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Trends in biosensor research and development

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Abstract

Trends in biosensor research and development are described. Micromachining techniques have been applied to construct biosensor systems. Several detection units for flow injection analysis have been fabricated. An electrochemical flow cell was fabricated, and both an enzyme immobilized column and an electrochemical detector were integrated. A chemiluminescence detector was also fabricated. Biosensor systems for clinical analyses in the toilet have been developed. A quartz crystal oscillator was used for protein detection, and a thermistor was employed as a thermal detector resulting from the enzyme reaction. An injectable biosensor for rapid measurement has been developed, and the determination of sugars in fruits was carried out. Glucose oxidase was immobilized for the determination of glucose, and three enzymes, invertase, mutarotase and glucose oxidase, were immobilized for the sucrose sensor. Microbiosensors using carbon fiber microelectrodes have been applied to neuroscience. Micro acetylcholine sensors based on acetylcholine esterase and choline oxidase were fabricated and characterized. Micro glutamate sensors were also fabricated using platinized carbon fiber electrodes which were modified with glutamate oxidase. The micro glutamate sensor was applied to the detection of glutamate released from the neurons.

Introduction

In the last 15 years, micro fabrication techniques based on IC technology, such as photolithography and etching, have been applied to other fields. These techniques which are used to make some small and efficient three-dimensional devices are called micromachining. Furthermore, some studies to make miniaturized chemical analysis systems have already been reported [1–3]. These analysis systems have many advantages, such as fast response, small amount of sample and low consumption of reagents, as compared with the conventional one. We have applied the micromachining technique to make a miniaturized enzyme-based sensor system.

The determination of organic compounds in urine is important in clinical analysis. Recently adult disease has increased because of the aging society. Easy methods to measure health index components in urine at home are required. Glucose in urine is an index component of diabetes and can be measured by the glucose oxidase (GOD)–hydrogen peroxide electrode system. Protein in urine is an indicator of kidney disease and a protein biosensor using quartz crystal oscillation has been developed. Other kinds of biosensors using thermistor, ISFET and photon counter have also been developed. When these biosensors are miniaturized and mass production is done on the basis of silicon fabrication technology, they will be inexpensive and disposable. By embodying these biosensors in a toilet stool, we can get a lot of information about our condition from urine. On transmitting these data to the hospital, they can be used for screening of adult diseases and for receiving advice on health control.

Harvested fruits are stored and allowed to ripen for a certain period before shipment or distribution to the market for consumption. During ripening, it is thought that changes occur in the balance of sugars, organic acids, free amino acids, pigments and fragrances in the fruits [4, 5]. It is important to monitor such changes for proper manufacturing, handling and processing of the fruits. An injectable biosensor for rapid measurement has been developed, and the determination of sugars in the fruits carried out.

An *in vivo* acetylcholine sensor would be a powerful tool for elucidating the action of the neuron. A smaller electrode causes less damage to the tissue during insertion. A carbon fiber electrode is considered to be one of the most useful transducers for *in vivo* biosensors. Acetylcholine is not directly oxidized on the carbon fiber electrode, and hence the immobilized enzymes are necessary to detect acetylcholine. In our work, electrochemical operations for pre-electrolysis and measurement were employed for the sensitive determination of hydrogen peroxide. A carbon fiber electrode was

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modified with acetylcholine esterase and choline oxidase to fabricate an acetylcholine sensor.

A platinized carbon fiber electrode has been fabricated and employed for the highly sensitive detection of hydrogen peroxide. Glutamate oxidase was immobilized on the platinized carbon fiber electrode and it was applied to the determination of glutamate, which is one of the most important neurotransmitters. Released by the presynaptic neuron, it binds to specific receptors on the postsynaptic neuron. An interaction between glutamate and glutamate receptor is strongly related to memory storage phenomena known as long term potentiation in the hippocampus and long term depression in the cerebellum [6, 7]. Quantitative analysis of released glutamate is needed to clarify the molecular mechanism especially in the long term depression phenomenon, *In vivo* and *in vitro* glutamate sensors are powerful tools to elucidate the action sites in the brain. In our study, a micro glutamate sensor was used for the determination of glutamate released from cerebellar neurons.

