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Ion chromatography for determination of nitrite and nitrate in seawater using monolithic ODS columns

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Abstract

A fast and highly sensitive ion chromatographic method using monolithic ODS columns was developed for the determination of nitrite (NO_2^-) and nitrate ($NO3^-$) in seawater. Two monolithic ODS columns (50 mm × 4.6 mm i.d. + 100 mm × 4.6 mm i.d.) connected in series were coated and equilibrated with 5 mM cetyltrimethylammonium chloride (CTAC) aqueous solution. The column efficiency with 0.5 M NaCl as the mobile phase did not decrease in spite of the increase in flow rate of the mobile phase. Thus, good chromatograms were obtained within 3 minutes for NO_2^- and NO_3^- in artificial seawater without interferences by coexisting ions. The detection limit (S/N = 3) with UV detection at 225 nm was 0.8 and 1.6 μg/L for NO_2^- and NO_3^-, respectively. The characteristics of the monolithic CTA^+-coated ODS columns were discussed. The present method was successfully applied to the fast and sensitive determination of NO_2^- and NO_3^- in real seawater samples.

Keywords: NO_2^- and NO_3^- in seawater; Monolithic ODS columns; Cetyltrimethylammonium chloride (CTAC); NaCl mobile phase; UV detection

1. Introduction

The concentration levels of nitrite and nitrate in water environments are important indicators of water quality and are also associated with pollution of eutrophication and blooms [1,2]. However, as the levels in seawater are generally low, sensitive method is necessary for quantification. Nitrite in seawater was determined by spectrophotometry [3,4] and fluorometry [5] of azo dyes produced by diazo and coupling reactions between nitrite and aromatic primary amines under acidic conditions. Whereas, nitrate was reduced to nitrite through a reductive column filled with copperized-cadmium [3–5] and by using a UV lamp [6] and was finally determined as the summation of nitrate and nitrite [3–6]. Those determinations were performed by flow injection analysis (FIA) [3–6] with higher sample throughput. However, the methods are not necessarily simple in the measurement system and problems with the stability and handling of the reduction column have been reported. Thus, the development of simple, fast and sensitive methods is still desirable for the simultaneous determination of nitrite and nitrate in seawater.

Ion chromatography (IC) and capillary zone electrophoresis (CE) based on the separation method have been examined for such simultaneous determination. In IC, it is important to remove the interferences due to large amounts of matrix anions such as Cl^-, SO_4^{2-}, and Br^- [7–12]. The use of cationic surfactant (cetyltrimetylammonium (CTA^+))-coated ODS column with higher anion-exchange capacity and 0.1 M sodium chloride as the eluent allowed the direct UV detection of nitrite and nitrate without the interferences by the matrix ions [7,8]. Similar method was examined using an anion-exchange column with high exchange-capacity [9]. The use of ODS column coated with zwitterionic surfactant (Zwittergent-3-14) and artificial seawater as eluent (20-fold dilution) was effective for the sensitive UV detection of nitrate and iodide in seawater, but nitrite could not be determined due to the interferences by Br^- etc [10]. When an anion-exchange column and a dilute sodium perchlorate eluent (pH 10) were used for the detection of nitrite, nitrate,

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and sulfide in saline (pore) waters, samples were 10 times diluted to ensure the separation, leading to lower sensitivity. Further, separation of the analytes from bromide ion was not so good [11]. Column-switching ion chromatography is useful for anion detection in saline solutions but is complicated in measurement system and operation, and is lower in sensitivity [12]. In those methods, the analytes were measured with longer retention times (15–17 min) to remove the interferences by matrix ions [7–9,11,12]. In CE, on the other hand, the matrix ion (chloride ion) in seawater did not interfere the analyte detection but work effectively as a leading ion for the on-line preconcentration technique using transient isotachophoresis (tITP) [13–15]. Time necessary for the measurement procedure containing capillary column pretreatment and the tITP procedure was 15–20 min [14,15].

