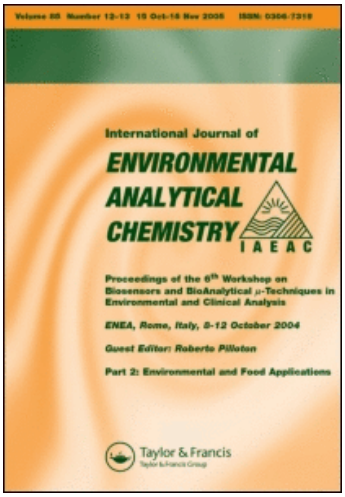

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Biochemical sniffer for odourless hydrogen peroxide vapour

Kimio Otsuka[a]; Teruyoshi Goto[b]; Hirokazu Amagai[b]; Naoko Ishii[c]; Hideaki Endo[c]; Kohji Mitsubayashi[a]
[a] Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo, Japan [b] Graduate School of Engineering, Tokai University, Hiratsuka, Kanagawa, Japan [c] Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, Minato-ku, Tokyo, Japan

To cite this Article Otsuka, Kimio , Goto, Teruyoshi , Amagai, Hirokazu , Ishii, Naoko , Endo, Hideaki and Mitsubayashi, Kohji(2006) 'Biochemical sniffer for odourless hydrogen peroxide vapour', International Journal of Environmental Analytical Chemistry, 86: 14, 1049 — 1056
To link to this Article: DOI: 10.1080/03067310600847336
URL: http://dx.doi.org/10.1080/03067310600847336

Intern. J. Environ. Anal. Chem.
Vol. 86, No. 14, 10 December 2006, 1049–1056

Taylor & Francis
Taylor & Francis Group

Biochemical sniffer for odourless hydrogen peroxide vapour

KIMIO OTSUKA†, TERUYOSHI GOTO‡, HIROKAZU AMAGAI‡, NAOKO ISHII§, HIDEAKI ENDO§ and KOHJI MITSUBAYASHI*†

†Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo, 101-0062, Japan
‡Graduate School of Engineering, Tokai University, 1117 Kitakaname, Hiratsuka, Kanagawa, 259-1292, Japan
§Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, Konan, Minato-ku, Tokyo, 108-8477, Japan

(*Received 19 December 2005; in final form 25 May 2006*)

A biochemical gas-sensor (bio-sniffer) was constructed for convenient measurement of odourless hydrogen peroxide (H_2O_2) vapour, which is harmful to skin and mucous membranes. An enzyme-immobilized membrane was fabricated by spreading the mixture of catalase and photo-crosslinkable polymer on a dialysis membrane. An H_2O_2 biosensor was constructed by attaching this catalase-immobilized membrane to the sensitive top of a Clark-type oxygen electrode, and the oxygen generation from the decomposition of H_2O_2 catalysed by catalase was measured amperometrically. This biosensor was first applied to the measurement of H_2O_2 solution and was able to quantify the concentrations of H_2O_2 solution from 0.02 to 10.0 mmol L^{-1}. Then, this biosensor was applied to gaseous phase as a bio-sniffer and was able to detect the odourless H_2O_2 vapour with the calibration range from 0.5 to 30 ppm, where the threshold limit value assigned by the American Conference of Governmental Industrial Hygienists (1 ppm) is covered.

Keywords: Hydrogen peroxide vapour; Gas sensor; Bio-sniffer; Continuous monitoring

1. Introduction

Hydrogen peroxide (H_2O_2) is a strong oxidative agent and has strong antimicrobial activity. Therefore, it is used for disinfection of food, medical, or biological equipments and for bleaching textiles, paper pulp, leather, hair, and teeth [1, 2]. Such reactivity also causes health risks such as burn or corrosion of skin, carcinogenicity, and chemical irritation of oral soft tissues with injudicious use of teeth bleach [1, 2]. Furthermore,

*Corresponding author. Fax: +81-3-5280-8094. Email: m.bdi@tmd.ac.jp

International Journal of Environmental and Analytical Chemistry
ISSN 0306-7319 print/ISSN 1029-0397 online © 2006 Taylor & Francis
DOI: 10.1080/03067310600847336

its odourless vapour causes irritation of the eyes, mucous membranes, and skin. Thus, the American Conference of Governmental Industrial Hygienists (ACGIH) has assigned H_2O_2 a threshold limit value of 1 ppm as a time-weighted average (TWA) for a normal 8 h workday and a 40 h working week [3]. This indicates the importance of monitoring the concentration of H_2O_2 vapour in the environment. H_2O_2 is also detected in expiration, and its concentration is increased in several inflammatory respiratory diseases such as asthma, chronic obstructive pulmonary disease, and cystic fibrosis [4–9]. Thus, the concentration of H_2O_2 vapour could be used in diagnosing such diseases.

