



HOKKAIDO UNIVERSITY

Title	Improvement of a Phosphate Ion-selective Microsensor Using Bis(dibromophenylstannyl)methane as a Carrier
Author(s)	Satoh, Hisashi; Miyazaki, Yuji; Taniuchi, Shou et al.
Citation	Analytical Sciences, 33(7), 825-830 https://doi.org/10.2116/analsci.33.825
Issue Date	2017
Doc URL	https://hdl.handle.net/2115/67039
Type	journal article
File Information	P04 microsensor 2017 AnalSci.pdf



Improvement of a Phosphate Ion-selective Microsensor Using Bis(dibromophenylstannyl)methane as a Carrier

Hisashi SATOH,^{*†} Yuji MIYAZAKI,^{*} Shou TANIUCHI,^{*} Mamoru OSHIKI,^{**}
Rathnayake M. L. D. RATHNAYAKE,^{***} Masahiro TAKAHASHI,^{*} and Satoshi OKABE^{*}

^{*}*Division of Environmental Engineering, Faculty of Engineering, Hokkaido University, North-13, West-8, Sapporo 060-8628, Japan*

^{**}*Department of Civil Engineering, National Institute of Technology, Nagaoka College, 888 Nishikatakaimachi, Nagaoka, Niigata 940-0834, Japan*

^{***}*Department of Civil Engineering, Faculty of Engineering, University of Peradeniya, Peradeniya 20400, Sri Lanka*

An ionophore-doped sensing membrane phosphate (PO_4) microsensor based on bis(dibromophenylstannyl)methane (Bis microsensor) is described. The Bis microsensor showed a Nernstian response. The response of the Bis microsensor was log-linear down to a monohydrogen phosphate ion (HPO_4^{2-}) concentration of $0.5\ \mu\text{M}$ (corresponding to $1.0\ \mu\text{M}$ of orthophosphate at pH 7.2), whereas the detection limit of PO_4 -microsensors based on trialkyl/aryl tin chloride was $50\ \mu\text{M}$ of HPO_4^{2-} . The Bis microsensor showed excellent selectivity for HPO_4^{2-} against nitrite, nitrate, chloride, bicarbonate and sulfate, as compared with PO_4 microsensors based on trialkyl/aryl tin chloride. Dissolved oxygen, which is known to interfere with the response of a previously developed cobalt-based potentiometric solid-state PO_4 microsensor, had no effect on the response of the ionophore-doped sensing membrane-type microsensors described herein. Only OH^- (*i.e.*, pH) interfered with the ionophore-doped sensing membrane-type microsensors.

Keywords Phosphate microsensor, ionophore-doped sensing membrane, bis(dibromophenylstannyl)methane, tip diameter

(Received January 14, 2017; Accepted March 27, 2017; Published July 10, 2017)

Introduction

Phosphate is an indispensable nutrient for RNA, DNA, and cellular metabolism of all life forms,¹ and controlling the growth rates of plants and microorganisms.² Hence, it has to be supplied to crops as a fertilizer in modern agriculture, and plays an important role in the performance of water-treatment processes.^{3,4} However, applying excessive amounts of phosphate fertilizer and discharging treated or untreated wastewater carrying phosphate should be avoided because runoff of phosphate into water bodies causes eutrophication.⁵ In order to determine adequate amounts of phosphate for sustainable agriculture and reliable water-treatment processes, the mechanisms of phosphate uptake by plants and the effect of phosphate on microorganisms must be elucidated.

For a better understanding of the phosphate uptake mechanism in these processes, *in situ* monitoring of phosphate is significantly important. The intake of orthophosphate (PO_4) in microorganisms and plants, the degradation of organophosphate into PO_4 by microorganisms, the adsorption of PO_4 into soil and natural organic matters, and the release of PO_4 from soil particles, all occur on the microscale. For example, these processes occur in rhizosphere,⁶ in microbial aggregates and

