

# Microfluidic Polymer Chip with an Embedded Ion-Selective Electrode Detector for Nitrate-Ion Assay in Environmental Samples

Takashi MASADOME,\*† Kazuki NAKAMURA,\* Daisuke IJIMA,\* Osamu HORIUCHI,\*  
Benjaporn TOSSANAITADA,\* Shin-ichi WAKIDA,\*\* and Toshihiko IMATO\*\*\*

\*Department of Applied Chemistry, Faculty of Engineering, Shibaura Institute of Technology,  
Toyosu, Koto, Tokyo 135-8548, Japan

\*\*Health Device Research Institute, Human Stress Signal Research Center (HSS), National Institute of  
Advanced Industrial Science and Technology (AIST), 1-8-31 Midorigaoka, Ikeda, Osaka 563-8577, Japan

\*\*\*Department of Applied Chemistry, Graduate School of Engineering, Kyushu University,  
Fukuoka 819-0395, Japan

A nitrate ion-selective electrode (NO<sub>3</sub><sup>-</sup>-ISE) has been developed based on tetradodecylammonium bromide as an anion exchanger and 2-nitrophenyl octyl ether as a plasticizer. The NO<sub>3</sub><sup>-</sup>-ISE shows an almost Nernstian response to nitrate ion over a concentration range between  $1.0 \times 10^{-6}$  and  $1.0 \times 10^{-1}$  M, with an anionic slope of  $-57.7 \pm 0.7$  mV/decade. The selectivity coefficients of the NO<sub>3</sub><sup>-</sup>-ISE for nitrate ion against chloride and sulfate ( $\log k_{\text{NO}_3^-, \text{Cl}^-}^{\text{pot}} = -2.42$  and  $\log k_{\text{NO}_3^-, \text{SO}_4^{2-}}^{\text{pot}} = -4.33$ ) were obtained. A microfluidic polymer chip was fabricated using polystyrene plates and stainless-steel wires as a template for the channel. The microfluidic polymer chip is composed of a mixing chip and a NO<sub>3</sub><sup>-</sup>-ISE detector chip. The microfluidic polymer chip, integrated with a NO<sub>3</sub><sup>-</sup>-ISE detector consisting of the NO<sub>3</sub><sup>-</sup>-ISE and a Na<sup>+</sup>-ISE as a reference electrode, showed an almost Nernstian response to nitrate ion over a concentration range between  $1.0 \times 10^{-5}$  and  $1.0 \times 10^{-1}$  M, with an anionic slope of  $-54.3 \pm 1.3$  mV/decade. The microfluidic polymer chip was then applied to the determination of nitrate ion in environmental water samples, such as a tap water, a well-water sample and water for agricultural use.

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## Introduction

Nitrates are widely contained in a tap water, soil, groundwater, seawater, surface water, rain water, food and so on. The most pervasive source of contamination by nitrate for water supplies is fertilizer runoffs to streams, and dairy farm feces that leach into groundwater.<sup>1,2</sup> The determination of nitrate in aqueous solutions is very important because nitrate ingestion above a critical level causes significant clinical problems, such as methemoglobinemia in infants, bladder cancer, gastric cancer, and leukocyte enzyme abnormality.<sup>2-4</sup> The determination method of nitrate ions, based on spectrometry,<sup>5-9</sup> fluorometry,<sup>10,11</sup> atomic absorption spectrometry<sup>12,13</sup> and ion chromatography,<sup>14</sup> requires expensive instrumentation. Furthermore, the procedures used in these methods are time-consuming and complicated. Nitrate ion-selective electrodes (NO<sub>3</sub><sup>-</sup>-ISE) and nitrate ion-sensitive field effect transistors (NO<sub>3</sub><sup>-</sup>-ISFETs) provide an attractive analytical method for the determination of nitrate ion, because the procedures are simple, and applied to colored and turbid samples.<sup>15-27</sup>

The microfluidic chip technologies,<sup>28-52</sup> such as integrated chemical analysis systems, micro total-analysis systems ( $\mu$ -TAS)

and "Lab-on-a-Chip", have attracted much attention because of a decreased use of reagents and lower sample volumes. However, the corresponding detection systems still remain large. Microfluidic chips integrated with potentiometric electrochemical sensors, such as ISEs and ISFETs, are expected to be candidates for realizing integrated chemical analyzing systems,  $\mu$ -TAS and Lab-on-a-Chip because the sensitivity of the ISEs and ISFETs does not depend on their size. To date, several potentiometric miniaturized ISEs have been reported. However, only a few studies regarding microfluidic chips with embedded miniaturized ISEs and ISFETs have been reported. For example, Oki *et al.*<sup>45,46</sup> have reported on the production of ISFET-based healthcare chips fabricated on a polyethylene terephthalate plate and based on a micromolding technique for the determinations of pH, Na<sup>+</sup> and K<sup>+</sup>. The use of a micromachining process for microfluidic chips is useful for mass production, but such a process is very complicated for chip design studies. Hassan *et al.*<sup>28</sup> reported a miniature chip with an all-solid state miniaturized NO<sub>3</sub><sup>-</sup>-ISE. However, the microfluidic chip on system uses a conventional Ag/AgCl reference electrode. Ibanez-Garcia *et al.*<sup>29</sup> reported potentiometric determinations of ammonium and nitrate ions using continuous-flow analytical microsystems based on low-temperature co-fired ceramic technology. Although liquid ion-exchanger and screen-printed reference electrodes were employed in the system, the microsystems were not applied to the determination of nitrate

† To whom correspondence should be addressed.  
E-mail: masadome@sic.shibaura-it.ac.jp

ion in real water samples. The lower detection limit of the microsystems for nitrate ion was  $7.9 \pm 0.2$  ppm (*ca.*  $1.6 \times 10^{-5}$  M).

