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Fukushi, Keiichi
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Wakida, Shin-ichi

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Optimum conditions for effective use of terminating ion in transient isotachophoresis for capillary zone electrophoretic determination of nitrite and nitrate in seawater using artificial seawater as the background electrolyte

**Nobuhiro Ishio¹, Keiichi Fukushi¹, Kanako Michiba¹, Sahori Takeda²,
Shin-ichi Wakida²**

**¹Research Institute for Marine Cargo Transportation, Kobe University of
Mercantile Marine, Kobe, Japan**

**²National Institute of Advanced Industrial Science and Technology, AIST
Kansai, Osaka, Japan**

Abstract We examined the transient isotachophoresis (ITP) conditions such as a kind of terminating ion, its concentration and injection procedure to improve the limit of detections (LODs) for the determination of nitrite and nitrate in seawater by capillary zone electrophoresis (CZE). Artificial seawater containing 3.0 mM cetyltrimethylammonium chloride (CTAC) was employed as a background electrolyte (BGE). After a sample was injected, 600 mM acetate was separately

injected into the capillary as the terminating ion for transient ITP. The LODs for nitrite and nitrate were 15 and 7.0 µg/L (as nitrogen), respectively, which were obtained at a signal to noise ratio (S/N) of 3. The values of the relative standard deviation (RSD) of peak area for nitrite and nitrate were 7.3 and 0.8%, and the RSDs of peak height were 5.7 and 1.2% when the concentration of nitrite and nitrate was 0.05 and 0.25 mg/L. The RSDs of migration time for these ions were 0.2%. The proposed method was applied to the determination of nitrite and nitrate in seawater samples. The results of nitrite were nearly agreed with those obtained by naphthylethylenediamine spectrophotometric analysis (SPA, correlation coefficient 0.9041).

Correspondence: Dr. Keiichi Fukushi, Research Institute for Marine Cargo Transportation, Kobe University of Mercantile Marine, 5-1-1 Fukaeminami-machi, Higashinada-ku, Kobe 658-0022, Japan (Tel & Fax: +81-78-431-6343; E-mail: fukushi@cc.kshosen.ac.jp)

Keywords Seawater analysis · Nitrate · Nitrite · Transient isotachopheresis · Terminating ion · Capillary zone electrophoresis

Introduction

Ion chromatography (IC) is commonly used for the determination of inorganic ions in environmental waters such as drinking water, surface water and rainwater **except for seawater samples**. Ito *et al.* [1] have performed the determination of nitrite and nitrate in seawater pretreated by passage through a Sep-Pak C₁₈ column by IC using a cetyltrimethylammonium (CTA⁺)-coated column with high anion-exchange capacity. Recently, electrostatic IC has been reported for the direct determination of UV-absorbing inorganic anions (such as bromide, nitrate and iodide) in saline matrixes [2]. However, the baseline separation of bromide and nitrate could not be obtained in an actual seawater sample. **There are not many papers** on the application of capillary zone electrophoresis (CZE) to seawater analysis [3-6] although it is a powerful separation method alternative to IC. We have demonstrated the determination of bromide, nitrite and nitrate in environmental water samples by CZE using the artificial seawater containing 3 mM cetyltrimethylammonium chloride (CTAC) as the background electrolyte (BGE). Any sample pretreatment without filtration was not necessary in our method for the determination of above anions in environmental waters including

seawater [7]. However, the CZE sensitivity was not high enough for the analysis of environmental waters. Several on-line preconcentration techniques have been proposed to enhance the CZE sensitivity [4]. It is difficult to apply the ionic-strength mediated stacking [8] *etc.* to seawater analysis because of its high salinity. Quite recently, CZE method has been established for the determination of nitrate in seawater using chloride-induced leading-type transient isotachophoresis (ITP) [9]. We have also demonstrated that transient ITP proved to be a suitable preconcentration procedure for the determination of nitrite and nitrate in seawater samples [10, 11]. Boden *et al.* [12] described that the transient ITP time of the analyte depends on the mobility difference of the matrix and analyte as well as of the matrix and co-ion, the length of the sample zone, the conductivity of the sample zone and the concentration of the co-ion. That is to say, the transient ITP time is dependent on the mobility and concentration of the terminating ion if other conditions are fixed. It is, therefore, important to select the proper terminating ion and to determine its concentration in order to obtain higher transient ITP concentration. In our papers chlorate has been adopted as the terminating ion as the result of simple investigation. Křivánková *et al.* [13] mentioned that a suitable weak acid or a weak base which effective mobility is higher than that of

hydroxide ion or hydrogen ion is generally used as a terminating ion. However, there are few papers concerning the determination of the most effective terminating ion for practical application of transient ITP.