biofilms,⁷ and at the sediment-water interface.⁸ Because submillimeter resolution is required for *in situ* analysis of such systems where steep concentration profiles of solutes occur,⁹⁻¹⁷ microsensors with a micrometer-sized tip are an ideal tool for *in situ* analysis. The use of microsensors has improved the understanding of many biological processes *in situ*, such as the physiology within tissues and cells of animal¹⁸ and plant species,¹⁹ and the microbial processes in sediments, microbial mats, and biofilms.⁹⁻¹⁷ The use of a PO_4 microsensor is also critical for the *in situ* analysis of PO_4 . In general, ion chromatography and spectrophotometric methods are used to determine PO_4 in bulk samples.²⁰ However, these classical methods are inadequate for *in situ* analysis due to their low spatial resolution. Lee *et al.*⁷ fabricated a cobalt-based potentiometric solid-state PO_4 microsensor and measured PO_4 microprofiles with the depths in microbial flocs taken from the enhanced biological phosphorus removal process. Although the response time of the microsensors was less than 1 min and the detection limit was $75\ \mu\text{M}$, the sensor response was inhibited by dissolved oxygen (DO) and common anions (*e.g.*, bicarbonate, chloride and sulfate). Since the DO concentration can dynamically fluctuate in environmental samples due to photosynthesis and microbial respiration,^{9,11,13} the development of a PO_4 microsensor that is insensitive to DO is critical for environmental analysis. However, the development of such a PO_4 microsensor has remained challenging. Thus far, many types of carrier-based ion-selective microsensors have been

[†] To whom correspondence should be addressed.
E-mail: qsatoh@eng.hokudai.ac.jp

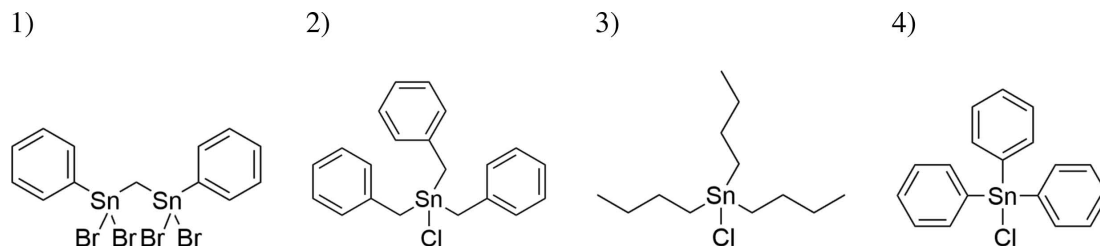


Fig. 1 Chemical drawing of the ionophores used. 1, Bis(dibromophenylstannyl)methane (Bis); 2, tribenzyltin chloride (TBeT); 3, tributyltin chloride (TBT); 4, triphenyltin chloride (TPT).

developed for *in situ* environmental analyses.^{21–24} In ion-selective microsensors based on ion carriers, the carriers are incorporated into liquid membranes. The membranes form an organic phase (called an ionophore-doped sensing membrane) that separates an aqueous sample solution from an aqueous internal filling electrolyte of the microsensor. Each carrier is capable of selectively extracting a specific ion from the aqueous sample solution into the membrane phase by acting as a translocation carrier.²¹ Ideally, ionophore-doped sensing membrane-type PO_4 microsensors should be insensitive to DO and other oxidative and reductive chemical species, and their selectivity should be improved by selecting an adequate PO_4 selective carrier.

In this paper, the construction procedure and characteristics of ionophore-doped sensing membrane-type microsensors for PO_4 are reported. Bis(dibromophenylstannyl)methane, tribenzyltin chloride, tributyltin chloride, and triphenyltin chloride were selected as PO_4 selective carriers. The performance of the microsensors, such as response, selectivity and stability, were investigated. The microsensors developed herein gave a fast response to PO_4 at low concentrations with no interference from DO. To our knowledge, this is the first report of a successful ionophore-doped sensing membrane-type microsensors for PO_4 .

Experimental

Microsensor preparation and apparatus

The basic design and preparation of a shielded ionophore-doped sensing membrane-type microsensor has been described previously.^{22,23} In short, green glass tubes (No. 8516; Schott, Mainz, Germany) were drawn to form microcapillaries and white glass tubes (AR glass, Schott) were pulled to a thickness of about 2 mm using a heating coil. The green glass was put over the tapered end of the white glass and fused using a small hot flame. The tip diameter and the tip opening of the capillary were adjusted to *ca.* 10 μm for microsensors. After the tip size was adjusted, the capillary was silanized by baking it in a 1.0-liter glass container with 0.25 mL of a silanizing agent (*N,N*-dimethyltrimethylsilylamine) at 150°C for 3 h. The capillary was then placed in a casing made from a Pasteur pipette, and the casing was glued to the capillary with epoxy glue. The filling, connection, and calibration of the ionophore-doped sensing membrane-type microsensors have been described in detail in previous papers.^{11,22,23}

