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Frequency Response of the Glucose Sensor Based on Immobilized Glucose Oxidase Membrane

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ABSTRACT

Frequency response of the glucose sensor based on the immobilized glucose oxidase membrane was investigated experimentally by giving the sinusoidal change of glucose concentration to the glucose sensor and observing its output signal. Observed values of gains and phase lags of the frequency response of the glucose sensor followed the frequency response model of the first-order with dead time; The time constant and also the dead time were estimated and found to decrease as the amount of enzyme immobilized in the membrane increased and the thickness of the membrane decreased.

Index Entries: Glucose sensor; immobilized glucose oxidase membrane; frequency response; time constant; dead time.

INTRODUCTION

Efforts on research of biosensors have been devoted mostly to the development of molecular recognition devices for which several types of biological functions and schemes have been tested and applied. In these research works, the performance characteristics of a biosensor are usually demonstrated by the calibration curves, which are the correlation between the concentration of the substrate to be measured and the steady-state output of a biosensor. The dynamic behavior of biosensors, however, will be one of major concerns when the practical use of these biosensors is to be considered. It seems, however, that studies on the dynamic characteristics of biosensors have rarely been reported. Experimental observations on the dynamic behavior of biosensors will be needed, and the theoretical discussions, such as modeling of experimental results will be necessary in order to accumulate knowledge of the dynamic behavior of biosensors. Experimental and theoretical studies on the dynamic behavior of biosensors will be one of the challenging topics in the development of biosensors.

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In order to investigate dynamic responses of biosensors, an apparatus that generates the input signals to be given to biosensors is needed. Typical input signals conventionally used for investigating dynamic characteristics of any system are stepwise and sinusoidal signals. In this work, the apparatus that generates continuous sinusoidal changes of substrate concentration was developed and used to investigate frequency responses of glucose sensors based on immobilized glucose oxidase membrane.

GENERATOR OF SINUSOIDAL SIGNAL OF SUBSTRATE CONCENTRATION

Continuous generation of sinusoidal changes of substrate concentration in the measuring cell, in which a biosensor was set, was achieved by the two sets of twin cylinders whose plungers were driven by electric stepping motors under the control of the actuating signals generated by a computer. An illustrative diagram of the sinusoidal signal generator is shown in Fig. 1. When one of the oppositely connected cylinders of a twin cylinder set is in the stroke of pushing out, the other is in the stroke of taking in. A three-way solenoid valve is connected to the top of each cylinder to control liquid flows. When the cylinder is in the stroke of pushing out, the three-way solenoid valve connected to the cylinder will open the liquid flowing from the cylinder to the heat exchanger (the "c → h" flow) and at the same time close the liquid flow flowing from the reservoir to the cylinder (the "r → c" flow). When the cylinder is in the stroke of taking in, the three-way valve will close the "c → h" flow and open the "r → c" flow. The reciprocating motion of twin cylinders can feed the liquid from each reservoir into a mixing cell continuously with the help of the cooperative operation of three-way solenoid valves.

Suppose that the one of two twin cylinder sets shown in Fig. 1 feeds, the liquid from a reservoir containing the substrate solution of concentration C_1 into a mixing cell at the flow rate of F_1 described by the following equation.

$$F_1 = F_0 + F_0 \sin(\omega t) \tag{1}$$

In Eq. (1), F_0 is the average flow rate and ω is the angular velocity of the sinusoidal function. As easily be understood by an inspection of Eq. (1), F_1 makes sinusoidal changes between zero and $2F_0$.

The other twin cylinder set will feed the liquid from a reservoir containing the substrate solution of concentration C_2 into a mixing cell at the flow rate of F_2 described by the following equation.

$$F_2 = F_0 - F_0 \sin(\omega t) \tag{2}$$

In Eq. (2), F_0 is again the average flow rate. F_1 changes between zero and $2F_0$ as F_1 does, but there is a 180° difference in phase angle between F_1 and F_2.

