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Note

Ion chromatography of nitrite, bromide and nitrate ions in brine samples using a chloride-form anion-exchange resin column

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In aquaculture research, maintenance of water quality is of great importance[1–4]. Recent improvements in fishpond management and fish culture systems, such as intensive feeding and fertilization, have created complex problems. Deterioration of water quality is primarily the resulting effect of these improvements. For example, high-protein feeds and nitrogen fertilizers applied in fishponds produce considerable amounts of nitrite in the water. Moreover, their synergistic effects cannot be ignored. Hence the routine determination of nitrite in fishpond water is required.

Since its introduction by Small *et al.*[5], ion chromatography has been widely used for the determination of ions in water. However, the presence of very high concentrations of chloride is the main obstacle in the analysis of seawater samples, affecting the separation and detection not only of nitrite but also of other anions.

Itoh and Shinbori[6] applied ion chromatography to the analysis of seawater using a 125-cm long column and a conductimetric detector. This technique provided a simple and sensitive analytial method for brine samples. However, nitrite could not be determined owing to the presence of a large chloride peak.

Attempts to determine nitrite in seawater also led to innovations in the methodology. Lee and Field[7] employed a post-column cerium fluorescence detection system to determine nitrite and nitrate in drinking water and seawater. The use of a pretreatment column in the silver form for removal of chloride has been reported[5].

Various detectors have been applied in ion chromatography in addition to the conductimetric detector. The UV detector has been shown to be useful detector for several kinds of inorganic anions[8]. Selective detection of specified inorganic ions can be achieved by tuning the wavelength of the UV detector in ion chromatography[9,10] and also in ion-exclusion chromatography[11]. The elimination of the chloride matrix

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interference in the sub-ppm determination of nitrite in seawater was achieved by a heart-cutting and recycling method using a dual detection system consisting of conductimetric and a UV detectors[1,2]. This method, however, requires valve switching during analysis and needs a long analysis time.

This paper describes a simple and rapid method for UV-absorbing anions, such as nitrite, nitrate and bromide, in brine samples using a chloride-form anion-exchange resin column combined with a UV detector.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a Model IC 100 ion chromatographic analyser, a Type 3300 strip-chart recorder (Yokogawa Electric, Tokyo, Japan), a Model 638 variable-wavelength UV monitor (Hitachi, Tokyo, Japan) operated at 210 nm and a Chromatopak C-R1A integrator (Shimadzu, Kyoto, Japan). The sample loop size of the Rheodyne Model 7125 loop injector was 50 μl.

A Yokogawa PAM 3-035 guard column (30 mm $\times$ 4.6 mm I.D.) and a SAM 3-125 separation column (125 mm $\times$ 4.9 mm I.D.) were used. The gel for the separation column was composed of a hydroxyalkylated polyacrylate matrix and covalently introduced quaternary ammonium residues, of particle diameter 10 μm and ion-exchange capacity 50 μequiv./ml. The column temperature was maintained at 40 $\pm$ 1°C. The suppresser system was removed.

Stainless-steel column, tubing and filter components were used, hence daily washing of the system by pumping pure water was required in order to prevent corrosion problems due to the chloride ions in the eluents.

Reagents

The eluent was prepared by dissolving the appropriate amount of potassium chloride (E. Merck, Darmstadt, F.R.G. in deionized water, and was filtered through a 0.45-μm membrane filter (Toyo Roshi, Tokyo, Japan) and degassed before use. The flow-rate of the eluent was 1.5 ml/min throughout. Sample solutions containing various concentrations of sodium chloride as matrix ions were prepared from Suprapur-grade reagent (E. Merck). Other reagents were of analytical-reagent grade from Nacalai Tesque (Kyoto, Japan) and Wako (Osaka, Japan).

Deionized water was prepared with a Nanopure water purification system (Barnstead, Newton, MA, U.S.A.).

Seawater samples were obtained from fish aquaria in the Seto Marine Biological Laboratory (Kyoto University, Wakayama, Japan). The sample from the Pacific Ocean was provided by Dr. Nakayama (Kyoto University).