The purpose of this work is to find out the most effective terminating ion and to investigate the effect of the concentration and the proper injection procedure for the CZE determination of nitrite and nitrate in seawater using transient ITP. The following injection procedures were carried out for the addition of terminating ion into the capillary: the terminating ion was directly added into a sample solution; the terminating ion was separately injected after the sample solution was injected. The proposed method was applied to the determination of nitrite and nitrate in real seawater samples. The CZE results for nitrite were compared with those obtained by naphthylethylenediamine spectrophotometric analysis (SPA) [14] which was conventionally used for nitrite and nitrate analyses.

Experimental

Apparatus

A Perkin-Elmer (Foster City, CA, USA) Model 270A and 270A-HT capillary electrophoretic analyzer with a UV-Vis absorbance detector were used. The rise-time for the detector was set at 0.50 s. A polyimide-coated fused-silica capillary (GL Sciences, Tokyo, Japan), 75 μm ID $\times$ 375 μm OD, served as the capillary electrophoresis column. The total length ($L_{\text{tot.}}$) of the column was 72 cm and the effective length ($L_{\text{det.}}$) was 50 cm, respectively. Peak area, peak height and migration time were measured using a Hitachi (Tokyo, Japan) Model D-2500 Chromato-Integrator. A Horiba (Kyoto, Japan) F-22 pH meter was used. A Shimadzu (Kyoto, Japan) Model UV-160A UV-Vis spectrophotometer was used for SPA.

Reagents

All reagents were of analytical-reagent grade and were used as received.

Cetyltrimethylammonium chloride (CTAC) was obtained from Tokyo Chemical Industry (Tokyo, Japan). Standard solutions of bromide (68 mg/L), nitrite (0.02-1.0 mg/L) and nitrate (0.1-1.0 mg/L) were prepared from 1000 mg/L potassium bromide (Wako, Osaka, Japan), sodium nitrite (Nacalai Tesque, Kyoto, Japan) and potassium nitrate (Wako) solutions. The concentrations of nitrite and nitrate were expressed as the nitrogen concentration. The following sodium compounds (Nacalai Tesque) were examined as a terminating ion for transient ITP: acetate, succinate, sulfite, formate, sulfate, bromate, chromate, chlorate, and thiosulfate. For the analysis by SPA method, *N*-1-naphthylethylenediamine dihydrochloride and sulfanilamide were purchased from Wako. Distilled, demineralized water, obtained from a Yamato Kagaku (Tokyo, Japan) Model WG220 automatic still and a Nihon Millipore (Tokyo, Japan) Milli-QII system, was used throughout. The preparation of artificial seawater (pH 7.9) was based on a Japanese Standard [15] without the bromide. The preparation procedure and the composition of the artificial seawater were described in our previous paper [16]. All solutions used in this work were filtered through a 0.45- μ m membrane filter before use.

Procedure

Seawater samples were filtered through a 0.45- μm membrane prior to the CZE analysis. Nitrite and nitrate in the filtered seawater samples were determined by the following procedure. The detection wavelength was set at 210 nm. 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 filled with BGE (artificial seawater containing no bromide and 3 mM CTAC, pH 7.9) by vacuum for 3 min. A sample solution was vacuum injected into the capillary for 4.5 s (*ca.* 95 nL). As the terminating ion, 600 mM acetate was injected for 19 s (*ca.* 400 nL) for the determination of nitrite and 15 s (*ca.* 315 nL) for nitrate. A voltage of 6 kV was applied, with the sample inlet side as the cathode. Each step was run automatically.

