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Highly sensitive capillary zone electrophoresis with artificial seawater as the background electrolyte and transient isotachophoresis as the on-line concentration procedure for simultaneous determination of nitrite and nitrate in seawater

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Abstract

Transient isotachopheresis (ITP)-capillary zone electrophoresis (CZE) method with artificial seawater as the background electrolyte (BGE) was improved to further lower the limit of detection (LOD) for determination of nitrite and nitrate in seawater. By lowering the pH of BGE, the difference between effective mobility of nitrite and that of nitrate increased, thereby permitting increased sample volumes to be tolerated and their LODs to decrease. Artificial seawater with pH adjusted to 3.0 using phosphate buffer was adopted as the BGE. To reverse electroosmotic flow (EOF), a capillary was flushed with 0.1 mM dilauryldimethylammonium bromide (DDAB) for 3 min before the capillary was filled with the BGE. Limits of detection (LODs) for nitrite and nitrate were 2.7 and 3.0 $\mu\text{g/l}$ (as nitrogen), respectively. The LODs were obtained at a signal to noise ratio (S/N) of three. Values of the relative standard deviation (RSD) of peak area for these ions were 2.0 and 0.75%, respectively, when nitrite concentration was 0.05 mg/l and that of nitrate was 0.5 mg/l. The RSDs of peak height were 4.4 and 2.3%. The RSDs of migration time for these ions were 0.19 and 0.17%. The proposed method was applied to determination of nitrite and nitrate in a proposed certified reference material for nutrients in seawater, MOOS-1, distributed by the National Research Council of Canada (NRC). Results agreed with the assigned tolerance interval. This method was also applied to determination of these ions in seawater collected around Osaka Bay. Results nearly agreed with those obtained by a conventional spectrophotometric method.

Keywords: Seawater analysis; Nitrogen speciation; Capillary zone electrophoresis;

Transient isotachophoresis; Nitrite; Nitrate

1. Introduction

Simultaneous detection and speciation of nitrite and nitrate within environmental, food, industrial, and physiological systems has gained increased interest as these ions are closely related to domestic life; however, a substantial degree of uncertainty remains [1]. From the marine environmental perspective, historical data have suggested that fish avoidance, physiological and reproductive anomalies, and mortality have been associated with eutrophication and blooms [2]. In addition, elucidation of the global carbon cycle is desired to predict weather in the near future [3]. Therefore, development of a simple and sensitive method for determination of seawater nutrients, including nitrite and nitrate, is desirable. Nitrite in seawater is generally determined by absorption detection of an azo chromophore; nitrate is reduced to nitrite using a cadmium-copper column, etc. and determined as the difference between the total nitrite and nitrite concentrations [4-7]. It is troublesome to prepare the cadmium-copper column and to treat cadmium-containing waste water. Fluorescence detection of a diazonium ion was also developed for determination of nitrite in seawater; nitrate was measured as excess nitrite formed when nitrate was reduced to nitrite [8]. There are few methods for simultaneous determination of nitrite and nitrate in seawater. Ito et al. [9] developed the ion chromatography (IC) procedure; it uses a cetyltrimethylammonium (CTA⁺)-coated column with high anion-exchange capacity for determination of nitrite and nitrate in seawater. The limits of detection (LODs) for nitrite and nitrate (1.2 and 1.8 µg/l, recalculated as nitrogen concentration) were sufficiently low for seawater analysis. However, seawater samples had to be pretreated by passage through a Sep-Pak C₁₈ column before IC analysis. In addition, detection had to be carried out at 225 nm because of the

unknown peak near nitrite at 215 nm, even though molar absorptivities for nitrite and nitrate at 215 nm were larger than those at 225 nm. Hu et al. [10] determined bromide, nitrate, and iodide in seawater by electrostatic IC with a 20-fold-diluted artificial seawater as the eluent. The LODs were excellent: 0.75 $\mu\text{g/l}$ for bromide, 0.12 $\mu\text{g/l}$ for nitrate (recalculated as nitrogen concentration), and 0.8 $\mu\text{g/l}$ for iodide. Unfortunately, baseline separation of bromide and nitrate could not be obtained, and nitrite could not be determined because of interference from matrix ions.