C_M, the substrate concentration of the combined flour of these two liquid flows, will be given by Eq. (3), and makes sinusoidal changes between C_1 and C_2.

$$C_M = (C_1 + C_2)/2 + (C_1 - C_2)\sin(\omega t)/2 \tag{3}$$

The more detailed structure and the operational characteristics of this sinusoidal signal generator were explained elsewhere *(1)*.

In order to monitor the time change of C_M, blue dextran (mol wt ~2,000,000, NYCOMED AS, Oslo, Norway), a water-soluble blue dye, was dissolved as a tracer

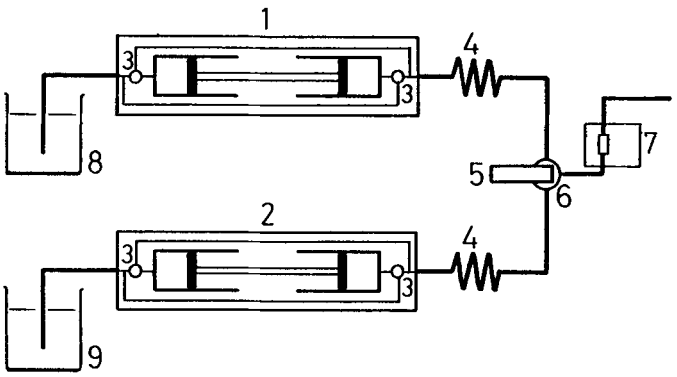

Fig. 1. Schematic diagram of sinusoidal signal generator 1 and 2: twin cylinders, 3: three-way solenoid valve , 4: heat exchanger, 5: biosensor, 6: mixing c ell, 7: spectrophotometer, 8, and 9: reservoirs.

into the solution stored in one of two reservoirs shown in Fig. 1. The combined flow was led into the flow cell of the spectrophotometer, and the blue dye concentration of the combined flow was continuously measured at the wavelength 350 nm. The concentration of blue dextran of the combined flow can easily be converted to the substrate concentration of the combined flow, C_M. The output signal of the biosensor responding to C_M is measured to obtain the frequency response data of the biosensor.

METHODS

Solubilized collagen fibrils was used as a supporting matrix for immobilizing enzymes *(2–4)*. One hundred milligrams of solubilized collagen (available as SCL from Nippi Co., Tokyo, Japan) were suspended in 20 mL of water at room temperature. Water suspension of SCL was, given a few drops of glacial acetic acid (guaranteed reagent, Hayashi Jyunyaku Kogyo Co., Osaka, Japan) and was stirred by a magnetic stirrer for the complete dissolution of SCL. The solution of SCL was then dialyzed for 1 d with distilled water. The SCL gel obtained was mixed well with 20 mg of glucose oxidase (GOx, from *Aspergillus niger*, Type II, Sigma, St. Louis, MO). The mixture was spread over the flat square plate of 8 × 4 cm and dried overnight at room temperature to make the GOx-SCL membrane. The 10 w/v% of glutaraldehyde solution (ca. 50% in water, Tokyo Kasei Kogyo Co., Tokyo, Japan) was spread over the surface of the dried GOx-SCL membrane for crosslinking. The GOx-SCL membrane was washed with deionized water and peeled off the plate. The GOx-SCL prepared membrane was then stored in deionized water at 4°C. The thickness of the GOx-SCL membrane was measured by a micrometer caliper at about 20 points on the membrane, and an arithmetic mean value of thickness was calculated. For each preparation of GOx-SCL membrane, the membrane thickness measured at each point was in the range of about ±10% deviation from an arithmetic mean thickness.