Four types of solutions were prepared as ionophore-doped sensing membranes for PO_4 microsensors according to previous reports (Fig. 1). First, a solution of 2% (w/w) bis(dibromophenylstannyl)methane (synthesized by the Center for Integrated Nanotechnology Support, Tohoku University) in 2-NPOE (Bis microsensor) was prepared.²⁵ Second, a solution of 3% (w/w)

tribenzyltin chloride²⁶ (Santa Cruz Biotechnology, Inc., USA) in 2-nitrophenyl octyl ether (2-NPOE, Fluka, Switzerland) to which *ca.* 3 volumes of tetrahydrofuran (THF, Selectophore quality, Wako Pure Chemical, Japan) was added to dissolve tribenzyltin chloride (TBeT microsensor) was prepared.²⁶ Third, a solution of 1.5% (w/w) tributyltin chloride (Wako Pure Chemical) and 25 mol% (relative to the carrier) sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB, Fluka, Switzerland) in 2-NPOE (TBT microsensor)²⁷ was prepared. Lastly, a solution of 1.5% (w/w) triphenyltin chloride (Wako Pure Chemical) and 25 mol% (relative to the carrier) NaTFPB in 2-NPOE (TPT microsensor) was prepared.²⁷

To a portion of each type of ionophore-doped sensing membrane, 10% (w/w) high-molecular-weight polyvinyl chloride (PVC, Wako Pure Chemical) was added. Thereafter, *ca.* 3 volumes of THF was added to each mixture.²² According to previous reports, the filling electrolytes used were prepared as follows; 100 mM potassium chloride (Wako Pure Chemical) for Bis microsensor,²⁵ 10 mM potassium dihydrogen phosphate (Wako Pure Chemical), adjusted to pH 7.2 for the TBeT microsensor,²⁶ and 100 mM sodium chloride (Wako Pure Chemical) for the TBT and TPT microsensors.²⁷ The filling solutions were degassed under a vacuum and filtered through a 0.2- μm pore size membrane (Advantec Co., Ltd., Japan). The silanized capillaries were filled with the filling electrolyte using a Pasteur pipette. Then, under microscopic inspection, the tips were dipped in an ionophore-doped sensing membrane, and suction was applied until an ionophore-doped sensing membrane with a thickness of *ca.* 300 μm for Bis, TBT and TPT microsensors and *ca.* 1000 μm for TBeT microsensor was introduced. Additionally, *ca.* 300 μm of the PVC-containing ionophore-doped sensing membrane was sucked to solidify the membrane. Finally, the casing was filled with a 300 mM KCl solution and connected to the reference by an Ag/AgCl wire.

For potentiometric measurements of PO_4 with microsensors, a pH/mV meter (HORIBA, F-23) was used. All tests were carried out with the same Ag/AgCl reference electrode (HORIBA, 2060A-10T). The PO_4 microsensors were calibrated in dilution series of PO_4 in 100 μM acetate buffer solutions, which was referred to as standard PO_4 solutions. A 100 μM portion of acetate buffer solution at pH 7.2 was prepared to add 100 μM of acetic acid into 100 μM of sodium acetate solution until the pH reached to 7.2. The microsensors were conditioned by placing them in the 10 mM standard PO_4 solution for 30 min before use. From the pH and total PO_4 (T- PO_4) concentration, which is defined as the sum of the phosphate species (H_3PO_4 , H_2PO_4^- , HPO_4^{2-} , and PO_4^{3-}) concentrations, each phosphate species concentration was calculated using the acidic constants (K_1 , K_2 and K_3) of phosphoric acid. Values of 2.16, 7.20, and 12.35 were used for $\text{p}K_1$, $\text{p}K_2$, and $\text{p}K_3$, respectively.²⁸ The concentrations of each phosphate species were plotted against

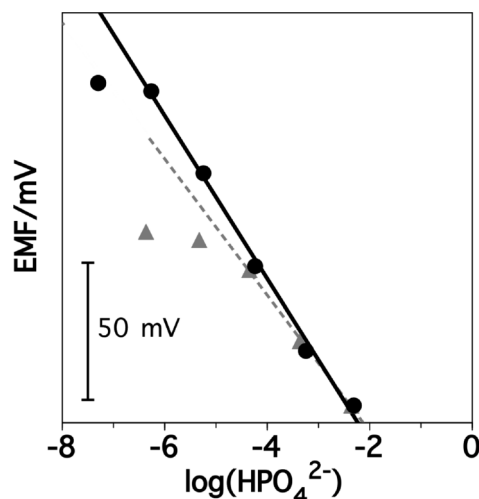


Fig. 2 Typical response curves of Bis (●) and TBeT (▲) PO₄ microsensors, plotted as EMF versus HPO₄²⁻ concentrations in a 100 μM acetate buffer solution.