Bachas *et al.*<sup>30</sup> reported on the microfabrication of screen-printed nanoliter vials with embedded surface-modified electrodes and its application to  $\mu$ -TAS for the potentiometric determination of nitrate ion. However, the  $\text{NO}_3^-$ -ISE showed a slope of  $-45.4 \pm 0.9$  mV/decade for the nitrate ion, and the detection limit was  $4 \times 10^{-4}$  M for the nitrate ion.

We have already developed microfluidic chips integrated with ISFETs fabricated on polystyrene plates by using stainless-steel wire as a template for the channel, for the determination of ammonium ion and ionic surfactants.<sup>53-55</sup> In this case, we used an Ag/AgCl wire electrode as a reference electrode in the polymer-based microfluidic chip, and carrier solutions containing  $1.0 \times 10^{-2}$  M chloride ion were also used for the determination of a baseline potential. The Ag/AgCl wire electrode responds to the chloride ion in the carrier solution. However, common nitrate-ISEs may not have sufficient selectivity to the chloride ion. If a carrier solution containing a high concentration of chloride ion is used for the determination of  $\text{NO}_3^-$  ion by a polymer-based microfluidic chip with embedded  $\text{NO}_3^-$ -ISE, the chloride ion will interfere with the response of the  $\text{NO}_3^-$ -ISE. Therefore, it is very important to find other reference electrodes for applying our microfluidic chip system integrated with ISFETs to the determination of nitrate ion. Furthermore, ISE is fabricated in a cheaper way than the ISFET.

In this research, we developed a new microfluidic polymer chip with embedded  $\text{NO}_3^-$ -ISE and  $\text{Na}^+$ -ISE as a reference electrode using a small-diameter wire as a template for the channel. Furthermore, the microfluidic chip that we developed was applied to the determination of environmental water samples, such as a tap water, a well water and water for agricultural use.

## Experimental

### Chemicals

Tetradodecylammonium bromide (TDAB) from Sigma-Aldrich and tetradecylammonium bromide (TAB) from Tokyo Chemical Industry Co., Ltd. were used as the anion exchangers for  $\text{NO}_3^-$ -ISEs. Sodium tetrakis[3,5-bis(trifluoromethyl)phenyl] borate (TFPB) used as the cation exchanger and bis(12-crown-4) (B12C4) used as a  $\text{Na}^+$  ionophore for a  $\text{Na}^+$ -ISE were obtained from Dojindo Laboratories. Poly(vinyl chloride) (PVC) (degree of polymerization: 1100) was obtained from Wako Pure Chemicals Co. 2-Nitrophenyl octyl ether (NPOE) from Dojindo Laboratories and 2-nitrophenyl dodecyl ether (NPDE) from Fluka were used as a plasticizer for  $\text{NO}_3^-$ -ISEs and  $\text{Na}^+$ -ISE. The purities of the NPOE and NPDE were more than 99.0%, according to the manufacturer's specifications. 2-Nitrophenyl phenyl ether (NPPE), used as a plasticizer, was obtained from Kanto Chemical Co., Ltd. All other reagents were of guaranteed grade.

### Fabrication of $\text{NO}_3^-$ -ISEs, $\text{Na}^+$ -ISEs and measurement of the potential response in a batch system

NPOE (2.0 mL), PVC (0.4 g) and an anion exchanger ( $2.0 \times 10^{-4}$  mol) were dissolved in 10 mL of tetrahydrofuran (THF) in a beaker. The coating of polyethylene at *ca.* 1 mm from the tip of BEAMEX-S wire (Standard type irradiated polyethylene wire from Furukawa Electric Co. Ltd.) was torn off. The resulting bare metal part of the tip of the wire was washed with ethanol, water and ethanol. Next, the washed wire was dried.

A  $\text{NO}_3^-$ -ISE was fabricated by coating the bare metal part of the BEAMEX-S wire with the THF solution several times. The fabricated  $\text{NO}_3^-$ -ISE was preconditioned with a  $10^{-1}$  M  $\text{KNO}_3$  solution for 1 day before use. A sensing membrane solution for  $\text{Na}^+$ -ISE was fabricated by dissolving NPOE (0.20 mL), TFPB (0.0046 g), B12C4 (0.010 g) and PVC (0.10 g) in 3.0 mL of THF solution. The preparation of the  $\text{Na}^+$ -ISE was performed in the same manner as that of the  $\text{NO}_3^-$ -ISE by using the BEAMEX-S wire.

The response of the  $\text{NO}_3^-$ -ISE and  $\text{Na}^+$ -ISE was examined by measuring the emf of the following electrochemical cell with a potentiometer (Model COM-20R, TOA-DKK, Tokyo) at room temperature.



A 1 M  $\text{CH}_3\text{COOLi}$  solution was used as a salt bridge solution for measuring the emf, because  $\text{CH}_3\text{COO}^-$  ion will not interfere with the response of  $\text{NO}_3^-$ -ISE compared with the  $\text{Cl}^-$  ion; also, a negligible junction potential will be generated for the  $\text{CH}_3\text{COOLi}$  solution due to the fact that the mobilities of  $\text{Li}^+$  ion and  $\text{CH}_3\text{COO}^-$  ion are almost identical.