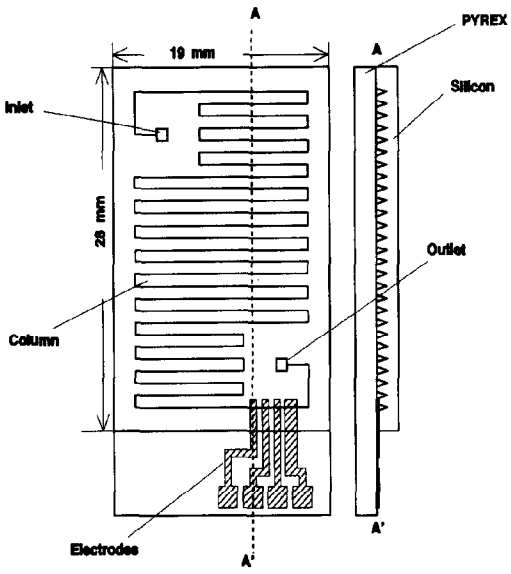

Fig. 2. Integrated glucose sensor.

Micromachined biosensors

Electrochemical flow cell

An electrochemical flow cell which has a very small inner volume, *c.* 20 nl, was fabricated (Fig. 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. GOD was immobilized onto the sample inlet hole of the cell using glutaraldehyde and bovine serum albumin (BSA). Glucose was calibrated in the range 30–1000 mg/dl when 0.2 μl of the sample was injected.

Integration of enzyme immobilized column and electrochemical flow cell

A long open-tubular column was fabricated on the silicon substrate. Figure 2 shows the structure of a glucose sensor which was integrated with an enzyme immobilized column and an electrochemical flow cell. The column was made by anisotropic silicon etching to be 100 μm wide, 70 μm deep, 1 m long, with a total volume of the column of 5 μl. Four gold electrodes were formed on the glass substrate. Both of the two substrates were anodically bonded. A connecting union to the pump or sample injector was glued on the inlet and outlet hole with epoxy resin. GOD was immobilized on the inner wall of the column using 3-aminopropyltriethoxysilane and glutaraldehyde. This device was put in a conventional FIA system.

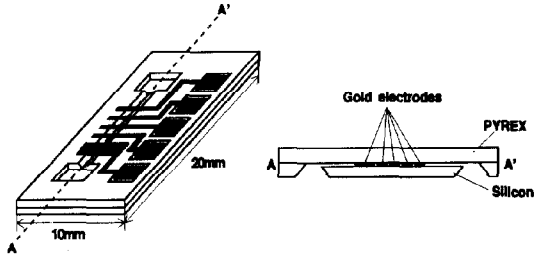

Fig. 1. Micro electrochemical flow cell.

Integration of enzymatic reactor and chemiluminescence detector

An enzymatic reactor and a chemiluminescence detector were integrated on the same chip. Figure 3 shows the structure of the device. It consists of a silicon and glass substrate. On the silicon substrate, an enzymatic reaction column, a mixing chamber and a spiral flow cell were made by anisotropic etching. The whole size of the measuring unit was 15 mm × 20 mm, and the total internal volume of the device was about 15 μl. Enzyme immobilized glass beads were packed into the column, and a photodiode was placed onto the spiral flow cell.

Using GOD immobilized glass beads, determination of glucose concentration was carried out. Glucose was calibrated in the range 10–300 mg/dl. Glucose contained in human serum and urine was measured by a chemiluminescence detector. The correlation coefficient between this chemiluminescence method and the conventional method was 0.99. Lactic acid contained in human serum was measured using the same procedure as for the glucose determination. Samples containing L-lactic acid at concentrations of 4 to 50 mg/dl can be

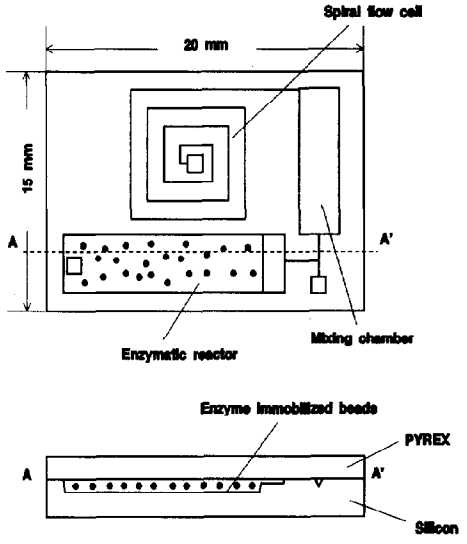

Fig. 3. Integrated chemiluminescence enzyme sensor.

