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Phosphate ion sensing using molecularly imprinted artificial polymer receptor

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Abstract A phosphate-selective molecularly imprinted polymer was prepared using 1-allyl-2-thiourea as a functional monomer, and a method of sensing for phosphate was suggested using an ion-sensitive field-effect transistor (ISFET) or pH meter as a transducer. The phosphate-selective polymer bound to phosphate selectively, and the ion concentration change or the pH shift in the analyte solutions after incubation with the imprinted polymer were measured in these systems. In using ISFET electrode, concentration of phosphate from 10 to 40 μM could be measured selectively.

Keywords Phosphate · Molecular imprinting · Thiourea group · Molecular recognition · Ion-sensitive field-effect transistor · pH meter

Introduction

Molecular imprinting has been known as a method of providing synthetic polymer receptors for given molecules in a “tailor-made fashion” [1–3]. The polymers prepared by this technique can be utilized for the materials of molecular recognition such as separation media in liquid chromatography [4, 5], chemosensors [6, 7], and sensor chips [8, 9]. The principle of the molecular imprinting technique is that the complex is formed between a target molecule (template molecule) and monomers capable of interacting with the template molecule (functional monomer) (Fig. 1a), followed by polymerization in the presence of a cross-linking agent (b). After removal of the template from the cross-linked polymer matrix, the functional groups in the resulting binding sites can be arranged to a suitable position for interacting with the template molecule (c).

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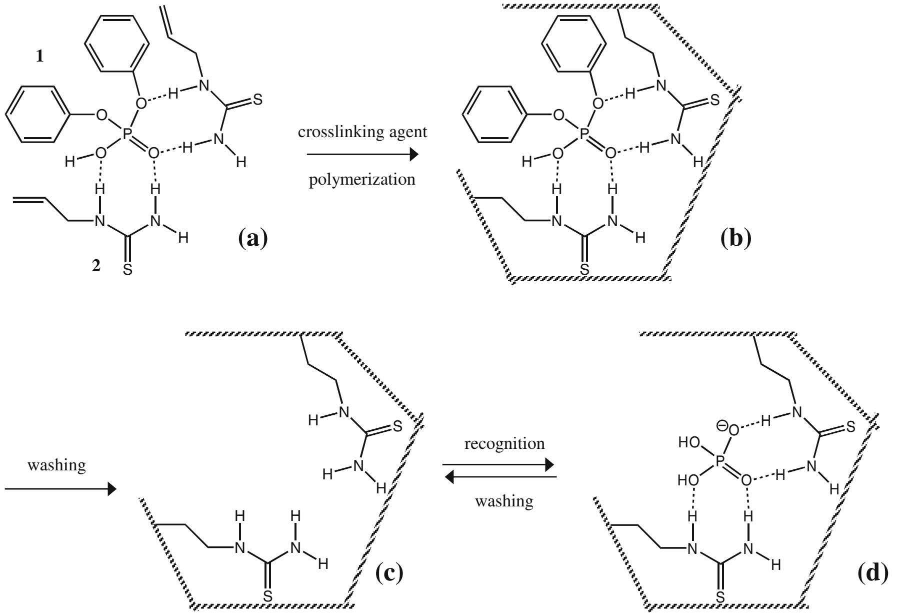


Fig. 1 Schematic illustration of molecular imprinting for phosphate. **a** Diphenyl phosphate **1** (template) and 1-allyl-2-thiourea **2** (functional monomer) were mixed in acetonitrile (solvent); **b** Polymerization with addition of ethyleneglycol dimethacrylate (EGDMA; crosslinking agent); **c** Resulting in formation of a complementary binding site for phosphate; **d** Rebinding of phosphate to the imprinted polymer

The analysis or recovery of environmental load substances would be a useful and desired application for molecularly imprinted polymers. Phosphate is a cause of eutrophication in storage dams, sewage treatment plants, rivers, and lakes, and is also a cause of the odor of mold during the summer season. We have reported that phosphate-selective polymers with thiourea groups at the binding sites can be prepared by the molecular imprinting technique and that they show high selectivity for phosphate in aqueous media; in addition, 1-allyl-2-thiourea is a good functional monomer for phosphate compounds imprinting [10]. The methods for the recovery of phosphate have also been demonstrated previously [11, 12]. In this study, a sensing system for phosphate was suggested that uses a phosphate-selective polymer as a molecular recognition material and an ion-sensitive field-effect transistor (ISFET) or pH meter as a transducer. The response and the selectivity of the sensors were evaluated in aqueous media.