Results and discussion

Effect of kind of terminating ion

In order to produce transient ITP at the initial stage of the CZE separation, a leading and terminating ion must be contained in a sample solution or a BGE. In case of artificial seawater BGE, it can be presumed that the chloride in the BGE acts as the leading ion. A terminating ion must be loaded in the capillary. The following anions were investigated to determine which one was more effective as a terminating ion: acetate, succinate, sulfite, formate, sulfate, bromate, chromate, chlorate and thiosulfate. In our previous papers [10, 11], the terminating ion was added into the sample solution for generating transient ITP. However, it should be noted that this procedure causes a dilution of the sample solution in real seawater analysis. In order to avoid the dilution, the terminating ion can be separately injected after the sample solution was injected. In addition to this advantage, the separate injection of the terminating ion leads to easier control of total amount of terminating ion injected into the capillary. Based on the above consideration, artificial seawater containing 68 mg/L bromide, 1.0 mg/L nitrite and 1.0 mg/L

nitrate was injected into the capillary as the sample solution for 2.0 s, and then 800 mM of each anion was injected as the terminating ion. In order to obtain the higher concentration effect, maximum amount of each terminating ion was injected into the capillary so that the baseline separation of nitrite and the terminating ion or that of nitrate and the terminating ion could be accomplished. The analytical results are summarized in Fig. 1. It was proved that the separate injection of the terminating ion was also effective as well as the addition of the terminating ion into the sample solution. The values on the ordinate show the ratio of the peak height for nitrite and nitrate to that without terminating ion. When acetate was used as the terminating ion, the largest peak height ratio for nitrite was obtained similar to when succinate, sulfite or formate was used. On the other hand, when acetate was used as the terminating ion the peak height ratio for nitrate was larger than those in case of other anions. The correlation coefficients between calculated mobilities [Hirokawa, T., personal communication] for several terminating ions and peak height ratios for nitrite and nitrate were -0.7847 and -0.9831, respectively. It can be presumed that the lower the mobility for a terminating ion, the larger the peak height ratios for nitrite and nitrate. This result could be explained by the following equation [12],

$$t = \frac{l_0 \cdot \kappa_s \cdot (\mu_M - \mu_E)}{i \cdot (\mu_M - \mu_A)^2} \quad (1)$$

where t , l_0 , κ_s , i , μ_M , μ_E , μ_A , is transient ITP time, the length of the sample zone, the electric conductivity of the sample zone, the current density, the mobility of matrix ion, the mobility of the co-ion and the mobility of analyte ion, respectively. In our artificial seawater BGE system, it is assumed that chloride in the BGE and the terminating ion corresponds to the matrix ion and the co-ion, respectively. The transient ITP time becomes longer when the terminating ion with lower mobility is used, as evident from Eq. 1. Acetate was, therefore, selected as the optimum terminating ion and its separate injection was adopted for subsequent experiments.

Effect of the concentration of acetate

Effect of the concentration of acetate as the terminating ion was examined. The concentration of acetate was increased over the range of 300-1000 mM. Maximum volume of each concentration of acetate was injected so that the baseline separation of nitrite and acetate or nitrate and acetate could be

accomplished. The peak height ratio for nitrite increased with acetate concentration up to 600 mM, but then slightly decreased, as shown in Fig. 2. The similar tendency was observed for nitrate. It was presumed that the transient ITP time became longer as the apparent length of the sample zone became longer when the higher concentration of acetate was used (see Eq. 1). At the acetate concentration of 700 mM or above, the analyte ions presumably reached to the detector before the transient ITP was not completely converted to the CZE. Therefore, 600 mM was adopted as the optimum concentration of acetate in subsequent experiments.

Effect of injection volume of sample solution

The easiest way for the sensitivity improvement is to increase the sample volume injected into the capillary. However, when artificial seawater containing 68 mg/L bromide, 1.0 mg/L nitrite and 1.0 mg/L nitrate as the sample solution was injected for 3.0 s (*ca.* 63 nL) without terminating ion, the baseline separation of nitrite and nitrate was not obtained, as shown in Fig. 3 (A). The peak shapes were also broadened. After the sample solution was injected for 3.0 s, 600 mM acetate was

injected into the capillary for 9.4 s and analyzed. The baseline separation of sharper nitrite and nitrate peaks was obtained, as shown in Fig. 3 (B). An increase in the sample volume is expected if the concentrations of nitrite and nitrate in the sample are lower than 1.0 mg/L. As the sample solution, artificial seawater containing 68 mg/L bromide, 0.1 mg/L nitrite and 0.1 mg/L nitrate was injected for 2.0-5.0 s. The injection period for 600 mM acetate was also increased according to the sample volume. As a result, the maximum injection period of sample solution was 4.5 s. The baseline separation of nitrite and nitrate was accomplished, as shown in Fig. 3 (C). Optimum conditions were summarized as follows: the injection period of sample solution was 4.5 s, the injection period of 600 mM acetate was 19 and 15 s for the determination of nitrite and nitrate, respectively. The unknown negative peaks were observed when acetate was used as the terminating ion, as evident in Figs. 3 (B) and (C). The negative peaks might be caused by the pH difference among the sample, BGE and terminating ion zones.

