Journal of Chromatography, 630 (1993) 195–200
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 686

Ion chromatography of nitrite and carbonate in inorganic matrices on an octadecyl-poly(viny1 alcohol) gel column using acidic eluents

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(First received July 7th, 1992; revised manuscript received October 13th, 1992)

ABSTRACT

Low levels of carbonate and nitrite contained in inorganic matrices were determined by ion chromatography on an Asahipak ODP-50 poly(vinyl alcohol) gel-based reversed-phase column. With an acidic mobile phase, inorganic matrix anions and cations eluted near the void volume of the column, whereas carbonate and nitrite were retained and separated completely from the matrix ions. After the separation column, the peak response was enhanced using a cation-exchange hollow fibre and 25 m*M* sodium sulphate or alkaline enhancers. Sea-water samples can be applied directly for the determination of carbonate and added nitrite at ppm levels. The maximum sample volume that can be loaded on the column without peak deformation depended on the pH of the sample solution and the sulphuric acid concentration in the eluent. A 50 μl sea-water sample was applicable with a 2.5 m*M* acid eluent.

INTRODUCTION

The determination of low levels of ions in water samples is very important, especially when the analytes co-exist with highly concentrated matrix ions.

Various suggestions for the determination of trace concentrations of inorganic ions in the matrix have been presented. Chloride matrix ion in an aqueous sample was selectively separated from chloride on an anion-exchange column and μg/ml levels of bromide, nitrate and nitrite ions were retained and completely separated [1,2]. The sensitive determination of carbonate by ion-exclusion chromatography using a conductivity detector and a pair of enhancement columns has been reported [3]. Carbonate was ionized during passage through the first enhancement column and in the second column the carbonate was converted into more sensitive hydroxide ion. Sample solutions including concentrated matrix ions, however, cannot be loaded over the capacity of the enhancement columns.

As an alternative method, an ion-exchange hollow-fibre suppressor system with an enhancer was applied to the determination of carboxylic acids and carbonate [4]. The ion-exchange hollow-fibre system is continuously regenerated by the outer flow of the enhancer. This system made it possible to inject samples consecutively.

Nitrite determination in sea-water samples has been achieved by a heart-cutting and recycling technique using ion chromatography on an anion-exchange resin column [5]. Kuchinicki et *al.* [6] reported the retention of nitrite on a reversed-phase column using acidified water as the eluent.

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0021-9673/93/$06.00

In previous papers we reported the retention behaviour of four UV-absorbing common anions using poly(vinyl alcohol) (PVA) gel-based columns with dilute sulphuric acid as the eluent [7,8]. At pH 2.3, bromate, bromide and nitrate eluted near the void volume of the column, whereas nitrite was retained, with a relatively large elution volume. It is of great importance in aquaculture and marine research to develop a sensitive and simple method for the determination of low levels of nitrite and carbonate in brine samples by direct injection. This paper reports the elution behaviour of nitrite and carbonate in ionic matrices on an octadecyl modified PVA gel column and its applicability to the analysis of brine sample.

EXPERIMENTAL

A Model IC 100 ion chromatographic analyser (Yokogawa Electric, Tokyo, Japan) equipped with an alkaline-compatible cation-exchange hollow-fibre suppressor system and a conductivity detector was employed [9]. A Model 638 variable-wavelength UV detector (Hitachi, Tokyo, Japan) was connected after the conductivity detector. The peak area was determined with a Chromatopak C-R1A integrator (Shimadzu, Kyoto, Japan). The wavelength of the UV detector was set at 210 nm and the column oven temperature was controlled at 40 ± 0.1°C. An Asahipak ODP-50 octadecyl-modified PVA gel column (150 mm × 4.6 mm I.D., particle diameter 5 μm) (Asahi Chemical Industry, Tokyo, Japan) was used as an analytical column. The ODP gel was prepared by reacting stearyl chloride with the hydroxy groups on the PVA gel [10,11]. A Model UV-3000 UV spectrophotometer (Shimadzu) was used for the measurement of the spectrum of nitrite solutions.

Sulphuric acid of concentrations from 0.05 to 5 mM as the eluent was prepared by dilution with water freshly deionized with a Nanopure water purification system (Barnstead, Newton, MA, USA). For the determination of carbonate, the eluent was degassed by bubbling helium through it followed by a degasser system to minimize the background level of carbonate. A laboratory-made degasser system composed of a thin-walled PTFE tube (2.5 m × 2.4 mm I.D.) installed in a vacuum bottle was connected between the eluent reservoir and a pump. The mobile phase flow-rate was maintained at 0.53 ml/min.