In the standard method of measuring atmospheric H_2O_2 vapour concentration, airborne H_2O_2 is collected using a midget-fritted glass bubbler (MFGB) containing $TiOSO_4$, and then the concentration is measured colorimetrically [10]. More conveniently, it is measured by gas-detector tubes. Colorimetric measurement requires a rather complicated procedure and expensive and large equipment. Gas-detector tubes provide a much more convenient measurement, but frequent measurements can be costly, and the calibration range is rather narrow (e.g. 0.05–3 ppm, hydrogen peroxide 0.1 a/1, order no. 81 01041, Dräger). Furthermore, neither of these methods is adequate for continuous measurement, so a convenient and low-cost measurement system for aerial H_2O_2 monitoring is desired. Several sensors for H_2O_2 vapour have been developed with semiconductor [11] or Nafion (R) membranes [12, 13]. Sensors with a semiconductor or those based on simple electrochemical reactions tend to show a low selectivity and so are inadequate for sensing multianalyte samples such as expiratory gas or industries [14]. On the other hand, sensors with biological recognition materials such as enzymes and antibodies (e.g. for ethanol, acetaldehyde, methyl mercaptan, trimethylamine, etc.) have been constructed [15–20]. Owing to the substrate specificity of enzymes and other biomaterials, these sensors show a high selectivity. We have been developing a large number of bio-sensors and bio-sniffers (bio-sensors for gaseous phase) using enzymes as well as antibodies [17, 18, 21]. Several biosensors for H_2O_2 have fabricated with catalase and have shown good selectivity [22–28].

The odourless and colourless properties of H_2O_2 may provide a new utility for this chemical. Many coding systems and security systems are dependent on the information which is latent or coded in visible substances such as fingerprint/palm-vein biometric security systems and bar codes. If information is coded as the presence/absence or the amount of odourless and colourless volatile chemicals like H_2O_2, even if the presence of the code is hard for an outsider to determine, the security of such a coding system should be high.

In this study, we developed a simple catalase-based biosensor for H_2O_2, whereby the decomposition of H_2O_2 by the reaction

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad (1)$$

was catalysed by catalase, and this O_2 was detected by a Clark-type oxygen electrode. We used a photo-crosslinkable polymer (PVA-SbQ), which is new to catalase-based H_2O_2 sensors, to immobilize catalase. The response and calibration properties of this sensor for both liquid and gaseous phases, and its selectivity, reproducibility, and dispersion, were evaluated and the sensor was found to have a wide calibration range, including the threshold limit value (1 ppm) assigned by ACGIH.

2. Experimental

2.1 *Chemicals*

All chemicals except those mentioned in the following sections were purchased from Wako Pure Chemical Industries, Ltd (Tokyo) and were of analytical grade. Hydrogen peroxide solution (30–35%wt) was diluted with distilled water to about 1 mol L^{-1}, 0.01 mol L^{-1}, and 0.001 mol L^{-1}, and the concentration of each solution was measured colorimetrially before the experiment.

2.2 *Construction of bio-sniffer for H_2O_2 vapour*

A biosensor for H_2O_2 was constructed by loading a catalase-immobilized membrane onto an oxygen electrode. To construct an enzyme-immobilized membrane, 2.0 mg of catalase (EC1.11.1.6: from bovine liver, 5000–13,000 U mg^{-1}, No. 039-12901; Wako Pure Chemical Industries, Ltd, Tokyo) was mixed with 200 mg of photocrosslinkable polyvinyl alcohol containing stilbazolium groups (PVA-SbQ (stilbazole Quaternized), Type SPP-H-13 (Bio), Toyo Gosei Co., Ltd, Tokyo). The mixture was spread on a 3.0 cm × 9.0 cm ultrathin dialysis membrane (thickness 15 μm, No. 157-0144-02, Technicon Chemicals Co., S.A., Orcq, Belgium), which was washed with distilled water and dried on a plastic plate before spreading the mixture. The dialysis membrane was placed in the dark below 10°C for 1.0 h and then irradiated with a fluorescent light for 30 min in order to photocrosslink the PVA-SbQ, thus immobilizing the enzyme on the dialysis membrane.

