

Short Communication

Ion interaction chromatography with nonylamine reagent for the determination of nitrite and nitrate in natural waters

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ABSTRACT

An ion interaction chromatography method for the determination of nitrate and nitrite using a reversed-phase C₁₈ column is described. The eluent was 2.0 mM nonylammonium phosphate of pH 6.5 and the UV detection wavelength was 205 nm. For a sample volume of 200 μ l detection limits of 2.0 and 1.5 μ g/l were obtained for nitrite and nitrate, respectively. The change of the detection wavelength to 190 nm enables also the quantitative detection of chloride. Results obtained for natural waters have been correlated with those obtained using standard spectrophotometric procedures.

INTRODUCTION

Among commonly used high-performance ion-chromatographic methods such as eluent-suppressed or single-column ion-exchange chromatography, ion exclusion chromatography or ion interaction chromatography, the last technique exhibits several advantages. Numerous examples of the use of all these techniques for nitrate and nitrite determination can be found in the literature [1]. The use of non-polar stationary phases covered with suitable ion-pairing reagents provides a wider variability of chromatographic parameters than other methods. In the past this method was widely employed for the separation of ionic and ionizable organic solutes, including biomolecules, and recently for the separation of inorganic ions. Because reversed-phase col-

umns are readily available this approach is attractive. Ion interaction chromatography can be carried out using conventional HPLC instrumentation with most common phases and with numerous detection methods, including UV detection.

In ion interaction chromatography of inorganic anions, the most frequently used ion interaction reagents (column modifiers) are quaternary ammonium salts with hydrophobic long-chain aliphatic or aromatic groups [2–6]. As was shown for the first time by Skelly [7], with the use of different reversed-phase columns salts of long-chain primary aliphatic amines can also be very satisfactory column modifiers. Among butylamine, hexylamine, octylamine and decylamine, the best results were obtained with octylamine. This reagent was subsequently used by several workers in ion interaction chromatography with direct UV detection [8–10]. In a comparative study of different amines as ion

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interaction reagents, an increase in the retention of inorganic anions with an increase in the length of the aliphatic chain was observed [11,12]. A comparison of heptylamine and octylamine indicated an increase in sensitivity of determination with the larger alkyl group [9].

In spite of preliminary modification of the column, the presence of an ion interaction reagent in the eluent was found to be necessary [7]. As counter ions in the amine salts used as column modifiers, perchlorate [11], salicylate [9] and most often weakly UV-absorbing phosphate [7,8,10] have been applied. The use of sulphonate as the eluting anion allows the indirect spectrophotometric detection of anions that do not absorbing UV radiation as chloride or sulphate [11].

Nonylamine has been used as a column modifier only by Dreux *et al.* [11]. Owing to the possibility of decreasing its concentration in the eluent in comparison with heptylamine or octylamine, better detectability with conductivity detection can be obtained. Using this ion interaction reagent a better sensitivity of UV detection at 204 nm than with conductivity was demonstrated.

The aim of this study was to optimize the ion interaction chromatographic determination of inorganic anions with the use of reversed-phase C_{18} columns modified with nonylamine with special emphasis on nitrite and nitrate, which are of significant importance for environmental protection. Results obtained for natural waters were verified using standard spectrophotometric procedures. Numerous other methods for nitrite and nitrate determination can be also found [12].

EXPERIMENTAL

Apparatus

The 3D HPLC system consisted of a Perkin-Elmer (Norwalk, CT, USA) Series 100 pump, a Rheodyne Model 7125 injection valve and a Tridet detector (Bacharach, Pittsburgh, PA, USA). For detection in the UV range a Knauer (Bad Homburg, Germany) variable-wavelength Type 87.00 monitor equipped with a 10-mm path-length flow-through cuvette of 10- μ l volume was used. A PR-18 (5 μ m) reversed-phase C_{18} column (250 \times 4.0 mm I.D.) from ZOCh (Lublin, Poland) was employed. For pH measurements a Radelkis (Budapest, Hungary)

OP-265 pH meter equipped with a combined glass-calomel electrode was used.

