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Journal of Chromatography A, 1003 (2003) 197–202
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Simultaneous determination of inorganic nitrogen species by microcolumn ion chromatography

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Received 25 February 2003; received in revised form 5 May 2003; accepted 6 May 2003

Abstract

Inorganic nitrogen species (nitrate, nitrite and ammonium ions) were simultaneously determined by microcolumn ion chromatography. Nitrate and nitrite were determined by UV detection at 206 nm, whereas ammonium ion was determined by fluorescence detection at excitation 410 nm and emission 470 nm. The latter fluorescence detection is based on the postcolumn reaction of ammonium ion with *o*-phthalaldehyde in the presence of 2-mercaptoethanol. Effects of the reagent concentration, pH, and other reaction conditions on the signal intensity were examined, and the optimum condition was explored. The present method allowed simultaneous determination of nitrate, nitrite and ammonium ions in river water.

Keywords: Derivatization, LC; Nitrate; Nitrite; Ammonium ion

1. Introduction

The degree of environmental pollution is increasing with rapid industrial and economic growth. Anthropogenic externally-supplied nitrogen is a key factor controlling primary production in *N*-limited environmental waters [1]. Nitrogen is found in human, industrial, and livestock drains. Increase use of these drains leads to eutrophication and environmental pollution.

Since nitrogen has five valence electrons and can take on oxidation states between +5 and −3, it has various chemical species. The nitrogen cycle also offers a variety of important biological and non-biological processes in the environment [2,3]. Thus, it is necessary to determine inorganic nitrogen species for the estimation of the degree of environmental pollution.

A considerable number of papers have dealt with the determination of nitrate, nitrite and ammonium ion. Nitrate and nitrite are determined sensitively by various methods [4,5]. There are many methods for the determination of ammonium ion. Earlier methods used Nessler reagent [6] and indophenol [7]. However, they have much interference such as dissolved organic matter, and the preparation of reaction reagents is complicated and time-consuming. Moreover, it is difficult to control the stability. Recently, the most often used analytical regent for the determination of ammonium ion and amino acids is *o*-phthalaldehyde (OPA) in the presence of 2-mercaptoethanol [8–10]. These assays are highly sensitive and selective to primary amines.

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0021-9673/03/$ – see front matter
doi:10.1016/S0021-9673(03)00836-7

There have been reported a few methods dealing with the simultaneous determination of nitrogen species [11–14]. One of these methods used cation-exchange and anion-exchange columns together with switching valves [11]. Other methods utilized bifunctional columns [12–14]. However, these methods provided less sensitivity and less selectivity.

In this paper, we report a method for the simultaneous determination of inorganic nitrogen species. Nitrate, nitrite and ammonium ions were separated by ion chromatography followed by direct UV detection of the anions and fluorimetric detection of ammonium ion.

2. Experimental

2.1. Apparatus

The apparatus employed for this work is shown in Fig. 1. The eluent and the postcolumn mixing reaction reagent solution were supplied by using a syringe pump (MF-2 Microfeeder, Azumadenki Kogyo, Tokyo, Japan) equipped with two MS GAN-050 gas-tight syringes (Ito, Fuji, Japan) at 4.2 μl/min, respectively. Samples were injected with a model 7520 valve injector (Rheodyne; Cotati, CA, USA). Anion-exchanger, IC-Anion SW (Tosoh, Tokyo, Japan) was employed as the stationary phase and it was packed into fused-silica tubing with 100× 0.32 mm I.D., according to the method previously reported [15]. The eluent and the postcolumn mixing reaction reagent solution were mixed at a Nano Y connector (Upchurch Scientific, Oak Harbor, WA, USA) [16], followed by passing into a fused-silica capillary tube (50 μm I.D.). Cations are not retained on the anion-exchange column, and non-retained ammonium ion reacts with the reaction reagent in the capillary tube. The effluent was first monitored by a UV-2070 Plus Intelligent UV–Vis detector (Jasco, Tokyo, Japan) and the effluent from the UV detector was merged by the postcolumn reaction reagent, followed by detection with an FP-1520 Intelligent fluorescence detector (Jasco). The UV detector was operated at 206 nm, whereas the fluorescence detector was operated at 410 nm for excitation (Ex) and 470 nm for emission (Em). Chromatopac C-R4AX (Shimadzu, Kyoto, Japan) was used as a data processor. Absorption spectra were measured by using a U-4000S spectrophotometer (Hitachi, Tokyo, Japan) in the stopped flow mode.

