0273-2289/93/4101-2—0011$03.00

Micromachined Detectors for an Enzyme-Based FIA

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ABSTRACT

Micromachining techniques were applied to construct biosensor systems. The micromachined biosensors have small size, low production cost, and good reproducibility. We made some detection units for flow injection analysis (FIA). An electrochemical flow cell was fabricated, and both the enzyme immobilized column and electrochemical detector were integrated onto the same chip. A chemiluminescence detector was also fabricated and applied to the determination of glucose and lactic acid contained in human serum and urine.

Index Entries: Micromachining; flow injection analysis; glucose sensor; lactic acid sensor.

MICRO ELECTROCHEMICAL FLOW CELL

An electrochemical flow cell that has very small inner volume, ca. 20 nL, was fabricated (*1*). This flow cell can be used as an electrochemical detector for liquid chromatography or FIA. When enzyme is immobilized on the cell, the flow cell can be employed as an electrochemical biosensor.

The structure of the device was shown in another abstract. Glucose oxidase (GOD) was immobilized onto the sample inlet hole of the cell using glutaraldehyde and bovine serum albumin (BSA). The cell was

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This report was presented at the US/Japan Workshop on Microfabrication and Biosensors, July 21–24, 1992, sponsored by the National Science Foundation.

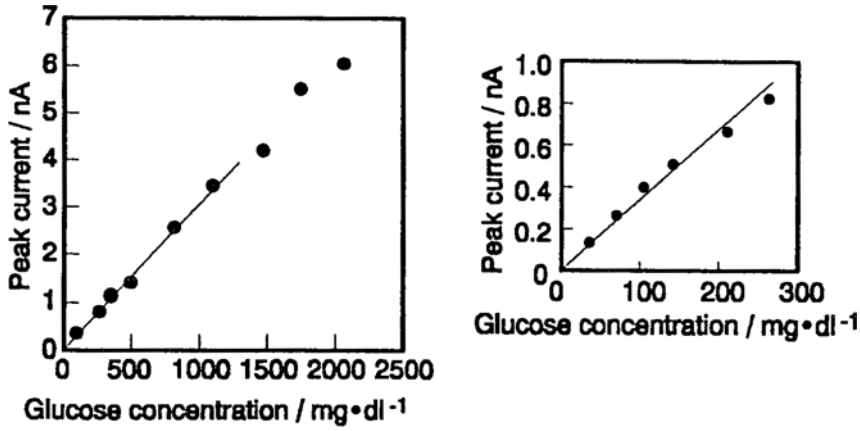

Fig. 1. Calibration curve of the microelectrochemical flow cell for glucose.

placed onto the cell holder, and a plunger pump and a sample injector were connected to it. Fig. 1 shows a calibration curve for glucose. A linear relationship was obtained in the range of 30 mg/dL to 1000 mg/dL when 0.2 μL of the sample was injected. It takes about 1 min to measure one sample. Good reproducibility was obtained using one device, however, the reproducibility was not as good between plural devices. This problem was caused by the GOD immobilization method used here. To solve this problem, we also fabricated an enzyme immobilized column with micromachining techniques.

INTEGRATION OF ENZYME IMMOBILIZED COLUMN AND ELECTROCHEMICAL FLOW CELL

A long open-tubular column was fabricated on the silicon substrate (*2*). The inner wall of the column was treated with silane coupling reagent, and then enzyme was immobilized on it. This type of column has some advantages, such as low pressure drop and less diffusion compared with concentional packed columns. Moreover, an electrochemical cell, whose structure is almost the same as described above, was integrated on the same chip.

Fig. 2 shows structure of a glucose sensor that was integrated with the enzyme immobilized column and electrochemical flow cell. The column was made by anisotropic silicon etching and was 100 μm in width, 70 μm in depth, and 1000 mm in length, with a total volume of 5 μL. Four gold electrodes were fabricated on the glass substrate. Both of the substrates were anodically bonded. GOD was immobilized on the inner wall of the column using 3-aminopropyltriethoxysilane and glutaraldehyde. The measurable range of this device was between 15 mg/dL and 450 mg/dL.

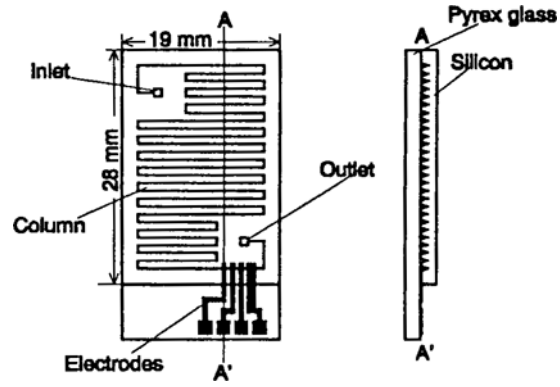

Fig. 2. Structure of the integrated glucose sensor.