The suppressor system for anion determinations is constructed from a perfluorosulphonic acid cation-exchange hollow-fibre membrane inserted coaxially in a PTFE tube [9]. In this study, the column eluate flowed through the inside of the hollow-fibre tube, and on the outside 25 mM sodium sulphate or an alkaline solution was pumped as a peak enhancer in the opposite direction to the eluate at a flow-rate of 2 ml/min [4].

Sodium hydrogencarbonate and sodium nitrite of analytical-reagent grade were purchased from Nacalai Tesque (Kyoto, Japan) and sodium chloride, sodium nitrate and potassium sulphate of Suprapur grade for the matrices were obtained from Merck (Darmstadt, Germany). All other chemicals were of analytical-reagent from Nacalai Tesque.

Stock solutions of 1 mg/ml carbonate (as CO_3^{2-}) and nitrite (as NO_2^-) were made using freshly deionized water. Aliquots of these stock solutions were diluted to the appropriate concentration with deioinized water or concentrated matrix solutions.

Sea-water samples were filtered through a 0.2-μm nylon membrane filter (Corning, Corning, NY. USA) before injection.

RESULTS AND DISCUSSION

Chromatography of nitrite and carbonate in matrix ions

Authentic sample mixtures of 20 μg/ml nitrite and 200 μg/ml carbonate containing various different matrix ions were injected on to the Asahipak ODP-50 column and eluted with 1 mM sulphuric acid. Inorganic anions and cations such as F^-, Cl^-, SO_4^{2-}, NO_3^-, HPO_3^-, Br^-, Li^+, Na^+, K^+ and NH_4^+ eluted around the void volume of the column, whereas nitrite and carbonate were retained and separated from each other, as shown in Fig 1. Both carbonate and nitrite can be detected with the conductivity detector after conversion into ionized form by increasing the eluate pH through the enhancer system. More sensitive detection of nitrite was performed with a UV detector connected after the conductivity detector.

The upper traces in Fig. 1 were obtained with the UV detector. The nitrite peak was clearly separated from all anion matrix peaks. As chloride ions do

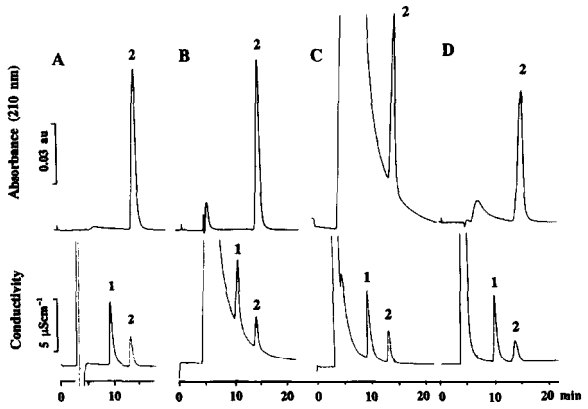

Fig. 1. Chromatograms of carbonate and nitrite in various matrix ions. Detection: upper traces, UV detector set at 210 nm; lower traces, conductivity detector. Peaks: 1 = carbonate (200 μg/ml); 2 = nitrite (20 μg/ml). Matrix ions: (A) none; (B) NaCl (30 mg/ml as Cl-); (C) $NaNO_3$ (10 mg/ml as NO_3^-); (D) K_2SO_4 (10 mg/ml as SO_4^{2-}). Eluent: 1 m*M* H_2SO_4. Sample volume: 20 μl. Enhancer: 25 m*M* Na_2SO_4.

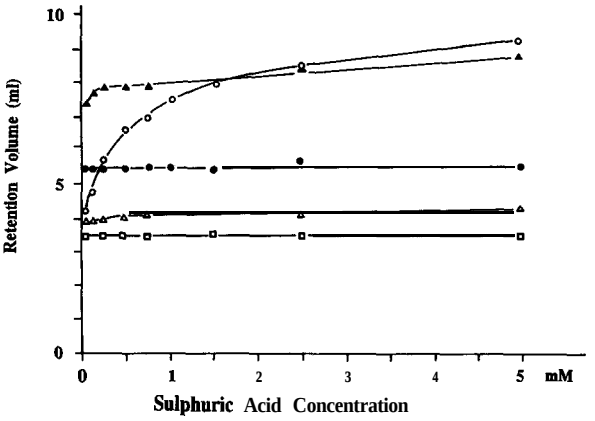

Fig. 2. Relationship between sulphuric acid concentration in the eluent and the retention volumes of nitrite, carbonate and three carboxylic acids. 0 = Nitrite; ● = carbonate; A = propionic acid; △ = acetic acid; □ = formic acid. Nitrite and carboxylic acids were monitored by UV detection, and carbonate by conductivity detection.

