Biosensors and Bioelectronics 24 (2009) 1877–1882

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Fabrication of a highly sensitive penicillin sensor based on charge transfer techniques

Seung-Ro Lee[a,*], M.M. Rahman[a], Kazuaki Sawada[a,b,c], Makoto Ishida[a,b,c]

[a] Department of Electrical and Electronic Engineering, Toyohashi University of Technology, 1-1, Hibari-gaoka, Tempaku-cho, Toyohashi 441-8580, Aichi, Japan
[b] Intelligent Sensing System Research Center, Toyohashi University of Technology, 1-1, Hibari-gaoka, Tempaku-cho, Toyohashi 441-8580, Japan
[c] Japan Science and Technology, CREST, 1-1, Hibari-gaoka, Tempaku-cho, Toyohashi 441-8580, Japan

ARTICLE INFO

Article history:
Received 6 June 2008
Received in revised form 20 August 2008
Accepted 11 September 2008
Available online 20 September 2008

Keywords:
Penicillin sensor
CTTPS
ISFET
ENFET
Cyclic voltammetry

ABSTRACT

A highly sensitive penicillin biosensor based on a charge-transfer technique (CTTPS) has been fabricated and demonstrated in this paper. CTTPS comprised a charge accumulation technique for penicilloic acid and H^+ ions perception system. With the proposed CTTPS, it is possible to amplify the sensing signals without external amplifier by using the charge accumulation cycles. The fabricated CTTPS exhibits excellent performance for penicillin detection and exhibit a high-sensitivity (47.852 mV/mM), high signal-to-noise ratio (SNR), large span (1445 mV), wide linear range (0–25 mM), fast response time (<3 s), and very good reproducibility. A very lower detection limit of about 0.01 mM was observed from the proposed sensor. Under optimum conditions, the proposed CTTPS outstripped the performance of the widely used ISFET penicillin sensor and exhibited almost eight times greater sensitivity as compared to ISFET (6.56 mV/mM). The sensor system is implemented for the measurement of the penicillin concentration in penicillin fermentation broth.

1. Introduction

Sensors based on field-effect principle in semiconductor structures have been extensively studied within the recent years (Caras and Janata, 1980, 1985; Van der Schoot and Bergveld, 1987; Seki et al., 1998; Hafeman et al., 1988; Osa, 1993). In this process, the enzyme molecules are immobilized on the surface of a semiconductor structure which converts the respective substrate to turn out a charged product. The product is significantly detected by an ion-sensitive surface layer of the sensor device and the resulting surface charge modules the space charge region at the insulator–semiconductor interfaces. The ENFET (Soldatkin et al., 1997), electrolyte–insulator–semiconductor (EIS) devices (Beyer et al., 1994; Menzel et al., 1995), electro-catalyst (Umar et al., 2008a,b; Rahman and Jeon, 2006; Rahman and Jeon, 2007) and light addressable potentiometric sensors LAPS (Inone et al., 1996) are three typical examples of such field-effect biosensors. The determination of various kinds of penicillin is very important in medicine, pharmaceutical production, environmental monitoring, and for biochemical process control. Recently, the potentiometric enzyme biosensors have been developed for the detection of Penicillin G, mainly for the analysis in fermentation broths (Wang, 1993; Zhong and Li, 1993; Wang et al., 1990; Li et al., 1998), where the determination of relatively high concentrations of penicillin is required. However, in many applications such as drug-control analysis of antibiotic tablets, clinical laboratories, food control, capsules and injections, the detection of small amounts of penicillin are needed.

Previously charge transfer type pH sensor based on Charge Coupled Device (CCD) technique was proposed and developed successfully by our group (Sawada et al., 1999, 2004). The ISFET type penicillin sensor is widely used (Poghossian et al., 2003). In this present work, CTTPS with high performances is proposed and demonstrated experimentally. An ISFET is an ion-sensitive field effect transistor used to measure ion concentrations in solution; when the ion concentration (such as pH) changes, the current through the transistor will change accordingly. Here, the solution is used as the gate electrode. A voltage between substrate and oxide surfaces arises due to an ions sheath. The surface hydrolyzation of OH groups of the gate materials varies in aqueous solutions due to pH value. Previously, Si_3N_4 (Cohen et al., 1978), Al_2O_3 (Chou et al., 2001, 2002), and Ta_2O_5 (Ito, 2000; Chou et al., 2000; Kosmulski, 2001) are used as typical gate materials. An ISFET's source and drain are constructed similarly as MOSFET. The gate electrode is separated from the channel by a barrier which is sensitive to hydrogen ions and a gap to allow the substance under test to come in contact with the sensitive barrier. An ISFET's threshold voltage depends on the pH of the substance in contact with its ion-sensitive barrier. An

* Corresponding author. Tel.: +81 532 44 6745; fax: +81 532 44 6757.
E-mail address: srlee@dev.eee.tut.ac.jp (S.-R. Lee).