In this study, ion chromatography using monolithic ODS columns was examined for the fast and sensitive determination of nitrite and nitrate in seawater. Our purpose of this study is to get good chromatograms with much shorter retention times, for example 3 min without deteriorating separability, thus leading to sensitive UV detection. Monolithic ODS columns with higher porosity allow higher flow rate of the mobile phase [16,17], and were used for ultra-fast anion separation [18–20]. Likewise, similar separation was performed on a short particulate ODS column [21]. In those studies, tetrabutylammonium salt [18] and dilaurylmethylammonium bromide (DDAB) [19–21] were used as the ion-interaction and the column-coating reagent, respectively. However, those previous methods cannot be applied to the trace analysis of nitrite and nitrates in seawater, due to the interferences by matrix anions in the sample. Our approach to detect the analytes in seawater utilizes monolithic CTA^+-coated columns with high anion-exchange capacity as the stationary phase and a concentrated sodium chloride solution as the mobile phase.

2. Experimental

2.1. Apparatus

The IC system used in this study consisted of: (1) an eluent delivery pump (a LC-6200 pump (Hitachi, Tokyo, Japan) or a LC-10A pump (Shimadzu, Kyoto, Japan)), (2) a sample injector (Rheodyne, Cotati, CA, USA) with a 200- or 500-μl sample loop, (3) a L-4200 UV-visible detector (Hitachi), and (4) a C-R8A chromato-processor (Shimadzu). Two monolithic C18 reversed-phase (ODS) columns which were just different in length were used: Chromolith Speed ROD RP-18e (50 mm × 4.6 mm i.d.) and Chromolith RP-18e (100 mm × 4.6 mm i.d.) (Merck KGaA, Darmstadt, Germany).

2.2. Reagent, mobile phase, seawater samples

All inorganic sodium salts were of analytical grade and were used for preparation of standard anionic solutions and mobile phases. Standard anionic solutions were prepared by mixing and diluting stock solutions of the salts (anion conc., 10 g/L). Stock solutions of 2 M NaCl, 0.1 M Na_2HPO_4, and 0.5 M NaH_2PO_4 were used for mobile phase preparation. The mobile phases prepared were passed through a membrane filter (cellulose-nitrate type; pore size, 0.1 μm) before use. Artificial seawater (35‰ salinity) was prepared according to the Lyman–Fleming formula [22]. Two surface seawater samples were collected around the coastal area of Kurahashi Island (Hiroshima prefecture, Japan) and subjected to the IC analysis after filtration through a membrane filter (pore size 0.45 μm).

2.3. Preparation of separation columns

Two monolithic C18 reversed-phase columns (50 and 100 mm long) were coated and equilibrated with 5 mM cetyltrimethylammonium chloride (CTAC) aqueous solution. CTAC was obtained from Nacarai tesque (Kyoto, Japan). The coating solution was delivered through the columns at a flow rate of 0.5 ml/min. Completion of column coating was confirmed by rapid increase in conductivity of the effluent, but the column coating was further continued for ca. 1–2 h to ensure the adsorption equilibrium. The coating solution was switched to water and then to 0.5 M NaCl containing 5 mM sodium phosphate buffer (pH 4.7) with or without 0.2 mM CTAC. The preparation of CTA^+-coated column was also checked from the retention times of NO_2^- (ca. 4 min) and NO_3^- (ca. 8 min) in artificial seawater at a flow rate of 1 ml/min for the 0.5 M NaCl mobile phase.

3. Results and discussion

3.1. Choice of IC conditions using monolithic columns

There are two methods for anion separation using ODS columns [7,8,10,18–21,23]: one approach is to use them as the columns dynamically coated with hydrophobic cationic modifiers such as tetrabutylammonium ion in the mobile phase (ion-interaction chromatography). The other is the use of ODS columns permanently coated with cationic surfactants such as CTAC and DDAB (ion-exchange chromatography). The second method was used in this study because higher ion-exchange capacity was necessary for the separation of nitrite and nitrate in seawater [7–9]. The separation of analytes was at first examined using a 50 mm-long column coated (equilibrated) with 5 mM CTAC aqueous solution and 0.1 M NaCl as the mobile phase. Two results were observed: (1) the broadening of chromatograms of nitrite and nitrate in 35‰ artificial seawater for 100 μl-sample injection and (2) slight decrease in the retention times of anions (iodate, nitrite, bromide, and nitrate) in deionized water in spite of good separation. The former indicates that anion-exchange capacity of the column was not enough for the separation of nitrite and nitrate in seawater. The latter suggests that the hydrophobic

