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Multifunctional Biocompatible Membrane and Its Application to Fabricate A Miniaturized Glucose Sensor with Potential for Use In Vivo

Chien-Yuan Chen,[*,1,2] *Kazuhiko Ishihara,*[3] *Nobuo Nakabayashi,*[3] *Eiichi Tamiya,*[2] *and Isao Karube*[2]

[1]*Department of Agricultural Chemistry, National Taiwan University, 1, Section 4, Roosevelt Rd., Taipei 106, Taiwan, R.O.C.*
[2]*Research Center for Advanced Science and Technology, University of Tokyo. 4-6-1, Komaba, Meguro-Ku, Tokyo 153, Japan*
[3]*Institute for Medical and Dental Engineering, Tokyo Medical and Dental University. 2-3-10, Kanda-Surugadai, Chiyoda-Ku, Tokyo 101, Japan*

Abstract. **A multifunctional membrane with biocompatibility, diffusion-limiting effect, and the ability to curtail the responses of an H_2O_2 electrode to ascorbate and urate was prepared. It was composed of MB, AB, and CTA, where MB is the copolymer of 2-methacryloyloxyethyl phosphorylcholine (MPC) and n-butyl-methacrylate (BMA), AB is the copolymer of acrylamide-2-methylpropane sulfonic acid (AMPS) and BMA, CTA is cellulose triacetate. Investigation of the biocompatibility of this membrane showed that, compared with CTA, relatively few platelets bound to it. The membrane was coated onto the working electrode of a needle-type glucose sensor on which immobilized glucose oxidase membrane has been coated. The sensor did not respond to ascorbate and urate at their concentration normally encountered in blood. Its response was not inhibited by metal ions in blood at usual concentration. The sensor exhibited superior thermostability in addition to a rapid response (< 90 seconds in batch operation), good reproducibility (RE < 5%), good stability (more than 36 hours continuously in heparinized whole blood), and a wide dynamic range (5–650 mg/dl glucose). The sensor was used to determine glucose in serum. The data obtained from the sensor showed good agreement with that from a clinical autoanalyzer (R = 0.973).**

Key Words. **MPC, BMA, AMPS, biocompatibility, cellulose triacetate, diffusion-limiting effect, glucose sensor, needle type, glucose oxidase, serum, whole blood**

Introduction

The measurement of glucose is of importance in many fields including clinical chemistry (Nishida et al., 1996; Ritter et al., 1996), food and bio industries (Olsson et al., 1990; Queinnec et al., 1992; Phelps et al., 1995), and environmental management (Stephens et al., 1997). Relevant processes to immobilize enzymes (Vreeke and Rocca, 1996; Liu et al., 1995, 1996a, 1996b; Wilke et al., 1997) or mediators (Liu and Deng, 1996c; Losada et al., 1997) for constructing glucose sensors have been intensively studied. The mathematical model (Krishnan et al., 1996) and dynamic behavior (Yamasaki et al., 1996) of glucose sensors have also been reported.

The application of glucose sensors in the medical field attracted more attention than in other fields. The sensors based on physically non-invasive (Spanner and Niessner, 1996a, 1996b) or enzyme-free (Benmairoha, 1996) principles have been reported for blood sugar determination. Mediated or unmediated enzymatic glucose sensors, however, played the major role for this purpose. Biosensors for medical applications are often shaped in planar (Wilke et al., 1997; Khan, 1997; Nagata et al., 1995a, 1995b; Steinkuhl et al., 1996) or needle-type (Nishida et al., 1996; Sakakida et al., 1996). The planar sensors are convenient for *in vitro* determination of biofluid samples. Recently the screen printing (Khan 1997; Nagata et al., 1995a, 1995b) and photolithographic (Steinkuhl, 1996) techniques are applied to construct them. The needle-type sensors, especially a miniaturized one (Sakakida et al., 1996; Amine et al., 1995), are good choice for implantable *in vivo* purpose (Lager et al., 1994; Thome-Duret et al., 1996).

Biocompatibility, diffusion-limiting effect and a diminished response to interfering materials are indispensable in developing a glucose sensor for determining undiluted blood and serum samples or for *in vivo* purpose. Biocompatibility is necessary to obviate blood clotting and immune reactions. The diffusion-limiting effect is used to extend the working range of the sensor to make the determination of glucose in undiluted serum or whole blood possible. A diminished response to

*Corresponding author Professor Chien-Yuan Chen, Department of Agricultural Chemistry, National Taiwan University, 1, Section 4, Roosevelt Rd., Taipei 106, Taiwan, R.O.C. Tel/Fax: +886-2-2366-1696 E-mail: chenyuan@ms.cc.ntu.edu.tw.

interfering materials is required for accurate measurement.

There are two kinds of biocompatible materials, active (positive) (Yui et al., 1989; Lenk et al., 1989) and inert (negative) (Murabayashi et al., 1985; Kottke-Marchant et al., 1989). Neither of them elicits an immune reaction but the formers have a disadvantage in that components of biological fluids adhere to them. These act as a barrier to the diffusion of analysate into the biological layer of a biosensor and consequently they reduce its response. 2-Methacryloyloxyethyl-phosphorylcholine (MPC) is an inert biocompatible material. It can copolymerize with methacrylate compounds, for example n-butylmethacrylate (BMA). The synthesis of MB, the copolymer of MPC and BMA, and its compatibility with blood has been described (Ishihara et al., 1990a, 1990b; Abe 1990). This polymer was selected as the component responsible for biocompatibility for the task of constructing a multifunctional membrane suitable for biosensors' application.

The dynamic range of a glucose sensor without a diffusion-limiting membrane was found less than 100 mg/dl (Chen et al., 1992). The glucose concentration of blood lies between 50 and 120 mg/dl for non-diabetics and can be as high as 600 mg/dl for diabetics (Kanai and Kanai, 1983). A diffusion-limiting membrane is necessary for extending the dynamic range of glucose sensors so that glucose can be determined directly in undiluted biological fluids. Cellulose acetate (Ikeda et al., 1987), polyurethane (Peteu et al., 1996), and their derivatives have been widely used for this purpose.

Ascorbate and urate are major interfering materials in blood to biosensors based on an H_2O_2-sensing electrode (Bindra and Wilson, 1989). The strong response of an H_2O_2 electrode based glucose sensor to ascorbate and urate, compared with glucose, has been reported as severe interference to such sensors (Bindra and Wilson, 1989). How to curtail the response of such biosensors to ascorbate and urate at the concentration they normally encountered in blood always attracts lots of attention (Van-Os et al., 1995). A polymer with negative charge, for example Nafion, has been used for this purpose (Kristensen et al., 1987). A negatively charged molecule, acrylamide-2-methylpropane sulfonic acid (AMPS) that can readily react with n-butylmethacrylate (BMA) to form a copolymer, AB, with good membrane-forming character was selected for this purpose in this study.

