmed in TTL control mode and signals were given by the LabView using PC's LPT port. Each channel was filled with glucose/lactate solution of different concentration level. The proposed VI has another button to switch off the perfusion control which would close all the channels and stop the fluidic movement.

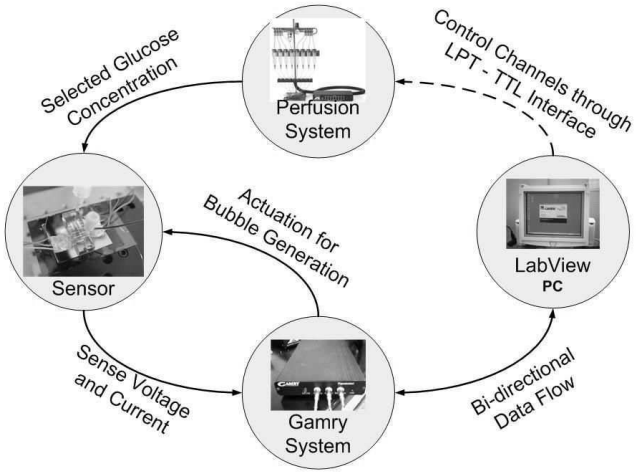

Fig. 6. Dataflow Diagram of Proposed Automated System.

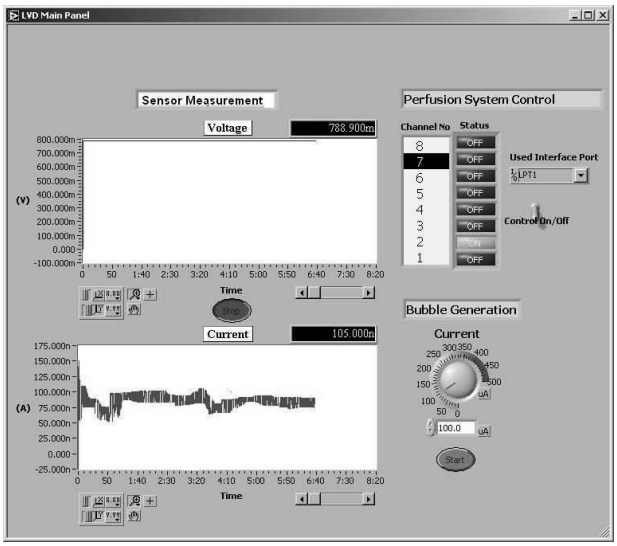

Fig. 7. Screen shot of the Virtual Instrument developed for automation.

Sensor Measurement

The platinum wire of the sensor was connected to the positive and stainless steel needle was connected to reference electrode of measurement unit. The basic steps of the measurement of current flows as follows. When the start button is pressed, the VI detects if PC750 is installed and active. Then it asks for the name of the file to which measurement data should be stored. This is the only input the user gives at the beginning of the measurement. Subsequently, the current level is acquired continuously at the rate of 5 Hz while maintaining a constant voltage level of 0.82v (i.e., potentiostat mode). The measured current value and the timing value are stored to the given file until the stop button is pressed. Later this data can be used for plotting and analysis.

Bubble Generation

To create a micro environment with oxygen and hydrogen bubbles, we need to supply a constant current to the actuator electrodes. The micro channel should be filled with the fluid with desired concentration and all the channels in the perfusion system should be closed to stop the fluidic movement in the channel. The desired current level (i.e., galvanostat mode) was calibrated using the control knob and the start button in the bubble generation control section was used to generate the bubbles. This was controlled by Gamry FAS1 and the advantage of having two electrochemical instruments is that both measurement and actuation for bubble generation can be executed in parallel.

B. Sensor Operation

In each measurement cycle, 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 (or lactate) solution under measurement from the glucose solution inlet which is connected to perfusion system. The required channel of the perfusion system was opened by clicking on the control buttons provided in the LabView VI.
2. The "Start" button in the bubble generation section is to actuate the embedded electrodes and generate both H_2 and O_2 bubbles in the micro-fluidic channel using water electrolysis. After generating the required size of the bubble, "Stop" is pressed to cease the actuation.
3. The current measurement is started by pressing the "Start" button which is in between the graphs as shown in Figure 7. The file name for the storage of the acquired data is given in the panel as well. The output response is measured in the air-saturated microenvironment in the channel.
4. The O_2-bubble is placed under the sensor by controlling the perfusion channel. Then, the output response can be measured in the O_2-saturated microenvironment in the micro channel.
5. The perfusion channel is opened once more so that the H_2-bubble is placed under the sensor. Then, the output response is measured in the O_2-depleted microenvironment in the channel.
6. The above process can be repeated with fluid of different concentration by using a different channel in the perfusion system.

In Figure 7, a screen capture of the LabView VI is shown, in which a constant input voltage level is being applied as shown in the top graph panel while corresponding sensor output is being displayed in the bottom graph panel.

V. FUTURE WORK

Although the proposed automated sensor system is anticipated to be used for continuous saccharoids monitoring purposes with minimal intervention, there are numerous technical challenges with the prototype system as discussed in the previous section. In order to address these issues properly, our future work will focus on the following tasks:

- The output current of the sensor for a fixed concentration of glucose and lactose can be different depending on the previous state of output current, thickness of the sensor, flow rate of the fluid and temperature. All the possible relationship can be established by gathering a large amount of data with the help of the proposed automation.
- Different materials (including silver needle) will be tested to enhance the responsiveness of the sensing element.
- The automation with LabView also provides the immense flexibility to modify the test environment. More instruments can be added to the current testbench in order to provide more advanced functions. For example, a bidirectional micro pump can be added for precise bubble position manipulation which is a difficult operation at the moment.
- Precise timing of sensing and actuation events in the measurement cycle is critical in continuous self-calibration and sensing. Large amount of preliminary data should be collected from the prototype sensor chip under development. The proposed VI will be extensively used to collect data sets with varying parameters. Also, the VI interface should be enhanced to provide more functions including closed-loop execution of multiple measurement cycles.

VI. CONCLUSION

This paper has presented novel glucose oxidase (GOD) and lactate oxidase (LOD)-coupled amperometric microsensors with integrated elctrochemical actuation system and a LabView-based automated control interface. Each of the proposed sensor systems have three embedded components: (1) fluidic channel for bidirectional glucose or lactate movement, (2) O_2-depleting/saturating built-in electrochemical actuator and (3) needle-type oxidase-coupled amperometric sensing element. Each measurement operation, three different output responses can be obtained via the proposed LabView VI interface: air-saturated, O_2-saturated and O_2-depleted. The O_2-depleted output response can be used for in situ one-point self-calibration and diagnosis. Also, the O_2-saturated output response can be used to achieve significantly enhanced measurement sensitivity. The proposed automated control interface manages the automatic control of the prototype sensor and can be used to collect large amount of data from the sensor chip that is being developed. So, more effective sensor characterization is possible while different sensor designs and enhancements can be rapidly applied and tested. Autonomous and continuous sensing and self-calibration with minimal human intervention is also envisioned.

ACKNOLEDGEMENT

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