interaction between octadecyl group of the column and cetyl group of CTA^+ is not so strong compared to conventional particulate ODS columns [7,8,23,24]. On the other hand, the higher concentration of NaCl as the mobile phase was effective to enforce the hydrophobic interaction with octadecyl group on the stationary phase [25]. Thus, the use of a coupled column (50 mm + 100 mm long columns connected in series) and higher concentration of NaCl were examined for further experiments. In the range of 0.1–1.5 M NaCl of mobile phase examined, good separation was obtained with 0.5 M NaCl for the analytes in 35‰ artificial seawater, whereas nitrate was partially overlapped with bromide when 1.5 M NaCl was used as the mobile phase. Further, the addition of CTAC in the mobile phase was examined in order to keep the retention times of analytes. However, the larger baseline fluctuation was rarely observed for 0.5 M NaCl with 0.2 mM CTAC, whereas a good baseline was always obtained for the mobile phase without CTAC. Thus, further study was carried out by 0.5 M NaCl without CTAC. Na_2SO_4 (2 M) with weak elution power (sulfate is the second major anion in seawater) was not enough for eluting nitrite and nitrate in artificial seawater (retention times were 13.3 and 32.3 min, respectively, at the flow rate of 1.0 ml/min).

3.2. Fast and sensitive detection of nitrite and nitrate in seawater

The major characteristics of a monolithic ODS column, a single piece of silica column with interconnected skeletons, are a larger ratio of through-pore size to skeleton-size and a high porosity (about 15% higher than particulate column) [16,17]. These allow higher flow rate of mobile phase due to lower backpressure and also limited band broadening compared to conventional particulate ODS column [18–20]. Fig. 1 shows the separation of nitrite and nitrate (50 μg/L of each) in 35‰ artificial seawater at flow rates of 1–3 ml/min for the mobile phase of 0.5 M NaCl. Good separation of the analytes was obtained at a flow rate of 3 ml/min and the column pressure was ca. 90 kg/cm^2 for the coupled columns. The theoretical plates per the columns were almost constant for each anion: nitrite (930, 1020, and 1030) and nitrate (1840, 2030, and 2210) for flow rates of 1, 2, and 3 ml/min, respectively. The dependence of the analytes separation on injected sample volume was examined in the range of 100–500 μl. Both the peak height and the peak area of nitrite and nitrate in 35‰ artificial seawater increased with the sample volume, but the peak height did not increase above 300 μl because seawater samples containing excess of salts also exerted as eluent. Thus, the sample injection volume of 200–300 μl was appropriate.

Fig. 2 shows the ion chromatograms of lower levels of nitrite and nitrate in 35‰ artificial seawater at UV 210 and 225 nm. The baseline stability at UV 225 nm was higher compared with that at UV 210 nm, although the sensitivity at UV 225 nm was lower. The detection limit (DL, S/N = 3) at 225 nm was 0.8 and 1.6 μg/L for nitrite and nitrate, respectively. The DLs were lower compared to previous IC by CTA^+-coated ODS (4 μg/L for nitrite and 8 μg/L for nitrate) [7], which could be ascribed to the increase in sample volume from 100 to 200 μL, the suppression of peak broadening due to the use of monolithic columns, the decreased sample self-elution effect on the columns with high anion-exchange capacity (ca. 0.4 mM equivalent/150 mm long column), etc. The values were also lower in comparison with IC (6.2 μg/L for nitrite and 10 μg/L for nitrate) [11], the column-switching method (300 μg/L for nitrite and 100 μg/L for nitrate) [12], and CE (8.9 μg/L for nitrite and 16 μg/L for nitrate) [15], but higher compared to IC by ODS-column coated with zwitterionic surfactant (0.5 μg/L for nitrate) [10]. Furthermore, successive sample injection every 4 min was possible without any troubles. This high sample throughput (15 samples/h) is comparable to those by FIA [4–6] (18, 45, 10 samples/h, respectively). The standard deviations ($n = 6$) of peak area, peak height, and retention time were 0.7, 0.9, and 0.2% for 50 μg/L NO_2^- and 3.4, 5.5, and 1.0% for 50 μg/L NO_3^-, respectively. Higher concentration (0.5 M NaCl) of the mobile phase suppressed the decrease in the retention times of nitrite and nitrate: for 7-h operation, for example, the rate of the decrease in the retention times was 0.12 and 0.42%/h for nitrite and nitrate, respectively at a flow rate of 3 ml/min and did not affect for the determination. Further