The GOx-SCL membrane was attached onto the top end of the dissolved oxygen sensor of galvanic type (Type OE-232A, Toa Denpa Kogyo Co., Tokyo, Japan, Ag cathode and Pb anode) to cover its poly-tetrafluoroethylene membrane. This

Table 1
Preparations of GOx-SCL Membrane

Membrane code	SCL, mg	GOx, mg	Membrane thickness, mm with ± 10% variance
20/100-A	100	20	55
20/100-B	100	20	59
10/100	100	10	55
30/200	200	30	102
20/200	200	20	104

setup of glucose sensor (GOx-SCL glucose sensor) decreases its output signal with an increase of the glucose concentration as described by Eq. (4).

$$C_6H_{12}O_6 + O_2 + 2H_2O \rightarrow C_6H_{12}O_7 + H_2O_2 \quad (4)$$

The glucose solution used for experiments was prepared by dissolving glucose (guaranteed grade, Hayashi Jyunyaku Kogyo Co., Osaka, Japan) into the buffer solution of pH 5.5 prepared from sodium acetic acid (guaranteed reagent, Kanto Kagaku Kogyo Co., Tokyo, Japan) and glacial acetic acid. The experimental temperature was maintained at 25°C throughout this work by holding reservoirs 1 and 2 shown in Fig. 1 in a constant temperature bath and by circulating water at 25°C through a jacket surrounding a mixing cell.

EXPERIMENTAL RESULTS

Preparations of GOx-SCL Membrane

Table 1 shows GOx-SCL membranes prepared for setting up the GOx-SCL glucose sensors. The code "10/100" appearing in Table 1, for instance, means the GOx-SCL membrane prepared from 10 mg of GOx and 100 mg of SCL.

Calibration of the GOx-SCL Glucose Sensor

The GOx-SCL glucose sensor output was measured with the dissolved oxygen meter (Type DO-1, Toa Denpa Kogyo Co., Tokyo, Japan). The meter reading at the zero glucose concentration was adjusted to a predetermined value beforehand. The glucose solution was prepared with a buffer solution of pH 5.5 saturated with air. As shown in Fig. 2 linear calibration curves were obtained for each setup of GOx-SCL glucose sensors for the range of glucose concentration up to 600 mg glucose/mL. The linearity of the glucose sensor output will be secured by the high enough enzyme activity of the immobilized enzyme membrane as discussed in the previous work *(5)*. Sample solution was stirred with a magnetic stirring equipment at 500 rpm where the speed ranging from 200–680 rpm (and probably higher) was found not to affect the sensor output. The existence of blue dextran in the sample solution did not interfere with the glucose sensor output at a concentration of 100 mg blue dextrose/L.

Sinusoidal Input and Output Signals

In generating sinusoidal input signals, C_1 was kept at 100, 200, or 300 mg glucose/mL, and C_2 was always kept at zero. Sinusoidal input signals and also

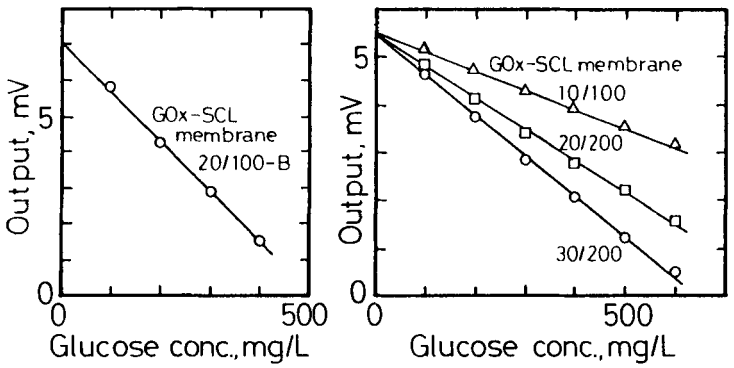

Fig. 2. Calibration curves of GOx-SCL glucose sensors, 25°C and pH 5.5.

sinusoidal output signals of GOx-SCL glucose sensors were examined for their fidelity to the theoretical sinusoidal wave forms calculated with given values of ω and amplitude. Observed waves of input and output signals were superimposed within 1% error by the corresponding calculated waves. The period of input signals and that of output signals coincided with each other within a 1% difference for each run.

