Characterization of an O_2 Sensor Using Microelectrodes

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***Abstract*—This paper describes the response characteristics of a Clark type oxygen sensor using microelectrodes under a variety of operating conditions. The silicon based microelectrodes were fabricated using a CMOS process with Pt surface using a lift-off process. The Clark type sensor was configured using a three-electrode system. A set of four operating conditions with varying activation voltages, the use of oxygen-permeable membrane, and the use of different types of electrolyte layer, were experimented to determine suitable configuration for future integrated oxygen sensor designs. The results show that using Nafion as a solid state electrolyte and oxygen-permeable membrane provides the best linearity and sensitivity. The device was shown to have a good linear response with respect to dissolved oxygen concentration over a range of 0% to 100% with a correlation coefficient of 0.88 to 0.98. The oxygen sensor achieved a response time of 2-4 seconds, and consistent results over a long continuous operating time of at least 80 hours.**

***Keywords*—** *Clark Type Oxygen Sensor, Amperometry, PDMS*

I. INTRODUCTION

During cell culture and microbial development oxygen consumption is one of the most important indicators of biological activities [1, 2]. The oxygen level affects cell function and viability via energy stress when adenosine triphosphate (ATP) is produced by oxidative phosphorylation. Activities of metabolic enzymes and signaling pathway [3], and the production of reactive oxygen species are all affected by the oxygen level in cells [4, 5]. Hypoxia state can results in cancer diseases through the increase in the production of specific factors such as angiogenesis and hypoxia-inducible factor 1a (HIF-1a) activity [6-9]. Abnormal level of O_2 is an indicator of defective metabolism and neurological disorders such as Alzheimer's disease [9, 10]. Abnormal level of O_2 can affect the efficiency of tumor, and chemo and radiation therapies [4, 6, 9, and 10].

The oxygen sensors that are currently used in medical applications are bulky devices with inconsistent performance and high cost [3]. Some commercial devices such as 782 oxygen meter (Strathkelvin instruments) and Oxygraph-2k (Oroboros Instruments) are widely used but they are expensive and are limited to measuring oxygen levels up to two samples at a time [11]. Increasing amount of effort over the last several decades has been devoted to making oxygen sensors more practical for medical applications in terms of its size and cost [12-18]. Miniaturization of oxygen sensors provides the ability to integrate with in vitro cell culturing platform for the purpose of instantaneous monitoring of cellular oxygen consumption [1, 11]. It also allows the integration of oxygen sensors with other types of sensors for multiplexed sensing applications.

Among various types of oxygen sensors, sensors based on membrane-covered oxygen electrodes first developed by Clark in 1956 are the most widely used oxygen sensors due to their simplicity and reliability for measuring partial pressure of oxygen in both gaseous and aqueous phases at ambient temperature [18]. With the rapid advances in semiconductor technology, various types of miniaturized Clark-type oxygen sensors have been proposed [11, 19]. The main difficulty of applying the semiconductor manufacturing process for microelectrode fabrication is the incompatibility of the internal electrolyte solution with the fabrication processes [15]. Miyahara et. al. [14] injected the electrolyte manually using a syringe for the charge transfer between electrodes as a post-processing step to add electrolyte. Niazi et. al. [19] designed microelectrodes with solid electrolyte membrane to improve electrical conductivity and eliminate the need for rehydration. Jobst et. al. [12] used a solid state proton conductive matrix PCM to increase sensor's lifetime and to eliminate the need for rehydration.

In this paper, the characteristics of an oxygen sensor using three Platinum-coated (Pt) microelectrodes for measuring dissolved oxygen under a variety of conditions are presented to elucidate its suitability for integration with other types of electrochemical sensors on the same substrate surface. The operating conditions include the use and the absence of oxygen permeable membrane, the use and absence of electrolyte solution including Nafion as a solid state electrolyte. The sensor characteristics were evaluated by measuring the change of output current as a function of dissolved oxygen concentrations ranging from 0% to 100%. The results show that using Nafion as solid-state electrolyte and oxygen-permeable membrane provides the best linearity and sensitivity. However, using Nafion as solid-state electrolyte as well as membrane also shows comparable linearity and sensitivity. The latter is more compatible with integration and multiplexed sensing applications using other sensing modals.