Using this catalase-immobilized membrane, a bio-sniffer (biosensor for gaseous phase) for H_2O_2 vapour was fabricated as follows. The catalase-immobilized membrane was cut into *c.* 10 × 10 mm. This cut was attached to the sensitive area of a commercial Clark-type dissolved oxygen electrode (Model BO-P, ABLE Corp., Tokyo) and then secured with a silicone O-ring. This bio-sniffer detects the H_2O_2 by monitoring the changes in oxygen concentration caused by the reaction described in equation (1).

2.3 *Evaluation set-up for liquid and gaseous phases*

The response and calibration property of this H_2O_2 biosensor were evaluated first in the liquid phase. In the preparatory experiment, the effect of pH on the output of this biosensor was evaluated. As a result, the sensor output against H_2O_2 solution (1.0 mmol L^{-1}) was almost similar between pH 4.5 and 8.0. As the optimal pH for catalase was described as pH 7.0 in the specification, all experiments were carried out with a phosphate buffer of pH 7.0 and 50.0 mmol L^{-1}. Ten millilitres of phosphate buffer was filled in a 20 mL beaker, and the sensitive area of the biosensor was set in the buffer. A solution of H_2O_2 was added to the buffer with a micropipette so that the concentration of H_2O_2 would reach the set point. The sensor was provided with a fixed potential of −700 mV *versus* Ag AgCl as a counter/reference by a computer-controlled potentiostat (Model 1112, Fuso Inc., Kawasaki, Japan). This potential was chosen according to the specification of the oxygen electrode. The output current of the sensor was sent to the computer via an A/D converter

(ADC-16, Pico Technology Co., Ltd, St Neots, UK). Then, the sensor output induced by the catalase reaction (equation (1)) was continuously monitored on a computer display and saved on the hard disk for later analysis. Since the applications of this biosensor were intended to monitor environmental H_2O_2 vapour and a 'chemical coding' under ordinary conditions, all the experiments were carried out at room temperature (*c*. 20°C).

Second, the selectivity, reproducibility, and dispersion of this biosensor were evaluated. To evaluate the selectivity of this biosensor, the output currents against ammonia, lactic acid, acetaldehyde, methylmercaptan, trimethylamine, ethanol, and acetone were compared with that against H_2O_2. The experimental set-up was the same as that for the liquid phase. The concentration of each substrate was 2.0 mmol L^{-1}. The reproducibility and dispersion of this biosensor were also evaluated for the 5.0 mmol L^{-1} H_2O_2 solution.

Third, the biosensor was evaluated for H_2O_2 vapour as a bio-sniffer. Various amounts (5.0–20.0 μL) of H_2O_2 solution were placed onto a 1.0 × 1.0 cm filter paper and then put into a gas sampling bag (880 mL, SAN GIP G-4, 200 × 140 × 0.04 mm, C.I. Sanplus Co., Ltd, Tokyo). The sampling bag was filled with air and kept under room temperature for 20 min. The sensitive area of the bio-sniffer was moistened with phosphate buffer (pH 7.0, 50.0 mmol L^{-1}) and put into the sampling bag. The output current of the bio-sniffer was monitored and saved in the same manner as in the liquid phase. The concentration of the H_2O_2 vapour of the sampling bag was measured with a gas-detector tube for H_2O_2 vapour (No. 32, Gastec Corp., Kanagawa, Japan) simultaneously.

3. Results and discussion

Figure 1 shows the response (inset figure) and calibration plot of this biosensor in the liquid phase. The output current of the sensor was stable before the injection of H_2O_2 solution, increasing abruptly after injection of the H_2O_2 solution and then reaching stable state. The response time to reach 90% of the steady current after applying H_2O_2 solution was approximately 25 s. This sensor was calibrated against H_2O_2 solutions from 0.02 to 10.0 mmol L^{-1} with a high correlation coefficient of 0.999, deduced from a regression analysis of the linear plots by a method of least squares according to the following equation:

$$\text{Sensor output } (\mu\text{A}) = 2.09\left[\text{H}_2\text{O}_2\ (\text{mmol L}^{-1})\right] + 0.24. \quad (2)$$

The selectivity of the biosensor is shown in figure 2. This catalase-immobilized biosensor showed little or no response for the solution of alkali (ammonia), acid (lactic acid), acetaldehyde, malodorants (methyl mercaptan and trimethylamine), alcohol (ethanol) or organic solvent (acetone). As observed in other biosensors, this high selectivity is due to the substrate specificity of the enzyme, catalase. The high selectivity of this biosensor will be useful in avoiding any false-positive alarms in monitoring environmental H_2O_2 vapour and avoiding contamination of information when H_2O_2 and this biosensor are used for coding information.