Capillary zone electrophoresis (CZE) has been studied recently as a new approach to seawater analysis. Mori et al. [11] used a background electrolyte (BGE) containing zwitterionic and non-ionic surfactants to suppress increased migration current resulting from high ionic strength of the BGE for determination of UV-absorbing anions in seawater. Relatively low LODs were obtained for bromide (64 $\mu\text{g/l}$) and nitrate (8.4 $\mu\text{g/l}$, recalculated as nitrogen concentration), but relatively high relative standard deviations (RSDs) resulted for the peak area (5.9-7.0%). A standard addition method was used for quantification. Tu et al. [12] determined nitrate in seawater by CZE with a relatively low concentration BGE and chloride-induced leading-type transient isotachophoresis (ITP) as the on-line concentration procedure. The LOD was a relatively low 7.9 $\mu\text{g/l}$ (recalculated as nitrogen concentration), but standard addition with 2.5-fold dilution of the sample was necessary to overcome peak height changes caused by possible chloride concentration variation in the sample. Nitrite and nitrate in real seawater samples have not been determined simultaneously by the CZE procedures described above. We developed a CZE procedure for simultaneous determination of nitrite and nitrate in seawater using artificial seawater as the BGE and transient ITP as the

on-line concentration procedure [13]. The method was verified through determination of nitrite and nitrate in a proposed certified reference material for nutrients in seawater, MOOS-1 [14], distributed by the National Research Council of Canada (NRC). It was proved that the LOD for nitrate (10 $\mu\text{g/l}$, recalculated for nondiluted seawater) was sufficiently low for analysis of MOOS-1, but further improvement of the LOD for nitrite (22 $\mu\text{g/l}$, recalculated for nondiluted seawater) was necessary.

In the present study, transient ITP-CZE with artificial seawater as the BGE was improved to lower LODs for nitrite and nitrate, especially for nitrite, in seawater. We examined effects of pH of the BGE, concentration of a phosphate buffer in the BGE, amounts of sample solution and the terminating ion injected into the capillary, applied voltage, etc. on separation and concentration of nitrite and nitrate. The proposed method was applied to determination of nitrite and nitrate in MOOS-1 to verify accuracy of the method. Moreover, seawater samples were taken from the surface and the seabed around a coastal area of Osaka Bay; nitrite and nitrate in samples were determined by the proposed CZE procedure and naphthylethylenediamine spectrophotometry (NS) [7] conventionally used for nitrite and nitrate analyses. Results were compared to examine applicability of the proposed procedure to actual seawater analysis.

2. Experimental

2.1. Apparatus

The capillary electrophoretic analyzer used throughout this study was a Perkin-Elmer 270A-HT with a UV-visible absorbance detector (Foster City, CA, USA). Rise-time for the detector was set at 0.50 s. A polyimide coated fused-silica capillary (GL Sciences, Inc., Tokyo, Japan), 75 μm i.d. \times 375 μm o.d., served as the capillary electrophoresis column. Total length of the column ($L_{\text{tot.}}$) was 72 cm; effective length ($L_{\text{det.}}$) was 50 cm. Peak area, peak height, and migration time were measured using a Hitachi (Tokyo, Japan) D-2500 Chromato-Integrator. Seawater samples were taken from the surface and seabed around the coastal area of Osaka Bay in July and August 2002 using a glass reagent bottle (1 l) with a thin rope and a Rigosha (Tokyo, Japan) Vandorn water sampler (2 l), respectively.

2.2. Reagents

The cationic surfactant dilauryldimethylammonium bromide (DDAB), used for reversing electroosmotic flow (EOF), was obtained from Tokyo Kasei Kogyo, Co., Inc. (Tokyo, Japan). All reagents were of analytical-reagent grade and were used as received. Standard solutions containing bromide (68 mg/l), nitrite (0.02-0.1 mg/l), and nitrate (0.1-0.5 mg/l) for calibration graphs were prepared from 1000 mg/l potassium bromide (Wako Pure Chem. Inds., Ltd., Osaka, Japan), sodium nitrite (Nacalai Tesque, Inc., Kyoto, Japan), and potassium nitrate (Wako) solutions, respectively. Concentrations of nitrite and nitrate were expressed as nitrogen concentration. Phosphate buffers were prepared with 1 *M* phosphoric acid (Nacalai Tesque) and 1 *M* sodium dihydrogenphosphate (Nacalai Tesque) to adjust pH of

artificial seawater BGE. Distilled, demineralized water, obtained from a Yamato Kagaku (Tokyo, Japan) WG220 automatic still and a Nihon Millipore (Tokyo, Japan) Milli-QII system, was used for all experiments. Preparation of artificial seawater was based on a Japanese Standard [15] without the bromide. Preparation procedure and composition of the artificial seawater are described in a previous paper [16]. All solutions used in this study were filtered through a 0.45- μ m membrane filter before use. A proposed certified reference material for nutrients in seawater, MOOS-1 [14], was obtained from NRC (Ottawa, Canada). The MOOS-1 preparation procedure is described in another previous paper [13].