0956-5663/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2008.09.008

ISFET and ENFET glucose sensors based on a new principle using the electrolysis of hydrogen peroxide, oxidation of glucose, and a Pt electrode actuators have been reported (Seo et al., 1997; Park et al., 2002).

Usually the glucose-sensitive ENFET is based on a local change in the pH of bio-membranes subsequent to the formation of gluconic acid (Yao et al., 2007; Luo et al., 2004a,b). The charge transfer type pH sensor, especially pH-CCD has been incorporated in two-dimensional pH image sensors (Hizawa et al., 2006). In this report, a highly sensitive penicillin sensor based on a charge transfer technique (CTTPS) has been fabricated and demonstrated. The CTTPS changed the depth of the appropriate potential using the charge transfer technique. The depth of the potential in the semiconductor is varied by detecting the change of penicilloic acid and H^+ ions produced from penicillin (with enzymatic reaction of penicillin oxidase) near the sensing region. We have demonstrated that the penicillin sensitivity of the CTTPS can be enhanced by a signal accumulation operation with phosphoric acid penicillin buffer solution. The charge accumulation operation is continued and controlled up to five cycles. It is expected that the sensor would be capable of improving the signal-to-noise ratio (SNR) (Sawada et al., 2004). As the most important characteristics of a penicillin sensor are sensitivity, linear dynamic range, span, linear detection limit, and repeatability. Hence, in the present work, two types of penicillin sensors (ISFET and CTTPS) are fabricated and studied in terms of their comparative sensitivity and performances individually.

2. Experimental

2.1. Materials

Penicilline oxidase (POx, EC 3526 Becillus cereus from Sigma, specific activity: 1650 U/mg protein), Triton X-100 (wetting agent), and penicillin were purchased from Sigma–Aldrich company and used as received without further purifications. Deionized (D.I.) water was used throughout the whole experiment for the preparation of the samples, buffers, and enzyme solutions. A 0.01 M phosphate buffer solution (PBS) was freshly prepared at pH 7.0 before performing experiments. The 0.01 M PBS was used as a supporting electrolyte by mixing solutions of KH_2PO_4 and K_2HPO_4 in appropiate proportions. POx enzyme solution was freshly prepared daily before the fabrication of the chips by dissolving the penicillin oxidase in the PBS buffer. The 0.1 M penicillin oxidase solution (25 μL) was immobilized on the sensing area of ISFET as well as CTTPS using the simple adsorption technique. Then it was dried for 30 min at room temperature. Penicillin solutions (1.56, 3.13, 6.25, 11.5, 25, 50, 100, 200, 400, and 800 mM) were prepared prior to conducting the measurements in the PBS system. For developing the ISFET and CTTPS type penicillin biosensors, all experiments were carried out into a cell containing 25.0 μL (0.01 M, pH 7.0) test solution at room temperature ($25 \pm 2\,^{\circ}C$). In the measurement, the penicillin sample was introduced into the chip of each sensor and allowed to remain for 3 min for the enzymatic reaction to take place. Finally, the output current was measured within the next 40 s.

2.2. Measurement principle

The proposed CTTPS is constructed by immobilizing the enzyme penicillinase onto the ion-sensitive membrane (Si_3N_4) of pH-CCD. The CTTPS detects variations in the H^+ ion concentration resulting from the catalysed hydrolysis of penicillin by the enzymatic reaction. The depth of the potential well in the semiconductor is varied by detecting the change of H^+ ions produced by the penicilloic acid near the sensing region, which is shown in Fig. 1A. The CTTPS changes the depth of the potential well to charge quantity by charge transfer technique (fill and spill techniques). The charge accumulation operation is continued for five "signal integration cycles" by the amount of penicillin in the sample solution, where the span and sensitivity were increased significantly.