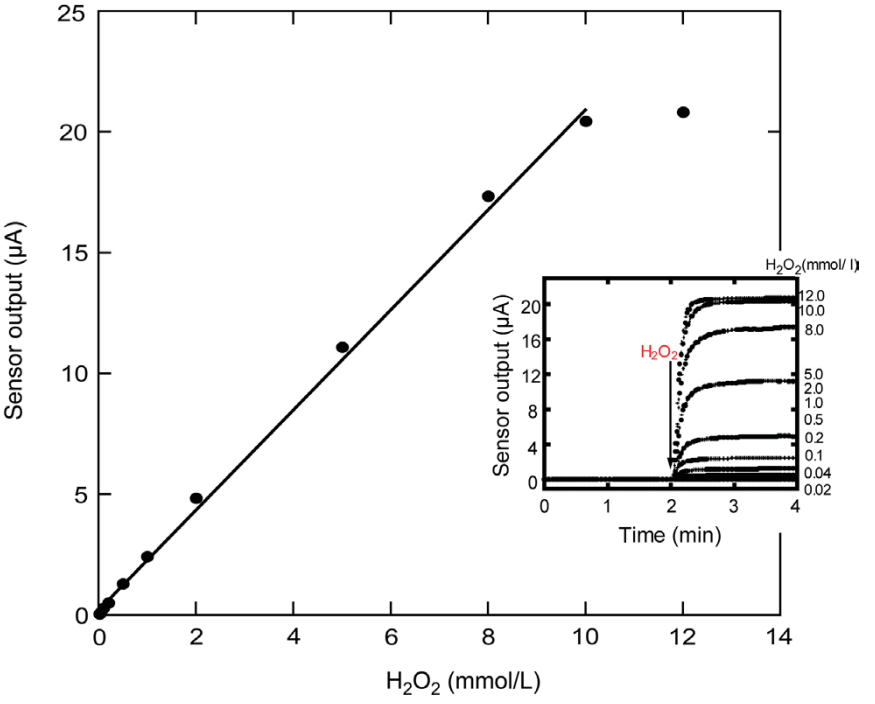

Figure 1. Typical responses of the catalase-immobilized biosensor against H_2O_2 solution (inset figure) and calibration curve.

The performance of this H_2O_2 biosensor against 5.0 mmol L^{-1} solution was reproducible over multiple measurements, showing a coefficient of variation of 1.31% (average = 10.37 μA, $n = 10$). This biosensor showed a small dispersion. The output current of six biosensors against 5.0 mmol L^{-1} of H_2O_2 solution showed a coefficient of variation of 2.73% (average = 10.18 μA, $n = 6$).

For H_2O_2 vapour, as shown in figure 2, a similar rapid response to that observed in the liquid phase was observed when this bio-sniffer was exposed to H_2O_2 vapour. The response was as fast as that for the H_2O_2 solution, and the response time to reach 90% of the steady current after applying H_2O_2 vapour was approximately 31 s. Figure 2 shows the calibration plot of this bio-sniffer against H_2O_2 vapour. This bio-sniffer showed a linear response to the concentration of H_2O_2 vapour and was calibrated against H_2O_2 vapour from 0.5 to 30.0 ppm with a correlation coefficient of 0.994, deduced from a regression analysis of the linear plots by a method of least squares according to the following equation:

$$\text{Sensor output } (\mu\text{A}) = -0.05 + 0.09\ [H_2O_2\ (\text{ppm})]. \tag{3}$$

The calibration range of this bio-sniffer covers the threshold limit value of ACGIH (1 ppm), suggesting that this sensor can provide a convenient method for monitoring the concentration of H_2O_2 in the work place. The upper limit of the calibration range (30 ppm) of this bio-sniffer was not where the output current showed saturation but was constrained by the upper limit of the detection tube. The output current increased to