not absorb UV light at 210 nm, no effect of the matrix ion on the nitrite peak was observed. When the matrix was 10 mg/ml nitrate, a sharp nitrite peak appeared on the tail of a large matrix ion peak. The lower traces were obtained with the conductivity detector. The tail part of the chloride and the nitrate matrix ion peaks overlap the carbonate peak, but the retention time of carbonate was not affected by the presence of the matrix ions.

When the matrix was 10 mg/ml sulphate ion, the matrix ion eluted in a narrow band but the nitrite peak was broadened and the retention was reduced slightly. The shape and retention of the carbonate peak was not affected by the matrix ions.

Effects of the concentration of sulphuric acid in the eluent

In Fig. 2 the retention of nitrite and carbonate are plotted as a function of the concentration of sulphuric acid in the eluent. The retention of nitrite increased rapidly with increasing concentration of sulphuric acid and approached a constant value asymptotically. As the pK_a value of nitrite is 3.15 [12], the fraction of the neutral molecule increases with increasing eluent concentration or decreasing pH. Hence the neutral nitrite molecules are retained on the column.

The retention time of carbonate was not affected by the eluent concentration throughout the range examined. Considering the pK_{a_1} value of 6.33 [12], carbonate molecules in an aqueous acidic solution are in the equilibrium: $CO_2 + H_2O \rightleftharpoons H_2CO_3$. This equilibrium lies largely to the left [13], and therefore most of the carbonate molecules are present in the CO_2 form and adsorbed on the gel. Carbonate was not retained with a pure water eluent.

The retention behaviour of three carboxylic acids is also shown in Fig. 2 for comparison. Under the present conditions, these carboxylic acids are present in the neutral form, because the pK_a values of these acids are higher than the eluent pH. The acids are retained on the column through hydrophobic interactions [14]. Therefore carboxylic acids eluted in order of increasing carbon number. The shorter retention time of these acids at lower eluent concentrations can be explained as the progress of the ionization of carboxylic groups at relatively higher pH. Formic acid eluted very close to the negative peak originating from water in the sample throughout the sulphuric acid eluent concentration.

Effects of the eluent concentration and the enhancer pH on the peak response

As the conductivity detector responds only to the ionized eluate components, the sensitivity for

carbonate was very low in the acidic eluate. Therefore, the peak enhancement system is essential for the detection of carbonate. When 25 m*M* sodium sulphate was employed as an enhancer, the response of the carbonate peak depended largely on the eluent concentration. The response of the carbonate peak decreased rapidly with increasing acid concentration in the eluent. Three causes can be considered to explain the relationship between the response and the eluent concentration. First, at higher eluent concentrations, a neutral enhancer does not have sufficient alkalinity to ionize carbonate molecules. Second, a higher concentration of the acid in the mobile phase resulted in a higher salt concentration in the eluate after neutralization by passage through the enhancement system and the ionization of the carbonate was suppressed, giving a lower detector response. Finally, as carbonate is present as carbon dioxide in the acidic eluent, these molecules are easy to release from the flow line to the outside through the wall of the cation-exchange hollow-fibre tube before conversion into the ionized form by the enhancer.

With the UV detector, the nitrite peak can be monitored before the enhancer, but the peak area decreases rapidly with increasing sulphuric acid concentration owing to the spectral change brought about by the neutralization of the nitrite molecules in the lower pH eluents. The effect of sulphuric acid concentration on the spectrum of nitrite is shown in Fig. 3. Below 0.5 m*M* sulphuric acid, where the pH of the solution is near the pK_a value of nitrite, ionization of nitrite progresses with strong absorption of UV radiation around 210 nm, whereas above 0.5 m*M* ionization of the molecules is suppressed and the UV absorption decreases with increase in acid concentration. Peak enhancement is also a useful technique for the determination of nitrite with UV detection.

Calibration graphs for carbonate and nitrite

Samples of 20 μl containing various concentrations of carbonate were injected. The eluent used was 1 m*M* sulphuric acid. In addition to a neutral salt enhancer, alkaline enhancers, ranging from 0.2 to 0.6 *M* in concentration, were examined to solve the previously mentioned shortcomings of the enhancement system.