The principle of this technique is the same as the basic principle for a CCD (Swart and Campbell, 1981). The CTTPS measurement principle is shown schematically in Fig. 1A, which is similar to that of an ISFET (Sohn et al., 1997). The CTTPS is normally based on the oxidation of penicillin according to the following reactions on the sensing regions,

$$\text{Penicillin} + H_2O \xrightarrow{\text{penicillinase}} \text{Penicilloic acid} + H^+$$

The penicillin oxidase converted penicillin to penicilloic acid, which released H^+ ions in the surrounding solution. These ions were detected by penicillin sensitive layer (Si_3N_4) on the top of the electrolyte insulator semiconductor substrates and the resulting sensor signal is measured from the penicillin concentration in the standard solution. The depth of the potential well in the semiconductor is varied by detecting the change of H^+ ions produced by the penicilloic acid near the sensing region. The proposed CTTPS is constructed by immobilizing the enzyme POx on to the ion-sensitive membrane (Si_3N_4) of the pH-CCD.

The operational procedure of the CTTPS was described using the potential diagram shown in Fig. 1B. The clockwise (indicated by arrow) cycle is initiated by turning off the output gate as shown in Fig. 1B-a, where the potential of the input diode is decreased and the electric charge is transferred into a potential well under the sensing area of the device. The potential φ of the input diode is briefly pulsed from high to low (Fig. 1B-b), then stable at higher pulse (Fig. 1B-c). The transfer gate is turned on and the charge is transferred to the floating diffusion part shown in Fig. 1B-d. The procedure from Scheme 1a to B-e is repeated several times, which is shown in B-f.

The CTTPS is operated in the signal integration mode. Charges corresponding to the penicillin concentration are transferred from a sensing part to the floating diffusion region in several times, where the signal charges are accumulated in the floating diffusion region. It is expected that the SNR of the penicillin concentration increases with accumulation of the signal charges. As the penicillin concentration signal S_{G0} is accumulated n times and the total signal S_G is described as follows:

$$S_G = nS_{G0}$$

The total noise N is described as follows:

$$N = \sqrt{N_1^2 + N_2^2 + \cdots + N_n^2}$$

where $N_1, N_2, \ldots, N_n$ are the noise component of each integration stage. If these components are the same, the above equation can be simplified as

$$N = \sqrt{nN_0^2}$$

Therefore, the total SNR is given by

$$\frac{S_G}{N} = \frac{nS_0}{\sqrt{nN_0^2}} = \sqrt{n}\frac{S_0}{N_0}$$

According to this equation, the SNR increases with $n^{0.5}$ times when the signal is integrated n times. The noise component ($1/f$) of the source follower circuit is not influenced because the input signal of the source follower circuit increases as a result of the integration (see supplemental information SI-1).