In this study, a miniaturized needle-type glucose sensor with three-electrode configuration was constructed. A piece of platinum wire, a piece of silver wire with its active surface converted into Ag/AgCl, and a stainless-steel hypodermic needle were used as the working, the reference, and the counter electrode, respectively. A glucose oxidase (GOD) membrane immobilized with PVA-SbQ and the multifunctional membrane (MAC) were coated onto the active surface of the working electrode. PVA-SbQ is a polyvinyl alcohol bearing styrylpyridinium group (Ichimura 1984). It is photocrosslinkable by the light with wavelength shorter than 460 nm. Enzymes (Matsumoto et al., 1984), organelles (Harrison et al., 1988), and microbial cells (Ichimura et al., 1987) have been immobilized in it. GOD was immobilized by a method combining chemical cross-linking with glutaraldehyde and entrapment by PVA-SbQ as described previously (Chen et al., 1992). The multi-functional membrane, MAC, was prepared by dip- or drop-coating of the mixture of MB, AB, and CTA and then air-dried at room temperature. Investigation of the biocompatibility of this membrane showed that, compared with CTA, relatively few platelets bound to it after contacting with platelet-rich plasma (PRP) (Ishihara et al., 1990a). This membrane was used to confer biocompatibility (from MPC), diminished response to ascorbate and urate (from AMPS) and a wider working range (from CTA) toward the sensor.

The reproducibility, selectivity and stability of the sensor along with the effects of temperature, pH, and metallic ions were investigated. The sensor was used to determine glucose in serum. The data obtained from the sensor was in good agreement ($r = 0.973$) with that from a clinical autoanalyzer.

Experimental

Materials and instrumentation

Glucose oxidase (EC 1.1.3.4) from *Aspergillus niger*, glucose, and imidazole were from the Sigma Chem. Co., Milwaukee USA. Cellulose triacetate was from Eastman Kodak Co., N.Y., USA. All the chemicals required for synthesizing MB and AB were obtained, purified, or prepared as described (Murabayashi et al., 1985; Kottke-Marchant et al., 1989; Ueda et al., 1991). Phosphate buffered saline (PBS) was prepared by dissolving 2.754 g NaCl, 2.081 g KH_2PO_4 and 0.477 g NaOH in 1000 ml distilled water and adjusting to pH 7.4 with 0.1 N NaOH solution (Perrin and Dempsey, 1981). Glucose solutions were prepared in PBS and allowed to stand for at least 24 hours to equilibrate the α and β anomer. Control serum was from Katayama Chem. Co., Osaka, Japan. The glucose analysis kit based on glucose oxidase was from Wako Chem. Co., Japan. All other chemicals used in this study were of the highest grade available and were used without further purification.

The batch-operating system is shown in Figure 1. It was composed of a potentiostat, a circulating water bath, a water-jacketed glass reactor, a magnetic stirrer with stirring bar, and a chart recorder. The hypodermic needle

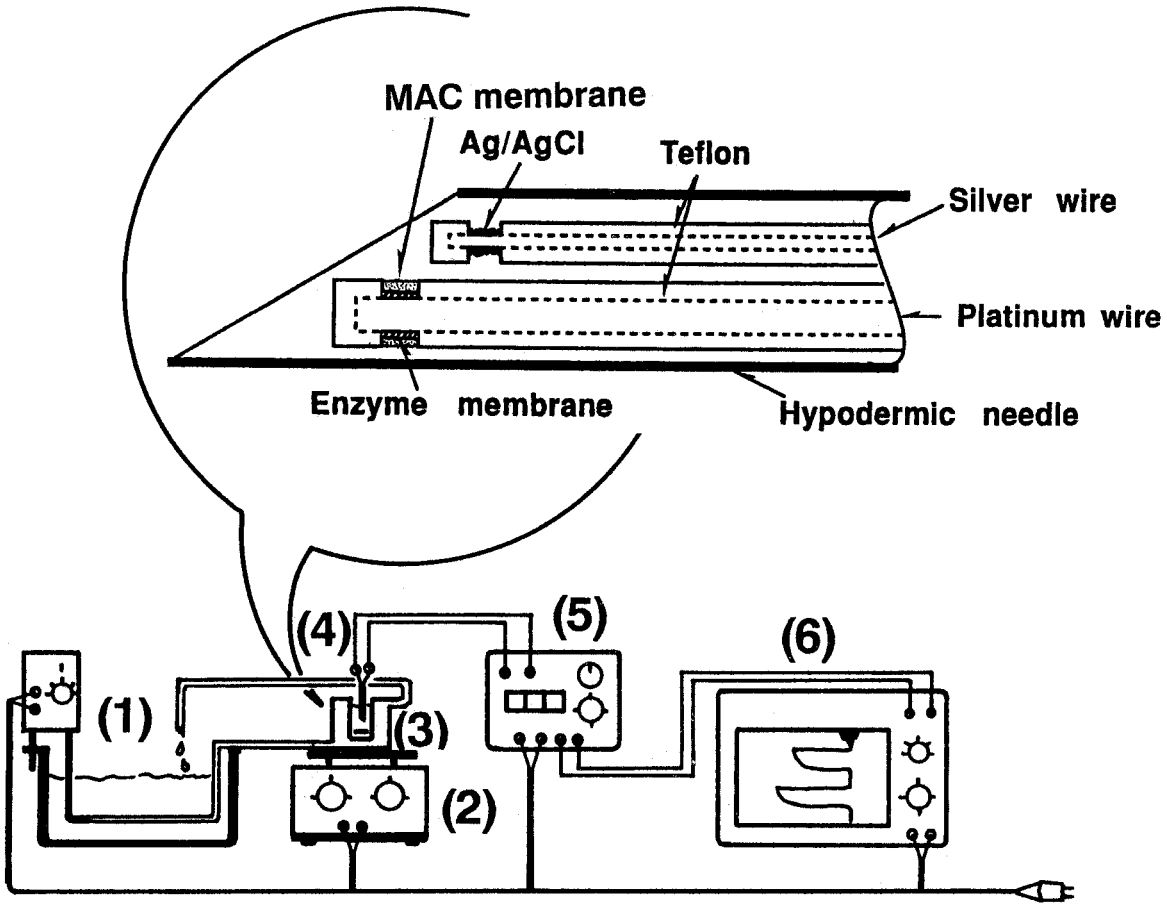

Fig. 1. *Schematic diagram of the batch-type operation system. Key: (1) Thermostat circulating water bath, (2) Magnetic stirrer, (3) Water-jacketed glass reactor, (4) Miniaturized glucose sensor, (5) Potentiostat, (6) Chart recorder.*

made of stainless steel (OD = 1.2 mm, ID = 1.0 mm) was obtained from Terumo Co., Tokyo, Japan. Platinum wire (D = 0.3 mm) was obtained from Tokuriki Co., Tokyo, Japan. The clinical autoanalyzer was a Beckman Glucose Analyzer II from the Beckman Instrument Co., CA USA. The electron microscope was a JSM-5400 Scanning Microscope from Joel Co., Tokyo, Japan.