II. EXPERIMENTAL SETUP

A. Sensor Setup

The microelectrodes array designed in [21] was used in the experiments. The microelectrodes were fabricated using a commercial CMOS process with Pt surface using a lift-off process. The array has twenty one sensors with different sizes and geometry and arrangement of working electrode (WE), reference electrodes (RE), and counter electrode (CE). The overall chip area is 9mm X 9mm with bonding pads on the exterior that were 160µmx160µm each [21]. For all experiments sensor 17 was used, it has four pairs of WEs with

area of 15µm² each and the CE and RE each has an area of 186 µm². Fig.1(a) shows the microphotographs of the microelectrodes used in the experiments. The electrodes are connected to a potentiostat (eDAC, Colorado Springs, CO) through a set of micromanipulator probes. Fig.1(b) is a close-up view of the setup with the microelectrodes on the chip.

(a) (b)

Fig. 1. (a) microphotographs of the microelectrodes used. (b) Close-up view of experiment setup with membrane and PDMS well.

B. Materials

Sodium sulfite was purchased from Eisen-Golden laboratories (Berkeley, California). 0.1M KCL electrolyte was purchased from HACH Company (Loveland, Colorado). Nafion perfluorinated resin was purchased from Sigma Aldrich (St. Louis, Missouri). A PCTE (Polycarbonate Track Etch Membrane) oxygen-permeable membrane was purchased from Sterlitech Corporation (Kent, Washington).

C. Activation Voltage

A range of activation voltages has been suggested in the literature from -0.6 to -0.8 [1, 13, 14, 15, 17, 20]. A low voltage is preferred to achieve the least interference from other molecules in the solution. The voltage range for measuring oxygen was determined using solutions of three different concentrations of 0%, 2.6%, and 100%, and measuring the current in the range from -0.4 to -0.8 V.

D. Sensor Operating Conditions

Experiments were carried out with four different operating conditions: 1) No electrolyte and no membrane; 2) with 0.1M KCL electrolyte and PCTE membrane; 3) with Nafion as solid electrolyte and PCTE membrane; 4) with Nafion as solid electrolyte, but no PCTE membrane. During experiments under each condition Na_2SO_3 was used as zero oxygen concentration and DI water as saturated oxygen concentration. Solutions of different oxygen concentrations were made by mixing Na_2SO_3 with DI water to achieve the desired concentration. All O_2 concentrations for measurement by the sensor were validated using the Oakton DO6+ dissolved oxygen meter. The oxygen sensor responses are plotted against the concentrations to determine sensor's sensitivity, linearity, and dynamic range.

E. Impact of PDMS Well on the Experiments

In all experiments pipetting was used to inject the solutions in the PDMS reservoir. To reduce the impact of oxygen diffusion through the PDMS reservoir, the time from the measurements by the oxygen meter to the measurements by the sensor with pipetting was controlled to be less than 10 seconds.

III. RESULTS AND DISCUSSIONS

A. Optimal Activaion Voltage

Based on the specific sensor geometry, a range of voltages from -0.4V to -0.8V was studied to determine the best tradeoff point for activation voltage and the reduction current. Fig.2 shows the relationship between the activation voltage applied at the WE vs. RE and O_2 reduction current. A voltage of -0.6V was chosen for all experiments.

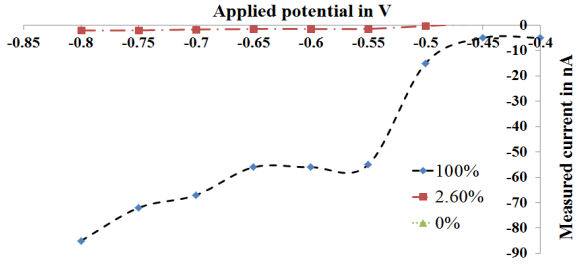
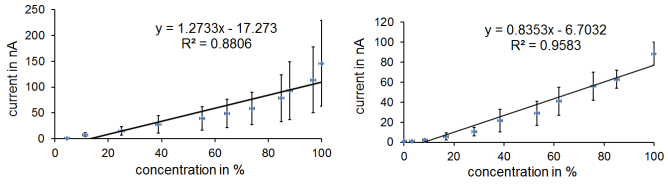


Fig.2. Amperometry measurements at a WE vs. RE voltage range between -0.4V and -0.8V.