RESULTS AND DISCUSSION

Principle of matrix ion evicted chromatography

The problems encountered in the determination of minor ions coexisting in a major matrix ion are the peak overlapping on the matrix ion peak and the peak broadening due to the overwhelming amount of the matrix ion.

In ion chromatography with conventional eluents, *e.g.*, carbonate solutions, the

fixed ions on the ion-exchange sites are replaced by the matrix ions as the ions are passed along the column. It requires a long time to recover the ion-exchange equilibrium after the matrix ion has eluted from the column. The chaotic state in the column makes the retention volumes fluctuate, and moreover the analyte peak shapes are badly distorted.

To prevent this disordered state in the column, we selected the counter ion in the eluent to be the same as the anion included in the matrix. By using neutral chloride salt solutions, such as potassium or sodium salts, the eluent contains only the matrix anion species, and therefore all the ion-exchange sites on the resin are occupied by the single ion species. Under these conditions, the matrix ion apparently is retained on the column and subsequently eluted at the void volume in a narrow peak, whereas analyte ions are retained and eluted in order of their ion-exchange selectivity. Using this system, sharp and symmetrical peaks of analyte ions were obtained.

Relationship between eluent concentration and capacity factors

Prior to the analysis, the column was thoroughly washed with potassium chloride solution for equilibration.

The logarithmic retention volume of the nitrite, bromide and nitrate peaks decreased linearly with increasing logarithmic chloride concentration in the eluent.

For the analysis of brine samples, the concentration of the potassium chloride eluent was set at 50 m*M*, considering the resolution and the analysis time.

Effects of the chloride matrix ion concentration on the chromatographic data

Chromatograms were obtained by the injection of synthetic brine samples with various chloride matrix ion concentrations and containing 1.0 μg/ml of nitrite, 5.0 μg/ml of bromide and 1.0 μg/ml of nitrate ions.

Chloride matrix ion eluted at the column void volume in a narrow peak. The retention volumes of the analyte peaks were independent of the matrix ion

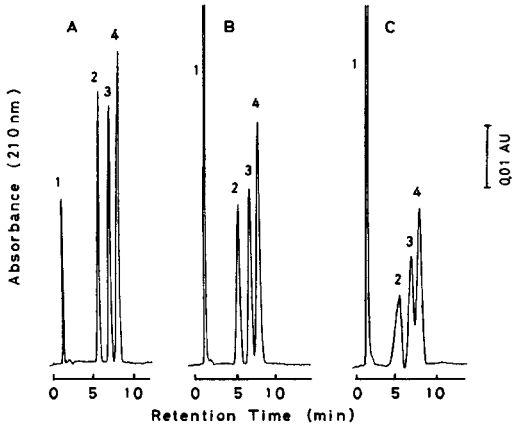

Fig. 1. Effect of chloride ion concentration in the sample solution on the peak shape of anions. Chloride ion concentrations: (A) 5; (B) 20; (C) 50 μg/ml. Peaks: 1 = Cl^-; 2 = NO_2^- (1 μg/ml); 3 = Br^- (5 μg/ml); 4 = NO_3^- (1 μg/ml). Eluent, 50 m*M* potassium chloride solution; flow-rate, 1.5 ml/min; column temperature, 40 ± 1°C; sample volume, 50 μl; detection, UV absorption at 210 nm.

concentrations up to 50 mg/ml. The peak shape of the analytes and the column efficiency varied with the matrix ion concentration. Fig. 1 shows the effect of the chloride matrix ion concentration on the peak shape and the resolution.

Below a matrix ion concentration of 5 mg/ml (141 m*M*), the peak shapes of three anions are symmetrical and well separated from each other. Above this level, the peak width of the analyte ions increased owing to leading of the peak and the peak height decreased with increasing matrix ion concentration. At 20 mg/ml ppm (563 m*M*) of chloride matrix, the peak became skewed, but acceptable resolution between neighbouring peaks were attained. This matrix concentration is equivalent to that in the ocean sample. The analyte anion peak area was not affected by the matrix ion, which implies that the system is applicable to the direct injection of the seawater samples for quantitative analysis.