The determination concentration range was defined as the Nernstian response concentration range. The lower detection limit was defined as the primary ion activity at which the potential deviated by 18 mV from extrapolation of the linear portions of the calibration curve. The emfs were measured for 12 min for one sample solution. We adopted emf values of which the variation with time reached 0.5 mV/min. In general, a stable emf was obtained within 5 min, and the variation of emf with time was very small. The reproducibility of the potential response was checked by the standard deviation of the average slope values obtained with multiple calibration ( $n = 3$ ).

### Microfluidic polymer chip integrated with a $\text{NO}_3^-$ -ISE detector and flow system for measuring nitrate ion

The microfluidic polymer chip is composed of a mixing chip and an ISE detector chip based on a  $\text{NO}_3^-$ -ISE, as shown in Fig. 1. The mixing chip consisted of a sample reservoir, a sample injection channel (a), a carrier solution channel (b) and a mixing channel. Channels of the polymer-based microfluidic chip were fabricated using polystyrene plates (ITEM 70128 500, Tamiya Co. Ltd., Japan) and stainless-steel wires (SUS304-W1, Waki Industrial Co. Ltd., Japan) according to almost the same method.<sup>41,53-55</sup> The chip design of a polymer-based microfluidic chip detector with an embedded  $\text{NO}_3^-$ -ISE and a  $\text{Na}^+$ -ISE as a reference electrode was similar to that shown in a previous paper.<sup>53-55</sup> However, the ISE embedded in the chip was improved to a cartridge-type ISE. The cartridge-type ISE can be easily replaced by a new ISE, in the case that the ISE shows a lower performance during long-term use. Figure 2 shows the schematic structure of the microfluidic chip with a  $\text{NO}_3^-$ -ISE based flow system. Nitrate ion solutions as the sample solution and a buffer solution as a carrier solution were flowed to the sample injection channel and the carrier solution channel in the microfluidic polymer chip with a syringe pumps (MD-1001, Ailis Co. Ltd.) at a flow rate of  $20 \mu\text{L min}^{-1}$ , respectively. A  $0.02 \text{ M KH}_2\text{PO}_4 + 0.0133 \text{ M Na}_2\text{HPO}_4$  phosphate buffer solution (pH 6.7), a  $1.77 \times 10^{-2} \text{ M}$  sodium citrate +  $1.42 \times 10^{-3} \text{ M}$  citric acid buffer solution (pH 6.1) and a  $2.65 \times 10^{-2} \text{ M CH}_3\text{COOH} + 2.65 \times 10^{-2} \text{ M CH}_3\text{COONa}$  buffer solution (pH 4.7) were used as the carrier solutions. The  $\text{Na}^+$ -ISE as a reference electrode responded to  $\text{Na}^+$  ion in the carrier solutions. The potential response between the  $\text{NO}_3^-$ -ISE and a  $\text{Na}^+$ -ISE as

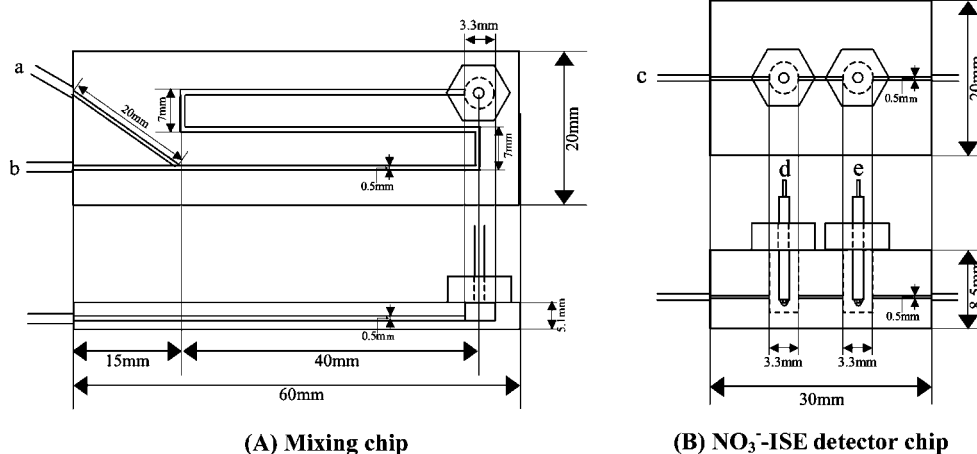


Fig. 1 Schematic structure of a fabricated microfluidic polymer chip consisting of a mixing chip and a  $\text{NO}_3^-$ -ISE detector chip. a) Sample solution, b) carrier solution, c) solutions from the mixing chip, d)  $\text{NO}_3^-$ -ISE, e)  $\text{Na}^+$ -ISE used as a reference electrode.

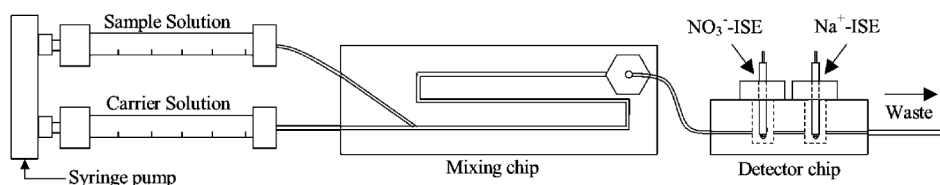


Fig. 2 Schematic structure of flow system based on the microfluidic chip with  $\text{NO}_3^-$ -ISE.