Calibration graphs

Calibration graphs for nitrite and nitrate were linear using both peak area and peak height. The regression equations relating area response (y, arbitrary units) to concentrations for nitrite (x, 0-0.1 mg/L) and nitrate (x, 0-0.5 mg/L) were $y=5.77 \times 10^4 x + 1100$ (correlation coefficient 0.9957), $y=9.12 \times 10^4 x + 1220$ (0.9999) respectively. The regression equations relating height response to concentrations for nitrite and nitrate were $y=1.42 \times 10^4 x + 412$ (0.9965), $y=2.96 \times 10^4 x + 4220$ (0.9996). The values of the intercept are due to the trace amount of nitrite and nitrate dissolved in the acetate solution as impurities. The reasons are as follows: the nitrite and nitrate peaks were observed even when artificial seawater containing no nitrite and nitrate was analyzed by CZE using transient ITP while the peaks were not appeared without transient ITP. The concentrations of nitrite and nitrate in 600 mM acetate solution were estimated to be 7.0 $\mu\text{g/L}$ and 43 $\mu\text{g/L}$, respectively. The values of the relative standard deviation (RSD) and the limit of detection (LOD, $S/N=3$) for nitrite and nitrate are summarized in Table 1. Whereas the RSDs of the migration time of nitrite and nitrate showed good reproducibility, RSDs of the peak area and peak height of nitrite were larger than

those of nitrate. It should be noted that the nitrite concentration was 1/5 of the nitrate concentration, and the sensitivity of nitrite was lower than nitrate at 210 nm. The concentrations of 0.05 mg/L for nitrite and 0.25 mg/L for nitrate were close to those in real seawater samples. The LODs for nitrite (15 µg/L) and nitrate (7.0 µg/L) were lowered to 1/4.2 and 1/4.7 of the LODs (63 µg/L for nitrite and 33 µg/L for nitrate) obtained using chlorate as the terminating ion in our previous paper [10], respectively.

Analysis of seawater samples

The proposed method was applied to the determination of nitrite and nitrate in seawater samples taken from Osaka Bay on November 2001. Figures 4 (A) and (B) represent electropherograms of the seawater sample (Table 2 (I)) for the determination of nitrate and nitrite, respectively. Nitrite concentration was also determined by SPA method. The results are summarized in Table 2. The CZE results were obtained using peak area measurements. The results of nitrite were nearly agreed with those obtained by SPA method (correlation coefficient 0.9041). Paired *t*-test [17] was also performed to examine the nitrite data obtained by both

methods statistically. The proposed CZE and SPA methods do not give significantly different results.

Conclusions

We found that 600 mM acetate was most suitable terminating ion for transient ITP-CZE determination of nitrite and nitrate in seawater using artificial seawater as the BGE. The most effective procedure for transient ITP was to inject the maximum amount of terminating ion separately after the sample solution was injected so that the baseline separation of the analyte and the terminating ion could be accomplished. The proposed method possesses relatively high accuracy for the determination of nitrite in seawater whose concentration is ca. 0.02-0.06 mg/L. Effect of the pH of the BGE is worthy of a further study to improve the LODs for the simultaneous determination of nitrite and nitrate in seawater although the LODs for these ions were considerably lowered.

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Fig. 1. Effect of kind of terminating ion on the concentration effect for nitrite and nitrate. Sample, artificial seawater containing 68 mg/L Br^- , 1.0 mg/L NO_2^- and 1.0 mg/L NO_3^- ; injection period, 2.0 s. Concentration of terminating ion, 800 mM; injection period, maximum period on condition that the baseline separation of the analyte and the terminating ion could be accomplished. h, peak height; h_0 , peak height without terminating ion; A, CH_3COO^- ; B, $\text{C}_4\text{H}_4\text{O}_4^{2-}$; C, SO_3^{2-} ; D, HCOO^- ; E, SO_4^{2-} ; F, BrO_3^- ; G, CrO_4^{2-} ; H, ClO_3^- ; I, $\text{S}_2\text{O}_3^{2-}$; $\square$, NO_2^- ; $\blacksquare$, NO_3^- . Electrophoretic conditions: BGE, artificial seawater without Br^- containing 3 mM CTAC; voltage, -6 kV; UV detection, 210 nm; capillary, $L_{\text{tot.}}=72$ cm, $L_{\text{det.}}=50$ cm, 75 μm ID.