Reagents

As ion interaction reagents nonylamine and cetyltrimethylammonium bromide (CTAB) from Koch-Light and tetrabutylammonium bromide (TBAB) from Fluka were used. All other reagents used were of analytical-reagent grade from POCh (Gliwice, Poland). Deionized water obtained using a Milli-Q system (Millipore) was used for preparing solutions.

Mobile phases consisted of the ion interaction reagent with the pH was adjusted with concentrated orthophosphoric acid or 0.1 M sodium dihydrogenphosphate solution.

The column was washed with 500 ml of acetonitrile–water (1:1) and then with 100 ml of deionized water pumped at 1 ml/min before use. Preliminary modification of the column was carried out using 500 ml of 10 mM nonylammonium orthophosphate solution of pH 6.5 delivered at 1.0 ml/min.

Reference determinations of nitrate and nitrite

As reference methods for the determination of nitrate and nitrite widely accepted standard spectrophotometric procedures were used. Determination of nitrate was based on reaction of nitrate with sodium salicylate in concentrated sulphuric acid [13]. Nitrosalicylic acid formed in this reaction gives in alkaline solution an ionized yellow product, the absorbance of which is measured at 410 nm. For determination of nitrite a highly sensitive and specific modified Griess method was employed, involving the formation of an azo dye by reaction of nitrite with sulphanilic acid and 1-naphthylamine at pH 2.0–2.5, with spectrophotometric detection at 520 nm [14].

RESULTS AND DISCUSSION

Optimization of chromatographic parameters

Owing to their strong absorption in the UV region, for the chromatographic detection of nitrite and nitrate direct UV detection can be utilized if the eluent does not contain too high concentrations of UV-absorbing species. In an earlier report on the use of columns, UV detection was used in the wavelength range 204–254 nm [7–11]. It was found in this study that maximum absorption of nonylamine

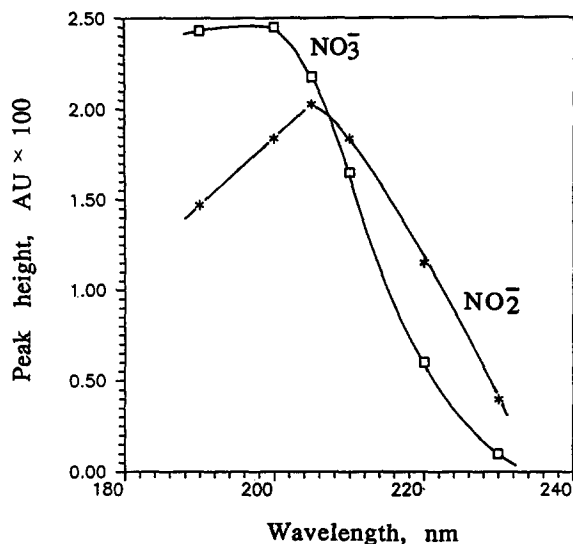


Fig. 1. Effect of wavelength on the detection of nitrite and nitrate by ion interaction chromatography using as eluent 2.0 mM nonylammonium phosphate (pH 6.5). Injection volume, 10 μ l of 10 mg/l nitrite and nitrate solutions; flow-rate, 1 ml/min.

occurs at 220 nm, and the dependence of the peak height for nitrite and nitrate on wavelength was examined (Fig. 1). For nonylammonium phosphate solution as eluent the optimum wavelength for determination of nitrite and nitrate was taken to be 205 nm, where the best detectability is observed for nitrite, usually present at much lower levels than nitrate in natural samples. Under those conditions almost 90% of maximum sensitivity can be obtained

TABLE I

EFFECT OF ELUENT COMPOSITION ON SEPARATION OF NITRITE AND NITRATE AT ELUENT pH 6.5

Injected sample volume, 20 μ l; detection wavelength, 205 nm.

Concentrations of eluent components (mM)		Retention time (min)	
Nonylamine	Phosphate	Nitrite	Nitrate
10	2	9.5	16.5
2	2	5.5	6.6
	3.5	3.8	4.8
0.5	2	4.6	5.8

for nitrate. The same wavelength has been already applied by other workers with the use of octylamine as modifier [8,11].