Experimental

Materials and preparation of imprinted polymer

1-Allyl-2-thiourea was purchased from Aldrich (St. Louis, MO, USA). Ethyleneglycol dimethacrylate (EGDMA), diphenyl phosphate, acetonitrile, and 2,2′-azobisisobutyronitrile were purchased from Wako Pure Chemicals (Osaka, Japan).

EGDMA and acetonitrile were distilled before use, and other chemicals were commercial reagents of the highest grade and used without further purification. ISFET electrode and equipment was purchased from BAS (Tokyo, Japan), and the pH meter WM-50EG was purchased from DKK-TOA Corp. (Tokyo, Japan).

The phosphate-selective polymer was prepared as follows; diphenyl phosphate (2.50 g, 10.0 mmol), 1-allyl-2-thiourea (11.6 g, 100 mmol), EGDMA (19.8 g, 100 mmol), and 2,2′-azobisisobutyronitrile as an initiator (200 mg, 1.2 mmol) were dissolved in acetonitrile (40.0 mL). The reaction mixture was degassed by sonication and then polymerized at 55 °C for 16 h, followed by heating at 80 °C for 3 h under a nitrogen atmosphere. The polymer was ground and sieved to yield a particle size of 80 µm, and the polymer particles were washed, first with a mixture of 500 mL of the methanol and triethylamine solvent (4:1, v:v), and then with methanol for 24 h by soxhlet extraction equipment [11].

Evaluation of ISFET sensor response

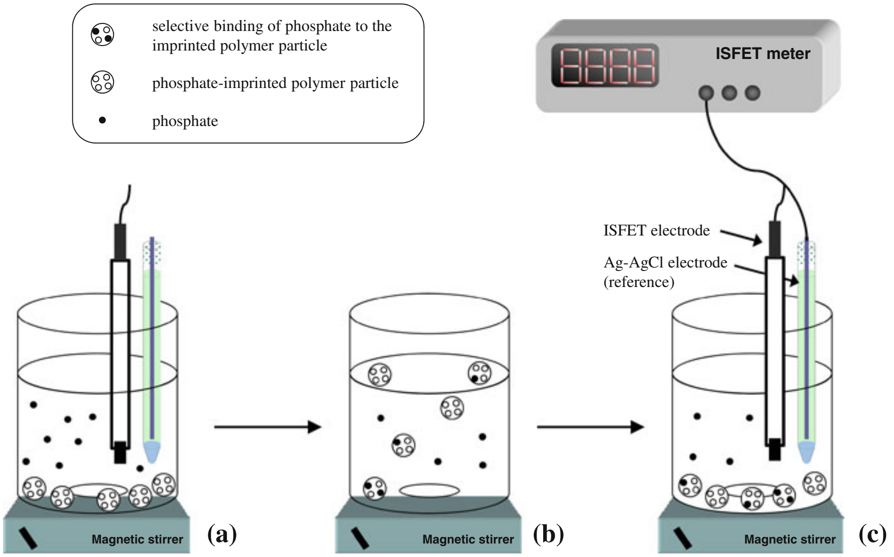
The ISFET electrode was placed in the ISFET Meter and an Ag–AgCl electrode was used as the reference electrode. These sensor electrodes were immersed in the 10 mL of each anion solution (NaH_2PO_4, NaF, NaCl, NaBr, $NaNO_2$, $NaNO_3$; each concentration was 20 µM) in distilled water. The solution was incubated for 30 min with 200 mg of imprinted polymer particles with continuous stirring at room temperature. The beaker was left for a short while and polymer particles were precipitated. The system enables the measurements of the source-gate voltage (V_{sg}), and the drain current (I_d) was set at 10 µA. The value of source-gate voltage (V_{sg}) was measured each before and after incubation with the polymer particles. The schematic illustration of the evaluation of sensor response is shown in Fig. 2. All measurements were carried out at room temperature. The response of the sensor was evaluated in triplicate.

Evaluation of pH electrode response

Polymer particles (200 mg) were mixed with 10 mL of each anion solution (NaH_2PO_4, NaF, NaCl, NaBr, $NaNO_2$, $NaNO_3$; each concentration was 20 µM) in distilled water. The solution was stirred for 30 min with magnetic stirrer at room temperature. The beaker was left for a short while and polymer particles were precipitated. The pH electrode was placed in the pH meter WM-50EG (DKK-TOA Corp., Tokyo, Japan), and the sensor electrode was immersed in the supernatant described above. The pH values were measured each before and after incubation with the polymer particles. All measurements were carried out at room temperature and the response of the sensor was evaluated in triplicate.