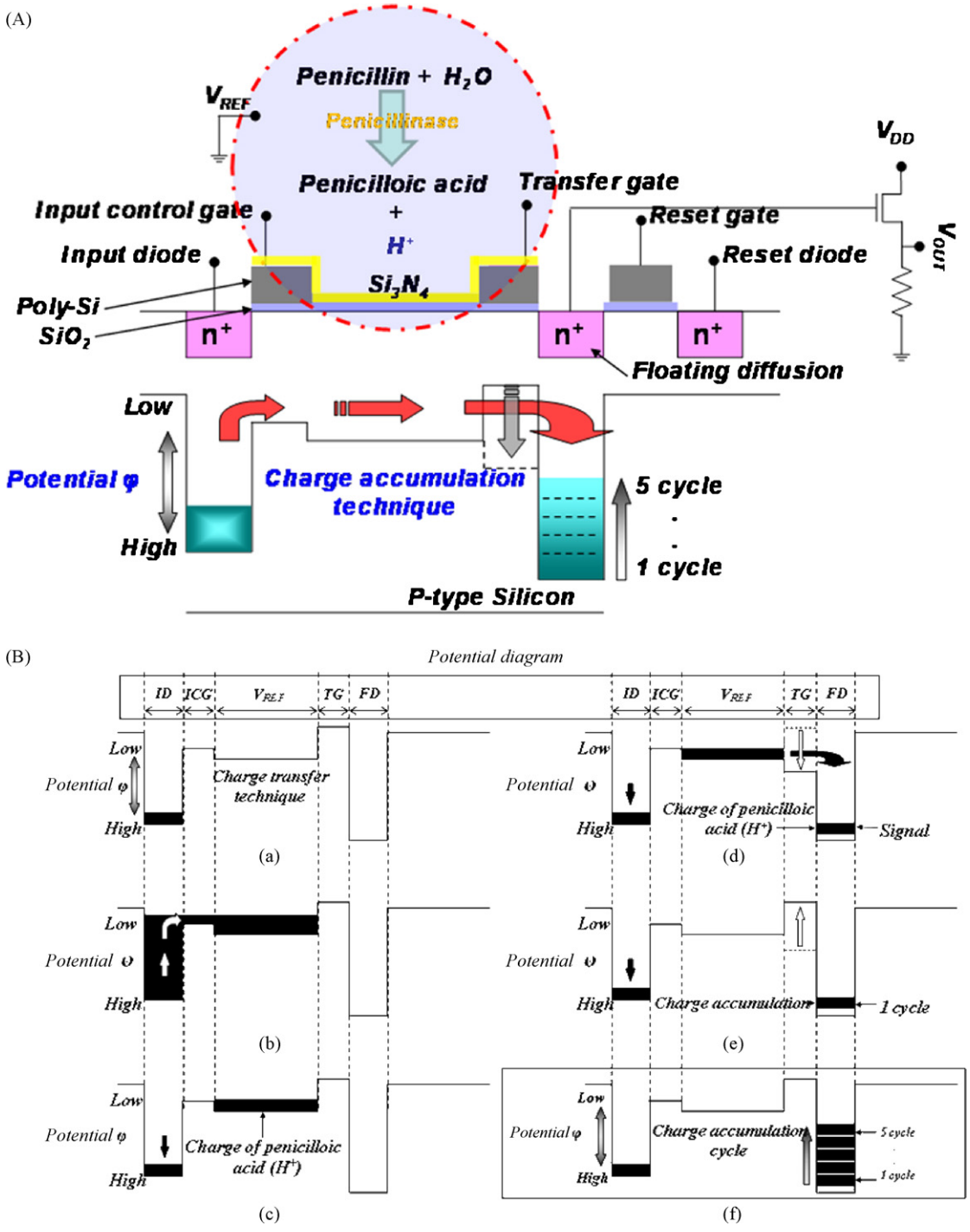

Fig. 1. (A) The schematic representation and (B) the operational procedure for the principle of the CTTPS.

2.3. CTTPS elements and structure

The CTTPS consists of seven elements, which is shown in Fig. 2A: an input diode (ID), an input control gate (ICG), an ion-sensing region, a transfer gate (TG), a floating diffusion (FD) region, a reset switch, and a source follower circuit. The penicillin-sensing part is constructed with an enzyme membrane consisting of POx/Si_3N_4 (ion-sensitive membrane)/SiO_2/p-type silicon substrate. The Si_3N_4 film acts as the penicilloic acid and H^+ ions sensitive membrane. The potential well is formed in the Si substrate surface under the sensing parts. As the concentration of the (penicilloic acid and H^+) ions in a solution is changed, the depth of the potential well is also changed. The depth of this potential well is increased due to the penicillin concentration on the sensing part is reduced. The potential of the input diode is decreased, where the electric charge is flown into a potential well under the sensing part. The depth of the potential well is determined by the value of the penicillin concentration. The ICG and TG electrodes are necessary to maintain the charge in the potential well of the ion-sensing region. The amount of charge stored in the potential well of the sensing region varies with the penicillin concentration. The stored charge in the potential well of the sensing region is transferred to the floating diffusion