2.3. Procedure

Nitrite and nitrate in MOOS-1 and real seawater samples were determined by the following procedure. Seawater samples were filtered through a 0.45- μ m membrane before analysis. No pretreatment procedures, including sample cleanup, were required except for filtration. The detection wavelength was set at 210 nm for CZE determination of nitrite and nitrate. The thermostat was maintained at 30°C. A new capillary was washed with 1 M sodium hydroxide for 40 min and then with water for 10 min. The capillary was rinsed with 0.1 mM DDAB [17] for 3 min to reverse the EOF. Subsequently, the capillary was filled with BGE (artificial seawater containing no bromide, adjusted to pH 3.0 with phosphate buffer) by vacuum for 3 min. After a sample (ca. 84 nl) was vacuum injected into the CE apparatus for 4 s the terminating ion solution, 600 mM acetate was injected for 17 s (ca. 357 nl). A

voltage of 8 kV was applied with the sample inlet side as the cathode. Each step was run automatically. Calibration graphs were prepared using synthetic standards.

Results and discussion

3.1. pH of BGE and concentration of phosphate buffer

In general, the simplest way to lower the LOD for an analyte is to increase the amount of sample injected into the capillary. We used artificial seawater (pH 7.9) as the BGE in our series of studies on CZE analysis of nitrite and nitrate in seawater. The amount of sample was limited because effective mobility of nitrite was very close to that of nitrate at pH 7.9. It is well-known that effective mobility for nitrite decreases with decreasing pH of acidic solutions, whereas effective mobility for nitrate is constant in the whole pH range: hence the difference between effective mobilities increases in acidic solutions [18]. Therefore, we expected to increase the amount of sample by using an acidic BGE. In addition, the concentration ratio with transient ITP was higher for nitrate than that for nitrite when the pH of the BGE was 7.9; nitrate was the neighborhood ion of acetate (terminating ion). Boden et al. [19] have reported similar results. We also expected to obtain a higher concentration ratio for nitrite because nitrite was the neighborhood ion of acetate when the acidic BGE was used. The pH of artificial seawater was varied in the range 2.5-3.5 using 40 mM phosphate buffer; the seawater was then used as the BGE. We used cetyltrimethylammonium chloride as the additive in the artificial seawater BGE

(pH 7.9) to reverse the EOF. Lucy et al. demonstrated that DDAB coatings of the capillary were more stable than CTAC in acidic conditions [20], and used it for rapid analysis of nitrite and nitrate by CZE [17]. They analyzed a mixture of 1.0 mM nitrite and nitrate using 20 mM phosphate BGE (pH 2.5) with counter-EOF and co-EOF with DDAB. Migration times for nitrite and nitrate with co-EOF were 1/4.5 and 1/1.8 of those with counter-EOF, respectively; absorbances for nitrite and nitrate with co-EOF were 1.7 and 1.8 times those with counter-EOF, respectively. Therefore, we adopted a pre-rinse of the capillary with DDAB solution to reverse the EOF. As a sample, artificial seawater containing 68 mg/l bromide, 0.05 mg/l nitrite, and 0.5 mg/l nitrate was injected into the analyzer for 4 s (84 nl). It is said that concentration of bromide in seawater is 68 mg/l [21]. Nitrite and nitrate concentrations were chosen by considering those concentrations in MOOS-1 (0.0386 mg/l for nitrite and 0.325 mg/l for the sum of nitrite and nitrate). Figures 1A-1D show those results. A low trapezoid peak was observed for nitrate, but nitrite was not observed without transient ITP (Fig. 1A). When pH was 2.5, the nitrite peak was small and the nitrate peak was not sufficiently stacked (Fig. 1C). The injection period for acetate (terminating ion) was 8 s (168 nl). Nitrite and acetate peaks overlapped when the injection period for acetate was increased to obtain the sharper nitrate peak. Both nitrite and nitrate peaks could not be thoroughly stacked when pH was 3.5 (Fig. 1D). The injection period for acetate was 12 s (252 nl). Baseline separation of nitrite and nitrate was not obtained when the injection period for acetate was increased. In contrast with results for pH 2.5 and 3.5, sufficiently stacked peaks for nitrite and nitrate with baseline separation were observed when the BGE with pH 3.0 was used (Fig. 1B). Peak heights for nitrite

and nitrate were 18-fold and 8-fold higher than those without transient ITP, respectively.

Two phosphate buffer concentrations, 10 and 40 mM, were also examined using BGE with pH 3.0. The RSDs of peak area and peak height for nitrate (0.7-2.3%) were relatively low in both buffer concentrations. On the other hand, RSDs of peak area and peak height for nitrite were 7.4 and 11.5%, respectively, when buffer concentration was 10 mM; they were 2.0 and 4.4% when buffer concentration was 40 mM. The RSDs of migration time for nitrite and nitrate were 0.1-0.3%, but migration time for nitrite tended to increase with the number of analyses when buffer concentration was 10 mM. When buffer concentration was higher than 40 mM, the instrument was liable to stop because of rapid increase of current. Optimum pH of the BGE and the phosphate-buffer concentration adopted in subsequent experiments were, therefore, 3.0 and 40 mM, respectively.