At 50 mg/ml (1.4 *M*) of chloride matrix ion, the anion peaks become skewed and the front part of the nitrate peak overlaps the tail of the bromide peak. Leading of the analyte peak caused the theoretical plate number, N, to decrease. The value of N for each peak was determined and the ratio N/N_0 is plotted in Fig. 2 as a function of the matrix ion concentration (N_0 is the N value obtained with a matrix-free sample). At higher matrix concentrations, N/N_0 decreased with increasing matrix concentration. The effect of the matrix on the column efficiency was greater for ions with shorter retention times. Peak broadening of the nitrite ion was particularly apparent among the three analyte peaks, but it was still separated from the bromide ion peak with up to 50 mg/ml of matrix coexisting, as shown in Fig. 1.

Determination of anions in seawater samples and contaminants in chemical reagents

Chromatograms obtained by the injection of 50 μl of seawater sample are demonstrated in Fig. 3. The seawater samples contained chloride ion at levels from 18 to 20 mg/ml. Trace A is for a sample taken from the Pacific Ocean, with a concentration of bromide ion of 65 μg/ml. Trace B is for a sample taken from an aquarium and included a considerable amount of nitrate ion produced by biological

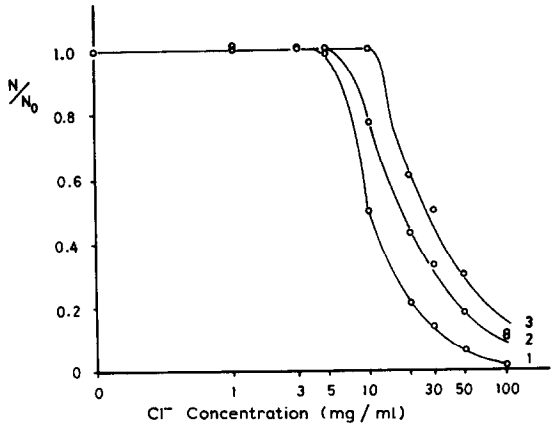

Fig. 2. Effect of chloride ion concentration in the sample solution on the column efficiency. 1 = NO_2^-;)2 = Br^-; 3 = NO_3^-. Conditions as in Fig. 1.

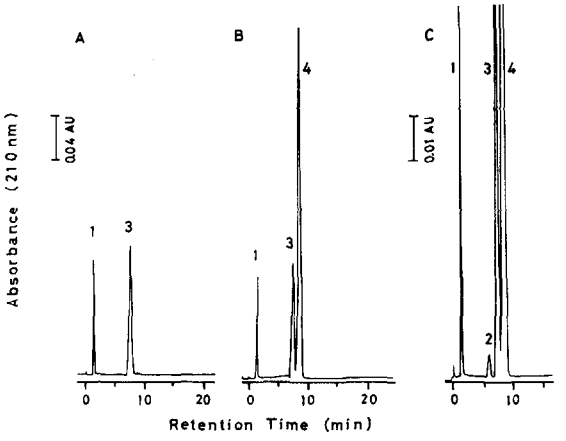

Fig. 3. Chromatograms of seawater samples. (A) Pacific Ocean; (B) aquarium; (C) sample B spiked with 0.5 μg/ml of nitrite. Peaks: 1 = Cl^-; 2 = NO_2^-; 3 = Br^-; 4 = NO_3^-. Conditions as in Fig. 1.

activities of fish, but nitrite was not detected. The result for an aquarium sample spiked with 0.5 μg/ml of nitrite is shown in the trace C.

The relationship between the peak area and the nitrite concentration was obtained at levels up to 20 μg/ml by injecting samples from the Pacific Ocean spiked with various concentrations of nitrite ion. The detection limit was 8 ng/ml (2.4 ng/ml as NO_2-N) for a signal-to-noise ratio of 2.

