



Separation of anions by ion-interaction chromatography with a novel cationic/zwitterionic eluent

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Abstract

Inorganic and organic anions can be separated on an ordinary silica C18 column using a mobile phase containing tetrabutylammonium hydroxide (TBAH) and an aminosulfonic acid zwitterion reagent (MOPS). The pH of this eluent is close to 7 and the background conductivity is about 50 μS , which is low enough to permit anion analyte detection by direct conductivity. Linear calibration curves were obtained for the six anions studied and detection limits ranged from 0.075 to 0.15 mg/l (ppm) for the five inorganic anions. The method was applied to the determination of water-soluble anions in aerosol samples at concentrations as low as 0.3 mg/l.

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1. Introduction

Anions in analytical samples are most commonly determined by ion chromatography (IC) using either suppressed or non-suppressed conductivity detection [1]. Suppressed IC generally requires an eluent containing a basic anion such as hydroxide or carbonate which can be converted to a species of very low conductivity by the acid associated with a membrane suppressor. Non-suppressed methods rely on the use of an

eluent containing a fairly large anion such as phthalate which has a lower conductance than the sample anions.

A very few reports have indicated that zwitterionic substances are effective eluents for anions and that a very low background conductance can be obtained after suppression. Ivey [2] reported that residual conductivities as low as 5–10 $\mu\text{S cm}^{-1}$ could be obtained when using salts of some N-substituted aminoalkyl-sulfonic acids with a packed suppressor. Irgum [3,4] separated anions with a 10 mM eluent concentration of an aminoalkylsulfonate zwitterion and obtained a background conductivity as low as 0.4 μS using membrane-suppressed detection. Gradient elutions

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could be run if the zwitterion used was sufficiently pure. Macka and Haddad [5] recently reported the use of histidine for an isoelectric ampholytic mobile phase for ion chromatography with unsuppressed conductivity detection. However, reports of zwitterionic eluents for on-suppressed IC are rare.

Mobile-phase ion chromatography (MPIC), also known as ion-pair chromatography or ion-interaction chromatography, is well suited for the determination of large molecules that carry a localized charge (surfactants, for example) and has also found some use in the chromatographic separation of other organic and inorganic anions. A reversed-phase silica column of the type commonly used in HPLC can be used for this type of IC with good efficiency. The present study describes a novel and effective separation method for anions using a standard silica C18 column with an eluent containing tetrabutylammonium hydroxide (TBAH) and a zwitterion. The eluent has a sufficiently low conductivity to permit direct conductimetric detection of sample anions with good sensitivity.

2. Experimental

2.1. Reagents

A 10% aqueous solution of tetrabutylammonium hydroxide and 3-(*N*-morpholino)-propane sulfonic acid (MOPS), both from Shanghai Chemical Reagents (Shanghai, China), were used to prepare the eluents used in this work. A Barnstead Easypure water system was used to further purify distilled water for all eluents and sample mixtures.

2.2. Samples and sample preparation

Aerosol samples were collected on 0.45 μm pore diameter Nucleopore glass fabric filters using an SK-11 sampling pump with a fixed flow rate of 75 l/min. The approximate volumes of terrestrial aerosols ranged from 18,000 to 36,000 l. An air volume of 108,000 l was used for marine aerosol sampling.

The collected samples were divided into four sections by boring (each area/total area = 15.4%). The collected aerosol was dissolved from one of the sections with 2.0 ml double-distilled water, then filtered to obtain the sample used for anion analysis by MPIC.

2.3. Instrumentation

A Dionex (Sunnyvale, CA, USA) series 4000i ion chromatograph was used in these experiments, equipped with a Dionex Analytical Pump with two pistons, an injection valve with a sample loop of 25 μl . A Waters (Milford, MA, USA) symmetry silica-based C18 column (250 mm \times 4.6 mm) and a Dionex conductivity detector were used. Neither the column nor the conductivity detector were heated; experiments were conducted at the ambient temperature of ca. 30 °C. There was temperature compensation in the detector with a default value of 1.7. Data were collected by using an N-2000 double-channel chromatogram workstation. Chinese software, CM 2.1 was used; it had a data acquisition rate of 10 data/s (10 Hz).