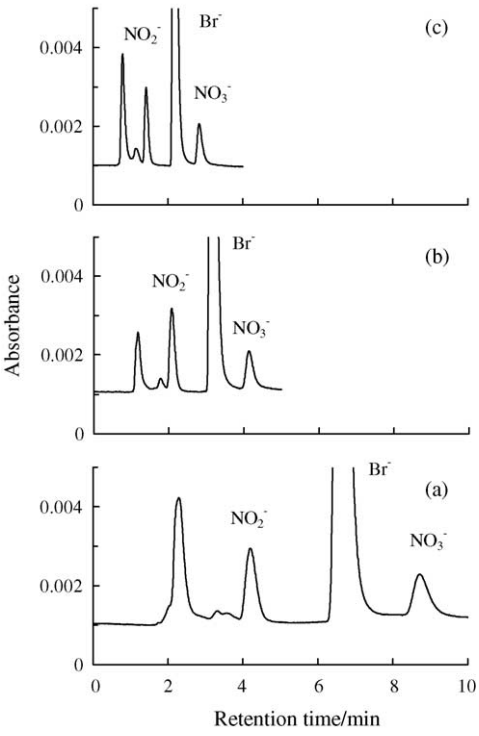

Fig. 1. Ion chromatograms of nitrite and nitrate (50 μg/L, each) in 35‰ artificial seawater. Conditions: separation columns, Chlomolith Speed ROD RP-18e (50 mm × 4.6 mm i.d.) and Chlomolith RP-18e (100 mm × 4.6 mm i.d.); mobile phase, 0.5 M NaCl + 5 mM sodium phosphate buffer (pH 4.7); flow rate of mobile phase: (a) 1.0 ml/min, (b) 2.0 ml/min, and 3.0 ml/min; detection, UV 225 nm; sample volume, 200 μl.

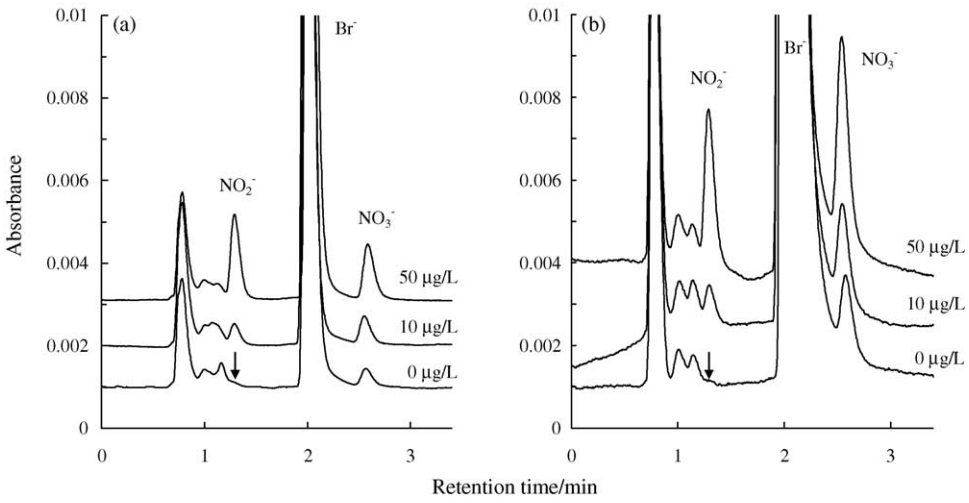

Fig. 2. Ion chromatograms of nitrite and nitrate in 35‰ artificial seawter at: (a) UV 225 nm and (b) UV 210 nm. Other conditions were same as Fig. 1. Flow rate of mobile phase, 3 ml/min.