Results and discussion

We have reported the phosphate-selective polymers prepared by molecular imprinting technique, and the imprinted polymers prepared with 1-allyl-2-thiourea



ISFET measurement before stirring polymer particles

Stirring

ISFET measurement after stirring polymer particles

Fig. 2 Evaluation of the ISFET sensor response. **a** ISFET and reference electrodes were placed in the ISFET meter and immersed in the analyte solution; **b** The solution and phosphate-selective polymer particles were stirred for 30 min; **c** Phosphate-selective polymer bound to phosphate selectively, and response of the ISFET sensor was evaluated. The system enables the measurements of the source-gate voltage (V_{sg}) and the drain current (I_d) was set at 10 μA

as the functional monomer showed high affinity for phosphate in aqueous media [10–12], and the dissociation constant, K_D, was estimated to be 10.3 μM. For the preparation of phosphate-selective polymer, diphenyl phosphate was used as the template molecule, instead of orthophosphoric acid because of the low solubility of orthophosphoric acid in the mixture solution of acetonitrile and EGDMA, but the imprinted polymer showed high affinity and selectivity for phosphate with no matter. Therefore, the use of diphenyl phosphate as a template molecule would be available for the preparation of phosphate-selective polymer. The time-dependent binding ability for phosphate in the phosphate-selective polymer has been also evaluated and more than 90% of phosphate bound to the polymer within 30 min, so the 30 min stirring time was selected.

In this article, the sensing systems for measuring phosphate in the combination of the molecularly imprinted phosphate-selective polymer as the molecular recognition material with ISFET or pH meter as the transducer were examined.

Evaluation of ISFET sensor response and pH change

An ISFET electrode can be fabricated in miniature using semiconductor technology and used to measure ion concentrations in solution. The change in ion concentrations is proportional to the phase boundary potential, and the ISFET electrode has been applied to biosensor technology [13, 14] and the sensors with molecularly imprinted polymer as the molecular recognition element [15].

In this article, the phosphate-selective polymer particles were incubated with analyte solutions. Because of the specific binding of phosphate to the phosphate-selective polymer, the phosphate concentrations in the solution decreased and, thereby, the pH shifted to alkaline in the supernatant. These changes in the supernatant were measured by an ISFET device or a pH meter in this system.

The source-gate voltage (V_{sg}) of the ISFET electrode was increased in the response of phosphate binding to the phosphate-selective polymer. The change of the value of source-gate voltage (ΔV_{sg}) was subtracted the value of after incubation with the polymer from the value of before incubation and is shown as the vertical axis, and the calibration curve for phosphate is shown in Fig. 3. The horizontal axis represents the initial concentration of phosphate. A linear relationship was obtained from 10 to 40 μM phosphate ($r^2 > 0.984$). A selectivity test was also conducted, and the ISFET sensor showed a selective response to phosphate, while the response to other analyte anions (NO_3^-, NO_2^-, F^-, Cl^-, and Br^-; each concentration was 20 μM) was lower (Table 1). The blank polymer which was prepared without a template molecule was used for the evaluation of the imprint effect and used as the control. The response to the sensor incubated with the blank polymer was remarkably low to each anion (Table 1). From the results of imprinted polymer and