Construction of the working electrode, the counter electrode, and the reference electrode of the needle type sensor

A stainless steel hypodermic needle (OD 1.2 mm, ID 1.0 mm) was cleaned with methanol and dichloromethane. It was further cleaned with an oxidative acid solution (conc. H_2SO_4 and 30% H_2O_2 in a 1:1 ratio) and an oxidative alkaline solution (conc. NH_4OH, 30% H_2O_2, and H_2O in a 1:1:6 ratio). It was thoroughly rinsed with distilled water after each cleaning step and was used as the counter electrode. A piece of platinum wire was insulated with Teflon tube. The Teflon tube was stripped to form a cavity (Bindra et al., 1991). This was achieved by making a circular cut on the Teflon tube 1 mm from the tip and then pull the tube out to create a cavity of 0.5 mm wide. It was used as the working electrode. A piece of Teflon coated silver wire (OD 0.127 mm) was created a cavity by the same operation (Bindra et al., 1991). The exposed surface of silver was converted to Ag/AgCl by dipping in an H_2O_2-HCl solution (1M H_2O_2 and 1M HCl at 1:1 ratio) and used as the reference electrode.

Preparation of the MAC solution

MPC was synthesized according to the method described previously (Ishihara et al., 1990a, 1990b; Abe, 1990). MB was prepared by copolymerization of MPC with BMA (Ishihara et al., 1990a, 1990b). The AMPS was obtained from Nitto Chem. Co., Tokyo, Japan and was used as supplied. AB was prepared by copolymerization of AMPS with BMA (Ueda et al., 1991). The structural formula of MB and AB are shown in Figure 2. MAC solution was prepared by mixing MB solution (10% w/v in methanol), AB solution (5% w/v in isopropanol) and CTA solution (2–4% w/v in 1,1,2,2-tetrachloroethane) at 1:1:2 ratio.

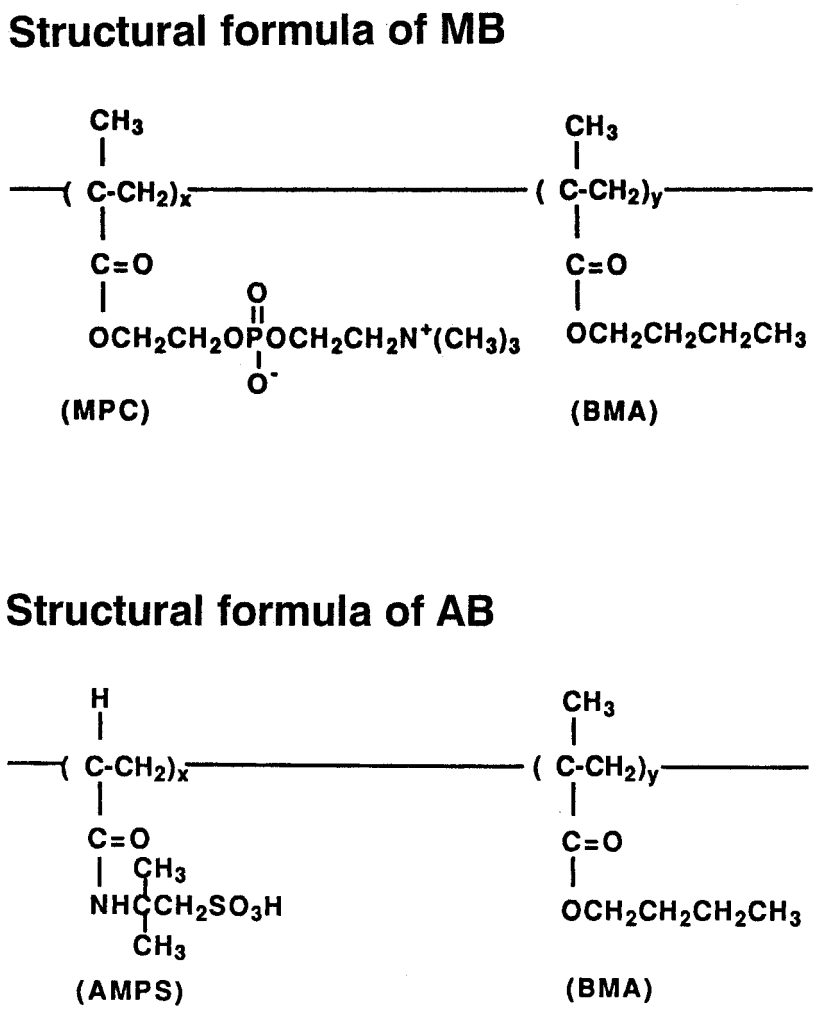

Fig. 2. The structural formula of MB, copolymer of 2-methacryloyloxyethyl-phosphorylcholine (MPC) and n-butylmethacrylate (BMA), and AB, copolymer of acrylamide-2-methylpropane sulfonic acid (AMPS) and BMA.

Drop-coating of the immobilized GOD membrane and the MAC membrane onto the working electrode

2 µl of the GOD solution composed of 1 mg GOD (138 U/mg), 5 mg BSA, 100 mg distilled water, and 100 mg PVA-SbQ was dropped on the active surface in the cavity of the working electrode. The electrode was placed in a sealed dark box with saturated glutaraldehyde vapor and kept at room temperature for 12 hours to complete the chemical crosslinking between GOD and glutaraldehyde. Exposed the electrode to a fluorescent lamp (10 W, 15 cm far) for 10 minutes to induce photocrosslinking of PVA-SbQ. Then, dropped 2 µl of the MAC solution over the enzyme membrane and air-dried for 30 minutes at room temperature.

Construction of the needle-type glucose sensor

The working electrode, after having been coated with GOD and MAC membranes on its active surface in the cavity, and the reference electrode were inserted into the interior of the counter electrode. Epoxy resin was used to fix the electrodes at their position. The configuration of the needle-type glucose sensor is shown in Figure 1. The sensor was stored dry at 4°C until use.

Evaluation of the biocompatibility of MAC membrane

The carotid artery of a rabbit weighing about 3 kg was canalled with PVC tubing. Fresh blood (ca. 90 ml) was collected in a disposable cylinder containing 10 ml of 3.8% (w/v) sodium citrate solution. Immediately afterwards the blood was centrifuged at 750 rpm (90 g) for 15 minutes. The supernatant was collected as platelet-rich plasma (PRP). The precipitate was centrifuged again at 2800 rpm (1250 g) for 10 minutes. The second supernatant contained less platelet and it was called platelet-poor plasma (PPP). The platelet content of PRP was diluted to approximately 10^8 cells/ml with PPP.

A CTA, an AB-CTA, and the MAC solution were cast on a piece of telephthalate disk (D 14 mm, Wako Chem. Co., Tokyo, Japan) individually and air-dried at room temperature for 1 hour. The disks were immersed in the PRP for 1 hour and then transferred to a saline solution containing 2.5% glutaraldehyde to immobilize the bounded platelet. The disks were rinsed, freeze-dried, coated with gold, and then the surface was investigated by scanning electron microscopy.

Determination of glucose concentration

The sensor was immersed in PBS solution for 2 hours to equilibrate the membrane system. A potential of + 650 mV was applied between the working and the reference electrode. The base current was measured and then glucose solution was injected into the PBS solution. The response current following injection was recorded with a chart recorder until a steady state current was observed. Magnetic stirring was used during the determination to ensure homogeneity of the solution. The difference between the base current and the steady state current was used to calculate the concentration of glucose in the sample according to a calibration graph.