When 25 m*M* sodium sulphate solution was used as an enhancer, in the detector cell carbonate is present as HCO_3^- ion and the peak appears in the direction of increasing conductivity. In contrast, with concentrated alkaline enhancers, carbonate shows a large negative peak from a very high background level [4,15]. The carbonate peak area increased with increasing concentration of the alkaline enhancer. For example, the peak area with 0.6 *M* NaOH was 1.5 times larger than that with 0.4 *M* NaOH as enhancer. With the addition of 1 *M* sodium sulphate to 0.4 A4 NaOH solution, the carbonate peak area was enhanced to become equivalent to that obtained with 0.6 A4 NaOH enhancer. With both enhancers, the eluate pH was increased to *ca.* 11.5, which is sufficiently high to ionize the carbonate to CO_3^{2-}, considering the carbonate pK_{a_2} value of 10.0 [12]. With 0.4 A4 NaOH and 1 *M* sodium sulphate enhancer, the peak area was three times larger than that obtained using a neutral salt enhancer. A relatively high concentration of sodium sulphate in the alkaline enhancer increases the penetration of the hydroxy ion into the inside of the cation-exchange hollow-fibre membrane to elevate the pH of the eluate. This salt effect is explained as suppression of the ionization of the sulphuric acid groups on the ion-exchange membrane tube, which reduces the ionic repulsion force of the Donnan potential against the hydroxy ions in the enhancer [16].

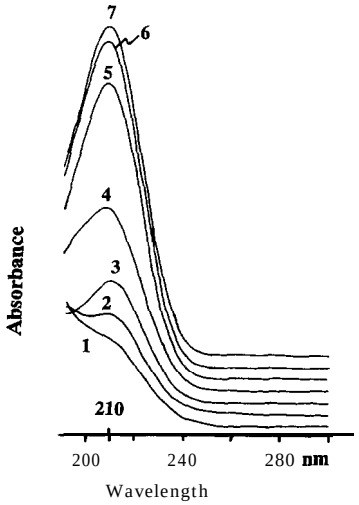

Fig. 3. Effect of the sulphuric acid concentration on the UV absorption spectrum of nitrite. Sample: 10 μg/ml nitrite solution. Sulphuric acid concentration in the solvent: 1 = 5; 2 = 2.5; 3 = 1; 4 = 0.5; 5 = 0.05; 6 = 0.005; 7 = 0 m*M*.

With both neutral and alkaline enhancers, a non-linear relationship between the peak area and the injected sample amount or concentration is observed. However, by plotting the logarithm of peak area against the logarithm of sample concentration, a linear relationship is obtained over the carbonate concentration range 5 μg/ml–1 mg/ml (0.1-20 μg as CO_3^{2-}) with both neutral and alkaline enhancers, as shown in Fig. 4. The linear relationship in Fig. 4 can be expressed as $A = BC^{0.95}$, where A represents the peak area, C the carbonate concentration and *B* is a constant. Below 5 μg/ml, the plots deviated from linearity. Alkaline enhancers yielded a larger peak area than a neutral enhancer, but the higher background with alkaline enhancers resulted in high noise levels, which impeded the improvement in the sensitivity for carbonate. Consequently, a very close dynamic range in the linear relationship between peak area and carbonate concentration is obtained with both alkaline and neutral enhancers.

A linear calibration graph for nitrite ranging from 0.2 to 100 μg/ml (from 2 ng to 2 μg as NO_2) is attained with the UV detector by applying a 20-μl aliquot of various concentrations of nitrite solutions.

Effects of sample volume on peak shape and retention

Sea water taken from the Pacific Ocean and spiked with 3.8 μg/ml of nitrite was injected. The sea water contained 20 mg/ml of chloride and 110 μg/ml of carbonate. When 0.75 m*M* sulphuric acid was used as the eluent, a symmetrical nitrite peak was obtained with up to 40-μl injections.

Significant band broadening, and even peak splitting, occurred when the sample was injected in a strong eluent, such as a neutral or basic salt solution, or when using large injection volumes. The explaination is that, in the column, the ionized solute moves faster than the neutral molecule in the band, the peak spreads toward the peak front and, in extreme cases, peak splitting takes place.

On the other hand, the shape and the retention volume of the carbonate peak at the conductivity detector were not affected by the sample volume with, up to 100-μl injections.