Table 1 Performance of several  $\text{NO}_3^-$ -ISEs

Quaternary ammonium salt	Plasticizer	Slope <sup>a</sup> /mV decade <sup>-1</sup>	Linear range/M
TAB	NPDE	$-58.5 \pm 1.7$	$3.2 \times 10^{-6} - 1.0 \times 10^{-1}$
TAB	NPOE	$-53.6 \pm 0.9$	$3.2 \times 10^{-6} - 1.0 \times 10^{-1}$
TAB	NPPE	$-53.4 \pm 0.4$	$3.2 \times 10^{-6} - 1.0 \times 10^{-1}$
TAB	FNDPE	$-55.3 \pm 0.6$	$3.2 \times 10^{-6} - 1.0 \times 10^{-1}$
TDAB	NPDE	$-58.5 \pm 1.6$	$1.0 \times 10^{-6} - 1.0 \times 10^{-1}$
TDAB	NPOE	$-57.7 \pm 0.7$	$1.0 \times 10^{-6} - 1.0 \times 10^{-1}$
TDAB	NPPE	$-54.4 \pm 0.9$	$1.0 \times 10^{-6} - 1.0 \times 10^{-1}$
TDAB	FNDPE	$-57.6 \pm 1.4$	$1.0 \times 10^{-5} - 1.0 \times 10^{-1}$

TAB, Tetradecylammonium bromide; TDAB, tetradodecylammonium bromide; NPOE, 2-nitrophenyl octyl ether; NPDE, 2-nitrophenyl dodecyl ether; NPPE, 2-nitrophenyl phenyl ether; FNDPE, 2-fluorophenyl 2-nitrophenyl ether.

a. Standard deviation of average slope values in multiple calibration ( $n = 3$ ).

a reference electrode in the microfluidic chip detector was measured with a potentiometer.

## Results and Discussion

### Response characteristics of the $\text{NO}_3^-$ -ISE and the $\text{Na}^+$ -ISE

The response characteristics of the several  $\text{NO}_3^-$ -ISEs to nitrate ion are summarized in Table 1. The dynamic range of the calibration curve of TDAB-based ISEs is wider than that of TAB-based ISEs, except for the TDAB and 2-fluorophenyl 2-nitrophenyl ether (FNDPE)-based ISE. TDAB-based ISEs

Table 2 Response characteristics of a TDAB-based  $\text{NO}_3^-$ -ISE

Parameter	Response characteristics
Slope <sup>a</sup> /mV decade <sup>-1</sup>	$-57.7 \pm 0.7$
Correlation coefficient, $\gamma$	0.9999
Linear range/M	$1.0 \times 10^{-6} - 1.0 \times 10^{-1}$
Lower detection limit/M	$3.6 \times 10^{-7}$

a. Standard deviation of average slope values in multiple calibration ( $n = 3$ ).

showed almost the same sensitivity to nitrate ion. The dynamic range of the calibration curve of TDAB and NPDE-based ISE, TDAB and NPOE-based ISE, TDAB and NPPE-based ISE is wider than that of the TDAB and FNDPE-based ISE.

From the results, TDAB and NPOE-based  $\text{NO}_3^-$ -ISE was used in subsequent experiments. Table 2 gives the response characteristics of the TDAB and NPOE-based  $\text{NO}_3^-$ -ISE to nitrate ion. The TDAB-based  $\text{NO}_3^-$ -ISE showed an almost Nernstian response with an anionic slope of  $-57.7 \pm 0.7$  mV/decade for nitrate ion in the concentration range from  $1.0 \times 10^{-6}$  to  $1.0 \times 10^{-1}$  M, as shown in Fig. 3. The lower detection limit for nitrate ion is  $3.6 \times 10^{-7}$  M. Figure 4 shows the emf-time profile of the  $\text{NO}_3^-$ -ISE. Based on the IUPAC definition of the response time as the time required from sample introduction to obtain 90% of the final signal,<sup>56</sup> the  $\text{NO}_3^-$ -ISE were found to have a response time of <2 min. As described in the introduction section, an Ag/AgCl wire electrode cannot be used as a reference electrode in the microfluidic polymer chip system. A  $\text{Na}^+$ -ISE based on B12C4 as a  $\text{Na}^+$  ionophore has high selectivity for  $\text{Na}^+$  ion over common cations, such as  $\text{Li}^+$ ,

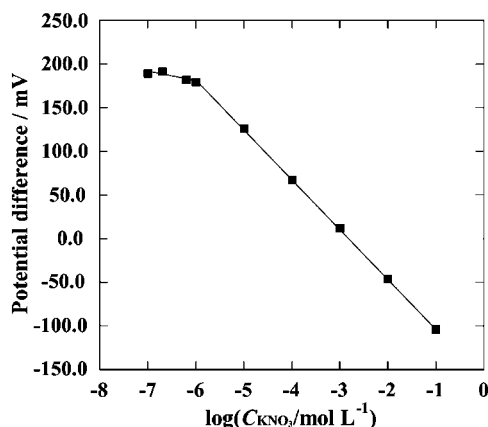


Fig. 3 Calibration curve for nitrate ion using a TDAB-based  $\text{NO}_3^-$ -ISE.

