Differential setup of light-addressable potentiometric sensor with an enzyme reactor

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1. Introduction

The light-addressable potentiometric sensor (LAPS) [1, 2] is a semiconductor-based chemical sensor that can measure the concentration of specific ions. The LAPS has an electrolyte – insulator – semiconductor (EIS) structure, and the insulator surface is in contact with the solution to be measured. Sensitivity and selectivity to various ions can be realized by modifying the sensing surface. Due to the field effect in the EIS structure, the width of the depletion layer responds to the potential on the insulator surface, which is a function of the ion concentration of the solution. In a LAPS, the semiconductor substrate is illuminated with a modulated light beam, and the induced photocurrent is measured to detect the variation of the capacitance of the depletion layer and to determine the ion concentration. Here, the measured area on the sensing surface is defined by illumination, and therefore, the LAPS is capable of spatially resolved measurement [3, 4].

Since LAPS can measure ions at arbitrary locations on the sensing surface, it can serve as a platform for building microfluidic devices with multiple measuring sites. An arbitrary shape of flow channels can be formed on the sensing surface, and the ion concentration can be measured anywhere within the flow channel. In addition, measurements at different locations can be carried out simultaneously by frequency division multiplex (FDM) [5, 6]. In FDM-LAPS, a plurality of light sources are employed to illuminate different locations with different frequencies, and the resulting photocurrent is decomposed into respective frequency components by Fourier analysis.

In this study, the LAPS was combined with a flow channel equipped with an enzyme reactor for biosensing applications and the principle of FDM-LAPS was applied to differential measurement to compensate for the drift during measurement.

2. Experimental

Combination of LAPS with an enzyme reactor

The LAPS was combined with a flow channel and an enzyme reactor as shown in Fig.1. The measurement cell was made of plexiglass and the enzyme reactor was an externally connected U-shaped silicone tube with an internal diameter of 1 mm and an inner volume of 33 μL. Two flow channels (width = 1 mm, height = 1.8 mm, length = 9 mm) were formed in the upstream and the downstream of the enzyme reactor, respectively, for differential measurement.

Immobilization of enzyme on glass beads

In this study, a differential LAPS system was constructed for detection of urea based on the enzymatic reaction of urease. Urease catalyzes hydrolysis of urea and produces ammonia. The concentration of urea can be determined by detecting the rise of pH value in the downstream of the enzyme reactor.

The enzyme reactor was loaded with glass beads (200 μm in diameter), on which urease was immobilized by silanization with aminopropyltriethoxysilane and covalent linking with glutaraldehyde. In a preliminary experiment by indophenol blue absorptiometry, urease molecules immobilized on 250 mg of glass beads catalyzed production of ammonia at a rate of 0.322 μmol/min. In the experiments below, 20 mg of glass beads were loaded in the enzyme reactor, which occupied 8 μL of the volume.

FDM-LAPS measurement

The middle point of each flow channel was illuminated with a laser beam (wavelength = 830 nm) guided by an optical fiber. The modulation frequencies for the two laser beams were 7100 and 9000 Hz, respectively. The resulting photocurrent was amplified and measured by PC at a sampling frequency of 100 kHz. The amplitudes at the two modulation frequencies were numerically calculated from the sampled data.
3. Results and Discussion

Single-channel measurement

Figure 2 shows an example of single-channel measurement in the constant-current mode [7], in which the potential was recorded only in the downstream of the enzyme reactor. The flow rate of $10^{-1}$ mol/L urea solution with 1 mM PBS was changed to various values. Since the reaction time was inversely proportional to the flow rate, the potential became lower (indicating higher pH) as the flow rate was decreased. In the single-channel measurement, however, the drift of the potential over time was relatively large.

![Fig. 2 Change of the potential recorded in the downstream of the enzyme reactor for various flow rates of $10^{-1}$ mol/L urea solution.](image)

Differential measurement

Figure 3 compares the results of (a) single-channel and (b) differential measurements, in which the flow rate was changed from 200 to 7.5 μL/min and again back to 200 μL/min. The sensor was operated in the constant-bias mode, and the resulting photocurrent was converted into pH change using the slope of the $I - V$ curve and the pH sensitivity obtained in advance by a calibration measurement. In the differential setup, the readout of the upstream was subtracted from that of the downstream to compensate for the drift over time. In Fig. 3(b), it can be seen that the drift was suppressed below 0.05 in pH by the differential setup.

![Fig. 3 Comparison of pH readouts for various flow rates of $10^{-1}$ mol/L urea solution in (a) single-channel and (b) differential measurements.](image)

Determination of urea concentration

The differential measurement was applied to determination of urea concentration. The concentration was changed in the range of 0 to $10^{-1}$ mol/L. For each solution, the flow rate was switched between 100 μL/min and 12.5 μL/min, and the readout of the upstream was subtracted from that of the downstream. Finally, $\Delta$pH was defined as the deference of readouts at these two flow rates. Figure 4 shows a calibration plot of $\Delta$pH versus urea concentration.

![Fig. 4 Calibration plot of $\Delta$pH obtained by differential measurement versus urea concentration.](image)

3. Conclusion

In this study, a flow channel with an enzyme reactor was constructed on the sensing surface of LAPS, in which the reaction time was controlled by the flow rate. A differential measurement was demonstrated to suppress the drift below 0.05 in pH and a calibration plot was obtained for urea concentration covering the clinical range.

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References