Effects of eluent concentration on peak shape and response

Higher eluent concentrations make it possible to inject larger volumes of sample for nitrite determination with UV detection but with a sacrifice of sensitivity to some extent. With higher sulphuric acid concentrations in the eluent a symmetrical peak shape is obtained with larger sample volumes. For example, when a 2.5 m*M* eluent was used, 50 μl of sea-water sample can be applied without peak deformation, as shown in Fig. 5.

With increasing eluent concentration, the sensitivity for carbonate with conductivity detection de-

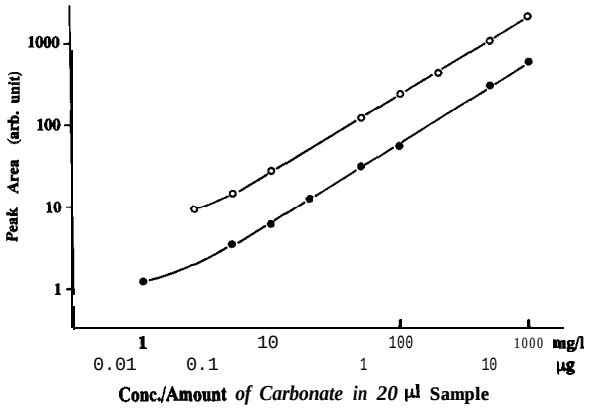

Fig. 4. Comparison of calibration graphs for carbonate obtained with neutral and alkaline enhancers. Eluent: 1 m*M* H_2SO_4. Enhancer: ● = 25 m*M* Na_2SO_4; 0 = 0.4 *M* NaOH + 1 *M* Na_2SO_4. Sample volume: 20 μl. Other conditions as in Fig. 1.

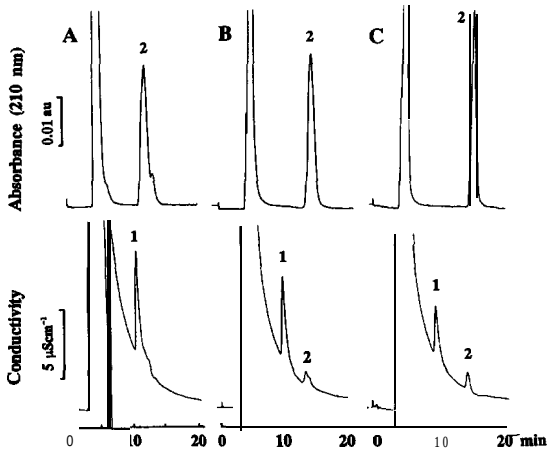

Fig. 5. Effect of the concentration of sulphuric acid in the eluent on the peak shape of carbonate and nitrite. Sample: sea water spiked with 3.8 μg/ml of nitrite. Sample volume: 50 μl. Sulphuric acid concentration in the eluent: (A) 0.5; (B) 1; (C) 2.5 m*M*. Peaks and other conditions as in Fig. 1.

creased whereas the background noise level increased. Therefore, sulphuric acid with concentrations over 2.5 mM is not applicable as an eluent with conductivity detection. As the pK_{a_1} value of carbonate is enough high for primary ionization in the present eluent, a 100-μl sea-water sample can be applied using dilute acid eluents such as 0.75 mM H_2SO_4 without any deformation of the peak shape.

The octadecyl-modified PVA gel column coupled with an acidic eluent provides a simple and sensitive method for the determination of nitrite and carbonate in inorganic matrix solutions. However, the retention mechanism of these ions cannot be explained clearly .

Carbonate can be separated from inorganic anion matrices also by ion-exclusion chromatography, where completely ionized inorganic anions are excluded from a strong cation-exchange resin column with an acidic eluent. Carbonate, being in the CO_2 form, behaves as a small inert molecule eluted at a retention volume $V_0 + V_i$, where V_0 is the interstitial volume and V_i is the internal volume of the column [3,14,17,18]. However, the present results with the ODP gel column showed that the retention volumes of carbonate and nitrite with an acidic eluent are much larger than the column volume of 2.5 ml. This means that the retention of these solutes is controlled not by the penetration into the pore of the gel beads as observed in ion-exclusion chromatography, and that some other interaction force between the solute and the gel plays an important role in the retention of the solutes.

Considering the very low dipole moment of carbon dioxide, the interaction between carbon dioxide and the stationary phase may be attributed to the hydrophobic interactions. Whereas the neutral nitrite, HONO, is a more polar compound, it is difficult to ascribe the large retention volume of nitrite to hydrophobic interactions. It can be considered that in sulphuric acid solutions, some interaction may take place between HONO molecules and alcoholic OH groups on the surface of the PVA gel beads.

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