A glycolyzed serum was obtained by incubating heparinized whole blood at 37°C for 18 hours to glycolyze it and then centrifuging it to collect the supernatant (Claremont 1986). When determining glucose in a serum sample, the base current was taken in the glycolyzed serum and then the sensor was transferred into the sample to obtain a steady state current. The concentrations of glucose in serum were calculated according to a calibration graph obtained in the glycolyzed serum.

Results and Discussion

Biocompatibility of the MAC membrane

Difference among a CTA, an AB-CTA and the MAC membrane in the quantity of platelets bounded on the surface is shown in Figure 3. After contacting with PRP for one hour, relatively few platelets adhered to the surface of the MAC membrane (Figure 3-C). By contrast, a relatively large number of platelet adhered to the surface of the cellulose triacetate (Figure 3-A) and the AB-CTA (Figure 3-B) membrane. These results in conjunction with previous work (Ishihara et al., 1990a, 1990b) suggest that the MAC membrane, owing to the MPC component, exhibited a high degree of compatibility to blood.

Diffusion-limiting effect of MAC membrane

Calibration graphs of the sensor coated with immobilized GOD membrane only and those further coated with MAC membranes containing various concentration of CTA are shown in Figure 4. The dynamic range of the electrode with immobilized GOD membrane only was 0.2–50 mg/dl glucose. The dynamic range of the sensor was extended to 2–200, 5–450 and 5–650 mg/dl glucose after being coated with MAC membranes prepared by using of 1, 2 and 3% (w/v) CTA solution. The ability to extend the dynamic range of the sensor demonstrated the

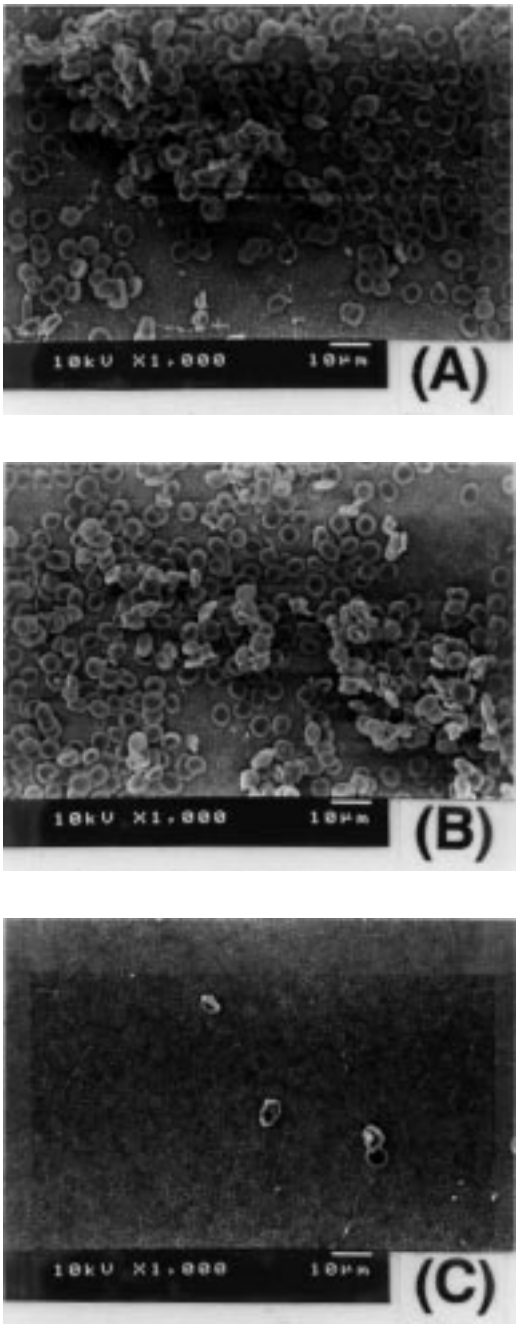

Fig. 3. *Scanning electron micrograph of (A) CTA, (B) AB-CTA, and (C) MAC membranes after being contacted with platelet rich plasma for one hour.*

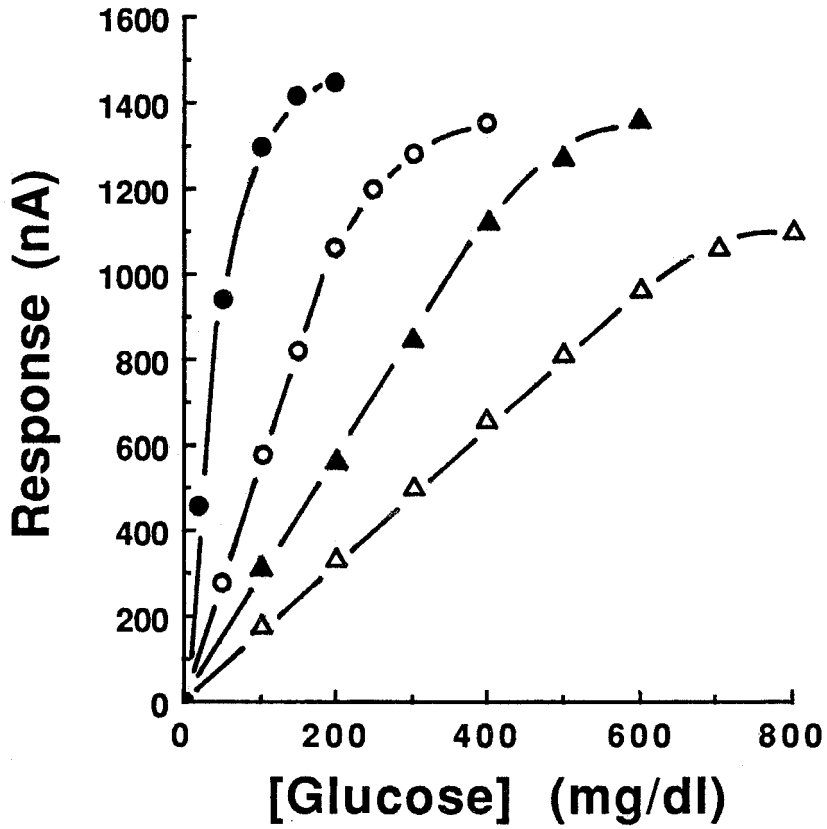

Fig. 4. Calibration curves of the glucose sensor coated with various membranes. Experiments were conducted at 37°C in phosphate buffer with saline (PBS), pH 7.4. Key: (●) immobilized glucose oxidase membrane only, (⊙,▲) immobilized glucose oxidase membrane and the MAC membrane prepared with (⊙) 1%, (▲) 2%, and (△) 3% CTA.

diffusion-limiting effect of the MAC membrane. The dynamic range can be adjusted according to the concentration of CTA used. The low solubility of CTA and the high viscosity of CTA solution make it unfavorable to use a CTA solution with concentration higher than 3%. The working range of the sensor using 2% CTA, 5–450 mg/dl, is wide enough for most clinical samples and was used for subsequent experiments in this study.