Fig. 2. Effect of the concentration of terminating ion. Terminating ion, acetate. $\square$, NO_2^- ; $\blacksquare$, NO_3^- . h, h_0 , injection period for terminating ion, electrophoretic conditions, sample and injection period as in Fig. 1.

Fig. 3. Electropherograms for the separation of bromide, nitrite and nitrate. Concentrations of bromide, nitrite and nitrate in artificial seawater as a sample solution: (A) and (B), 68 mg/L Br^- , 1.0 mg/L NO_2^- and 1.0 mg/L NO_3^- ; (C), 68

mg/L Br^- , 0.1 mg/L NO_2^- and 0.1 mg/L NO_3^- ; injection period, (A) and (B), 3.0 s; (C) 4.5 s. Terminating ion, 600 mM acetate; injection period, (A), 0 s; (B), 9.4 s; (C) 15 s. Identification of peaks: a, Br^- ; b, NO_2^- ; c, NO_3^- ; d, unknown; e, CH_3COO^- . Electrophoretic conditions as in Fig. 1.

Fig. 4. Electropherograms of seawater collected from Osaka Bay. Sample, sample (I) in Table 2; injection period, 4.5 s. Terminating ion, 600 mM acetate; injection period, (A), 15 s; (B), 19 s. Electrophoretic conditions as in Fig. 1. Identification of peaks as in Fig. 3.

Table 1 Precision and detection limit of determination of nitrite and nitrate

	RSD (%)			LOD ($\mu\text{g/L}$, S/N=3)
	Area	Height	Time	
NO_2^-	7.3	5.7	0.2	15
NO_3^-	0.8	1.2	0.2	7

a) Sample: artificial seawater containing 68 mg/L Br^- , 0.05 mg/L NO_2^- , and 0.25 mg/L NO_3^- , eight determinations

b) Analytical conditions as in Fig. 4

Table 2 Analytical results for nitrite and nitrate in seawater^{a)}

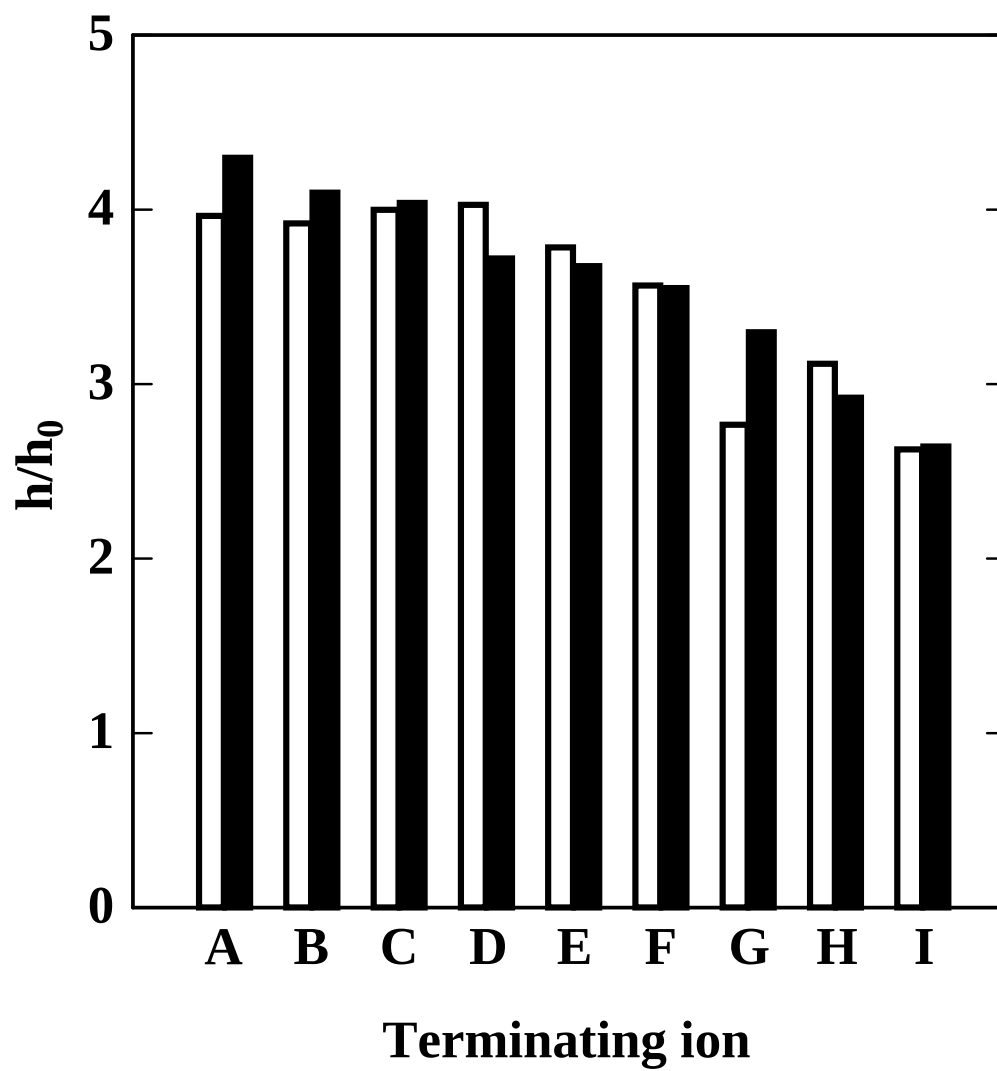
Sample	NO ₂ ⁻ (mg/L) ^{b)}		NO ₃ ⁻ (mg/L) ^{b)}
	CZE ^{c)}	SPA ^{d)}	CZE ^{c)}
(I)	0.047	0.058	0.25
(II)	0.062	0.054	0.24
(III)	0.058	0.061	0.30
(IV)	0.059	0.062	0.28
(V)	0.053	0.059	0.29
(VI)	0.045	0.051	0.27
(VII)	0.022	0.027	0.17