the microsensor signal (the electromotive force; EMF) for calibrations. T-PO₄ concentrations were measured by the ascorbic acid method (APHA 4500-P E).²⁹

The selectivity coefficient (K_{ij}^{pot}) of the microsensors to PO₄ over other anions was quantified according to the equation:³⁰

$$K_{ij}^{\text{pot}} = e^{\frac{(E_j^0 - E_i^0)z_i F}{RT}}$$

where E_j^0 is EMF extrapolated to 1 M activity for the interfering ion (V), E_i^0 is EMF extrapolated to 1 M activity for the phosphate ion (V), z_i is the charge of the phosphate ion, F is Faraday's constant, R is the molar gas constant, and T is the absolute temperature. The EMF values for PO₄ and the interfering ion were determined in pure single electrolyte solutions at pH 7.2, which included NaHCO₃, NaNO₃, NaNO₂, Na₂SO₄, or NaCl as common interfering ions. The interfering effect of buffer solutions was investigated in standard PO₄ solutions at pH 7.2, including acetic acid, citric acid, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), or bis-Tris-HCl as the buffer components by the SSM (acetic acid) and the fixed interference method (FIM) (citric acid, HEPES and bis-Tris-HCl).

Results and Discussion

Microsensor response

Figure 2 shows typical response curves (*i.e.*, calibration curves) of Bis and TBeT PO₄ microsensors plotted as EMF versus HPO₄²⁻ concentrations in 100 μM acetate buffer solutions at pH 7.2 ± 0.1. The responses of the PO₄ microsensors were linear from 0.5 μM to 5 mM and from 50 μM to 5 mM of HPO₄²⁻, with a determination coefficient of 0.995 and 0.999, and with a slope of 29.8 mV p(HPO₄²⁻)⁻¹ for the Bis microsensor and 24.9 mV p(HPO₄²⁻)⁻¹ for the TBeT microsensor, respectively. Totally, 18 Bis microsensors have been constructed in our laboratory, among which 12, corresponding to 67%, may be suitably applicable to the measurement of environmental samples, since the slopes of their calibration curves are steep (>10 mV p(HPO₄²⁻)⁻¹). For TBeT microsensors, 61% of the

Table 1 Average responses (±standard deviation) of the PO₄ microsensors based on 4 types of ionophore-doped sensing membrane

Ionophore	<i>n</i>	Slope/mV p(HPO ₄ ²⁻) ⁻¹	Linear range
Bis	12	32.0 ± 16.7	0.5 μM to 5 mM
TBeT	48	25.5 ± 8.7	50 μM to 5 mM
TBuT	8	12.8 ± 6.4	50 μM to 5 mM
TPT	5	<5	N.D.

n: The number of sensors analyzed.

N.D.: not detected.

microsensors had a steep slope. In contrast, the slopes of the remaining 33% Bis microsensors are lower than 10 mV p(HPO₄²⁻)⁻¹, which would not be sufficient to apply these microsensors to environmental samples due to the low signal-to-noise (*S/N*) ratio.

The average responses (slopes and linear ranges of calibration curves) of the PO₄ microsensors based on 4 types of ionophore-doped sensing membrane are summarized in Table 1. The slopes of Bis and TBeT microsensors were similar to the theoretical Nernstian slope (29.6 mV (decade for a divalent ion)⁻¹), in contrast, those of TBuT and TPT microsensors were far from the theoretical one. It should be noted that the *S/N* ratio of a sensor becomes higher when the slope of its response (*i.e.*, sensitivity) is higher. Thus, it was concluded that in view of the slope of the response curve and the linear range, the best result was obtained for the Bis microsensor; therefore, the Bis microsensors were used for further analyses.

The slopes of Bis microsensors were approximately comparable to a theoretical Nernstian slope, although it is known that in practice such ideal Nernstian behavior is usually not observed for ionophore-doped sensing membrane-type microsensors.²¹ Hence, we could conclude that Bis microsensors predominantly respond to HPO₄²⁻ among various PO₄ species (*i.e.*, H₂PO₄⁻, HPO₄²⁻, and PO₄³⁻).

The various PO₄ ion-selective electrodes described so far in previous studies responded partly to HPO₄²⁻^{20,26,31–35} and partly to H₂PO₄⁻.^{27,35} Because of the possible interference by OH⁻ and the fact that the fraction of each PO₄ species is determined by the pH value of the solution,²⁸ it is not always simple to unequivocally decide based on the electrode response alone which phosphate species is being sensed. Several researchers have applied a method of varying the pH of the standard PO₄ solution while keeping the T-PO₄ concentration in the solution unchanged to determine to which species a sensor responds.^{26,31} However, we could not apply this method to the ionophore-doped sensing membrane-type PO₄ microsensors in this study because the microsensors were sensitive to OH⁻ (see below).