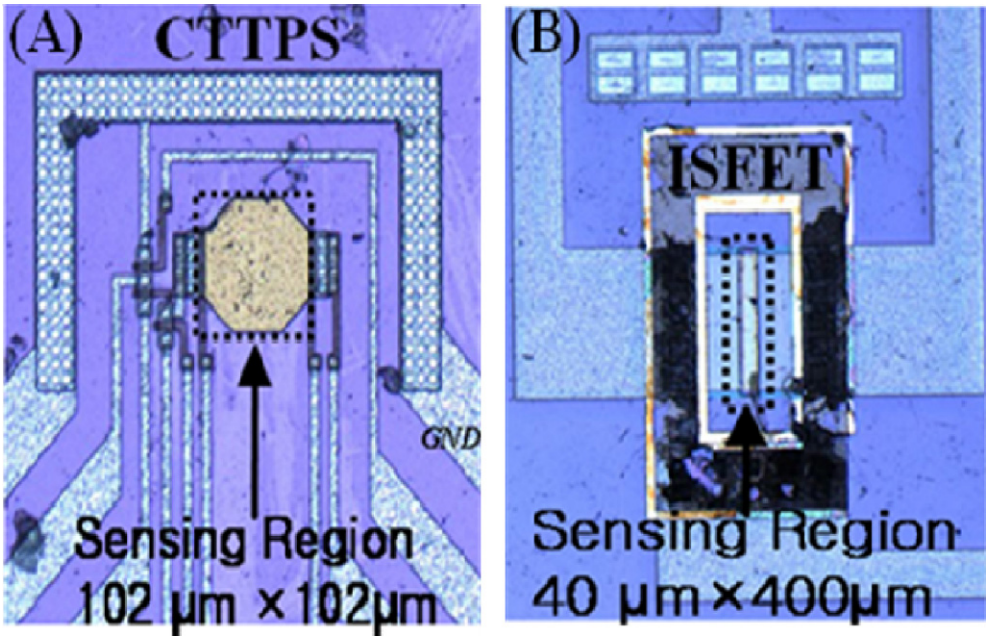

Fig. 2. (A) Photographs of the proposed charge transfer type penicillin sensor (CTTPS). (B) Photographs of the ISFET type penicillin sensor: Si_3N_4 gate size (40 μm × 400 μm; 16 000 μm^2).

region and read out using the source follower circuit, as shown in supplemental information (SI-2A).

2.4. CTTPS sensing signal amplifier

The sensing signal is amplified when the charge is transformed and read. The amplification is given in the equation below,

$$A = \frac{C_{SENS}}{C_{FD}} A_{SF}$$

where C_{SENS} is the capacitance of the sensing region, C_{FD} is the capacitance of the floating diffusion region, and A_{SF} is the gain of the source follower circuit. This procedure is repeated for five times. Each cycle is called a "signal integration cycle." As the signal integration cycle is repeated, the potential in the floating diffusion part is decreased and accumulated. By this integration operation, the potential under the ion sensing part is integrated in the floating diffusion part, which is connected to a gate electrode of the source follower circuit. Then the potential of the floating diffusion part is read from the V_{OUT} node of the source follower circuit. After the signal is measured, the floating diffusion part is rested using a reset transistor. In this study, the CTTPS is changed the depth of the potential well using the charge transfer fill and spill techniques. Since the charge accumulation operation is continued for five "signal integration cycles" the sensitivity and the repeatability are increased (see supplemental information SI-3).

3. Results and discussion

3.1. ISFET type penicillin sensor

The ISFET is a useful solid-state-type penicillin sensor. In this work, ISFET type penicillin sensors with a Si_3N_4 gate (40 μm × 400 μm; 16 000 μm^2) are designed and fabricated. A photograph of the ISFET penicillin sensor is shown in Fig. 2B. Cross-sectional view of the ISFET penicillin is shown in supplemental information (SI-1B). The enzyme membrane (POx) is immobilized onto the ISFET sensing area using the adsorption method. The drain current (I_D) is determined for the gate potential (V_G: −4 ∼ +2 V) and source-drain potential (V_D: 50 mV). The fabricated ISFET penicillin sensor has a sensitivity of about 6.56 mV/mM in the clinical concentration range of 0–12.5 mM standard penicillin solution (pH 7.0) with a lower detection limit of about 0.01 mM and an upper detection limit of about 100 mM, as shown in Fig. 3A and B, respectively. The pH sensitivity of the ISFET type penicillin sensor has a sensitivity of about 45 mV/pH in the range of 2–10 pH. The sensitivity and resolution are sufficient enough for clinical applications.

3.2. CTTPS type penicillin sensor

The CTTPS with a charge accumulation technique is fabricated using a 5-μm single–poly single-aluminum complementary metal-oxide-semiconductor (CMOS) process and is shown in Fig. 2A. The CTTPS consists of a penicillin-sensitive Si_3N_4/SiO_2/silicon structure on which a layer of the enzyme penicillinase is immobilized, so the structure of the sensiting region becomes $POx/Si_3N_4/SiO_2$/silicon. The thicknesses of the gate oxide (SiO_2) and ion-sensing membrane (Si_3N_4) are 65 and 100 nm, respectively. Si_3N_4 was deposited by thermal low-pressure chemical vapor deposition (LP-CVD). The reason why Si_3N_4 was used for the ion-sensing membrane, because it is easy to integrate with the CMOS process. The sizes of the sensing region and floating diffusion area are 10 580 and 795 μm^2, respectively. In this work, a reference electrode (Ag/AgCl) is used to fix the potential of the solution. All the measurements are carried out at room temperature. The prepared penicillin solution is measured using a pH meter (D-52, Horiba).