3.2. Amount of sample and terminating ion injected into the capillary

The injection period for the same sample solution as used in the previous section was varied in the range of 3-5 s (63-105 nl) using the artificial seawater adjusted to pH 3.0 as the BGE. The injection period for 600 mM acetate was fixed to 17 s (357 nl). The nitrate peak height and peak area increased with increased injection period up to 5 s (105 nl), but a slightly fronting peak was observed when the injection period was 5 s (105 nl). This shows that the nitrate zone was not thoroughly stacked with 5 s (105 nl) of the injection period. The nitrite peak height and peak area increased with increasing injection period up to 4 s (84 nl), but then almost leveled

off. It could be inferred that a part of the nitrite zone was included into the nitrate zone with 5 s (105 nl) of the injection period.

To achieve a better concentration effect with transient ITP, the maximum amount of terminating ion should be injected into the capillary to achieve baseline separation of nitrate and nitrite. It also separates the neighborhood ion and the terminating ion. The injection period for 600 mM acetate was also varied in the range of 11-17 s (231-357 nl) when fixing the injection period for the sample solution at 4 s (84 nl). Figure 2 shows those results. Values on the ordinate show ratios of the peak area and peak height for nitrite and nitrate to those for 17 s injection period (357 nl). Peak-area ratios for both nitrite and nitrate were approximately constant over the range of 11-17 s injection period (231-357 nl); RSDs of peak-area ratios for nitrite and nitrate were 5.2 and 1.4%, respectively. The peak-height ratio for nitrite increased with an increase in the injection period for 600 mM acetate up to 13 s (273 nl); subsequently, it almost leveled off. The peak-height ratio for nitrate increased linearly with increasing injection period for the acetate. When the sample amount is fixed, increase of peak-height ratios for nitrite and nitrate with the constant peak-area ratios proves stacking [22]. Baseline separation of nitrite and nitrate was not obtained when the injection period was longer than 17 s (357 nl). Consequently, optimum injection periods for the sample solution and 600 mM acetate were 4 and 17 s (84 and 357 nl), respectively.

3.3. Applied voltage

In general, a higher applied voltage is preferred to obtain shorter analytical time

along with higher efficiency and resolution [23]. Effects of applied voltage on migration time, peak height, and peak area for nitrite and nitrate were investigated using the applied voltage of 6-10 kV. When 8 kV was applied, migration times for nitrite and nitrate were shorter by 3 min than those when 6 kV was applied. The peak height and peak area for nitrite were 1.7-fold larger than those obtained with 6 kV; the peak height and peak area for nitrate were 1.2 and 1.4-times larger than those obtained with 6 kV, respectively. A similar phenomenon to that occurring with higher buffer concentration was observed when applied voltage exceeded 8 kV. Therefore, 8 kV was adopted as optimum voltage for nitrite and nitrate determination.

3.4. Calibration graphs

Standard solutions for nitrite and nitrate containing 68 mg/l bromide were prepared. Calibration graphs for nitrite and nitrate were linear using both peak area and peak height. Regression equations relating area response to concentration for nitrite (x, 0-0.1 mg/l) and nitrate (x, 0-0.5 mg/l) were $y=2.51 \times 10^4 x + 566$ (correlation coefficient, 0.9963) and $y=6.31 \times 10^4 x + 352$ (0.9999), respectively; those relating peak height were $y=3.10 \times 10^4 x + 900$ (0.9901) and $y=2.92 \times 10^4 x + 197$ (0.9999). Smaller correlation coefficients for nitrite result from lower concentrations of nitrite in sample solutions. Table 1 summarizes values of RSD and LOD for nitrite and nitrate. The RSDs of peak areas, peak heights, and migration times for nitrite and nitrate were not larger than 2.0, 4.4, and 0.19%, respectively. It is noteworthy that nitrite concentration was 1/10 that for nitrate.

The LODs, 2.7 $\mu\text{g/l}$ for nitrite and 3.0 $\mu\text{g/l}$ for nitrate in the proposed procedure, were lowered to 1/8 and 1/3 of the LODs (22 $\mu\text{g/l}$ for nitrite and 10 $\mu\text{g/l}$ for nitrate) obtained by the procedure described in our previous paper [13], respectively. The LODs for nitrite and nitrate in our method were almost equal to the LODs, 1.2 $\mu\text{g/l}$ for nitrite and 1.8 $\mu\text{g/l}$ for nitrate, reported by Ito et al. [9]. The LOD for nitrate in our method (3.0 $\mu\text{g/l}$) was superior to the LODs reported by Mori et al. (8.4 $\mu\text{g/l}$) [11] and Tu et al. (7.9 $\mu\text{g/l}$) [12], but inferior to the LOD (0.12 $\mu\text{g/l}$) reported by Hu et al. [10]. However, as mentioned in the introduction, these methods are subject to some interference from coexisting components in seawater; hence it is impossible to determine nitrite simultaneously using some of these methods.