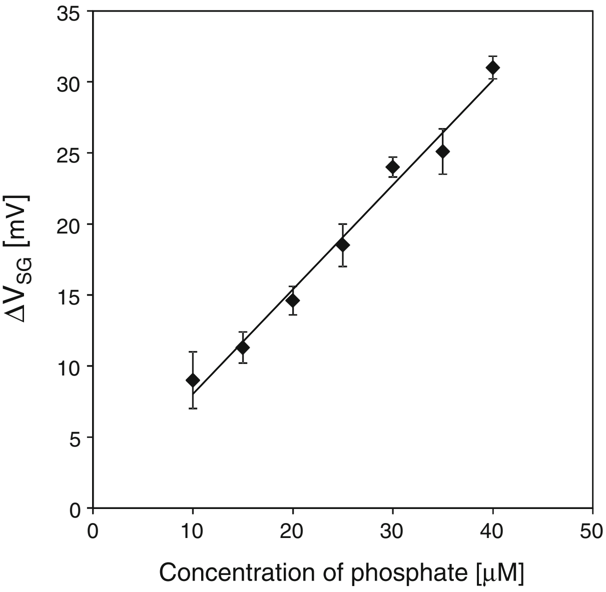


Fig. 3 Calibration curve in the ISFET sensor for phosphate. ISFET sensor electrodes were immersed in the 10 mL of each concentration of phosphate in distilled water. The solution was incubated for 30 min with 200 mg of imprinted polymer particles with continuous stirring at room temperature. The beaker was left for a short while and polymer particles were precipitated. The drain current (I_d) was set at 10 μA. The horizontal axis represents the initial concentration of phosphate. The data represent average values of three measurements and the standard deviation is indicated by *error bars*

Table 1 Response of the ISFET sensor to phosphate and reference compounds

	Source-gate voltage change of the ISFET (ΔmV)					
	$H_2PO_4^-$	NO_3^-	NO_2^-	F^-	Cl^-	Br^-
Imprinted polymer	14.5	6.3	1.2	3.0	1.1	5.6
Blank polymer	2.7	3.3	1.5	2.5	0.8	2.7

ISFET sensor electrodes were immersed in the 10 mL of each 20 μM anion solution (NaH_2PO_4, NaF, NaCl, NaBr, $NaNO_2$, $NaNO_3$) in distilled water. The solutions were incubated for 30 min with 200 mg of polymer particles with continuous stirring at room temperature. The beaker was left for a short while and polymer particles were precipitated. The drain current (I_d) was set at 10 μA. The response of the sensor was evaluated in triplicate

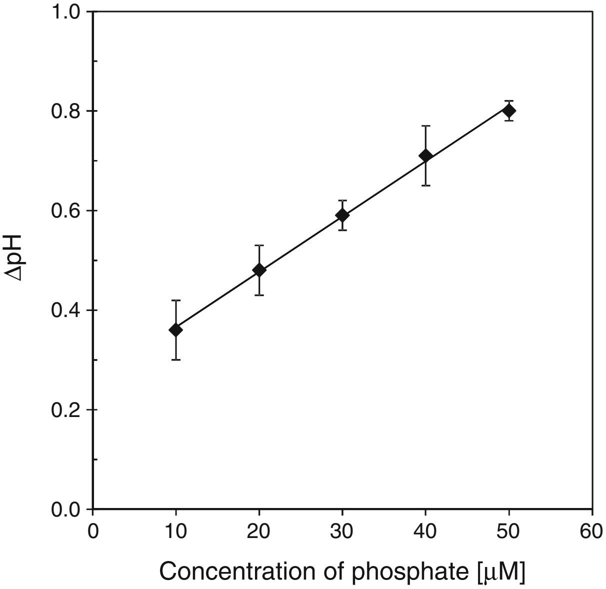


Fig. 4 pH change in the pH sensor for phosphate. Polymer particles (200 mg) were incubated with 10 mL of each concentration of phosphate in distilled water for 30 min with continuous stirring at room temperature. The beaker was left for a short while and polymer particles were precipitated and the supernatant was analyzed by pH meter. The pH shifts of analyte solutions after incubation with the polymer particles were measured. The horizontal axis represents the initial concentration of phosphate. The data represent average values of three measurements and the standard deviation is indicated by *error bars*

blank polymer, the binding activities of the polymers were related to the source-gate voltage of the ISFET sensor.

We also examined other approach, the pH shift of the supernatant before and after incubation with the imprinted polymer was also evaluated with the use of pH meter, because of the specific binding of phosphate to the phosphate-selective polymer, the phosphate concentrations in the solution decreased and the pH shifted to alkaline in the supernatant. The calibration curve is shown in Fig. 4 and from 10 to 50 μM of phosphate could be measured quantitatively ($r^2 > 0.998$). Selective

Table 2 pH shift in the sensing system for phosphate and reference compounds

	pH shift (ΔpH)					
	$H_2PO_4^-$	NO_3^-	NO_2^-	F^-	Cl^-	Br^-
Imprinted polymer	0.48	0.24	0.11	0.09	0.04	0.22
Blank polymer	0.13	0.12	0.09	0.07	0.03	0.11

Polymer particles (200 mg) were incubated with 10 mL of each 20 μM anion (NaH_2PO_4, NaF, NaCl, NaBr, $NaNO_2$, and $NaNO_3$) in distilled water for 30 min with continuous stirring at room temperature. The beaker was left for a short while and polymer particles were precipitated and the supernatant was analyzed by pH meter. The pH shifts of analyte solutions after incubation with the polymer particles were measured

response was also shown in the pH measurement system (Table 2). Phosphate selectively bound to the phosphate-selective polymer, by the imprint effect, which is the molecular recognition element of the sensor, and we thus demonstrated an effective sensing system for measuring phosphate.