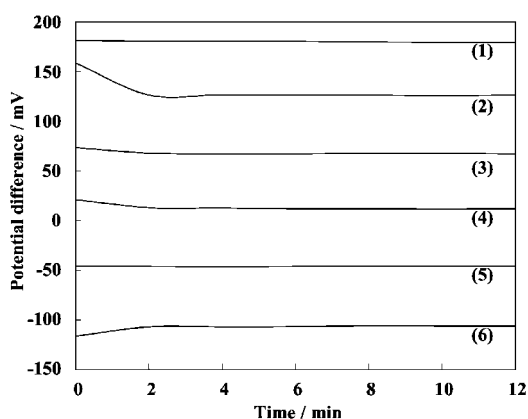


Fig. 4 Emf-time profile of the TDAB-based  $\text{NO}_3^-$ -ISE. Sample solution: (1)  $1.0 \times 10^{-6}$ , (2)  $1.0 \times 10^{-5}$ , (3)  $1.0 \times 10^{-4}$ , (4)  $1.0 \times 10^{-3}$ , (5)  $1.0 \times 10^{-2}$ , (6)  $1.0 \times 10^{-1}$  M  $\text{KNO}_3$ .

$\text{K}^+$ ,  $\text{Ca}^{2+}$  ions and so on.<sup>57</sup> Therefore, the  $\text{Na}^+$ -ISE is expected to be used as a reference electrode in the polymer-based microfluidic chip with embedded  $\text{NO}_3^-$ -ISE, if a carrier solution containing a high concentration of  $\text{Na}^+$  ion is used. Here, the response characteristics of the  $\text{Na}^+$ -ISE were measured in order to examine whether the ISE was used as the reference electrode or not, in the polymer-based microfluidic chip.

Irrespective of the kind of hydrophilic counter anions ( $\text{Br}^-$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ) of  $\text{Na}^+$  ion,  $\text{Na}^+$ -ISE showed almost the same Nernstian response as a cationic slope of  $54.5 \pm 0.1$  mV/decade for  $\text{Na}^+$  ion in the concentration range from  $1.0 \times 10^{-5}$  to  $1.0 \times 10^{-1}$  M. The electrode potential of the  $\text{Na}^+$ -ISE to  $\text{KNO}_3$  in the concentration range from  $1.0 \times 10^{-6}$  to  $1.0 \times 10^{-1}$  M in the presence of  $2.0 \times 10^{-2}$  M  $\text{Na}^+$  ion remained almost constant; the potential change was within *ca.* 5 mV.

These facts indicate that the  $\text{Na}^+$ -ISE can be used as a reference electrode in a microfluidic chip for the determination of nitrate ion in environmental water samples because the concentration of lipophilic anions, such as  $\text{SCN}^-$  ion contained in the environmental water samples, is very low.

#### The selectivity of the TDAB-based $\text{NO}_3^-$ -ISE

The selectivity of the TDAB-based  $\text{NO}_3^-$ -ISE with respect to nitrate ion was examined using a separate solution method.<sup>58,59</sup>

Table 3 Selectivity coefficients of a TDAB-based  $\text{NO}_3^-$ -ISE

Interfering ion, X	$\log k_{\text{NO}_3^-, \text{X}}^{\text{Pot}}$
$\text{Cl}^-$	-2.42
$\text{Br}^-$	-1.15
$\text{SCN}^-$	1.75
$\text{I}^-$	1.28
$\text{SO}_4^{2-}$	-4.33
$\text{H}_2\text{PO}_4^-$	-3.33
$\text{CH}_3\text{COO}^-$	-2.89
$\text{NO}_2^-$	-1.08
Citrate ion	-3.80
$\text{F}^-$	-4.03

To determine the selectivity coefficients of TDAB-based  $\text{NO}_3^-$ -ISE ( $k_{\text{NO}_3^-, \text{X}}^{\text{Pot}}$ ), we measured the TDAB-based  $\text{NO}_3^-$ -ISE potential separately for  $1.0 \times 10^{-2}$  M sodium nitrate and for  $1.0 \times 10^{-2}$  M solutions of common inorganic-interfering anions. The selectivity coefficients of the TDAB-based  $\text{NO}_3^-$ -ISE ( $k_{\text{NO}_3^-, \text{X}}^{\text{Pot}}$ ) were calculated from the equation

$$\log k_{\text{NO}_3^-, \text{X}}^{\text{Pot}} = (E_j - E_i)/S + \log c_i - \log c_j^{(-1/z_i)},$$

where  $E_i$  and  $E_j$  represent the measured emf values for nitrate ion and for the interfering ions, respectively;  $S$  is the theoretical slope of the  $\text{NO}_3^-$ -ISE ( $-59.2$  mV at 298 K);  $c_i$  and  $c_j$  are the concentrations of nitrate ion and the interfering ion, respectively; and  $z_j$  is the charge on the interfering ion (for example, in case  $\text{X}^-$  is  $\text{Cl}^-$  ion,  $z_j = -1$ ).

The selectivity coefficient values ( $\log k_{\text{NO}_3^-, \text{X}}^{\text{Pot}}$ ) exhibited in Table 3 indicate that the selectivity of the  $\text{NO}_3^-$ -ISE is in agreement with the Hofmeister series, which is correlated with the hydration energies of the anions.