2.2. Reagents and materials

All reagents were obtained from Nacalai Tesque (Kyoto, Japan), unless otherwise noted. All of the reagents were used as received. *o*-phthalaldehyde (OPA) and 2-mercaptoethanol were dissolved in methanol. Purified water was prepared in the laboratory by using a GS-590 water distillation system (Advantec, Tokyo, Japan).

The reaction reagent is composed of phosphate buffer, OPA and 2-mercaptoethanol in water–methanol (4:1, v/v). Although it is reported that the

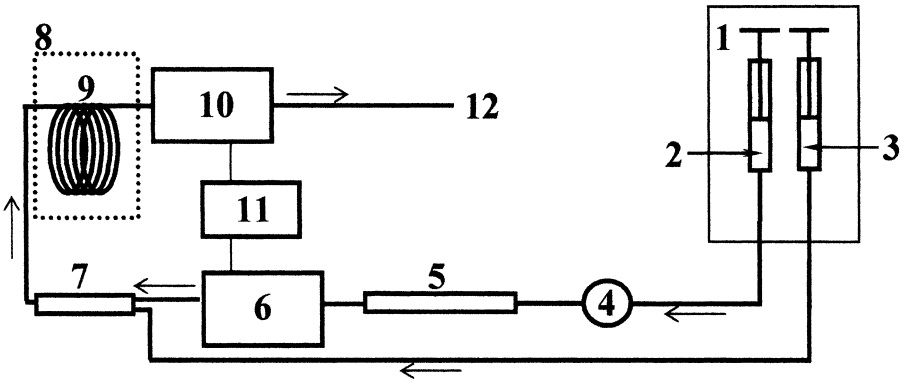

Fig. 1. Experimental apparatus. 1, syringe pump; 2, eluent; 3, reaction reagent solution; 4, sample injector; 5, separation column; 6, UV detector; 7, Nano Y connector; 8, water-bath; 9, reaction coil; 10, fluorescence detector; 11, data processor; 12, drain.

reaction reagent is prepared about 1 day ahead of use at room temperature [8,9], we freshly prepared the reaction reagent just before use.

Water samples were filtered with a 0.45-μm membrane filter, and hydrophobic components were then removed by passing into a laboratory-made pre-column (10×0.5 mm I.D.) packed with hydrophobic Develosil C30 packing material for LC (15–30 μm particle diameter; Nomura Chemicals, Seto, Japan).

3. Results and discussion

3.1. Absorption and fluorescence spectra

Fig. 2 shows emission and excitation spectra of the reaction reagents in the presence or absence of ammonium ion. These spectra were observed 30 min after the reaction at room temperature. The excitation spectra of the reaction reagent are given when the emission wavelength is fixed at 470 nm. It is observed that the excitation wavelength providing the maximum fluorescence intensity is 410 nm. On the other hand, the emission spectra of the reaction reagent are given where the excitation wavelength is fixed at 410 nm. It should be noted that the blank signals are observed in Fig. 2. It is presumed that the background signals are due to the intermediate product formed between OPA and 2-mercaptoethanol. Considering the results in Fig. 2, the fluorescence detector was operated at Ex=410 nm and Em=470 nm in the following experiments. In addition, nitrite and nitrate were detected at 206 nm in this work considering their absorptivity.

3.2. Effect of the reaction reagents on the signal intensity of ammonium ion

The effect of the phosphate buffer pH on fluorescence intensity of OPA–NH_4^+ product was examined in the pH region between 6.2 and 7.0. It was observed that the highest fluorescence intensity was observed at pH 6.4. Taylor et al. [8] reported that a few significant interferences were observed involving amino acids, peptides, amides or amines in neutral pH regions, therefore, it is important to adjust the pH for better determination of ammonium ion.