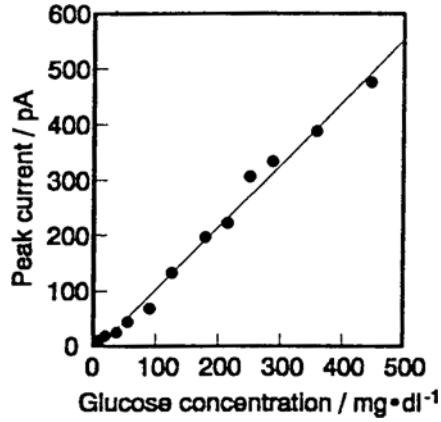

Fig. 3. Calibration curve of the integrated glucose sensor.

INTEGRATION OF ENZYMATIC REACTOR AND CHEMILUMINESCENCE DETECTOR

The flow injection analysis using chemiluminescence reaction has high sensitivity. The luminol reaction has particularly long been known for its use in hydrogen peroxide determination. When the enzyme reaction that generates hydrogen peroxide combines with luminol reaction, substrate can be measured by chemiluminescence. We, therefore, integrated an enzymatic reactor and a chemiluminescence detector on the same chip (3). The structure of the device was shown in another abstract.

Using GOD immobilized glass beads, determination of glucose concentration was carried out. Fig. 3 shows response curves for glucose standard solution. Good reproducibility was obtained. It takes about 1 min to measure one sample. Fig. 4 shows a calibration curve for glucose. A linear relationship was obtained in the range of 10 mg/dL–500 mg/dL.

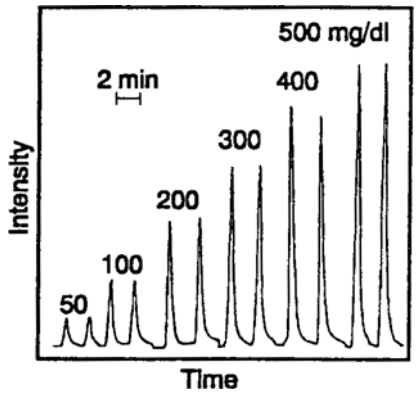

Fig. 4. Response curves of chemiluminescence detector for glucose.

Determination of Glucose Obtained in Human Serum and Urine

Glucose contained in human serum and urine was determined by a chemiluminescence detector. Glucose was added to the controlled human serum and controlled human urine, to adjust the glucose concentration to between 10 mg/dL–350 mg/dL. These samples were injected with no pretreatment apart from filtration. For a reference, the samples were also determined by a clinical inspection kit (GOD–POD method). The correlation coefficient between the chemiluminescence method and the conventional method was 0.99.

Determination of Lactic Acid Contained in Human Serum

Lactic acid contained in human serum was determined using the same procedure as the glucose determination. The sample containing L-lactic acid at the concentration from 4 mg/dL to 50 mg/dL can be measured (Fig. 5). The correlation coefficient between the chemiluminescence method and the conventional method was 0.98.

CONCLUSION

The detection units for enzyme-based FIA were fabricated using micromachining techniques. These micromachined devices are batch-processed, therefore, these can be made at low cost, and have good reproducibility. The signal from the detector becomes weaker as its size becomes smaller, but the electrochemical or chemiluminescence method is more sensitive than the spectroscopic method, therefore, the measurable range of the micromachined detector is almost the same as the conventional method.

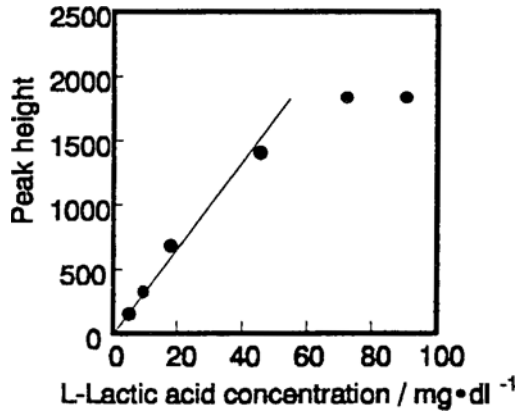

Fig. 5. Calibration curve of the chemiluminescence detector for L-lactic acid.

Additionally, the measurement can be carried out at the flow rate of reagent <50 μL/min. Then the pressure drops on these devices, except for the enzyme immobilized column which is <0.1 atm. These data indicate the possibility of applying the micromachined pump. Thus, the conventional plunger pump and sample injector used in the abovementioned experiments will be replaced by micromachined devices in the future.

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