Selectivity

The responses of Bis microsensors to various pure solutions, including HPO₄²⁻ or each interfering anion, are shown in Fig. 3. Environmentally important anions (*e.g.*, SO₄²⁻, HCO₃⁻, Cl⁻, NO₃⁻, and NO₂⁻) were selected as the interfering anions. Among the six types of anions studied, the EMF was lowest for HPO₄²⁻. The selectivity coefficients, K_{ij}^{pot} , obtained from the results shown in Fig. 3, are summarized in Table 2. The selectivity pattern of the Bis microsensor is HPO₄²⁻ >> NO₂⁻ > NO₃⁻ > Cl⁻ > HCO₃⁻ >> SO₄²⁻. These results indicate that the microsensor is highly selective for HPO₄²⁻ over the other 5 anions. This trend was similar to ionophore-doped sensing membrane-type

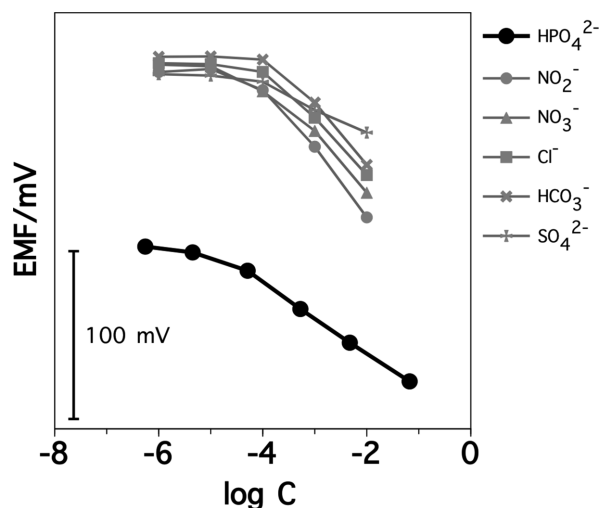


Fig. 3 Typical response curves of the Bis microsensor, plotted as the signal *versus* concentrations of HPO_4^{2-} and various interfering anions in 100 μM acetate buffer solutions.

Table 2 Average selectivity coefficients, $\log K_{ij}^{\text{pot}}$, for the Bis microsensor ($n = 4$) with comparison to literature values

	Bis microsensor	Bis(dibromophenylstannyl)methane ²⁵	Tributyltin chloride ²⁷
NO_2^-	-2.6	N.D.	-1.3
NO_3^-	-4.3	-3.55	-2.5
Cl^-	-5.4	-4.15	-1.5
HCO_3^-	-5.5	N.D.	-0.3
SO_4^{2-}	-10.0	-4.80	-2.7

N.D.: not detected.

PO_4 sensors based on bis(dibromophenylstannyl)methane (Table 2)²⁵ and bis(tribenzyltin) oxide ($\text{HPO}_4^{2-} > \text{NO}_3^- > \text{Cl}^- > \text{SO}_4^{2-}$).²⁶ K_{ij}^{pot} of the Bis microsensor and the sensors based on bis(dibromophenylstannyl)methane were much better than those of the sensors based on tributyltin chloride (Table 2). Thus, the use of bis(dibromophenylstannyl)methane as a carrier could improve the selectivity of an ionophore-doped sensing membrane-type PO_4 microsensor.

The responses of the microsensor to HPO_4^{2-} were compared at various pH (pH values of 5.0, 7.2, and 9.0). The slopes at pH values of 5.0, 7.2, and 9.0 were $30.4 \pm 2.7 \text{ mV p}(\text{HPO}_4^{2-})^{-1}$ ($n = 2$), $25.5 \pm 8.7 \text{ mV p}(\text{HPO}_4^{2-})^{-1}$ ($n = 48$), and $1.9 \pm 1.8 \text{ mV p}(\text{HPO}_4^{2-})^{-1}$ ($n = 4$), respectively. The poor response of the microsensor under alkaline conditions is due to interference from OH^- ions. Thus, the pH was an interfering factor for the Bis microsensor. The $\log K_{ij}^{\text{pot}}$ of a Bis microsensor for OH^- was determined by SSM to be 3.1. Therefore, the Bis microsensor should preferably be used under neutral pH conditions, and pH profiles should be measured with a pH microsensor²² simultaneously on a PO_4 profile measured, especially for microbial consortia in which the pH is known to gradually be changed over the depth.^{22,24} Of course, the pH profiles must be measured so as to calculate the T- PO_4 concentration from the measured HPO_4^{2-} concentration.