3.5. Analysis of MOOS-1 and seawater samples

The proposed method was applied to determination of nitrite and nitrate in MOOS-1. Table 2 summarizes those results: duplicate analyses were performed on each of three bottles and average values were calculated. Results for nitrite and the sum of nitrite and nitrate almost agreed with assigned tolerance intervals which were determined by NRC. That is to say, the method was found to be accurate. Figure 3A depicts an electropherogram of MOOS-1. Sharp peaks for nitrite and nitrate with baseline separation were detected within 7 min. The method was also applied to determination of these anions in seawater samples taken from the surface and the seabed around the coastal area of Osaka Bay on 11 July and 1 August, 2002. Seawater samples were also analyzed by naphthylethylenediamine spectrophotometry (NS) [7], which is conventionally used for nitrite and nitrate

analyses. Table 3 summarizes those results: values are the average of duplicate analyses. The CZE results for nitrite and nitrate almost agreed with those obtained by the NS method (correlation coefficients were 0.9647 for nitrite and 0.9790 for nitrate). It was interesting that concentrations of nitrite and nitrate in the seawater sample taken from the seabed in the Rokko Island on 11 July were approximately equal to those concentrations in the seawater sample taken from the same sampling site on 1 August. Nitrite and nitrate concentrations in seawater samples taken from other sampling sites on 11 July were higher than those on 1 August. Salinity of coastal seawater samples differs depending on the sampling site and depth because of the effects of river water and other factors. Salinity was not measured this time. Two-fold diluted artificial seawater containing 68 mg/l bromide, 0.05 mg/l nitrite, and 0.5 mg/l nitrate was analyzed by the proposed procedure with 8 s (168 nl) of the injection period to examine the effect of salinity on the peak height and peak area for nitrite and nitrate. The peak height and peak area for nitrite and nitrate were equal to those obtained with 4 s (84 nl) of the injection period for the nondiluted sample containing same concentrations of nitrite and nitrate. The proposed CZE method was not affected by salinity in sample solutions and was applicable to real seawater analysis. Figure 3B represents an electropherogram of surface seawater from the pond at our university (Kobe University of Mercantile Marine).

4. Conclusions

We demonstrated that the LODs of the transient ITP-CZE method for

determination of nitrite and nitrate in seawater could be lowered using artificial seawater BGE with pH 3.0 and DDAB as the EOF modifier to reverse EOF. The proposed CZE method is simple (sample cleanup and preparation of the special column are necessary for the IC method) and possesses sufficient detection power and precision with relatively short analysis time to be useful for simultaneous determination of nitrite and nitrate in seawater.

Acknowledgments

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Fig. 1. Effect of pH of background electrolyte on separation and concentration of nitrite and nitrate with transient isotachophoresis. Electrophoretic conditions: capillary, $L_{\text{tot.}}=72$ cm, $L_{\text{det.}}=50$ cm, $75\text{ }\mu\text{m i.d.}\times 375\text{ }\mu\text{m o.d.}$, pre-rinsed with 0.1 mM DDAB; BGE, artificial seawater without Br^- adjusted to pH 2.5 (C), 3.0 (A, B), and 3.5 (D) with 40 mM phosphate buffer; voltage, -8 kV ; wavelength for detection, 210 nm . Sample, artificial seawater containing 68 mg/l Br^- , 0.05 mg/l NO_2^- , and 0.5 mg/l NO_3^- ; vacuum injection period, 4 s (84 nl). Terminating ion, 600 mM acetate; injection period, A, 0 s (0 nl); B, 17 s (357 nl); C, 8 s (168 nl); D, 12 s (252 nl). Identification of peaks: a, Br^- ; b, NO_3^- ; c, NO_2^- ; d, CH_3COO^- .

Fig. 2. Effect of amount of terminating ion on concentration of nitrite and nitrate. pH of the BGE, 3.0; injection period of 600 mM acetate; 11-17 s (231-357 nl). Solid line: a , peak area; a_0 , peak area for the injection period of 17 s (357 nl); dotted line: h , peak height; h_0 , peak height for the injection period of 17 s (357 nl); \bigcirc , NO_2^- ; \triangle , NO_3^- . Sample, vacuum injection period, and other electrophoretic conditions as in Fig. 1.

Fig. 3. Electropherograms of MOOS-1 and surface seawater from the pond at Kobe University of Mercantile Marine. (A) Sample, MOOS-1; (B) Sample, surface seawater from the pond at KUMM. pH of the BGE, 3.0; injection period of 600 mM acetate; 17 s (357 nl). Other electrophoretic conditions and identification of peaks are as in Fig. 1.