In the proposed CTTPS, which is accumulation method H^+ ion perception system, the quality of output signal with "signal integration cycles" is high. The fabricated CTTPS possesses a high span penicillin sensitivity of about 47.852 mV/mM in the linear concentration range from 0 to 25 mM penicillin solution, a low detection limit of about 0.01 mM, and an upper detection limit of about 200 mM as shown in Fig. 3(B) and 4. The CTTPS is better than ISFET penicillin sensor in terms of span, sensitivity, and the linear range.

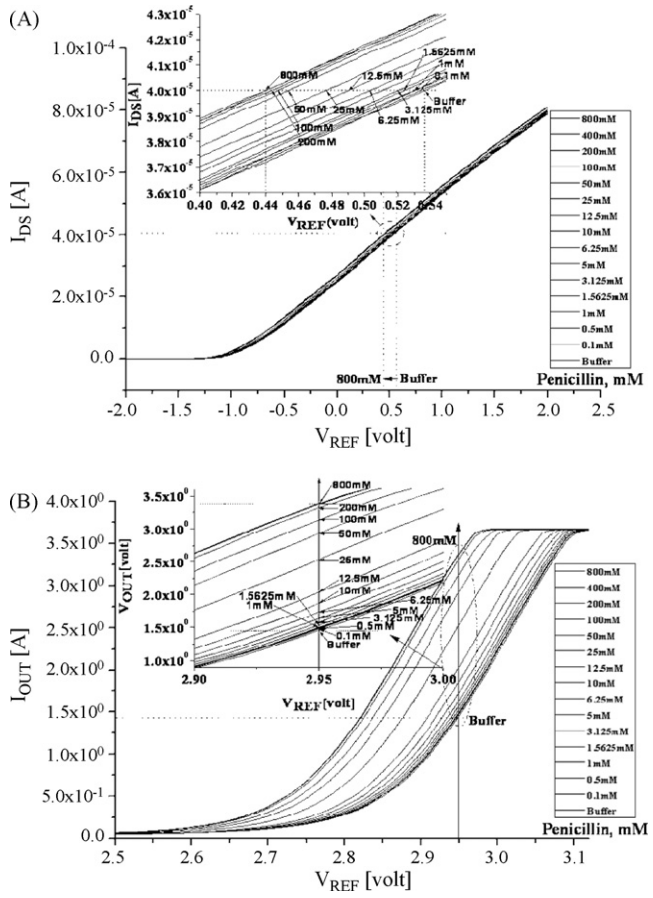

Fig. 3. (A) Characteristics of the ISFET type penicillin sensor using solutions, with penicillin concentration from buffer to 800 mM. (B) Characteristics of the proposed CTTPS using solutions, with penicillin concentration from buffer to 800 mM.

The proposed CTTPS is constructed by immobilizing the enzyme POx onto the ion-sensitive membrane (Si_3N_4) of pH-CCD, which is operated in a signal integration mode. Charges corresponding to the penicillin concentration are repeatedly transferred from a sensing part to the floating diffusion region, where the signal charges are accumulated. With the proposed CTTPS which uses an accumulation H^+ ion perception method, the quality of the output signal with five signal integration cycles is high. The CTTPS is compared with the ISFET in terms of span, linearity, and sensitivity. The sensitivity obtained from the CTTPS is 47.852 mV/mM according to Fig. 4. The sensitivity is closely followed the linear equation $Y = 0.0441X + 0.0048$ with $R^2 = 0.9961$ as shown in Fig. 4 (inset). The CTTPS type has excellent span and range compared with the ISFET type sensor, which is shown in Fig. 3A. The performances of both the fabricated penicillin sensors, presented in this paper, are summarized in Table 1. The sensitivity of CTTPS type is eight times higher compared to the ISFET type penicillin sensor. The characteristics of CTTPS are repeated 60 times in the 0–25 mM range as shown in Fig. 3B. The total non-linearity error is obtained less than 1% and excellent reproducibility has been achieved. The interferences of the fabricated CTTPS sensor assessed with biological active biomolecules have been checked.