Goyal et al. [10] reported that the increase in the ionic strength of the reagent solution lead to an increase in the reactivity of OPA. Actually, the buffer concentration affected the signal intensity of ammonium ion in this work. It was found that the higher the concentration of phosphate buffer, the better the sensitivity was achieved for ammonium ion.

The concentration of OPA also affected the signal intensity of ammonium ion. It was found that the fluorescence intensity of ammonium ion increased with increasing OPA concentration in the reaction

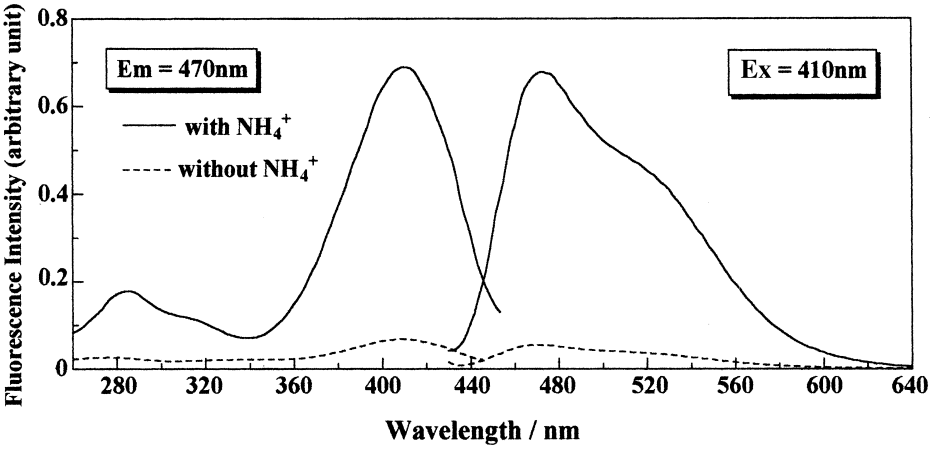

Fig. 2. Excitation and emission spectra of the reaction reagent in the presence or absence of ammonium ion. The reaction reagent: 0.25 *M* phosphate buffer (pH 6.4) containing 15 m*M* OPA, and 3.75 m*M* 2-mercaptoethanol. Concentration of ammonium ion: 0 or 5.0 μ*M*.

reagent solution up to 40 m*M*. At the higher concentration, the fluorescence signal leached plateau.

2-Mercaptoethanol is usually employed as a reducing agent for the derivatization of amino compounds with OPA [8–10]. The effect of 2-mercaptoethanol concentration on the signal intensity was also examined in this work. It was observed that 2-mercaptoethanol should be contained in the reaction reagent solution at the concentration al least 2 m*M*. At higher concentrations, slight increase in the fluorescence intensity was observed.

According to these examinations, 0.8 *M* phosphate buffer (pH 6.4), 50 m*M* OPA, and 10 m*M* 2-mercaptoethanol were selected as the optimum reaction reagent condition.

3.3. Effect of the reaction coil and temperature on the signal intensity

Fig. 3 shows the effect of the reaction coil conditions on the signal intensity. The reaction times of the reaction coil length of 0.5, 1 and 2 m were about 14, 28 and 56 s, respectively. It is found that the longer the reaction coil and the higher the reaction temperature, the better the sensitivity is achieved for ammonium ion. These results indicate that the reaction rate for OPA–NH_4^+ is not so fast, and longer reaction time and higher reaction temperature are favored for this reaction.