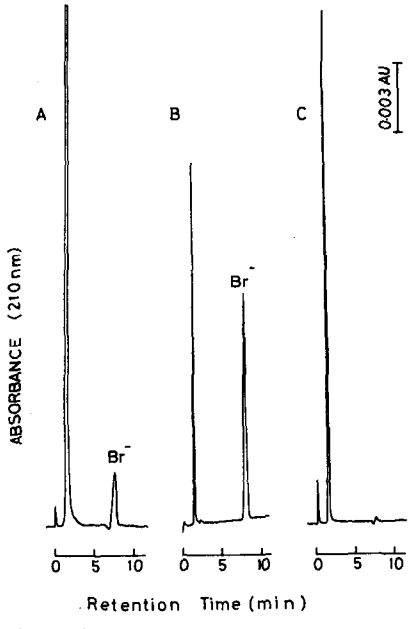

Fig. 4. Chromatograms of chemical reagents. (A) 1.0 *M* NaCl (brand A); (B) 0.2 *M* KCl (brand A); (C) 0.3 *M* KCl (brand B). Conditions as in Fig. 1.

Contaminants in commercially available sodium chloride and potassium chloride reagents were determined by this method. Fig. 4 shows the peak of the bromide ion present as a contaminant in the analytical-reagent grade reagents.

When the detector attenuation was set at high sensitivity, a small negative peak was observed just before the bromide peak. Fig. 4A shows this negative peak when 50 μl of 1.0 *M* sodium chloride solution (35.5 mg/ml as Cl^-) was injected. The negative peak is not observed in Fig. 4B, where a lower concentration of a 0.2 *M* potassium chloride (7.1 mg/ml Cl^-) was applied. The size of the negative peak increased with increasing concentration of chloride ion in the sample solutions. The origin of this negative peak is unknown, but the tail of the nitrite peak and the front of the bromide peak overlapped with it. This peak yields the error in the quantitative determination of nitrite and bromide ions at low concentration levels. The problem with the determination of nitrite was solved by the addition of 5–10% of methanol to the eluent, but the negative peak still overlapped the bromide peak.

Effect of the pH of the sample solution

The effect of the pH of the sample solution on the retention behaviour of analytes was studied. The pH of the sample solution was adjusted by adding dropwise a 0.05 *M* sulphuric acid or a 0.1 *M* sodium hydroxide solution. The retention volume and peak area of the three anions did not vary when the pH of the sample solutions ranged from 2.0 to 11.8, except for the peak area of the nitrite ion, which below pH 2.8 decreased with decrease in the sample pH. This may caused by the decomposition of nitrite ion in an acidic solution; however, no significant increase in the nitrate peak was observed in the sample pH range examined.

Separation of monocarboxylic acids from inorganic acids

Small monocarboxylic acids such as formic or acetic acid are often found as contaminants in practical samples. As carboxylic acids can be detected with a UV detector[13], such compounds sometimes interfere in the analysis of inorganic anions.

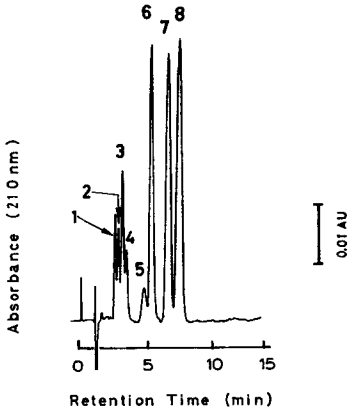

Fig. 5. Chromatogram of a mixture of monocarboxylic acids and inorganic anions. Peaks: 1 = unknown; 2 = acetic acid (20 μg/ml); 3 = formic acid (20 μg/ml) + *n*-propionic acid (20 μg/ml); 4 = *n*-butyric acid (20 μg/ml); 5 = *n*-valeric acid (20 μg/ml); 6 = NO_2^- (1 μg/ml); 7 = Br^- (5 μg/ml); 8 = NO_3^- (1 μg/ml). Conditions as in Fig. 1.

Fig. 5 shows the chromatogram of a mixture of five monocarboxylic acids and three inorganic anions using 50 m*M* potassium chloride solution as eluent. The five acids were eluted before the nitrite peak and did not interfere in the analysis of the inorganic anions. Acetic acid eluted first, followed by formic and *n*-propionic acid. Baseline resolution between *n*-valeric acid and nitrite was obtained when 30 m*M* potassium chloride solution was employed as the eluent.

The present method is also applicable to the analysis of monocarboxylic acids in a solution with a chloride ion matrix.

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