Table 1
Specifications of the newly developed penicillin sensor (CTTPS).

Penicillin sensor	ISFET(P)	CTTPS
Lower detection limit (mM)	0.10	0.01
Upper detection limit (mM)	100	200
Span (V)	0.096	1.861
Linear range (mM)	0–12.5	0–25.0
Sensitivity (mV/mM)	6.560	47.852
Linearity, R^2	0.9875	0.9961
Response time (min)	0.5–3	0.5–3

A series of successive measurements of 0.05 M penicillin in 0.1 M PBS yielded a good reproducible signal at CTTPS sensor with relative standard deviation (RSD) of 4.9%. The sensor-to-sensor and run-to-run reproducibility for 0.05 M penicillin detection are found to be 1.34 and 1.21%, respectively. To examine the long-term storage stabilities, the response for the CTTPS sensor is examined with respect to the storage time. After each experiment, the CTTPS sensor is washed with the buffer solution and stored in a 0.1 mM PBS at 4 °C. The long-term storage stability of the sensor was tested every 5 days. The sensitivity retained 98% of initial sensitivity up to 30 days. After 30 days, the response is gradually decreased, which might have been due to the loss of the enzyme activity. The above results clearly suggested that the CTTPS sensor can be used for 1 month without any significant loss in sensitivity.

Finally, it is concluded that sensor-to-sensor, run-to-run reproducibility, and long-term storage stability for the fabricated

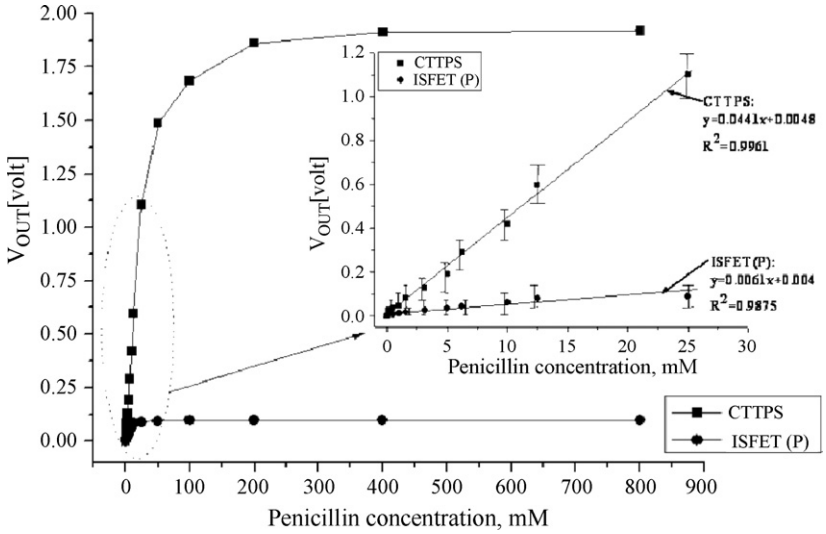

Fig. 4. Linearity of ISFET and CTTPS penicillin sensor.

penicillin sensors are excellent. The simplicity in fabrication procedures, ease of the detection step, high sensitivity, and good reproducibility of the sensor offer a good promise for practical penicillin analysis. Therefore, the CTTPS is a suitable technology in biotechnological processes as well as in food control.

4. Conclusions

An H^+ ion accumulation sensitive penicillin biosensor has been proposed and developed on the basis of ion accumulation method using charge transfer techniques. The CTTPS excels in performance over the widely used ISFET type penicillin sensor due to the stability and ion accumulation technique. The CTTPS reliability is tested by repeating the measurements 60 times in the range (0–25 mM). The obtained results are sufficient as the error ratio for the proposed CTTPS is less than 1%, which is acceptable for clinical application and implementation. With this performance, the developed CTTPS type penicillin sensor could be a new device in the healthcare field. The sensor was applied to the real sample analyses and satisfactory results were obtained. Finally, the performance of the proposed CTTPS type penicillin sensor is excellent in terms of the sensitivity, selectivity, response time, stability, and reproducibility.

Acknowledgements

This work was supported by "The Toyohashi University of Technology Global COE" Program. "Frontiers of Intelligent Sensing" Supported by Japan's Ministry of Education, Culture, Sports, Science and Technology (MEXT).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.09.008.