Effect of the MAC membrane to curtail responses to ascrobate and urate

The responses to ascorbate (2 mg/dl) and urate (10 mg/dl) of the bare electrode and the sensor with various membranes are shown in Figure 5. The bare electrode responded strongly to ascorbate and urate. The responses to ascorbate and urate were reduced to 21 and 39%, respectively, after the working electrode was coated with the immobilized GOD membrane. Both the responses to ascorbate and urate were reduced to zero after the working electrode was further coated with the MAC membrane. The concentrations of ascorbate and urate usually encountered in blood are 0.8–1.5 mg/dl and 4.5–8 mg/dl, respectively (Kanai and Kanai, 1983). The results obtained in this section ensured the capability of the MAC membrane to prevent the sensor from being interfered with ascorbate and urate in blood. This action effectively promoted the accuracy of the sensor when being applied to determine glucose in blood.

Characterization of the sensor

Response curves and the effect of temperature on response of the sensor. The response curves of the sensor to 100 mg/dl glucose at various temperatures are shown in Figure 6. The response intensity of the sensor increased with temperature from 2–50°C. The response intensities at 2, 10, 20, 30, 33, 40, 45 and 50°C, respectively, were 22.8, 35.3, 53.9, 77.8, 86.7, 113.1, 134.9 and 159.8% to that at 37°C. The response intensity of the sensor at lower temperature remained unchanged after being treated at higher temperature. Figure 6 also shows the time required for reaching a steady response current. At all temperatures the time needed for reaching 95% response was less than 90 seconds.

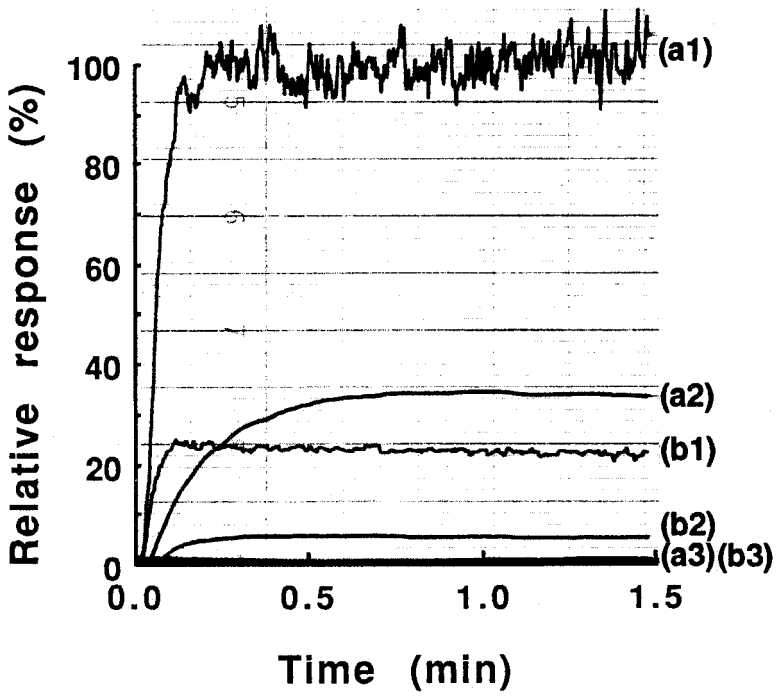

Fig. 5. *Response of the electrode to (a) 10 mg/dl urate and (b) 2 mg/dl ascorbate using (1) bare platinum, (2) platinum coated with MB-CTA, and (3) platinum coated with MAC as the working electrode. Experiments were conducted at 37 °C in PBS, pH 7.4.*

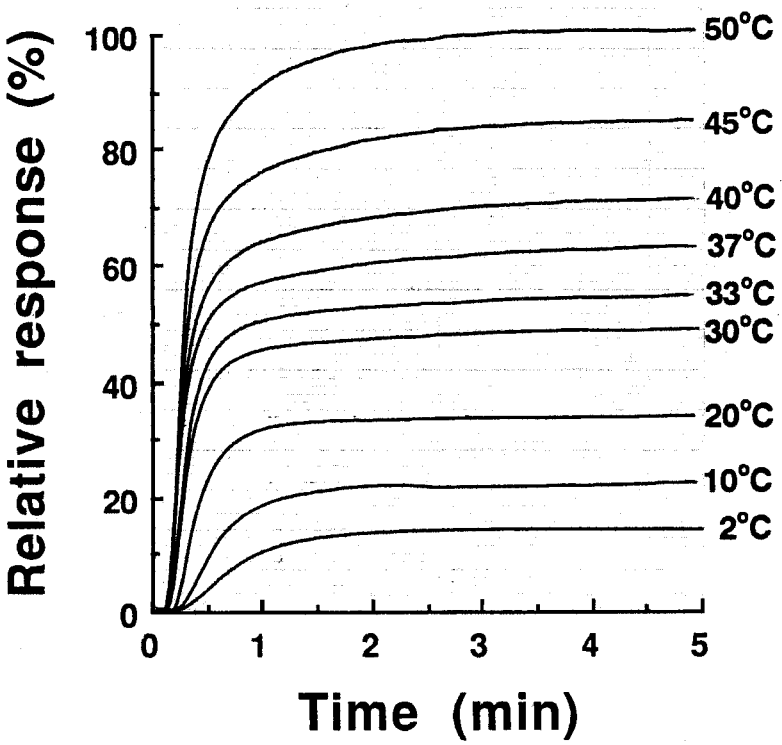

Fig. 6. *Effect of temperature on response of the glucose sensor. Experiments were conducted in PBS, pH 7.4 using 100 mg/dl glucose solution as the sample.*

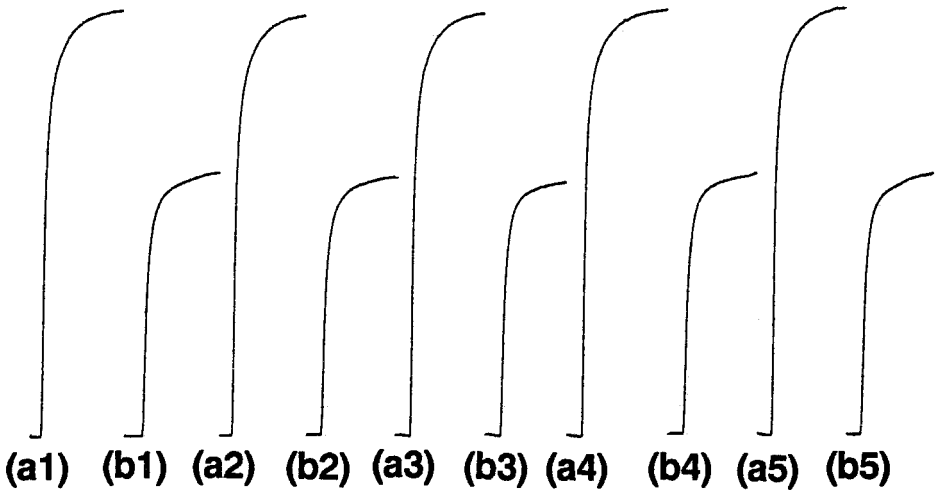

Fig. 7. Response curves of the glucose sensor at (a) 50 °C and (b) 37 °C alternatively for five repeats. Experiments were conducted in PBS, pH 7.4 using 100 mg/dl glucose solution as the sample.