The use of a buffer solution is beneficial to maintain a constant pH value in the samples, and to diminish the effects of any pH change. Acetic acid, citric acid, 2-[4-(2-hydroxyethyl)-1-

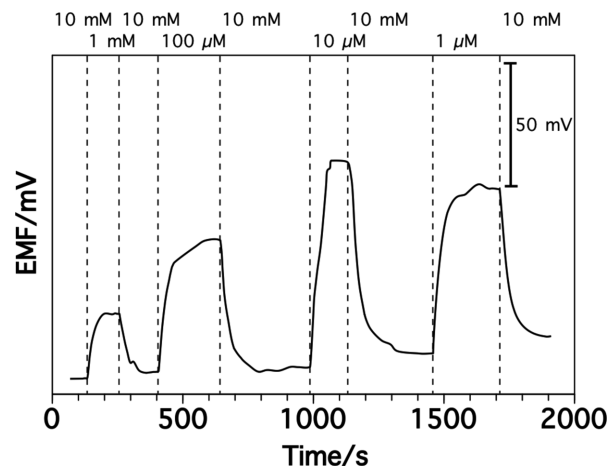


Fig. 4 Dynamic response time of the Bis microsensor for step changes in the concentration of total phosphate.

piperazinyl]ethanesulfonic acid (HEPES), and bis-Tris-HCl were selected as the buffer components, and their interfering effects were evaluated by the SSM and FIM. The microsensor did not respond to acetic acid when its concentration was lower than 1 mM. In contrast, when the Bis microsensors were calibrated against HPO_4^{2-} in the buffer solutions of 100 μM of citric acid, HEPES, or bis-Tris-HCl at pH 7.2, the detection limit increased to 100 μM of T- PO_4 , which was higher than that (1.0 μM of T- PO_4) in 100 μM acetate buffer solution (Fig. 2). The $\log K_{ij}^{\text{pot}}$ of a Bis microsensor for acetic acid, citric acid, HEPES, and bis-Tris-HCl were determined to be -0.56, 4.7, 9.8 and 10.0, respectively. Consequently, we concluded that acetate buffer solution was the best choice for a Bis microsensor.

DO inhibited the cobalt-based potentiometric solid-state PO_4 microsensor developed by Lee *et al.*⁷ In contrast, the EMF of the Bis microsensor was $74.4 \pm 2.3 \text{ mV}$ in 1 mM of T- PO_4 solution at DO concentrations of 10–70 μM (Fig. S1, Supporting Information). Theoretically, DO should have no effect on the response of an ionophore-doped sensing membrane-type microsensor.²¹ Therefore, the slight change in the EMF of the microsensor might be mainly due to potential drift.

The Bis microsensor was calibrated against hydrogen sulfide ion (HS^-) ranging from 0.5 to 50 μM in 100 μM acetate buffer solution at pH 7.1 ± 0.1 . EMF at 0.5 and 5.0 μM of HS^- was identical to those without HS^- , whereas EMF at 50 μM of HS^- decreased by about 100 mV as compared with that without HS^- . Therefore, the application of a Bis microsensor to a severe sulfidic environment^{9,17,36} should be avoided. When the microsensor was calibrated again after the exposure of 50 μM of HS^- for 10 min, the response curve was almost identical to that obtained before the exposure of HS^- , indicating that inhibition of the Bis microsensor by HS^- is a reversible process.

Dynamic response and stability

The dynamic response time is an important factor for a microsensor in terms of fast sensor response. In this study, the practical response times of a Bis microsensor were monitored by changing the T- PO_4 concentrations in the standard PO_4 solutions between 1 μM and 10 mM alternately. The change in the actual EMF over time showed that a Bis microsensor gave fast responses (Fig. 4). Generally, the 90% response time (defined as the time needed to reach 90% of the end value upon a concentration change)²¹ for the Bis microsensor was within

100 s. From the data, the potential drift of the Bis microsensor was estimated to be 10 mV h^{-1} when the microsensor was dipped in a standard PO_4 solution at 10 mM of T- PO_4 for 1 h, indicating that frequent recalibration is needed for accurate analysis.

For testing of long-term stability, a Bis microsensor was calibrated over three days after microsensor construction. The slope of the calibration curve for the microsensor was 24.5 and 24.9 mV p(HPO_4^{2-}) $^{-1}$ at the beginning of day 1 and day 3, respectively. Thus, the response of the microsensor was stable for at least 2 d, although the Y-intercept of the response curve shifted. However, some of Bis microsensors no longer responded to HPO_4^{2-} upon 5 h after microsensor construction. The reason for such a short lifetime is still unknown.