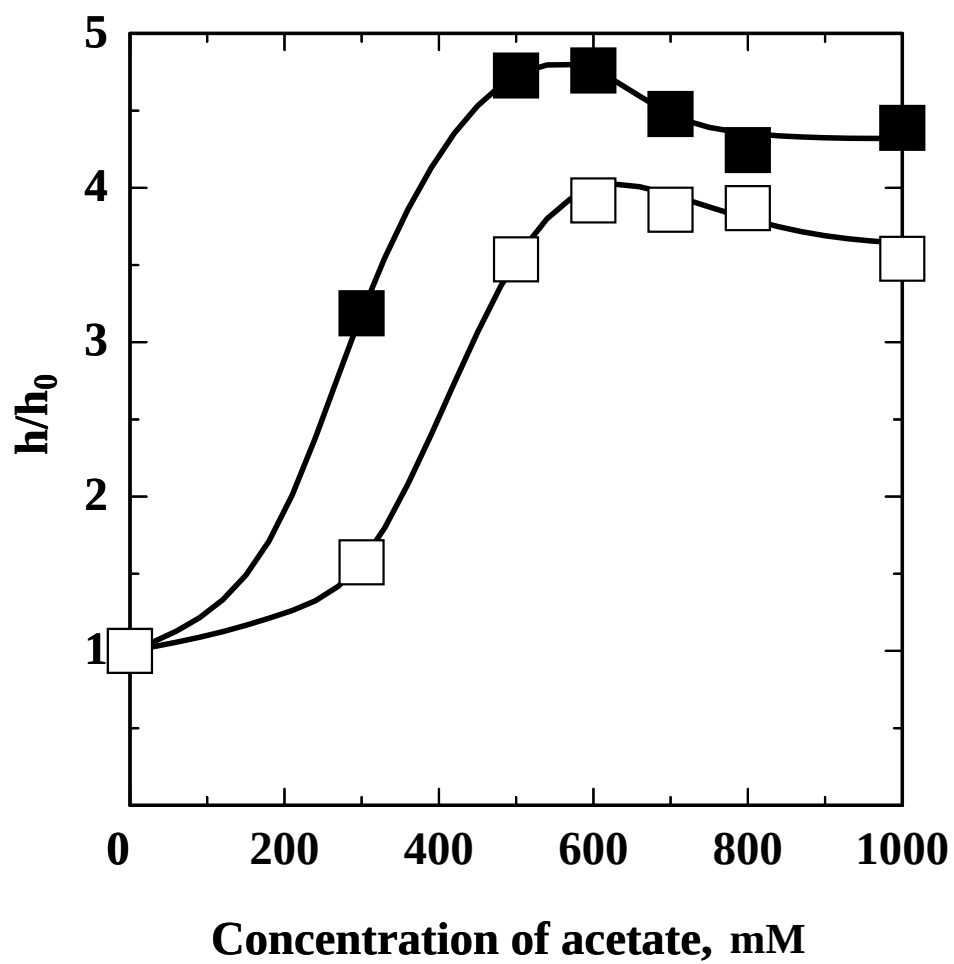
a) Sampling, November 2001; analytical conditions as in Fig. 4