Thermal stability of the sensor. Figure 7 presents the response graphs of the sensor at 50°C and 37°C alternatively for five repeats. The response graphs were almost the same during the alternative heating and cooling processes. The response of the sensor remained unchanged after being incubated in 50°C waterbath for 1 hour. In contrast, the free glucose oxidase solution lost more than 90% of its original activity under the same treatment. These results indicated the thermostability of the enzyme was significantly improved by the protective effect of the MAC membrane and the immobilization process used in this study. Experiments have been done to try to investigate the thermostability of the sensor coated with the immobilized enzyme membrane but without the MAC membrane. The PVA-SbQ membrane, however, was broken during the prolonged heating process. This made it impossible to distinguish between the immobilization process and the MAC membrane for the responsibility of improving the thermostability of the sensor.

Effects of pH and metallic ions on response of the sensor. The relative response of the sensor at various pH levels was investigated. Citrate and phosphate buffers covering the pH range from 4 to 9 were used. The optimal pH of GOD in solution is 5.6 and there is a rapid and permanent loss of activity at pHs lower than 2 or higher than 8 (Maruo 1982). The highest response of the sensor was found at pH 7.4 in phosphate buffer. The difference among responses of the sensor at pH 7.2–7.6, the pH range of human blood (Kanai and Kanai, 1983), was less than 3%. The elevation of the optimal pH from 5.6 to 7.4 enhanced the response and the sensitivity of the sensor in blood.

The effects of some metallic ions is shown in Table 1. Calcium, magnesium, iron, zinc and copper are the main metallic ions encountered in serum (Kanai and Kanai, 1983). Of these metallic ions, only calcium(II), at a concentration slightly higher than that normally found in serum, caused a reduction of 6.9% to the response of the sensor. Inhibition of immobilized GOD by silver(II) and mercury(II) has been studied previously (Liu et al., 1981). However, they caused no reduction to the response of the sensor prepared in this study. The free enzyme in solution, as a comparison, lost 86.2% of its original activity under the same concentration of Ag(I).

Selectivity, reproducibility, and stability of the sensor. Response of the sensor to various mono- and disaccharide is shown in Figure 8. This sensor did not respond to fructose, lactose and sucrose. The relative response of the sensor to maltose, galactose and mannose were less than 4% of glucose at the same concentration. Reaction of GOD with galactose, maltose and mannose has been reported (Keilin and Hartree, 1952). Sugars

Table 1. *Effect of metallic ions on response of the sensor*

Metallic ions	Metallic salts	Concentration (μM)	Relative response (%)
None			100.0
Ca^{2+}	$Ca(NO_3)_2$	2750*	93.1
Mg^{2+}	$Mg(NO_3)_2 \cdot 6H_2O$	1235*	97.0
Fe^{3+}	$Fe(NO_3)_3$	30*	98.5
Zn^{2+}	$ZnSO_4 \cdot 7H_2O$	30*	99.3
Cu^{2+}	$CuSO_4 \cdot 5H_2O$	25*	100.7
Pb^{2+}	$Pb(NO_3)_2$	25	100.0
Ag^{+}	$AgNO_3$	25	99.6
Hg^{2+}	$Hg(NO_3)_2$	25	99.2

*Slightly higher than those normally found in serum. Experiments were conducted in 20 mM imidazole buffer, pH 7.0, at 37°C.

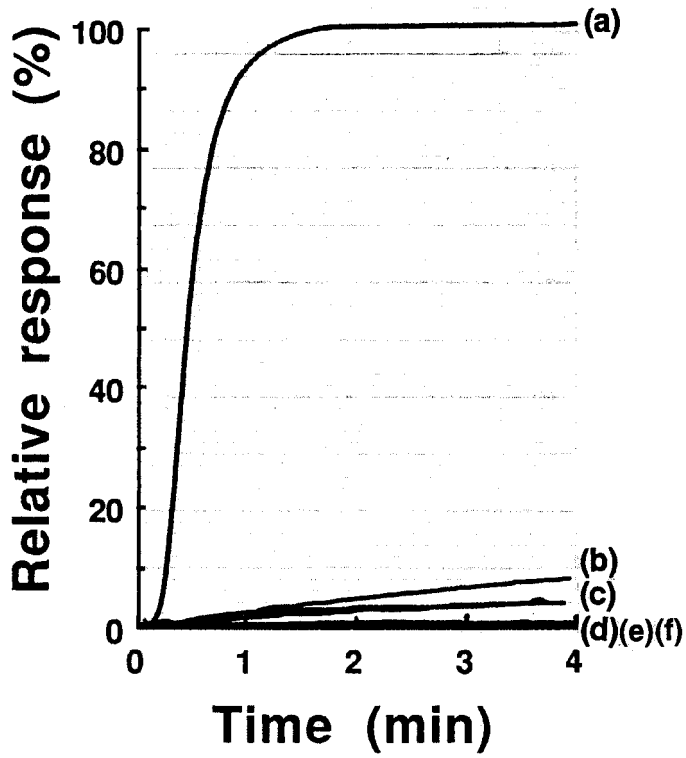

Fig. 8. *Response of the glucose sensor to 5.55 mM of (a) glucose, (b) mannose, (c) galactose, (d) fructose, (e) lactose, and (f) sucrose. Experiments were conducted in PBS, pH 7.4.*

other than glucose, however, are only present at a very low concentration in biological fluids. Therefore, any error caused by them would be negligible.

The sensor was used to assay the same glucose solution repeatedly to investigate its reproducibility. The relative error for a sample of 100 mg/dl glucose was 3.6% for 32 successive determinations.

To examine the stability in continuous determination, the sensor was located in a 37°C glass reactor containing 5 ml of the glycolyzed whole blood with 0.1% (w/v) sodium azide (NaN_3) as preservative. After 2 hours, glucose was injected to bring to a concentration of 100 mg/dl. After 16 hours, another injection was carried out. The response of the sensor was recorded continuously for 36 hours. On the other hand 10 µl samples were taken from the reactor at 2 hours' interval and analyzed by the GOD kit. The results are shown in Figure 9. The response of the sensor remained stable for 36 hours. During which period data from the sensor showed good agreement with that from GOD kit.

Determination of glucose in serum

Serum samples were assayed for glucose with both the sensor (y) and an autoanalyzer (x). Figure 10 shows the correlation between the data from the two methods. The correlation coefficient was 0.973 ($y = 0.925x + 21.133$, $n = 13$).

Conclusion

A multifunctional membrane suitable for biosensors' application that can endow the sensor with biocompatibility, a wider working range and the ability to prevent from being interfered by ascorbate and urate was prepared. The biocompatibility was mainly donated from the MPC molecule. The ability to escape from being interfered with ascorbate and urate was obtained from the negatively charged AMPS molecule. The capability to extend the working range was offered by the lower diffusion rate of glucose through CTA membrane. The combination of those constituents succeeded a membrane that can confer several good characteristics to medically applicable biosensors.