Effect of stirring

The effect of stirring on the response of a Bis microsensor to HPO_4^{2-} was investigated in standard PO_4 solutions under different flowing conditions (with and without stirring). A magnetic stirrer was used for agitation at 100 rpm. Theoretically, a flow rate should have no significant effect on the recorded EMF of ionophore-doped sensing membrane-type microsensors, except in terms of the response time.²¹ With stirring, the change in EMF was less than 1 mV (Fig. S2, Supporting Information), corresponding to a change of 3% in the HPO_4^{2-} concentration; its response time was somewhat shortened. Hence, it can be concluded that the calibration curve remained unchanged under different flow conditions. The stable response of a microsensor, independent of the hydraulic regime, is critical for *in situ* measurements of PO_4 concentrations in microbial consortia (e.g., biofilms, activated sludge flocks, and sediments) because the hydraulic regime inside and outside of them is significantly different.³⁷

Comparison between the ionophore-doped sensing membrane-type PO_4 microsensor and a solid-state PO_4 microsensor

A PO_4 microsensor, which is a cobalt-based potentiometric solid-state PO_4 microsensor, has already been developed by Lee *et al.*⁷ This microsensor showed excellent selectivity for orthophosphate ions (HPO_4^{2-} and H_2PO_4^-) under various environmental conditions. In contrast, the microsensor developed in this study is classified as an ionophore-doped sensing membrane-type microsensor. The ionophore-doped sensing membrane-type microsensor had advantages of insensitivity to DO and of no need for frequent reconditioning after corrosion over the cobalt-based potentiometric PO_4 microsensor. The interference of DO to the cobalt-based potentiometric PO_4 microsensor might restrict its application to anoxic microbial consortia. In addition, ionophore-doped sensing membrane-type microsensors are generally preferred as compared with a solid-state microsensor because they are easy to build at a lower cost. Moreover, an ionophore-doped sensing membrane-type microsensor can be made to smaller dimensions (e.g., tip diameter of approximately 1 μm) compared with a solid-state microsensor, therefore allowing it to measure concentration profiles to an extremely high spatial resolution (ca. 5 μm). On the other hand, the ionophore-doped sensing membrane-type PO_4 microsensor has the disadvantages of a short lifetime (usually a few days).

Conclusions

Ionophore-doped sensing membrane orthophosphate microsensors based on bis(dibromophenylstannyl)methane and

trialkyl/aryl tin chloride as the ion carrier could be constructed. The microsensor based on bis(dibromophenylstannyl)methane showed a Nernstian response toward HPO_4^{2-} with a detection limit of 0.5 μM of HPO_4^{2-} . The response time was shorter than 100 s, the potential drift was within 10 mV h^{-1} , and the lifetime of the microsensor was at least 2 d. The microsensor was insensitive to DO. The new ionophore-doped sensing membrane-type PO_4 microsensor described in this study improved the selectivity and detection limit, and can allow us to measure PO_4 concentration profiles in the oxic-anoxic interface in the rhizosphere, biofilms, and sediments.

Acknowledgements

This research was financially supported by Core Research of Evolutional Science & Technology (CREST) for “Innovative Technology and System for Sustainable Water Use” from Japan Science and Technology Agency (JST). A part of this work was supported by “Nanotechnology Platform” (project No. S-14-TU-0011) of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan, at the Center for Integrated Nanotechnology Support, Tohoku University.

Supporting Information

This material is available free of charge on the Web at <http://www.jsac.or.jp/analsci/>.