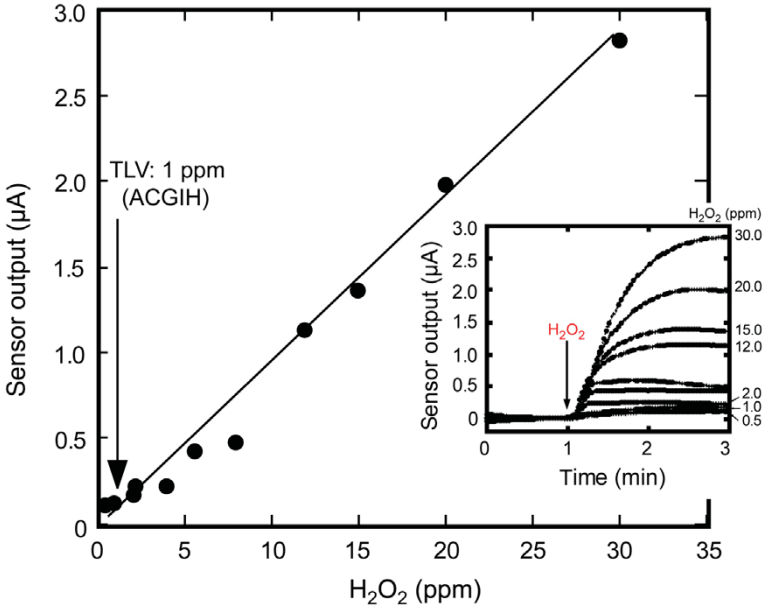

Figure 2. Typical responses of the catalase-immobilized bio-sniffer against H_2O_2 vapour (inset) and calibration plots of the catalase-immobilized bio-sniffer against H_2O_2 vapour.

Table 1. Selectivity of the H_2O_2 biosensor (showing output current against the 2.0 mmol L^{-1} solution of each substance).

Applied substances	Sensor output (%)[a]
H_2O_2	100
Ammonia	0.26
Lactic acid	0.25
Acetaldehyde	0.25
Methyl mercaptan	0.17
Trimethylamine	nr
Ethanol	nr
Acetone	nr

[a] Relative to that against H_2O_2 (H_2O_2: 100%).
nr: no response observed.

more than 10 μA in the liquid phase. When the calibration equation of this bio-sniffer was extrapolated to 10 μA, the concentration of H_2O_2 vapour increased above 110 ppm, suggesting that the calibration range of this bio-sniffer exceeds 2 magnitudes.

The biosensor for H_2O_2 with catalase showed a high selectivity and sufficient sensitivity against odourless H_2O_2 vapour, suggesting that this sensor might provide a convenient method for monitoring H_2O_2 vapour (table 1). Since the buffer added to this bio-sniffer dried out within 10 to 20 minutes, the bio-sniffer is not adequate for long-term monitoring. However, this disadvantage can be overcome by using a kind of flow-cell in which the buffer is supplied continuously to the enzyme-immobilized membrane as in some other biosensors [17]. Toniolo *et al.* developed an amperometric sensor for H_2O_2 vapour based on ion-exchange membranes (Nafion(R)) [12]. Their sensor showed

a high sensitivity (40 ppb) and was able to monitor the concentration of H_2O_2 vapour for more than 3 months, but they suggested that it might be affected by other substances such as SO_2, NO_x, and H_2S. Although our H_2O_2 bio-sniffer was not tested for these substances, the high specificity due to the enzyme might provide selectivity against these substances. The photo-crosslinkable polymer, PVA-SbQ, has been used to immobilize enzymes for various biosensors [29] and has worked well in this catalase-based bio-sniffer.

As the mechanism of such enzyme-based bio-sniffers as the H_2O_2 sniffer in this study is different from the our own olfactory mechanism, in which receptor peptides combine with chemicals, it should be possible to construct bio-sniffers for other odourless substances. Using several pairs of odourless substances and bio-sniffers with a high selectivity, a kind of 'chemical coding system' can be constructed where combinations of such odourless substances work as a code.

4. Conclusions

A biosensor for H_2O_2 vapour (bio-sniffer) was constructed with a catalase-immobilized membrane and a Clark-type oxygen electrode. This biosensor was first used to measure H_2O_2 solutions and was able to quantify H_2O_2 solution concentrations from 0.02 to 10.0 mmol L^{-1}. This biosensor also showed a high selectivity, high reproducibility, and low degree of dispersion. This biosensor was applied to the gaseous phase as a bio-sniffer and was able to detect the odourless H_2O_2 vapour within the calibration range 0.5–30.0 ppm, which covers the threshold limit value for ACGIH.

Acknowledgements

This study was supported in part by JSPS (Japan Society for the Promotion of Science) Grants-in-Aid for Science Research System, by the Hoso-Bunka Foundation Inc. Assistance Grants, and by SECOM Science and Technology Foundation for Research Grants.