Frequency Response of GOx-SCL Glucose Sensors

Based on the stepwise response experiments, the dynamic response of the glucose sensor based OIL the GOx-SCL membrane was expressed by the model of the first-order with the dead time *(6,7)*. The same model was again used to model the experimental results of this work.

The gain of the biosensor, G, is given by the ratio of the amplitude of output signal to that of input signal as defined by Eq. (5).

$$G = (\text{Amplitude})_{\text{Output signal}} / (\text{Amplitude})_{\text{Input signal}} \tag{5}$$

ϕ, the phase lag of the biosensor in the unit of degree, is given by $t_{\text{Max input signal}}$ and $t_{\text{Max output signal}}$ as described by Eq. (6), where $t_{\text{Max input signal}}$ and $t_{\text{Max output signal}}$ are the time in the unit of seconds at which input and output signals reach their respective maximums.

$$\phi = (180\omega/\pi)\,(t_{\text{Max output signal}} - t_{\text{Max input signal}}) \tag{6}$$

The theoretical values of the gain, G_{1st}, and the phase lag, ϕ_{1st}, of the first-order system are given by Eqs. (7) and (8), respectively *(8)*.

$$G_{1st} = 1/[1 + (\omega T)^2]^{1/2} \tag{7}$$

$$\phi_{1st} = (180/\pi)\tan^{-1}(-\omega T) \tag{8}$$

In Eqs. (7) and (8), T designates "time constant" of the first-order system in the unit of seconds.

In Fig. 3, observed G values were shown against C_1 for the glucose sensor using the 20/100-A membrane. G was independent of C_1 as shown in Fig. 3, which suggests the linearity of the glucose sensor response and supports the first-order model for modeling the sensor output.

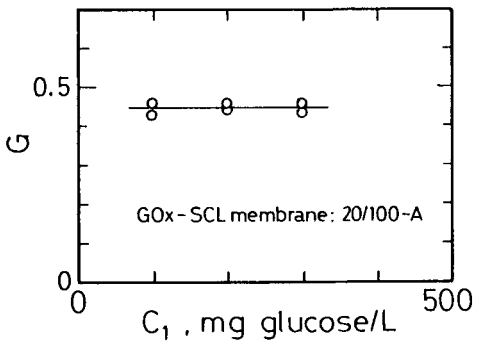

Fig. 3. Gain of GOx-SCL glucose sensors against C_1. Membrane 20/100-A, ω = 0.34 radian/s, 25°C and pH 5.5.

In Fig. 4A–D, G and ϕ are shown against ω. In the upper part of Fig. 4A, G was shown against ω for the glucose sensor using the 20/100-B GOx-SCL membrane. The value of T best fit to these data was estimated as 16.1 s by the curve-fitting technique of the Gauss-Newton method *(9)*. The theoretical curve of G_{1st} calculated for T of 16.1 s was depicted by the real line in the upper figure of Fig. 4A. In the lower figure of Fig. 4A, ϕ was shown against ω and the theoretical curve of ϕ_{1st} calculated for T of 16.1 s was also given by a real line. The observed value of phase lag, ϕ, was found to be much higher than the theoretical value, ϕ_{1st}, and the difference between observed and theoretical values, $\phi - \phi_{1st}$ called the "dead time." Although it is expressed in the unit of degree, the term $\phi - f_{1st}$ is easily converted to the unit of time by being multiplied by the term $\pi/(180\omega)$. During the dead time, the GOx-SCL, glucose sensor does not respond to the change in glucose concentration of the ambient solution. Similarly, G, G_{1st}, ϕ, and ϕ_{1st} are shown for the GOx-SCL membrane of the membrane code 10/100, 30/200, and 20/200 in Fig. 4B, C, and D, respectively. It may be concluded that the first-order system with dead time describes the frequency response of the glucose sensors based on the GOx-SCL membrane fairly well even, although the best-fit curves of G vs ω have a common tendency of crossing the experimental plots.