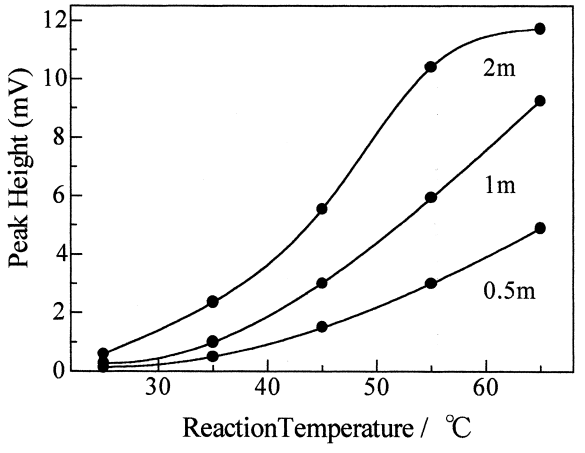

Fig. 3. Effect of the reaction coil condition. Eluent: water. Reaction reagent: 0.5 *M* phosphate buffer solution containing 30 m*M* OPA and 10 m*M* 2-mercaptoethanol. Sample: 1 m*M* ammonium ion. Wavelengths: Ex=410 nm and Em=470 nm. Reaction coil length: 0.5, 1 or 2 m. Reaction temperature: as indicated.

It was expected that the peak width and the pressure drop across the reaction coil increase with increasing reaction coil length. However, these effects were not significant. Considering these results, a 2-m length and 65 °C reaction coil temperature were selected as the optimum conditions.

3.4. Effect of the eluent and its concentration in the mobile phase

The effect of the eluent on the determination of nitrogen species was examined for sodium sulfate or sodium perchlorate. The higher the concentration of the eluent, the smaller the retention of nitrate and nitrite ions, and the better sensitivity was achieved for nitrate, nitrite and ammonium ions. Finally, sodium sulfate was used at the eluent in this study because sodium perchlorate absorbs UV light in the low wavelength, and gives about three-fold baseline noise than sodium sulfate. Therefore, 20 m*M* sodium sulfate (pH 5.7) is used as the eluent for the determination of nitrogen ions.

3.5. Determination of nitrogen ions

Fig. 4 demonstrates the detection of nitrogen-containing ions (nitrate, nitrite, and ammonium) under the optimum conditions. The sample concentration is 0.1 m*M* for each analyte, (6.2, 4.6, and 1.8 mg/l for nitrate, nitrite and ammonium ion, respectively). The reason for the slight tailing of ammonium ion is caused when it passes through the anion-exchange column.

Table 1 shows the relative standard deviations (RSDs) of the retention time, peak area, and peak height of the nitrogen-containing ions at 0.1 m*M* each. It is found that good repeatability can be achieved under the optimum condition in Fig. 4.

Table 2 summarizes results of the calibration data for the nitrogen containing ions. Good linear relationships between the peak height and the analyte concentration were observed at the concentration ranging from 0.02 to 0.1 m*M* for nitrate, nitrite, and ammonium ion, respectively. The limits of detection at signal-to-noise ratio (S/N) 3 were 1.6, 2.3 and 17 μM for nitrate, nitrite and ammonium ion,

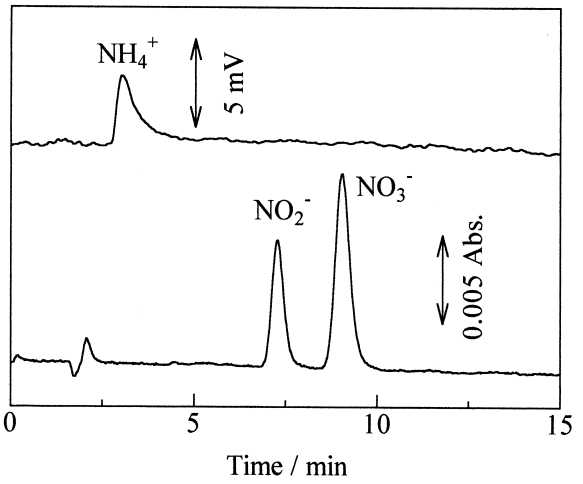

Fig. 4. Determination of nitrogen-containing ions under the optimum conditions. Eluent: 5 m*M* sodium sulfate. Reaction reagent: 0.8 *M* phosphate buffer (pH 6.4) containing 50 m*M* OPA and 10 m*M* 2-mercaptoethanol. Sample: 6.2 mg/l nitrate, 4.6 mg/l nitrite, and 1.8 mg/l ammonium ion. Column: IC-Anion SW (10 cm×0.32 mm I.D.). Wavelengths of detection: 206 nm for UV; Ex=410 nm and Em=470 nm for fluorescence. Reaction coil: 2 m×50 μm I.D. Reaction temperature: 65 °C.