References

[1] B.E. Watt, A.T. Proudfoot, J.A. Vale. *Toxicol. Rev.*, **23**, 51 (2004).
[2] L.J. Walsh. *Aust. Dent. J.*, **45**, 257, quiz 289 (2000).
[3] ACGIH. *1994–1995 threshold limit values for chemical substances and physical agents and biological exposure indices*. Paper presented at the Cincinnati American Conference of Governmental Industrial Hygienists (1994).
[4] I. Horvath, L.E. Donnelly, A. Kiss, S.A. Kharitonov, S. Lim, K.F. Chung, P.J. Barnes. *Am. J. Respir. Crit. Care Med.*, **158**, 1042 (1998).
[5] A. Emelyanov, G. Fedoseev, A. Abulimity, K. Rudinski, A. Fedoulov, A. Karabanov, P.J. Barnes. *Chest*, **120**, 1136 (2001).
[6] S.A. Kharitonov, P.J. Barnes. *Biomarkers*, **7**, 1 (2002).
[7] P.P. Rosias, E. Dompeling, H.J. Hendriks, J.W. Heijnens, R.A. Donckerwolcke, Q. Jobsis. *Pediatr. Allergy Immunol.*, **15**, 4 (2004).

[8] P. Montuschi, P.J. Barnes. *Trends Pharmacol. Sci*., **23**, 232 (2002).
[9] P. Montuschi. *Nat. Rev. Drug Discov*., **1**, 238 (2002).
[10] OSHA. *Computerized Information System*, US Department of Labor, Occupational Safety and Health Administration, Washington, DC (1994).
[11] I. Taizo, A. Sinichi, K. Kawamura. *PDA J. Pharm. Sci. Technol*., **52**, 13 (1998).
[12] R. Toniolo, P. Geatti, G. Bontempelli, G. Schiavon. *J. Electroanal. Chem*., **514**, 123 (2001).
[13] H.L. Huang, P.K. Dasgupta, Z. Genfa, J. Wang. *Anal. Chem*., **68**, 2062 (1996).
[14] K. Mitsubayashi, H. Matsunaga, G. Nishio, S. Toda, Y. Nakanishi. *Biosens. Bioelectron*., **20**, 1573 (2005).
[15] I. Karube. *Tanpakushitsu Kakusan Koso*, **30**, 277 (1985).
[16] K. Mitsubayashi, K. Yokoyama, T. Takeuchi, I. Karube. *Anal. Chem*., **66**, 3297 (1994).
[17] K. Mitsubayashi, Y. Hashimoto. *Sens. Act. B: Chem*., **83**, 35 (2002).
[18] K. Mitsubayashi, H. Matsunaga, G. Nishio, S. Toda, Y. Nakanishi, H. Saito, M. Ogawa, K. Otsuka. *Sens. Act. B: Chem*., **108**, 660 (2005).
[19] K. Mitsubayashi, Y. Kubotera, K. Yano, Y. Hashimoto, T. Kon, S. Nakakura, Y. Nishi, H. Endo. *Sens. Act. B Chem*., **103**, 463 (2004).
[20] J.H. Yu, H.X. Ju. *Anal. Chim. Acta*, **486**, 209 (2003).
[21] K. Mitsubayashi, T. Kon, Y. Hashimoto. *Biosens. Bioelectron*., **19**, 193 (2003).
[22] C.H. Fan, H.Y. Wang, D.X. Zhu, G. Wagner, G.X. Li. *Anal. Sci*., **17**, 273 (2001).
[23] L.B. Shang, Z.Y. Sun, X.W. Wang, G.X. Li. *Anal. Sci*., **19**, 1537 (2003).
[24] W.J. Zhang, G.X. Li. *Anal. Sci*., **20**, 603 (2004).
[25] Y. Xu, W.L. Peng, X.J. Liu, G.X. Li. *Biosens. Bioelectron*., **20**, 533 (2004).
[26] X. Gan, T. Liu, J. Zhong, X.J. Liu, G.X. Li. *Chembiochem*., **5**, 1686 (2004).
[27] X.J. Liu, Y. Xu, X. Ma, G.X. Li. *Sens. Act. B: Chem*., **106**, 284 (2005).
[28] H. Zhou, X. Gan, J. Wang, X.L. Zhu, G.X. Li. *Anal. Chem*., **77**, 6102 (2005).
[29] K. Mitsubayashi. *Ann. Chim. Sci. Mat*., **29**, 103 (2004).