In Fig. 5, observed values of dead time, $\phi - f_{1st}$ were found to be well correlated with ω by an empirical correlation of the form of Eq. (9).

$$(\text{Absolute value of } \phi - \phi_{1st}) = \alpha\omega^{\beta} \tag{9}$$

Estimated values of α and β are summarized in Table 2 together with estimated values of T.

CONCLUSION

Frequency response of the glucose sensors based on the GOx-SCL membrane was investigated with the different preparations of the GOx-SCL membrane. As in the case of the stepwise response, the frequency response of the glucose sensor based on the GOx-SCL membrane was modeled by the first-order system with dead time. For each of the GOx-S membrane, the gain of the glucose sensor was found to be fairly well expressed by the model of the first-order system with the time constant estimated by the curve-fitting technique. The observed phase lag was taken as the

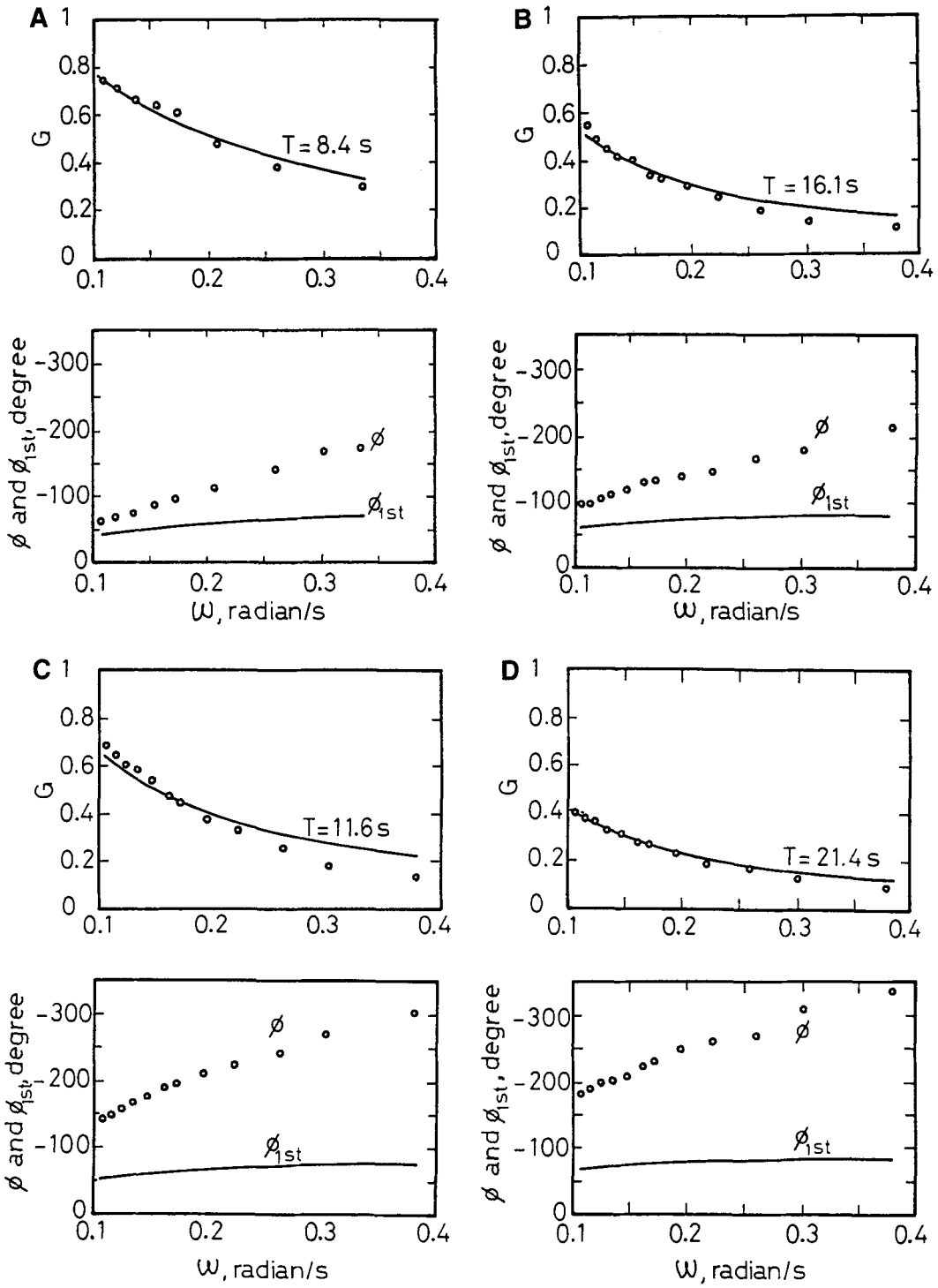

Fig. 4. Gain and phase lag of GOx-SCL glucose sensor against ω. Membrane **(A)** 20/100-B, 25°C and pH 5.5. **(B)** 10/100, 25°C and pH 5.5. **(C)** 30/200, 25°C and pH 5.5. **(D)** 20/200, 25°C and pH 5.5.

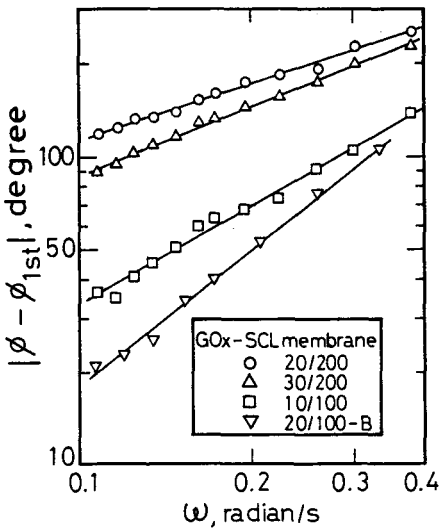

Fig. 5. Absolute value of dead time $|\phi - \phi_{1st}|$, against ω. 25°C and pH 5.5.

Table 2
Estimated Time Constants of First-Order Model and Empirical Exponents of Eq. (9)

Membrane code	T,s	α	β
20/100-B	8.4	560	1.5
10/100	16.1	317	0.95
30/200	11.6	480	0.75