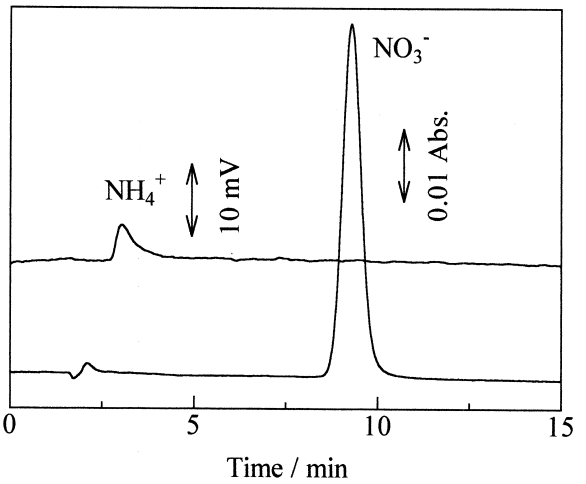

Fig. 5. Determination of nitrogen-containing ions in a river water sample. Eluent: 5 m*M* sodium sulfate. Sample: 0.2 μl of river water sample. Other operating conditions as in Fig. 4.

Table 1
Repeatability of nitrogen ions (RSDs, %; $n=5$)

Analyte ions	Retention time	Peak area	Peak height
NO_3^-	0.15	0.84	0.36
NO_2^-	0.21	0.68	0.51
NH_4^+	0.81	1.79	0.79

Analyte concentration: 0.1 m*M* each.

respectively. On the contrary, the limits of determination at $S/N=10$ were 5.5, 8.1 and 53 μ*M* for nitrate, nitrite and ammonium ion, respectively.

The present method could be applied to the determination of nitrogen-containing ions in river water samples. Fig. 5 shows the chromatogram for a river water sample. The river water samples were passed through a short column packed with Develosil C30 to remove hydrophobic compounds. It can be seen that nitrate and ammonium ion were determined sensitively, whereas nitrite was not detected. The determination results were 27.3 mg/l for nitrate and 1.98 mg/l for ammonium ion, respectively. The recovery of nitrate in river water sample was 88%.

The results were nearly the same as the ones obtained by ion chromatography. The ion chromatography was consisted of a PU-980 HPLC pump (Jasco), and a Model-5095 loop injector with injection volume of 20 μl (Rheodyne, Cotati, CA, USA), two Model-7000 six-port switching valves (Rheodyne), a UV-2070 ultraviolet detector (Jasco), a CM-8020 conductivity detector (Tosoh). The anion-exchange column used was a 4.6×50 mm TSK_{gel} IC-Anion-SW (Tosoh) and the cation-exchange column was a 4.6×150 mm TSK_{gel} Super IC-Cation (Tosoh). Nitrate was determined by UV detection, where as ammonium ion was determined by conductimetric detection. In addition, the limits of detection

Table 2
Calibration data for the nitrogen-containing ions at 0.02–0.1 m*M* concentrations

Analyte ions	Equation[a]	Correlation coefficient (r^2)
NO_3^-	$Y = 1.11 \times 10^5 C - 11.0$	0.999
NO_2^-	$Y = 7.83 \times 10^4 C - 30.8$	0.999
NH_4^+	$Y = 3.81 \times 10^4 C - 47.3$	0.998

[a] C = concentration of analyte in m*M*.

at $S/N=3$ achieved by the above ion chromatographic system were around 1 μM for nitrate and nitrite, whereas those for ammonium ion were around 3 μM.

4. Conclusions

Sodium sulfate was suitable as the eluent to elute nitrate and nitrite in a reasonable time. Ammonium ion was derivatized with OPA in the presence of 2-mercaptoethanol followed by fluorimetric detection. Under the neutral conditions, there was no interference with organic nitrogen species. The longer the reaction coil and the higher the reaction temperature, the better is the sensitivity for ammonium ion. Good repeatability was achieved under the optimum conditions. The determination of the inorganic nitrogen-containing ions in environmental river water sample was possible.

Acknowledgements

The authors gratefully acknowledge the Takeda Foundation for the financial support.

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