when more than 70 samples were injected, no problems have occurred for determination. Thus, the coated-columns were useful at least for 1-day measurements. Before the next measurements, the column was conditioned with 0.5 M NaCl with 0.2 mM CTAC (ca. 200–300 ml). This conditioning can easily recover the retention times of analytes, and the eluent can be switched to the original mobile phase without CTAC. Owing to these procedures, we could always get good separation for more than one year using the same coupled column.

Fig. 3 shows the chromatograms of real seawater samples and the samples spiked with nitrite and nitrate of 50–200 μg/L. Good separation was obtained without any interferences by matrix ions in seawater. Determination (concentration, C: μg/L) was carried out by peak area method: $y = 2.40 \times 10^2 C + 56$ ($r^2 = 0.9998$) and $y = 1.29 \times 10^2 C + 1.0 \times 10^3$ ($r^2 = 0.9999$) ($n = 6$, 0–1000 μg/L) for nitrite and nitrate, respectively. The concentration was 38.5 ± 1.2 μg/L and 189 ± 1 μg/L ($n = 3$) for nitrite and nitrate (Fig. 3a), respectively, and 10.8 ± 0.5 μg/L and 21.3 ± 0.5 μg/L ($n = 3$) for nitrite and nitrate (Fig. 3b), respectively. The recovery found for the added analytes of 50, 100 and 200 μg/L was $100.5 \pm 1.9\%$ and $103.3 \pm 5.2\%$ ($n = 9$) for nitrite and nitrate (Fig. 3a), respectively, and $99.7 \pm 1.7\%$ and $97.3 \pm 6.7\%$ ($n = 9$) for nitrite and nitrate (Fig. 3b), respectively.

In conclusion, the CTA^+-coated monolithic columns with higher porosity allowed higher flow rate of mobile phase, leading to higher sample throughput without the loss of separation efficiencies. Thus, the fast and sensitive determination

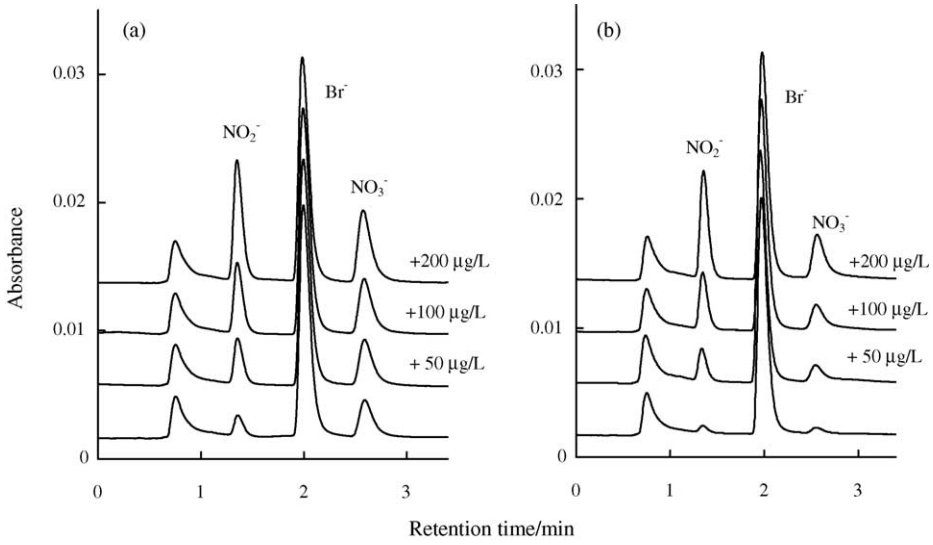

Fig. 3. Ion chromatograms of real seawater samples at UV 225 nm. Other conditions were same as Fig. 2. Surface seawater samples: (a) and (b) (the coastal area of Kurahashi Island).

for nitrite and nitrate in seawater was possible by ion chromatography with UV detection.

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