20/200	21.4	492	0.65

sum of the phase lag of the first-order system and the dead time. The dead time was found to be proportional to the exponential of ω for each membrane tested.

A higher amount of immobilized enzyme decreased the time constant of the glucose sensor. This effect was offset by the increase of membrane thickness. The dead time was predominantly affected by the membrane thickness. The time constant and the dead time might be controlled by an overall effect of the enzymatic activity and the mass-transfer resistance of the immobilized enzyme membrane.

REFERENCES

1. Kim C. (1995), Dynamic behavior of biosensors based on immobilized enzyme membrane, Master's thesis, Fukuoka Institute of Technology, Fukuoka, Japan.
2. Gondo, S., Osaki, T., Koya, H., and Morishita, M. (1981), *Artificial Organs* **5**, 732–734.
3. Morishita, M., Osaki, T., and Gondo, S. (1981), *Kagaku Kogaku Ronbunshu* **7**, 320–323.
4. Gondo, S., Osaki, T., and Morishita, M., (1981) *J. Mol. Catalysis* **12**, 365–374.
5. Gondo, S. and Morishita, M. (1986), *Ann. NY Acad. Sci.* **469**, 190–193.
6. Gondo, S. and Morishita, M. (1981), *Artificial Organs* **5**, 307,308.
7. Gondo, S. and Morishita, M. (1983), *Ann. NY Acad. Sci.* **413**, 225–227.
8. Ceaglske, N. (1956), *Automatic Process Control for Chemical Engineers*. John Wiley, New York.
9. Kuester, J. L. and Mize, J. H. (1973), *Optimization Techniques with Fortran*. McGraw-Hill, New York.