A needle-type glucose sensor using a platinum wire, an Ag/AgCl wire, and a stainless steel hypodermic needle as the working electrode, the reference electrode, and the counter electrode, respectively, was constructed. Glucose oxidase was chemically crosslinked with glutaraldehyde and then entrapped by a photocrosslinkable polyvinyl alcohol with styrylpyridinium group, PVA-SbQ. This procedure immobilized high density of the enzyme firmly in the membrane. High response and good stability of the sensor was consequently achieved. Over the immobilized enzyme membrane on the working electrode was further coated with the MAC

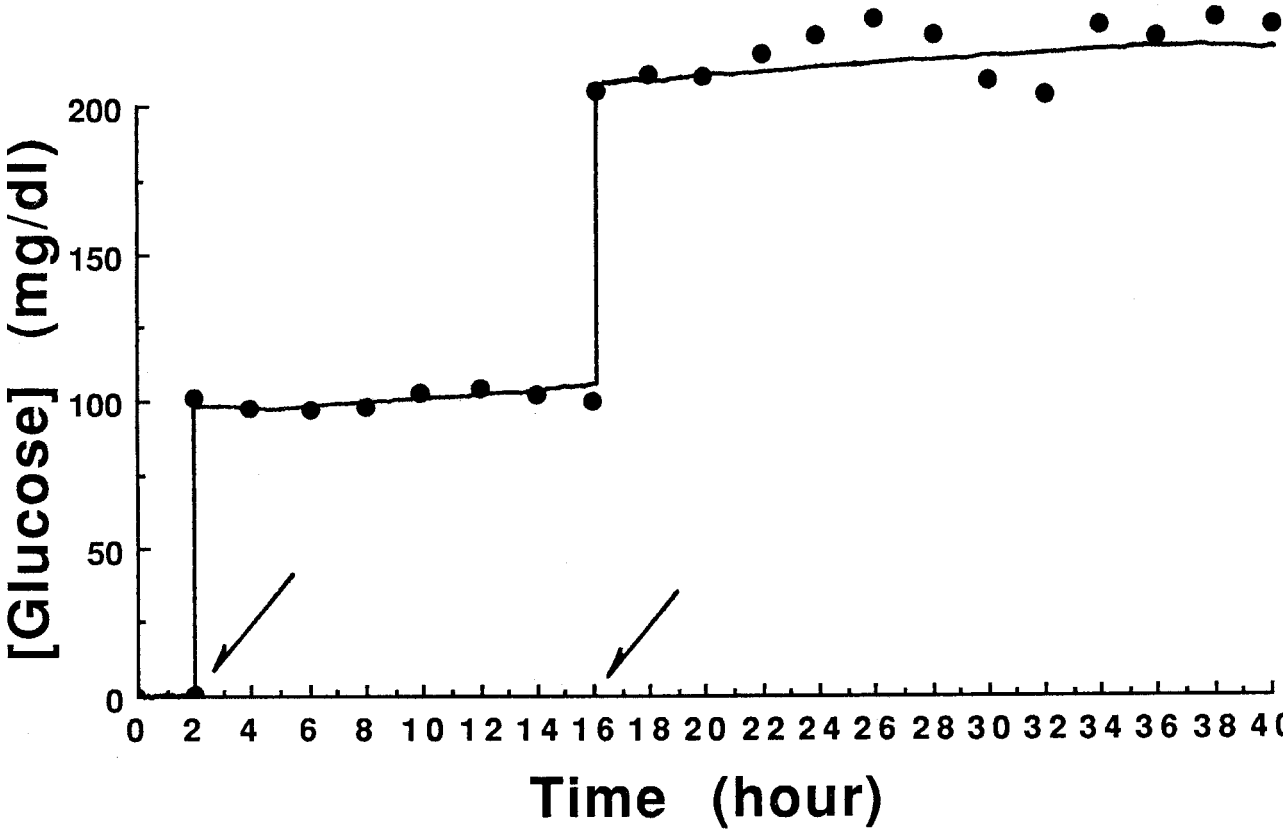

Fig. 9. Response of the glucose sensor in glycolyzed whole blood at 37°C (-) and the data from GOD assay kits (•). The arrows show the injection of 100 mg/dl glucose.

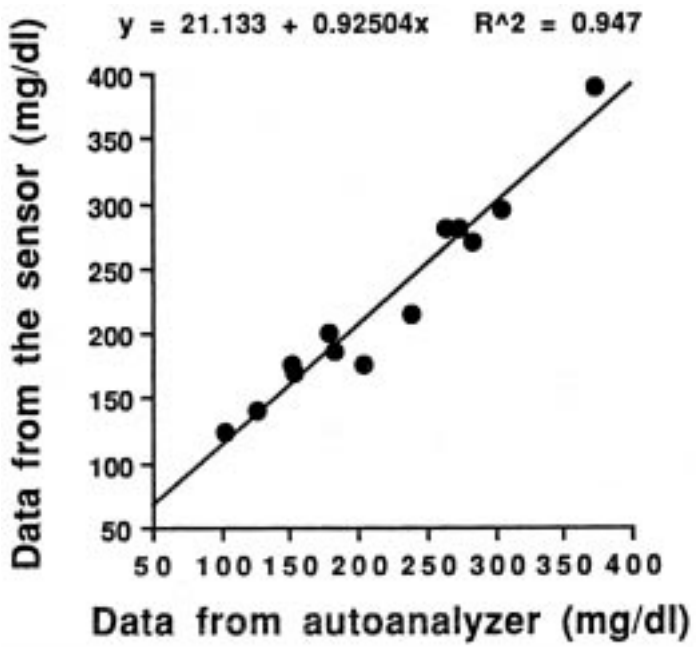

Fig. 10. Correlation between the data from the glucose sensor (y) and an autoanalyzer (x) of glucose concentration in serum samples ($r = 0.973, n = 13$).

multifunctional membrane. A miniaturized needle-type glucose sensor with characteristics suitable for medical application was thus completed.

Adsorption of blood proteins and platelet on surface of foreign materials has been reported as one of the initiation steps of blood clotting and immune reactions (Vroman and Leonard, 1977). The fact that platelet hardly adsorbs on the MAC membrane certified its blood compatibility. The MAC membrane being coated over the immobilized enzyme membrane but did not react with the enzyme is expectable to be applied to construct other biosensors.

The sensor showed characteristics such as biocompatibility, good reproducibility, good thermal stability and the ability to escape from being interfered with constitutes of blood. These characteristics revealed the possibility of the sensor to be applied for *in vivo* purposes. Those *in vivo* studies and the application of the MAC membrane to other biosensors are now progressing in this laboratory.