#### Effect of the structure of a microfluidic polymer chip on the mixing efficiency between a sample solution and a carrier solution

The performance of a microfluidic polymer chip depends on the mixing efficiency between a sample solution and a carrier solution. Therefore, the effect of the structure of a microfluidic polymer chip and the flow rate of the sample and the carrier solutions on the mixing efficiency between a sample solution and a carrier solution was examined. Figure 5 shows a schematic diagram of three fabricated microfluidic polymer chips. A green dye solution (solution 1) and a red dye solution (solution 2) were flowed to channel a and channel b in the microfluidic polymer chip with a syringe pump, respectively. If solution 1 and solution 2 are mixed completely, the color formed by mixing solution 1 with solution 2 is dark green at an outlet tube connected with a detector chip. Table 4 shows the effect of the structure of a microfluidic polymer chip on the mixing efficiency between solution 1 and solution 2. When a flow rate of  $1 \mu\text{L min}^{-1}$  was used, mixing was complete for all microfluidic polymer chips. At a flow rate of more than  $1 \mu\text{L min}^{-1}$ , the mixing efficiency decreased as the flow rate increased. In the case of a flow rate of  $20 \mu\text{L min}^{-1}$ , mixing was complete for only the type-C chip. In the case of a flow rate of  $50 \mu\text{L min}^{-1}$ , incomplete mixing or no miscibility between solution 1 and solution 2 for all types of microfluidic polymer chips was observed. In subsequent experiments, the type-C chip at a flow rate of  $20 \mu\text{L min}^{-1}$  was used by taking into account of mixing efficiency and the analysis time.

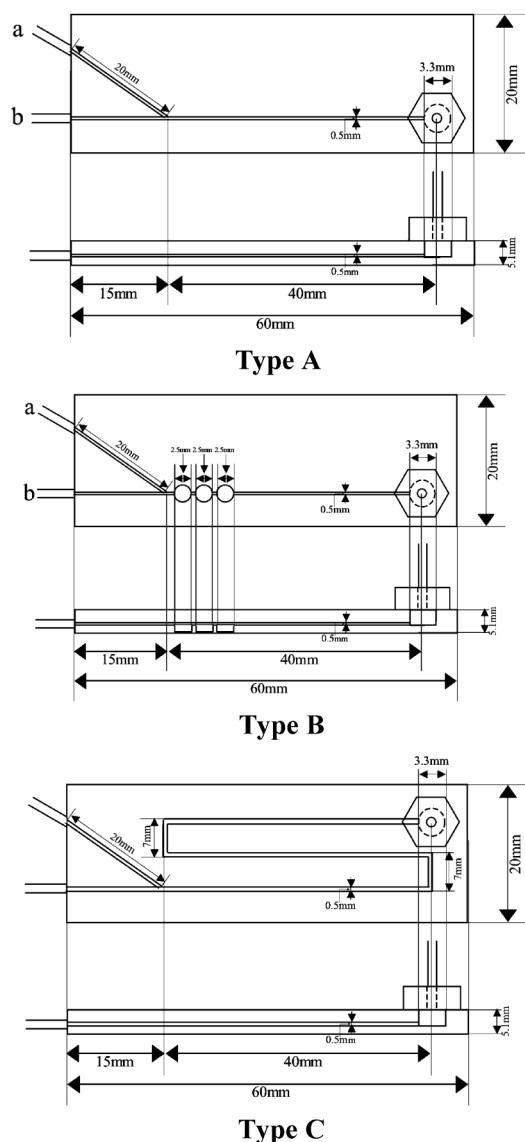


Fig. 5 Schematic structure of microfluidic polymer chips fabricated for evaluating the mixing efficiency between a sample solution and a carrier solution. a) A green solution, b) a red solution.

Table 4 Effect of the structure of a microfluidic polymer chip and the flow rate of the sample and carrier solutions on the mixing efficiency between a sample solution and a carrier solution

Type of a chip	Flow rate/ $\mu\text{L min}^{-1}$		
	1	20	50
A	a	c	c
B	a	c	c
C	a	a	c

a, Complete mixing; b, incomplete mixing; c, no miscibility.

#### Response characteristics of a microfluidic polymer chip integrated with a $\text{NO}_3^-$ -ISE and a $\text{Na}^+$ -ISE as a reference electrode

At first, the effect of the kind of carrier solution on the response characteristics of a microfluidic polymer chip integrated with a  $\text{NO}_3^-$ -ISE and a  $\text{Na}^+$ -ISE as a reference electrode to nitrate ion was examined, because several ions in

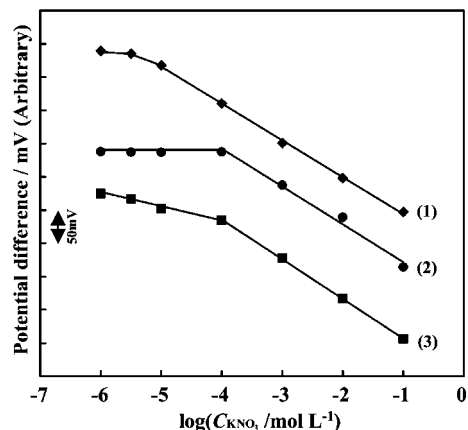


Fig. 6 Effect of the kind of carrier solution on the response characteristics of a microfluidic polymer chip integrated with a  $\text{NO}_3^-$ -ISE and a  $\text{Na}^+$ -ISE as a reference electrode to nitrate ion. Carrier solution: (1) 0.02 M  $\text{KH}_2\text{PO}_4$  + 0.0133 M  $\text{Na}_2\text{HPO}_4$  phosphate buffer solution (pH 6.7), (2)  $1.77 \times 10^{-2}$  M sodium citrate +  $1.42 \times 10^{-3}$  M citric acid buffer solution (pH 6.1), (3)  $2.65 \times 10^{-2}$  M  $\text{CH}_3\text{COOH}$  +  $2.65 \times 10^{-2}$  M  $\text{CH}_3\text{COONa}$  buffer solution (pH 4.7).