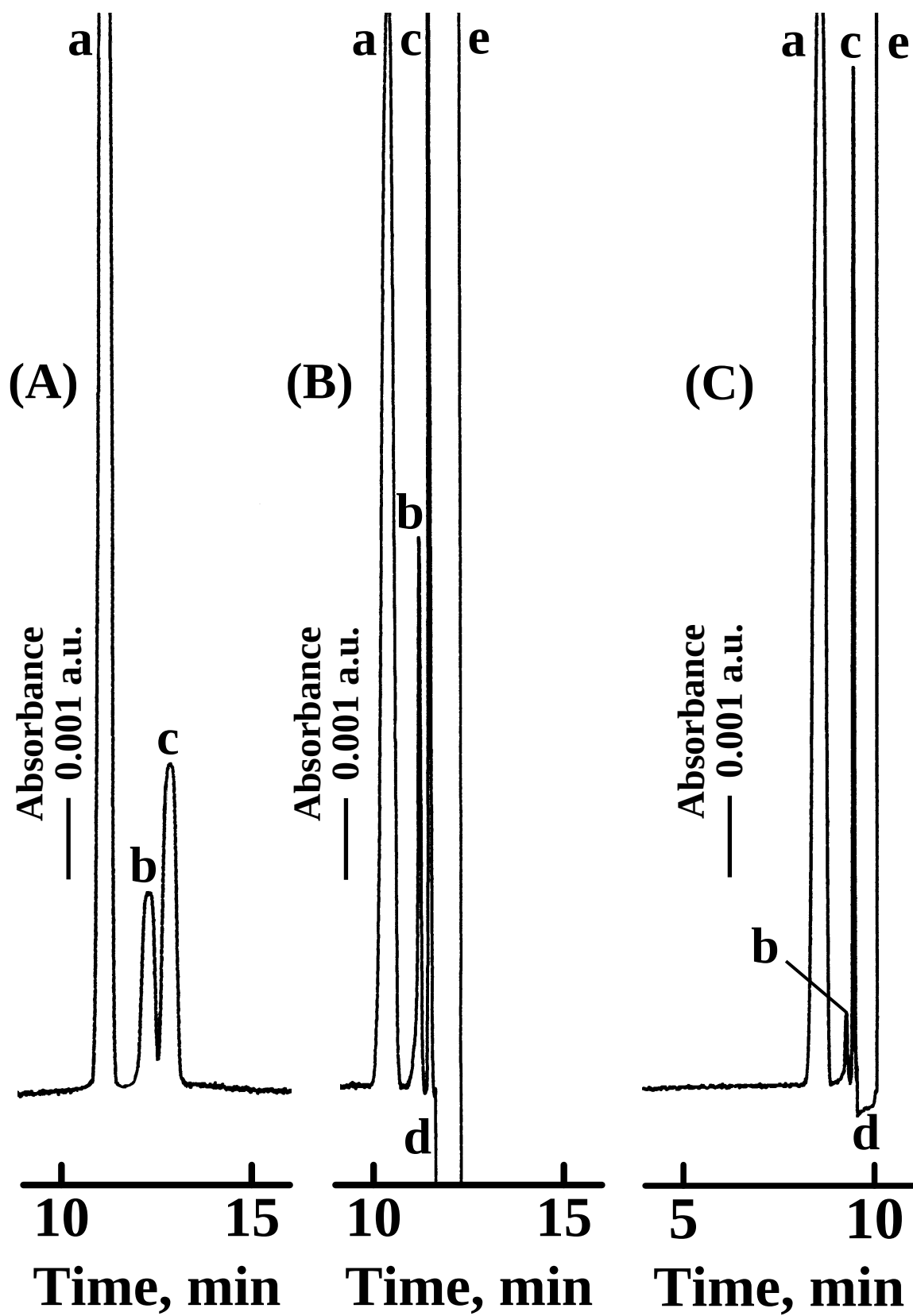
b) Concentrations of nitrite and nitrate were shown as nitrogen