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Reference

L. Abe, *Bachelor Thesis* (Toho University, Japan, 1990).
A. Amine, K. Digua, B. Xie, and B. Danielson, Analytical Letters **28**, 2275 (1995).
Y. Benmairoha, I. Christie, and P. Vadgama, Analytical Communications **33**, 23 (1996).
D.S. Bindra and G.S. Wilson, Anal. Chem. **61**, 2566 (1989).
D.S. Bindra, Y. Zhang, and G.S. Wilson, Anal. Chem. **63**, 1692 (1991).
C-Y Chen, M. Goto, H. Makino, Y-C Su, E. Tamiya, and I. Karube, Anal. Chim. Acta **265**, 5 (1992).
D.J. Claremont, C. Penton, and J.C. Pickup, J. Biomed. Bng. **8**, 272 (1986).
D.J. Harrison, R.F.B. Turner, and H.P. Baltes, Anal. Chem. **60**, 2002 (1988).
K. Ichimura, J. Polym. Sci., Polym. Chem. Ed. **22**, 2817 (1984).
K. Ichimura, K. Mishima, and A. Watanabe, Yosui To Haisui **29**(8), 742 (1987).
S. Ikeda, M. Ishida, K. Ito, K. Ichikawa, T. Yukawa, K. Ohkura, A. Nakao, H. Ichihashi, T. Kondo, and H. Kojima, The Chem. Soc. Japan **3**, 507 (1987).
K. Ishihara, R. Aragaki, T. Ueda, A. Watanabe, and M. Nakabayashi, J.J. Biomed. Mater. Res. **24**, 1069 (1990b).
K. Ishihara, T. Ueda, and N. Nakabayashi, Polym. J. **22**, 355 (1990a).
I. Kanai and M. Kanai, *Clinical Laboratory Methods and Diagnosis* (Kobunsha Publishing Co. Seoul, Korea, 1983).
D. Keilin and E.F. Hartree, Biochem. J. **50**, 331 (1952).
G.F. Khan, Electroanalysis **9**, 325 (1997).
K. Kottke-Marchant, J.M. Anderson, Y. Umemura, and R.E. Merchant, Biomaterials **10**, 141 (1989).
R. Krishnan, P. Atanasov, and E. Wilkins, Biosensors and Bioelectronics **11**, 811 (1996).
E.W. Kristensen, W.G. Kuhr, and R.M. Whightman, Anal. Chem. **59**, 1752 (1987).
W. Lager, I. V-Lucadou, H. Nischik, T. Nowak, W. Preidel, L. Ruprecht, M.J. Stanzel, and V. Tegeder, Hormone and Metabolic Research **26**, 526 (1994).
T.J. Lenk, B.D. Ratner, R.M. Gendreau, and K.K. Chittur, J. Biomed. Mater. Res. **23**, 549 (1989).
C.C. Liu, F.M. Fryburg, and A.K. Chen, Bioelectrochem. Bioenerg. **8**, 703 (1981).
H. Liu, Y. Liu, J. Qian, T. Yu, and J. Deng, Microchemical Journal **53**, 241 (1996a).
H. Liu, J. Qian, Y. Liu, T. Yu, and J. Deng, Bioelectrochemistry and Bioenergetics **39**, 303 (1996b).
H. Liu and J. Deng, Biosensors and Bioelectronics **11**, 103 (1996c).
Y. Liu, H. Liu, J. Qian, J. Deng, and T. Yu, Journal of Chemical Technology and Biotechnology **64**, 269 (1995).
J. Losada, I. Cuadrado, M. Moran, C.M. Casado, B. Alonso, and M. Barranco, Analytica Chimica Acta **338**, 191 (1997).
B. Maruo and N. Tamiya, *Enzyme Handbook* (Asakura Publishing Co., Tokyo, Japan, 1982).
K. Matsumoto, H. Mizuguchi, and K. Ichimura, Kobunshi Ronbunshu **41**(4), 221 (1984).
S. Murabayashi, H. Kambic, H. Harasaki, T. Morimoto, R. Yozu, and Y. Nose, Trans. Am. Soc. Artif. Intern. Organs **31**, 50 (1985).
R. Nagata, K. Yokohama, H. Durliat, M. Comtat, S.A. Clark, and I. Karube, Electroanalysis **7**, 1027 (1995a).
R. Nagata, K. Yokohama, S.A. Clark, and I. Karube, Biosensors and Bioelectronics **10**, 261 (1995b).
K. Nishida, M. Sakakida, K. Ichinose, S. Shimoda, Y. Konno, T. Uemura, M. Uehara, M. Shichiri, K. Ishihara, and N. Nakabayashi, Japanese Journal of Artificial Organs **25**, 144 (1996).
L. Olsson, C.F. Mandenius, and J. Volc, Analytical Chemistry **62**, 2688 (1990).
D.D. Perrin and B. Dempsey, *Buffers for pH and Metal ion Control* (Kodansha Publishing Co., Tokyo, Japan, 1981).
S.F. Peteu, D. Emerson, and R.M. Worden, Biosensors and Bioelectronics **11**, 1059 (1996).
M.R. Phelps, J.B. Hobbs, D.G. Kilburn, and R.F.B. Turner, Biotechnology and Bioengineering **46**, 514 (1995).
I. Queinnec, C. Destruhaut, J.B. Pourciel, and G. Goma, World Journal of Microbiology and biotechnology **8**, 7 (1992).
C. Ritter, H. Kontschieder, A. Dolezal, and H. Marsoner, J. Scandinavian Journal of Clinical and Laboratory Investigation **56**, 129 (1996).
M. Sakakida, K. Nishida, K. Ichinose, Y. Konno, S. Shimoda, T. Uemura, M. Uehara, M. Shichiri, K. Ishihara, and N. Nakabayashi, Japanese Journal of Artificial Organs **25**, 139 (1996).
G. Spanner and R. Niessner, Gresenius' Journal of Analytical Chemistry **335**, 327 (1996a).
G. Spanner and R. Niessner, Gresenius' Journal of Analytical Chemistry **354**, 306 (1996b).
R. Steinkuhl, C. Dumschat, C. Sundermeier, H. Hinkers, R. Renneberg, K. Cammann, and M. Knoll, Biosensors and Bioelectronics **11**, 187 (1996).
S.K. Stephens, I.E. Tothill, P.J. Warner, and A.P.F. Turner, Water Research **31**, 41 (1997).
V. Thome-Duret, M.N. Gangnerau, Y. Zhang, G.S. Wilson, and G. Reach, Diabetes and Metabolism **22**, 174 (1996).

T. Ueda, K. Ishihara, and N. Nakabayashi, Kobunshi Ronbunshu **48**(1), 289 (1991).
P.J.H.J. Van-Os, A. Bult, and W.P. Van-Bennekom, Analytica Chimica Acta **305**, 18 (1995).
M.S. Vreeke and P. Rocca, Electroanalysis **8**, 55 (1996).
L. Vroman and E.F. Leonard, eds., Ann. N.Y. Acad. Sci. **283**, 65 (1977).
D. Wilke, H. Mueller, and N. Kolytsheva, Fresenius' Journal of Analytical Chemistry **357**, 534 (1997).
A. Yamasaki, C. Kim, M. Morishita, Y. Tajima, and S. Gondo, Biosensors and Bioelectronics **11**, 823 (1996).
N. Yui, K. Kataoka, Y. Sakurai, T. Aoki, K. Sanui, and N. Ogata, Biomaterials **9**, 225 (1989).