References

1. N. Gilbert, *Nature*, **2009**, 461, 716.
2. J. J. Hudson, W. D. Taylor, and D. W. Schindler, *Nature*, **2000**, 406, 54.
3. M. N. Shafqat and G. M. Pierzynski, *Environ. Sci. Technol.*, **2011**, 45, 8217.
4. B. E. Rittmann and P. L. McCarty, “*Environmental Biotechnology: Principles and Applications*”, **2001**, McGraw-Hill Higher Education, New York.
5. Z. Dai, J. Du, X. Zhang, N. Su, and J. Li, *Environ. Sci. Technol.*, **2011**, 45, 223.
6. P. Hinsinger, G. R. Gobran, P. J. Gregory, and W. W. Wenzel, *New Phytol.*, **2005**, 168, 293.
7. W. H. Lee, Y. Seo, and P. L. Bishop, *Sens. Actuators, B*, **2009**, 137, 121.
8. X. Ding, M. Behbahani, C. Gruden, and Y. Seo, *J. Environ. Manage.*, **2015**, 160, 193.
9. Y. Nakamura, H. Satoh, S. Okabe, and Y. Watanabe, *Water Res.*, **2004**, 38, 2439.
10. Y. Nakamura, H. Satoh, T. Kindaichi, and S. Okabe, *Environ. Sci. Technol.*, **2006**, 40, 1532.
11. S. Okabe, H. Satoh, and Y. Watanabe, *Appl. Environ. Microbiol.*, **1999**, 65, 3182.
12. S. Okabe, H. Satoh, and T. Kindaichi, *Methods Enzymol.*, **2011**, 496, 163.
13. H. Satoh, Y. Nakamura, H. Ono, and S. Okabe, *Biotechnol. Bioeng.*, **2003**, 83, 604.
14. H. Satoh, H. Ono, B. Rulin, J. Kamo, S. Okabe, and K. I. Fukushima, *Water Res.*, **2004**, 38, 1633.
15. H. Satoh, Y. Nakamura, and S. Okabe, *Appl. Environ. Microbiol.*, **2007**, 73, 1341.
16. H. Satoh, Y. Miura, I. Tsushima, and S. Okabe, *Appl. Environ. Microbiol.*, **2007**, 73, 7300.

17. H. Satoh, M. Odagiri, T. Ito, and S. Okabe, *Water Res.*, **2009**, *43*, 4729.
 18. A. Jo, H. Do, G. J. Jhon, M. Suh, and Y. Lee, *Anal. Chem.*, **2011**, *83*, 8314.
 19. N. P. Revsbech, J. P. Jacobsen, and L. P. Nielsen, *Ecol. Eng.*, **2005**, *24*, 447.
 20. M. R. Ganjali, P. Norouzi, M. Ghomi, and M. Salavati-Niasari, *Anal. Chim. Acta*, **2006**, *567*, 196.
 21. D. Ammann, “*Ion-Selective Microelectrodes*”, **1986**, Springer Berlin Heidelberg, Berlin, Heidelberg.
 22. D. De Beer, A. Schramm, C. M. Santegoeds, and M. Kühl, *Appl. Environ. Microbiol.*, **1997**, *63*, 973.
 23. A. Gieseke and D. de Beer, “*Molecular Microbial Ecology Manual*”, **2008**, Vol. 8.1.3., Springer Netherlands, Dordrecht, 3483.
 24. D. de Beer, A. Bissett, R. de Wit, H. Jonkers, S. Köhler-Rink, H. Nam, B. H. Kim, G. Eickert, and M. Grinstain, *Limnol. Oceanogr. Methods*, **2008**, *6*, 532.
 25. J. K. Tsagatakis, N. A. Chaniotakis, and K. Jurkschat, *Helv. Chim. Acta*, **1994**, *77*, 2191.
 26. D. Liu, W. C. Chen, R. H. Yang, G. L. Shen, and R. Q. Yu, *Anal. Chim. Acta*, **1997**, *338*, 209.
 27. S. Sasaki, *Talanta*, **2004**, *63*, 131.
 28. M. Benjamin, “*Water Chemistry*”, **2001**, McGraw-Hill, New York.
 29. APHA, “*Standard Methods for the Examination of Water and Wastewater*”, 21st ed., **2005**, Washington, D.C.
 30. E. Bakker, E. Pretsch, and P. Bühlmann, *Anal. Chem.*, **2000**, *72*, 1127.
 31. C. M. Carey and W. B. Riggan, *Anal. Chem.*, **1994**, *66*, 3587.
 32. T. Le Goff, J. Braven, L. Ebdon, and D. Scholefield, *Anal. Chim. Acta*, **2004**, *510*, 175.
 33. F. Kivlehan, W. J. Mace, H. A. Moynihan, and D. W. M. Arrigan, *Electrochim. Acta*, **2009**, *54*, 1919.
 34. M. Fibbioli, M. Berger, F. P. Schmidtchen, and E. Pretsch, *Anal. Chem.*, **2000**, *72*, 156.
 35. S. O. Engblom, *Biosens. Bioelectron.*, **1998**, *13*, 981.
 36. S. Okabe, T. Itoh, H. Satoh, and Y. Watanabe, *Appl. Environ. Microbiol.*, **1999**, *65*, 5107.
 37. D. De Beer, P. Stoodley, and Z. Lewandowski, *Biotechnol. Bioeng.*, **1994**, *44*, 636.
-