The concentration of modifier in the eluent and the concentration of the eluting anion have a substantial influence on the resolution of anions by ion interaction chromatography. The latter can be adjusted by changing the total concentration of the anion in the eluent solution or, for anions of weak acids, by changing the pH of the eluent. In this study the optimization of the eluent composition was carried out at a constant pH of 6.5 and as orthophosphate was used as the counter anion (Table I). From examination of a wide range of nonylamine concentrations, 2.0 mM was adopted as the optimum at the same total concentration of phosphate in the eluent solution. Under those conditions, the detection limits for a signal-to-noise ratio of 2 were calculated to be 2.0 μ g/l for nitrite and 1.5 μ g/l for nitrate for a sample volume of 200 μ l. A further improvement in detectability can be obtained by increasing the

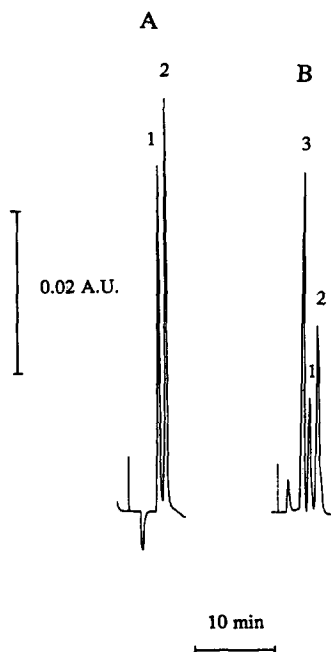


Fig. 2. Ion chromatograms obtained for injection of 200 μ l of a mixture of 0.5 mg/l nitrite and nitrate and 20 mg/l chloride solutions, obtained (A) at 205 nm using as eluent 2.0 mM nonylamine solution (pH 6.5) containing 3.5 mM orthophosphate and (B) at 190 nm using as eluent 2.0 mM nonylammonium phosphate (pH 6.5). Peaks: 1 = nitrite; 2 = nitrate; 3 = chloride.

TABLE II

CHARACTERISTICS OF ION INTERACTION CHROMATOGRAPHY OF NITRITE AND NITRATE USING DIFFERENT ION INTERACTION REAGENTS IN ELUENTS UNDER OPTIMIZED CONDITIONS

Separation parameter	Ion interaction reagent ^a		
	CTAB	TBAB	Nonylamine
Resolution (R_s)	1.0	2.2	3.7
Theoretical plate number (N) for nitrate	280	2800	2800
Eluent	0.25 mM KHP– 0.1 mM CTAB (pH 6.5)	5.0 mM KPH– 0.2 mM TBAB (pH 6.5)	2.0 mM NPH (pH 6.5)
Detection wavelength (nm)	254	215	205
Retention time (min)			
Nitrite	3.7	3.8	5.5
Nitrate	5.8	4.3	6.6
Chloride			4.1
Bromide			4.8
Iodide			7.2
Thiocyanate			13.3
Sulphate			19.7

^a CTAB = Cetyltrimethylammonium bromide; TBAB = tetrabutylammonium bromide; KHP = potassium hydrogenphthalate; KPH = potassium orthophosphate; NPH = nonylammonium orthophosphate.

phosphate concentration in the eluent, but it is accompanied by a loss of baseline resolution of the nitrite and nitrate peaks.

For the modifier used in this study it was confirmed that a single preliminary modification of the non-polar stationary phase is not sufficient for obtaining long-term stable values of retention times. For eluents containing nonylamine the deviations of the retention times during 5 days did not exceed ± 0.2 min. The precision of determination based on peak-height measurements, expressed as relative standard deviation for $n = 10$, was 1.5% for 0.5 mg/l of nitrate and 1.1% for 0.5 mg/l of nitrite.

Because of planned environmental application of the developed method, the effect of the presence of excess of chloride and sulphate on the determination of nitrite and nitrate was also examined. A 100 mg/l concentration of those two anions did not affect results. It was found, however, changing the detection wavelength from 205 to 190 nm permits the determination of chloride, as illustrated in Fig. 2.

The results obtained by ion interaction chromatography with nonylamine as modifier were compared with those obtained under optimum condi-

tions and with the same instrumental set-up for other frequently used modifiers, CTAB and TBAB (Table II). This comparison illustrates well the effectiveness of the method developed in this study. In order to complete the characteristics of the system in Table II, retention times are included for several other anions.