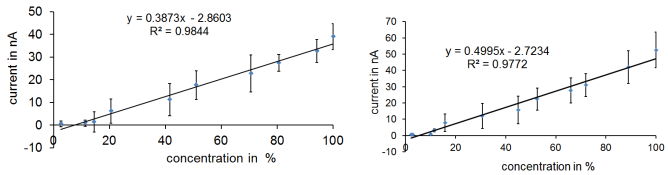
B. Linearity

Figs.3-4 show the calibration curves for the Clark sensor under four different conditions. Under each condition, the sensor output current increases with increase in O_2 concentration in a linear relationship with the correlation coefficients between 0.88 and 0.98. The error bars represent the standard deviation between four data points for the same condition. Several factors can contribute to variations of the measurement results. They include variations in pipetting speed and direction, electrochemical crosstalk between electrodes, and atmospheric oxygen diffusing through PDMS (Polydimethylsiloxane) well. Variations can be larger under the condition without electrolyte and membrane because the pipetted solution came in direct contact with the electrode surface causing disturbance in the immediate area of the electrode surface. This is why the error bars are larger.



(A) (B)

Fig.3. Calibration curve: (A) without electrolyte and membrane; (B) with 0.1M KCL electrolyte and PCTE membrane.



(A) (B)

Fig.4. Calibration curve: (A) with Nafion and PCTE membrane; (B) with Nafion but without PCTE membrane.

C. Sensor Response Time

The sensor results demonstrated fast response time of 2-4s. Fig.5 shows the results of adding DI water and Na_2SO_3 in a squential cycle for the case when the Nafion-only configuration was used. Comparing to the results published in [11, 16, 19], the sensor showed a good reproducibility with 91nA mean and 1.95nA standard diviation in the saturated oxygen level (21%), and 0.69nA mean and 0.06nA standerd diviation in the minimum oxygen level (1.7%).

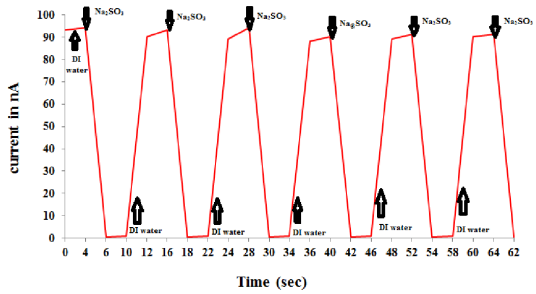


Fig.5. Time response and reproducibility of the Clark sensor

D. Discussion

Comparing the results of the O_2 sensor in this paper with previously published results, this sensor shows a good linearity, reproducibility, and fast response. The range of linearity of the sensor is within the range of linearity achieved by other groups [1, 11, 12, 13, 14, 15, 16]. The linearity of the sensor configurations with Nafion in this paper is significantly better than the ones without Nafion. One of the factors that could affect linearity is electrochemical crosstalk between electrodes [1, 13, 16]. Electrochemical crosstalk increases when the distance between WE and CE are small. Furthermore, due to the use microelectrodes and the fact that O_2 induced current is linearly proportional to the electrode area [23], the impact of process variation [22] on linearity can be more pronounced for sensors using microelectrodes. The peak to peak response time of the sensor between 2-4s is significantly shorter than the reported response time of 6.8-56s [1, 11, 12, 13, 14, 15, 16]. Response time could be affected by different factors such as type of the membrane, membrane thickness, area of the WE, and distance between WE and membrane [11].

IV. Conclusion

This paper presented the characterization results of a Clark-type oxygen sensor based on the Pt microelectrodes under four different operating conditions. The sensor without electrolyte and membrane has the worst linearity compared to other operating conditions. Although the results from the condition of using both Nafion as solid electrolyte and PCTE membrane produced the best overall results in terms of linearity, and response time. O_2 sensors in this condition are better suited as stand-alone sensors. The condition with Nafion but without PCTE membrane produced comparable results and is a better tradeoff for integrating O_2 sensors with other sensors with different sensing modal.

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