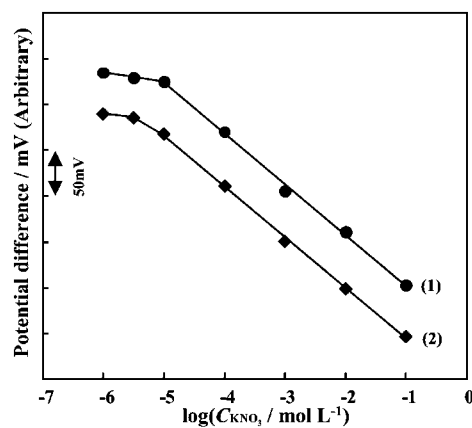


Fig. 7 Potential response of TDAB-based  $\text{NO}_3^-$ -ISE to the nitrate ion in a batch system and in the microfluidic polymer chip with the ISE fabricated using a stainless-steel wire with a diameter of 0.5 mm. (1)  $\text{NO}_3^-$ -ISE in a batch system. (2)  $\text{NO}_3^-$ -ISE in microfluidic polymer chip based microflow system. Potassium nitrate solutions as the sample solution and 0.02 M  $\text{KH}_2\text{PO}_4$  + 0.0133 M  $\text{Na}_2\text{HPO}_4$  phosphate buffer solutions (pH 6.7) as the carrier solutions were flowed to a sample injection channel and a carrier solution channel in the microfluidic polymer chip with a syringe pump at a flow rate of  $20 \mu\text{L min}^{-1}$ , respectively.

the carrier solution may interfere with the response characteristics of the  $\text{NO}_3^-$ -ISE, such as the dynamic range of the calibration curve and the slope sensitivity. The results are shown in Fig. 6. In the case that a 0.02 M  $\text{KH}_2\text{PO}_4$  + 0.0133 M  $\text{Na}_2\text{HPO}_4$  phosphate buffer solution as a carrier solution was used among the three carrier solutions examined, the microfluidic polymer chip showed the best dynamic range of the calibration curve and the slope sensitivity. In subsequent experiments, a 0.02 M  $\text{KH}_2\text{PO}_4$  + 0.0133 M  $\text{Na}_2\text{HPO}_4$  phosphate buffer solution was used as the carrier solution.

Figure 7 shows the potential response of the  $\text{NO}_3^-$ -ISE to the nitrate ion in a batch system using a  $\text{Na}^+$ -ISE as a reference electrode and in a microfluidic polymer chip (type-C) with a  $\text{NO}_3^-$ -ISE and  $\text{Na}^+$ -ISE as a reference electrode. The  $\text{NO}_3^-$ -ISE

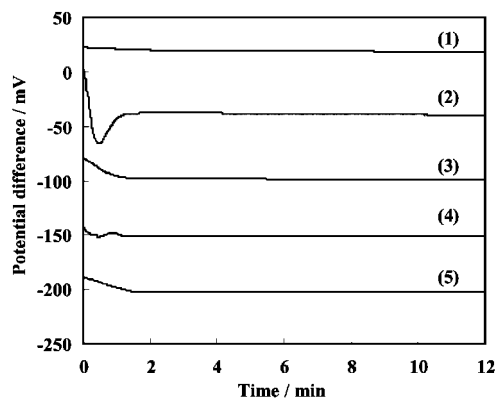


Fig. 8 Emf-time profile of the  $\text{NO}_3^-$ -ISE in the microfluidic polymer chip. Sample solution: (1)  $1.0 \times 10^{-5}$ , (2)  $1.0 \times 10^{-4}$ , (3)  $1.0 \times 10^{-3}$ , (4)  $1.0 \times 10^{-2}$ , (5)  $1.0 \times 10^{-1}$  M  $\text{KNO}_3$ . Concentrations of the nitrate ion are shown as those in a detector position. Other experimental conditions are the same as those in Fig. 7, line (2).

in a batch system shows an almost Nernstian response with an anionic slope of  $-54.9 \pm 0.8$  mV/decade ( $\gamma = 0.999$ ) to the  $\text{NO}_3^-$  ion in a 0.02 M  $\text{KH}_2\text{PO}_4 + 0.0133$  M  $\text{Na}_2\text{HPO}_4$  phosphate buffer solution in the concentration range from  $1.0 \times 10^{-5}$  to  $1.0 \times 10^{-1}$  M. Both results presented in Table 2 or Fig. 3 and those in Fig. 7, line (1) are based on the batch system; however, the linear ranges are different, the former spans five orders of magnitude in concentration; the latter is only four orders. The difference between the results presented in Table 2 and those in Fig. 7, line (1) may be due to interference of phosphate anion in a phosphate buffer solution for the response of the  $\text{NO}_3^-$ -ISE in a batch system using a  $\text{Na}^+$ -ISE as a reference electrode. On the other hand, the  $\text{NO}_3^-$ -ISE in the polymer-based microfluidic chip shows an almost Nernstian response with an anionic slope of  $-54.3 \pm 1.3$  mV/decade ( $\gamma = 1.000$ ) to  $\text{NO}_3^-$  ion in the concentration range from  $1.0 \times 10^{-5}$  to  $1.0 \times 10^{-1}$  M. In this case, the concentration of nitrate ion is shown as that at the detector position. From the results of Fig. 7, the  $\text{NO}_3^-$ -ISE in a batch system and in the polymer-based chip showed similar performance at low detection limits, dynamic range of the calibration curve, and the slope sensitivity. Figure 8 shows an emf-time profile of the  $\text{NO}_3^-$ -ISE in the microfluidic polymer chip. Based on the IUPAC definition of the response time as the time required from introduction of the sample solution and the carrier solution to obtain 90% of the final potential values,<sup>56</sup> the  $\text{NO}_3^-$ -ISE in the polymer-based microfluidic chip was found to have a response time of  $<1$  min. This fact indicates that the  $\text{NO}_3^-$ -ISE in the polymer-based microfluidic chip can be applied to the continuous-flow analysis of nitrate ion because the  $\text{NO}_3^-$ -ISE in the polymer-based microfluidic chip shows good stability of the potential and a fast response.