Determination of nitrite and nitrate in natural waters

The chromatographic literature contains already several examples of the use of ion interaction chromatography with direct UV detection for the determination of nitrite and nitrate in waters with the use of tetraalkylammonium salts as modifiers [4–6] and long-chain primary amines [7–10]. Using tetrapentylammonium fluoride, satisfactory results for nitrate determination in the range 21–134 mg/l in well waters were obtained [4]. In the determination of nitrate using tetramethylammonium phosphate, the results obtained differed from those given by the standard UV method [5]. Using a C_{18} column modified with cetyltrimethylammonium chloride with UV detection for nitrate and amperometric detection for nitrite, satisfactory recovery results

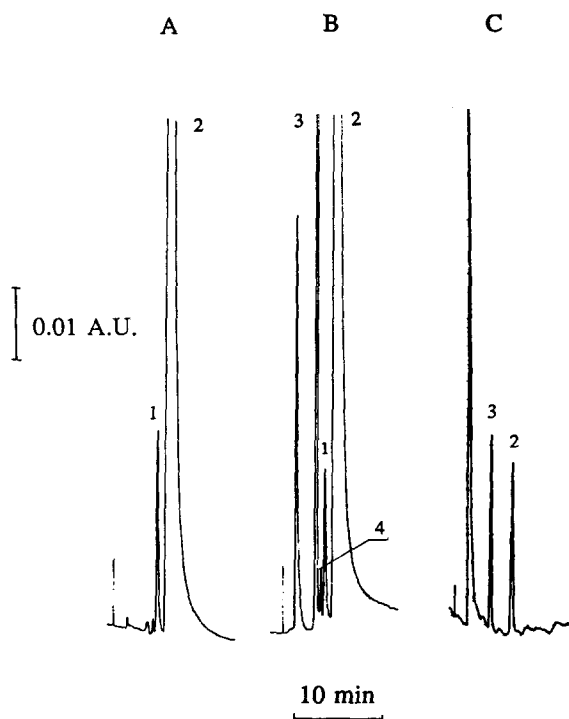


Fig. 3. Ion chromatograms obtained for a well water sample using direct UV detection at (A) 205 and (B) 190 nm and (C) conductivity detection. Eluent, 2.0 mM nonylammonium phosphate (pH 6.5). Sample volume, 10 μ l. Peaks: 1 = nitrite; 2 = nitrate; 3 = chloride; 4 = bromide.

were obtained for the determination of both species in sea water [6].

In earlier studies in which octylamine was used as

a modifier and chromatograms of natural waters [9,10] or plant extracts [7,8] were shown, the correlation with standard reference methods was not reported, hence it seemed to be valuable to carry out such a study for the present system with nonylamine as stationary phase modifier.

The reference spectrophotometric methods used for the determination of nitrate and nitrite were outlined under Experimental. Natural water samples were analysed soon after collection and filtered using a 0.45- μ m filter. Fig. 3 shows chromatograms obtained for a well water sample at different detection wavelengths. For comparison, a chromatogram obtained with the same eluent with conductivity detection is shown. These results clearly demonstrate the much better detectability of UV detection under given chromatographic conditions.

The results given in Table III demonstrate satisfactory agreement between the results obtained by ion interaction chromatography and the reference spectrophotometric methods.

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TABLE III

RESULTS OF ION INTERACTION CHROMATOGRAPHIC DETERMINATION OF NITRITE AND NITRATE IN NATURAL WATER SAMPLES AT 205 nm USING AS ELUENT 2 mM NONYLAMMONIUM ORTHOPHOSPHATE (pH 6.5)

Injected sample volume, 10 μ l.

Sample	Nitrite found (mg/l)		Nitrate found (mg/l)	
	Ion interaction chromatography	Spectrophotometry	Ion interaction chromatography	Spectrophotometry
River water I	0.082	0.081	10.8	11.5
River water II	0.27	0.26	10.4	11.4
Lake water	ND ^a	0.009	1.4	1.4
Tap water	ND	ND	5.5	5.3

^a ND = Not detected.

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