c) Calculated by peak area measurements

d) Naphthylethylenediamine spectrophotometric method







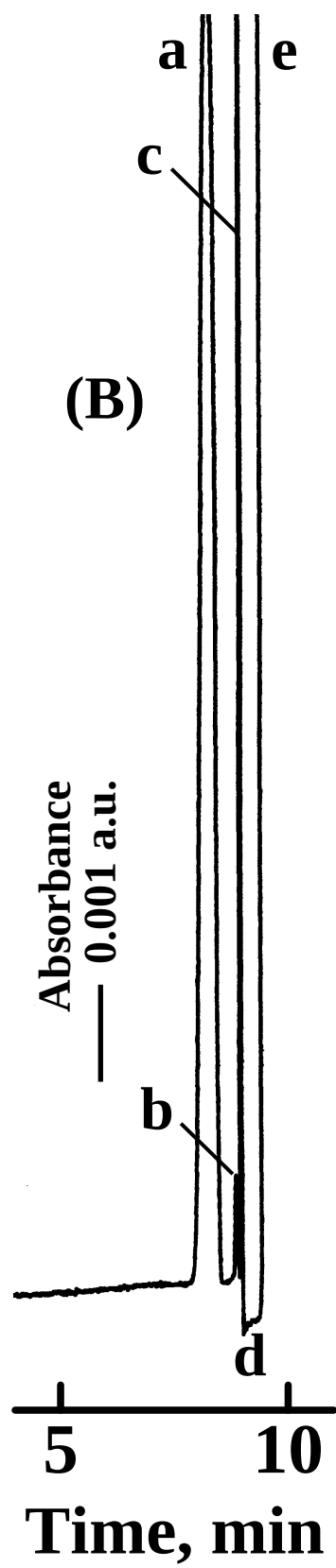
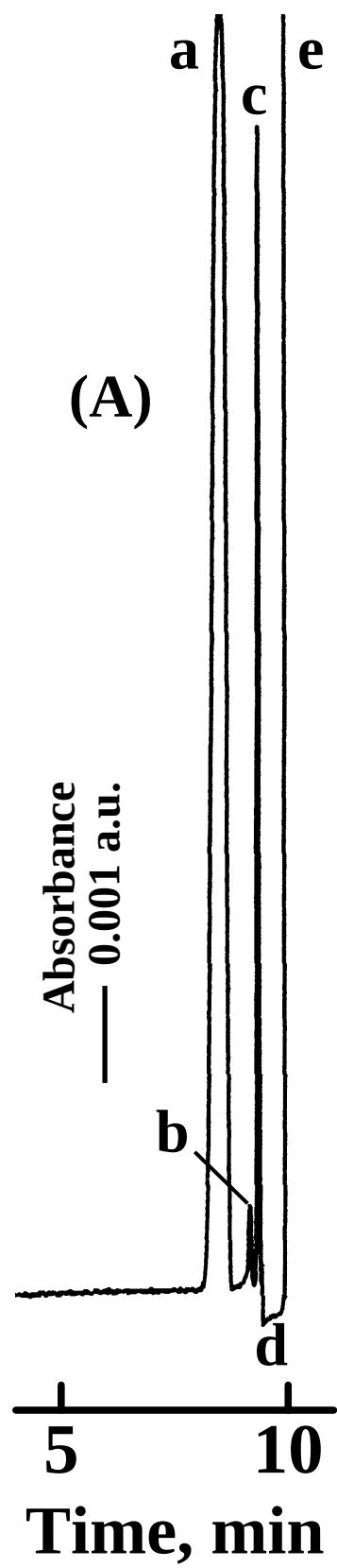


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	CZE ^{c)}	SPA ^{d)}	CZE ^{c)}
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(II)	0.062	0.054	0.24
(III)	0.058	0.061	0.30
(IV)	0.059	0.062	0.28
(V)	0.053	0.059	0.29
(VI)	0.045	0.051	0.27
(VII)	0.022	0.027	0.17

a) Sampling, November 2001; analytical conditions as in Fig. 4

b) Concentrations of nitrite and nitrate were shown as nitrogen

c) Calculated by peak area measurements

d) Naphthylethylenediamine spectrophotometric method