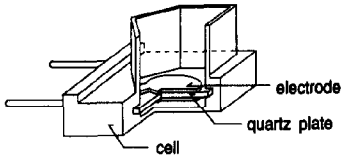

Fig. 4. Protein sensor system based on a quartz crystal oscillator.

measured. The correlation coefficient between this chemiluminescence method and the conventional method was 0.98.

Clinical analyses in toilet

Glucose in urine is an index component of diabetes and can be measured by the GOD–hydrogen peroxide electrode system. Protein in urine is an index component of kidney disease and a protein measuring biosensor using a quartz crystal oscillator was developed. An AT-cut quartz crystal with a resonant frequency of 9 MHz was utilized, as illustrated in Fig. 4. The measurement system was based on sedimentation by the turbidimetric procedure, and protein in urine can be determined on the basis of the decrease in the resonant frequency resulting from the mass change of the electrode surface. Either sulfosalicyclic acid or trichloroacetic acid was employed as precipitating reagent. A correlation coefficient of 0.996 was obtained using sulfosalicyclic acid. Other kinds of biosensors using thermistor, ISFET and photon counter have also been developed for the determination of substances in urine.

Needle-type biosensors for sugar in fruits

An injectable biosensor for rapid measurement has been developed, and the determination of sugars in fruits was carried out. The needle type biosensors were fabricated and combined with the conventional three-electrode system. The enzymes were immobilized on the working electrode using BSA and glutaraldehyde. The immobilized enzymes were GOD for the determination of glucose, and three enzymes, invertase, mutarotase and GOD, were immobilized for the sucrose sensor. For the standard calibration data using the glucose substrate, the response time was within 40 s, and linearity was obtained up to 200 mg/l glucose concentration. The glucose and sucrose needle type sensors were applied to the determination of sugars in fruits. The result was compared to that of the HPLC. The correlation coefficient between the biosensor and the HPLC was 0.9895 for glucose analysis and 0.9856 for sucrose analysis.

Micro acetylcholine sensors using carbon fiber electrodes

Carbon fiber microelectrodes were applied to the monitoring of neurotransmitters. A novel polarization technique was applied for analyzing hydrogen peroxide. The potential profile is shown in Fig. 5. An activation signal consisting of an anodic-cathodic triangular wave was introduced immediately before every measuring pulse. The parameters for measuring pulses were determined. The current intensity was defined as the difference in current between the first and second pulses.

Figure 6 shows the relationship between the current intensity and the hydrogen peroxide concentration. A single pulse of 750 mV was not available for hydrogen peroxide determination because the current fluctuated randomly against the hydrogen peroxide concentration. The pulse of operation gave an excellent calibration for hydrogen peroxide. Activation using the triangular potential is necessary to obtain a reproducible response. The subsequent response gradually diminished in the

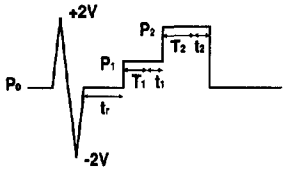

Fig. 5. Potential profile for measurement. P_0: start potential, −175 mV; P_1: potential of first pulse, 750 mV; P_2: potential of second pulse, 1100 mV; T_1+t_1: duration of first pulse, 1160 ms; T_2+t_2: duration of second pulse, 1160 ms; t_1: measuring time, 160 ms, t_2: measuring time, 160 ms, t_r: resting time, 2 s.