Fig. 1

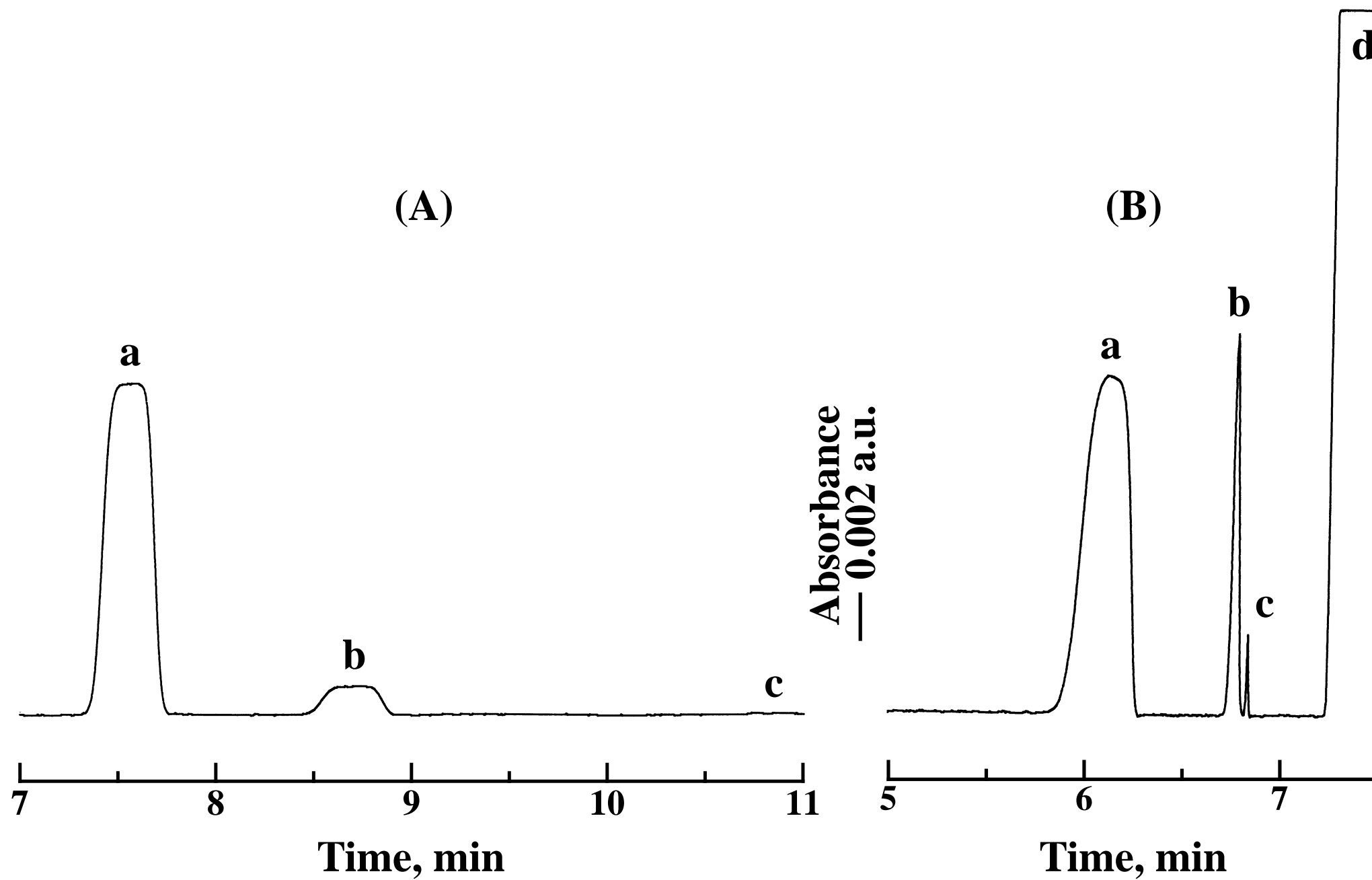


Fig. 2

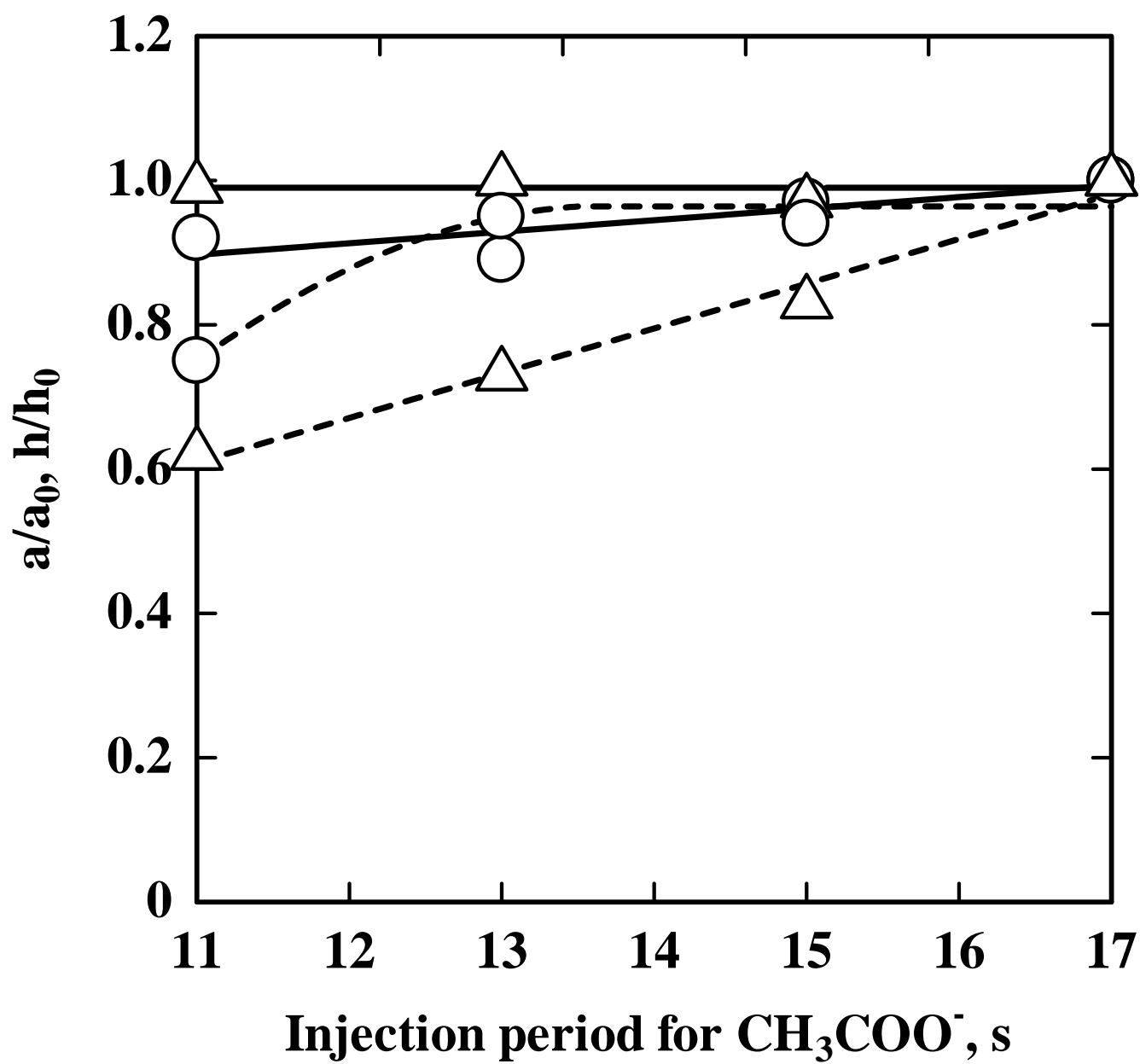


Fig. 3

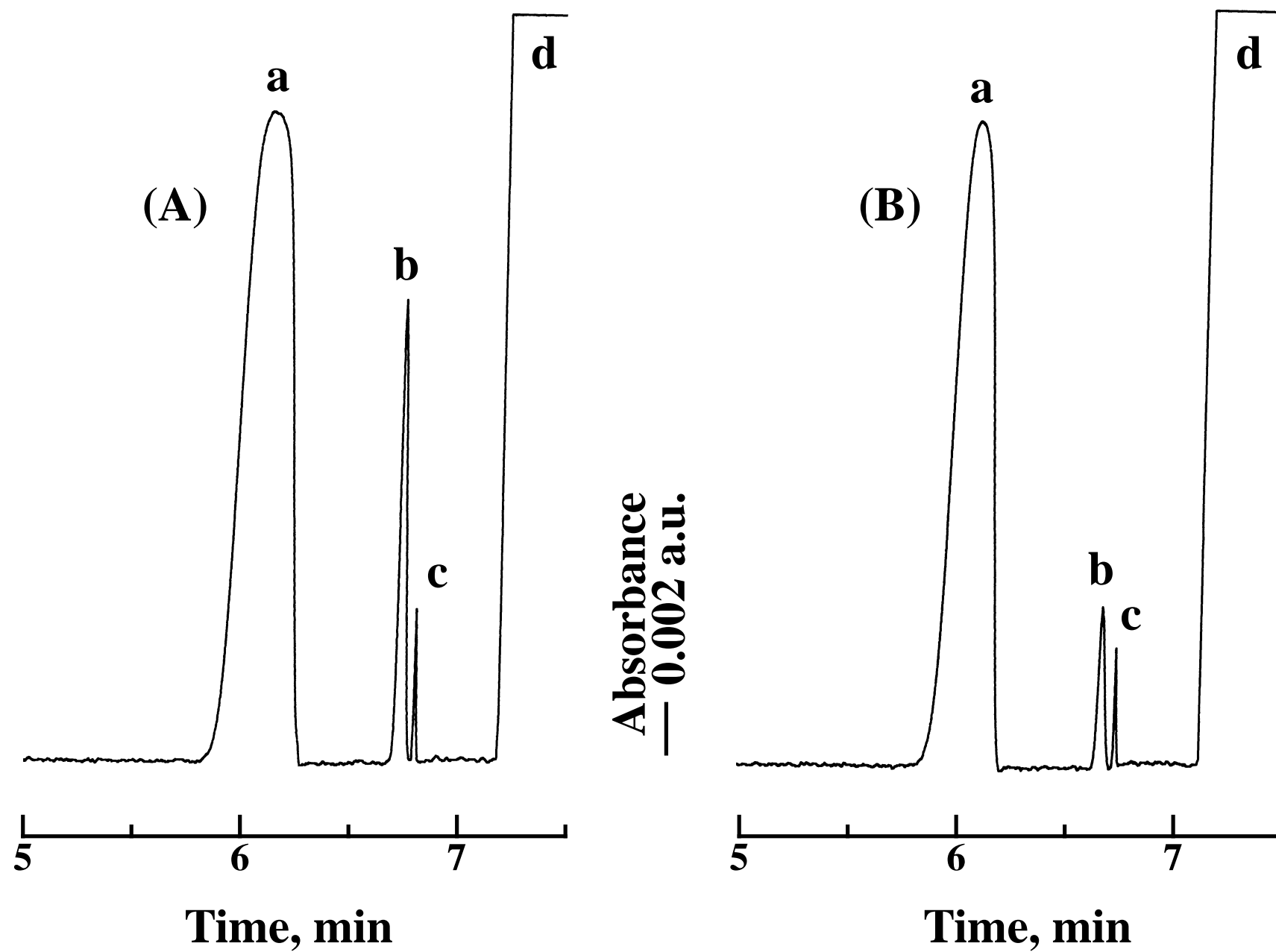


Table 1 Precision and detection limits of determination of nitrite and nitrate^a

	RSD^b, %			LOD
	Area	Height	Time	(S/N=3), µg/l
NO₂⁻	2.0	4.4	0.19	2.7
NO₃⁻	0.75	2.3	0.17	3.0

^aBGE, artificial seawater (pH 3.0); other electrophoretic conditions as in Fig. 1. ^bSample, artificial seawater containing 68 mg/l Br⁻, 0.05 mg/l NO₂⁻, and 0.5 mg/l NO₃⁻, eight determinations.

Table 2 Analytical results^a for nitrite and nitrate in MOOS-1^b

Bottle No.	NO₂⁻ (mg/l)	NO₃⁻ (mg/l)	NO₂⁻ + NO₃⁻ (mg/l)
1	0.0469	0.277	0.324