#### Application of the microfluidic chip to the determination of nitrate ion in environmental water samples, such as a tap water, well-water and water for agricultural use

Table 5 gives the results of a recovery test for nitrate ion added to environmental water samples by using a calibration curve with  $\text{KNO}_3$  solutions in the developed microfluidic chip. For all of the samples examined in this work, the recovery of the nitrate ion was *ca.* 96–105%. This result shows that the microfluidic chip can be applied to determine the level of nitrate ion in environmental water samples, such as tap water, well-water and water for agricultural use. However, the developed

Table 5 Recovery tests of nitrate ion added to several environmental samples

Sample	Added/mol L <sup>-1</sup>	Found <sup>a</sup> /mol L <sup>-1</sup>	Recovery <sup>b</sup> , %
A (1.25-fold dilution)	0	$(9.29 \pm 0.26) \times 10^{-5}$	
	$8.00 \times 10^{-5}$	$(1.75 \pm 0.02) \times 10^{-4}$	$102.2 \pm 0.6$
B (1.25-fold dilution)	0	$(8.39 \pm 0.03) \times 10^{-5}$	
	$1.60 \times 10^{-4}$	$(2.49 \pm 0.04) \times 10^{-4}$	$97.6 \pm 1.4$
C (2-fold dilution)	0	$(1.68 \pm 0.03) \times 10^{-4}$	
	$8.00 \times 10^{-5}$	$(2.37 \pm 0.05) \times 10^{-4}$	$104.6 \pm 0.3$
D (5-fold dilution)	0	$(8.62 \pm 0.66) \times 10^{-5}$	
	$4.00 \times 10^{-5}$	$(1.27 \pm 0.05) \times 10^{-4}$	$101.8 \pm 0.3$
	$8.00 \times 10^{-5}$	$(1.64 \pm 0.08) \times 10^{-4}$	$97.3 \pm 0.1$
	0	$(9.57 \pm 0.01) \times 10^{-5}$	
	$1.00 \times 10^{-4}$	$(1.96 \pm 0.04) \times 10^{-4}$	$100.4 \pm 3.1$
	$2.00 \times 10^{-4}$	$(2.98 \pm 0.08) \times 10^{-4}$	$101.2 \pm 3.8$

Sample: A and B, tap water; C, water for agricultural use; D, well-water.

a. Standard deviation of average concentration values in multiple measurements ( $n = 3$ ).

b. Standard deviation of average recovery values in multiple measurements ( $n = 3$ ).

polymer-based microfluidic chip may not be applied to sample solutions containing a high concentration of  $\text{Na}^+$  ion (0.1–1.0 M), because the potential of the  $\text{Na}^+$ -ISE used as a reference electrode may vary with the concentration of  $\text{Na}^+$  ion in a sample solution.

## Conclusions

A  $\text{NO}_3^-$ -ISE based on TDAB as an anionic exchanger shows good performance in terms of high selectivity for nitrate ion over small inorganic anions. A plastic polymer chip with the embedded  $\text{NO}_3^-$ -ISE and  $\text{Na}^+$ -ISE as a reference electrode was also fabricated. The microfluidic channel was fabricated by using a small-diameter wire as a template. The ISEs embedded in the chip are cartridge-type ISEs. The cartridge-type ISEs can be easily replaced by new ISEs, in the case that they show a lower performance during long-term use. A fabricated microfluidic polymer chip with the  $\text{NO}_3^-$ -ISE and  $\text{Na}^+$ -ISE as a reference electrode showed a similar performance to that of the  $\text{NO}_3^-$ -ISE in a batch system with respect to the slope sensitivity and the stability of its potential response. The advantages of the fabrication method used here are very ease fabrication of the microfluidic chip and very low cost. Furthermore, in the present microfluidic polymer chip, a mixing chip is separated from an ISE detector chip based on a  $\text{NO}_3^-$ -ISE. In the case that the mixing chip or the ISE detector chip is troublesome, the troublesome chip is easily replaced by a new chip. The sensitivity and lower detection limit of the present microfluidic chip is inferior to that of the other methods based on spectrometry,<sup>5-9</sup> fluorometry,<sup>10,11</sup> atomic absorption spectrometry<sup>12,13</sup> and ion chromatography.<sup>14</sup> However, the present microfluidic chip method has the advantages of a decreased use of reagent and lower sample volumes over the other methods. Furthermore, the present microfluidic chip method requires no expensive instrumentations and no complicated procedures. The lower detection limit of the present microfluidic chip for nitrate ion is superior to that of other microfluidic chips.<sup>29,30</sup> The level of nitrate ion in environmental water samples, such as a tap water, well-water and water for agricultural use was determined by the developed microfluidic chip with the embedded  $\text{NO}_3^-$ -ISE.

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