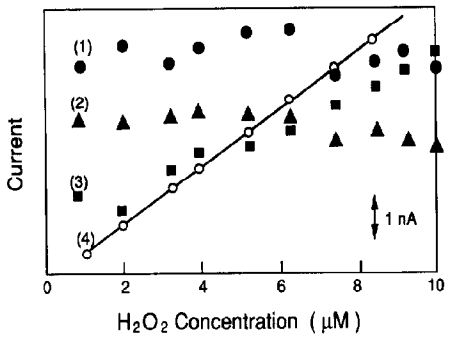

Fig. 6. Relationship between current intensity and hydrogen peroxide concentration under various kinds of pulse operation. Single pulse (1) P_1: 750 mV, T_1: 0.5 s. Double pulse (2) P_1: 750 mV, P_2: 850 mV, T_1: 0.5 s, T_2: 0.5 s; (3) P_1: 600 mV, P_2: 950 mV, T_1: 1 s, T_2 1 s; (4) P_1: 750 mV, P_2: 1100 mV, T_1: 1 s, T_2: 1 s. Currents of (1), (2), (3) and (4) in 1 μm hydrogen peroxide are 16.5, 1,2, 6.2 and 10.5 nA, respectively.

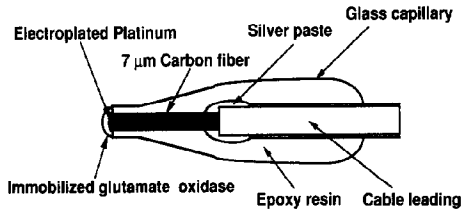

Fig. 7. Structure of a micro glutamate sensor.

absence of activation potential. A linear relationship was obtained between the current and the hydrogen peroxide concentration in the range 0.1 μM to 1 mM; the slope, *y*-intercept and linear correlation coefficient were 0.638 nA/μM, 10.3 nA and 0.9997, respectively. Reproducibility was tested by repeated 25 μl injections of 50 mM hydrogen peroxide solutions. The relative standard deviation for a set of ten injections was 0.68%. The current saturated above 4 mM.

The acetylcholine microsensor gave a linear calibration plot for the range 0.1 to 1.0 mM with a linear correlation coefficient of 0.9842. The sensitivity with a PVA-SbQ membrane is better than that obtained by using immobilized enzymes by crosslinking them with albumin and glutaraldehyde. The determination time for acetylcholine was 5 s. The determination limit of our sensor is 0.05 mM ($S/N=2$).

Micro glutamate sensors for neuroscience

A micro glutamate sensor has been used for the determination of glutamate released from cerebellar neurons. The structure of a micro glutamate sensor is shown in Fig. 7. After polishing the tip of the electrode, the surface of the carbon fiber electrode was platinized electrochemically. Glutamate oxidase was immobilized on the platinized carbon disk electrode.

Cerebellar tissues consist of four layers: molecular layer, Purkinje cell, granule cell layer and white matter (order from outside). The micro glutamate sensor was placed onto the molecular layer where synapses are located between parallel fibers, climbing fibers and Purkinje cells. The membrane potential of the presynaptic membrane was depolarized and was followed by release of neurotransmitters. The response to potassium stimulation was investigated. A rapid increase was observed to a peak value, followed by a gradual decrease to a background current within 90 s. The delay in the appearance of peak responses was due to taking approximately 2 min to replace high potassium solution in the flow chamber. Depolarization-induced release of glutamate, in either the presence or the absence of calcium ion, was investigated using our micro glutamate sensor. A perfusate solution was changed from normal Ringer solution to high potassium solution containing calcium ions. No glutamate was detectable in blank normal Ringer solution which gave only an increase of background current. The difference between depolarization-induced glutamate release under high calcium ion conditions was significant in the cerebellar tissues. These results support the suggestion that the voltage dependent calcium ion channel is related to glutamate release from cerebellar synaptic junctions. Using a calibration curve, the first peak current was shown to correspond to approx. 400 μM. This method has a huge potential for application to analyses of interactions between glutamate and a glutamate receptor which are related to neuronal functions and phenomena like excitatory transmission, synaptic plasticity and ischemic neuronal damage.

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