2	0.0423	0.262	0.305
3	0.0432	0.268	0.311
Assigned tolerance interval	0.0386±0.0081	—	0.325±0.034

^aBGE, artificial seawater (pH 3.0); other electrophoretic conditions as in Fig. 1; results for duplicate analyses using peak area. ^bMOOS-1, a proposed certified reference material for nutrients in seawater distributed by the National Research Council of Canada (NRC).

Table 3 Analytical results for seawater nitrite and nitrate

Sampling site	Depth (m)	NO ₂ ⁻ (mg/l)		NO ₃ ⁻ (mg/l)	
		CZE ^a	NS ^b	CZE ^a	NS ^b
Port of Kobe ^c	0	0.016	0.011	0.023	0.038
Port of Kobe ^d	0	—	0.006	0.005	0.003
Port of Kobe ^c	9.5	0.015	0.010	0.012	0.019
Port of Kobe ^d	8.0	0.019	0.019	0.023	0.017
Rokko Island ^c	0	0.016	0.014	0.103	0.111
Rokko Island ^d	0	0.008	0.004	0.026	0.025
Rokko Island ^c	11	0.019	0.017	0.014	0.029
Rokko Island ^d	11	0.019	0.018	0.014	0.012
Pond at KUMM ^{c,e}	0	0.031	0.033	0.093	0.119
Pond at KUMM ^{d,e}	0	0.010	0.006	0.012	0.008
Pond at KUMM ^{c,e}	5.0	0.002	0.003	0.002	0.005
Pond at KUMM ^{d,e}	4.5	—	0.002	—	0.002

^aBGE, artificial seawater (pH 3.0); other electrophoretic conditions as in Fig. 1; results for duplicate analyses using peak area. ^bNS, naphthylethylenediamine spectrophotometric method. Sampling dates: ^c11 July, 2002; ^d1 August, 2002.

^eKUMM, Kobe University of Mercantile Marine.