Conclusions

The sensing systems in combination with phosphate-selective polymer as the molecular recognition material and ISFET or pH meter as the transducer were demonstrated, and the phosphate concentrations were measured based on the change in ion concentrations in the analyte solution before and after incubation with the phosphate-selective polymer. By the selective binding of the phosphate-selective polymer, the phosphate concentrations decreased and the pH shifted to alkaline in the supernatant, and concentrations of phosphate could be measured selectively. The measureable range demonstrated in this study is satisfactory for the measurement of environmental waters. The development of methods for long-term measurement of phosphate concentrations is very much desired in storage dams, sewage treatment plants, rivers, and lakes. The imprinted polymer has good stability and could be supplied at low cost and in large quantities. We are now continuing development of an easy sensing system based on the combination of imprinted polymer with ISFET electrode. Thereby the methods utilizing molecularly imprinted polymers as recognition elements would be valuable for future environmental analysis in future.

References

1. Vlatakis G, Andersson LI, Muller R, Mosbach K (1993) Drug assay using antibody mimics made by molecular imprinting. Nature 361:645–647
2. Wulff G (1995) Molecular imprinting in cross-linked materials with the aid of molecular template. Angew Chem Int Ed Engl 34:1812–1832
3. Haupt K (2003) Imprinted polymers—tailor-made mimics of antibodies and receptors. Chem Commun 2:171–178
4. Sambe H, Hoshina K, Hosoya K, Haginaka J (2005) Direct injection analysis of bisphenol A in serum by combination of isotope imprinting with liquid chromatography-mass spectrometry. Analyst 130:38–40

5. Moller K, Nilsson U, Crescenzi C (2001) Synthesis and evaluation of molecularly imprinted polymers for extracting hydrolysis products of organophosphate flame retardants. J Chromatogr A 938:121–130
6. Turkewitsch P, Wandelt B, Darling GD, Powell WS (1998) Fluorescent functional recognition sites through molecular imprinting. A polymer-based fluorescent chemosensor for aqueous cAMP. Anal Chem 70:2025–2030
7. Wandelt B, Mielniczak A, Cywinski P (2004) Monitoring of cAMP-imprinted polymer by fluorescence spectroscopy. Biosens Bioelectron 20:1031–1039
8. Kugimiya A, Takeuchi T (2001) Surface plasmon resonance sensor using molecularly imprinted polymer for detection of sialic acid. Biosens Bioelectron 16:1059–1062
9. Matsui J, Akamatsu K, Nishiguchi S, Miyoshi D, Nawafune H, Tamaki K, Sugimoto N (2004) Composite of Au nanoparticles and molecularly imprinted polymer as a sensing material. Anal Chem 76:1310–1315
10. Kugimiya A, Takei H (2006) Preparation of molecularly imprinted polymers with thiourea group for phosphate. Anal Chim Acta 564:179–183
11. Kugimiya A, Takei H (2008) Selectivity and recovery performance of phosphate-selective molecularly imprinted polymer. Anal Chim Acta 606:252–256
12. Kugimiya A, Takei H (2008) Selective recovery of phosphate from river water using molecularly imprinted polymers. Anal Lett 41:302–311
13. Satoh I, Karube I, Suzuki S, Aikawa K (1979) Neutral lipid sensor based on immobilized lipase and a flow-through pH electrode. Anal Chim Acta 106:369–372
14. Gotoh M, Tamiya E, Karube I, Kagawa Y (1986) A microsensor for adenosine-5′-triphosphate pH-sensitive field effect transistors. Anal Chim Acta 187:287–291
15. Pogorelova SP, Bourenko T, Kharitonov AB, Willner I (2002) Selective sensing of triazine herbicides in imprinted membranes using ion-sensitive field-effect transistors and microgravimetric quartz crystal microbalance measurements. Analyst 127:1484–1491