References

Beyer, M., Menzel, C., Quack, R., Scheper, T., Schugerl, K., Treichel, W., Voigt, H., Ulrich, M., Ferretti, R., 1994. Biosens. Bioelectron. 9, 17–21.
Caras, S., Janata, J., 1980. Anal. Chem. 52, 1935–1937.
Caras, S., Janata, J., 1985. Anal. Chem. 57, 1917–1925.
Chou, J.C., Li, Y.S., Chiang, J.L., 2001. Sens. Actuator B 80, 290–291.
Chou, J.C., Weng, C.Y., Tsai, H.M., 2002. Sens. Actuator B 81, 152–157.
Chou, J.C., Li, Y.S., Chiang, J.L., 2000. Sens. Actuator B 71, 73–76.
Cohen, R.M., Huber, R.J., Janata, J., Ure, R.W., Moss, S.D., 1978. Thin Solid Film 53, 169–173.
Hafeman, D., Parce, J., McConnell, H., 1988. Science 240, 1182–1185.
Hizawa, T., Sawada, K., Takao, H., Ishida, M., 2006. Sens. Actuator B 117, 509–515.
Inone, S., Nakao, M., Yoshinobu, T., Iwasaki, H., 1996. Sens. Actuator B 32, 23–26.
Ito, Y., 2000. Sens. Actuator B 64, 152–155.
Kosmulski, M., 2001. Sens. Actuator B 80, 292–293.
Li, J., Liang, L., Li, G., Han, R., Chen, K., 1998. Biosens. Bioelectron. 13, 1023–1028.
Luo, X.L., Xu, J.J., Zhao, W., Chen, H.Y., 2004a. Biosens. Bioelectron. 19, 1295–1300.
Luo, X.L., Xu, J.J., Zhao, W., Chen, H.Y., 2004b. Sens. Actuator B 97, 249–255.
Menzel, C., Lerch, T., Scheper, T., Schugerl, K., 1995. Anal. Chim. Acta 317, 259–264.
Osa, T., 1993. Appl. Biochem. Biotechnol. 41, 41–49.
Park, K.Y., Choi, S.B., Lee, M., Sohn, B.K., Choi, S.Y., 2002. Sens. Actuator B 83, 90–97.
Poghossian, A., Schultze, J.W., Schoning, M.J., 2003. Sens. Actuator B 91, 83–91.
Rahman, M.M., Jeon, I.C., 2006. J. Organomet. Chem. 691, 5648–5654.
Rahman, M.M., Jeon, I.C., 2007. J. Braz. Chem. Soc 18, 1150–1157.
Sawada, K., Mimura, S., Tomita, K., Nakanishi, T., Tanabe, H., Ishida, M., 1999. IEEE Trans. ED 46, 1846–1849.
Sawada, K., Shimada, T., Ohshina, T., Takao, H., Ishida, M., 2004. Sens. Actuator B 98, 69–72.
Seki, A., Ikeda, S., Kubo, I., Karube, I., 1998. Anal. Chim. Acta 379, 9–13.
Seo, H.I., Kim, C.S., Sohn, B.K., Yeow, T., Son, M.T., Haskard, M., 1997. Sens. Actuator B 40, 1–5.
Sohn, B.K., Cho, B.W., Kim, C.S., Kwon, D.H., 1997. Sens. Actuator B 41, 7–11.
Soldatkin, A.P., Gorchkov, D.V., Martlet, C., Jaffrezic-Renault, N., 1997. Mater. Sci. Eng. C 5, 35–40.
Swart, P.L., Campbell, C.K., 1981. J. Microelectron. 12, 5–10.
Umar, A., Rahman, M.M., Kim, S.H., Hahn, Y.B., 2008a. Chem. Commun., 166–169.
Umar, A., Rahman, M.M., Kim, S.H., Hahn, Y.B., 2008b. J. Nanosci. Nanotechnol. 8, 3216–3221.
Van der Schoot, B.H., Bergveld, P., 1987. Biosensors 3, 161–186.
Wang, Z.X., 1993. Sens. Actuator B 13/14, 568–569.
Wang, Z.X., Li, S., Zhong, L.C., Li, G.X., 1990. Chin. J. Biotechnol. 6, 149–156.
Yao, K., Zhu, Y., Wang, P., Yang, X., Cheng, P., Lu, H., 2007. Mater. Sci. Eng. C 27, 736–740.
Zhong, L., Li, G., 1993. Sens. Actuator B 13/14, 570–571.