2.4. Procedure

Aqueous mobile phase (pH 7.15) containing 1 mM TBAH and 2 mM MOPS was pumped through a silica C18 column at a flow rate of 1.0 ml/min until a steady baseline was obtained (1–2 h) with direct conductivity detection. Then, a 25 μl anion sample was injected and data for the chromatographic separation were collected electronically.

3. Results and discussion

In the separation of anions by ion-interaction chromatography, the mobile phase usually contains a fairly bulky organic cation and a counter anion that is different from any of the sample anions. An equilibrium is established in which the eluent cation partitions between the column stationary phase and the liquid mobile phase. The eluent anion and sample anions then undergo a competitive pairing with the positively-charged sites on the stationary phase. Separation of sample anions is based on differences in their affinities for the positive sites on the stationary phase. Retention times of sample anions are strongly affected by the chemical structure and concentration of the ion pairing reagent and by the anion of the mobile phase. Addition of an organic modifier, such as methanol or acetonitrile, to the mobile phase can be used to shorten analyte retention times.

Table 1
Retention properties of fixed TBAH and MOPS ratio and changed total concentration

| Anions | 0.5 mmol/l TBAH and 2.5 mmol/l MOPS (pH = 6.55) TC = 21.5 μ S | | | 1 mmol/l TBAH and 5 mmol/l MOPS (pH = 6.35) TC = 51 μ S | | | 2 mmol/l TBAH and 10 mmol/l MOPS (pH = 6.5) TC = 91 μ S | | |
|----------|---|--------|------------|---|--------|------------|---|--------|------------|
| | T_r (min) | Plates | Resolution | T_r (min) | Plates | Resolution | T_r (min) | Plates | Resolution |
| Fluoride | 29.4 | 360 | 1.36 | 9.88 | 800 | 1.23 | 8.38 | 200 | 1.38 |
| Chloride | 27.0 | 270 | – | 11.9 | 6200 | 3.25 | 9.78 | 9500 | 4.71 |
| Nitrate | 38.8 | 1230 | 0.47 | 14.6 | 5600 | 1.43 | 12.1 | 6400 | 2.07 |
| Bromide | 37.8 | 6400 | – | 15.6 | 8900 | 1.21 | 13.4 | 8800 | – |
| Pyruvate | 45.3 | 740 | – | 17.8 | 1700 | 1.1 | 14 | 1300 | 5.22 |
| Nitrate | 46.0 | 5400 | – | 19.4 | 8300 | – | 18.2 | 8200 | – |

Spectrophotometric or suppressed conductivity detection is normally used in MPIC. However, we found that an aqueous mobile phase containing tetrabutylammonium hydroxide plus a zwitterionic reagent can be used to separate common anions on a bonded phase silica C18 column, and further, that the background conductivity is low enough for effective detection of sample anions by direct conductivity. Several zwitterionic reagents were tried including amino acids (glycine, cysteine, glutamic acid, and taurine) and aminosulfonic acids, 3-(*N*-morpholino)-propane sulfonic acid and 3-(cyclohexylamino)-1-propane sulfonic acid (CAPS). Of these, MOPS was the most satisfactory with regard to the quality of separations and because the pH could be close to 7 with only a small concentration of MOPS in the eluent.

Table 1 compares the retention times of the six sample anions as a function of TBAH and MOPS concentration in the mobile phase. At a concentration of 0.5 mM TBAH and 2.5 mM MOPS, the retention times were very long and peaks were very broad. The concentrations in the mobile phase were apparently too low to establish a stable equilibrium. However, at the

two higher concentrations of TBAH and MOPS listed in the table, the peaks were well formed and the sample anions were well separated. A TBAH concentration of 1.0 mM and a MOPS concentration of 5.0 mM gave the best resolution and had a relatively low baseline conductance of 51 μ S. This is a much lower background conductance than conventional eluents used in non-suppressed ion chromatography. For example, conductivities of 5 mM sodium benzoate and 5 mM sodium phthalate were measured as 476 and 455 μ S, respectively.

In preparing the eluent, the hydroxide ion of TBAH neutralizes the proton of the MOPS zwitterion to form the negatively charged sulfonate form of MOPS. The role of excess MOPS (zwitterionic form) in the eluent is not clear. Reduction of the MOPS concentration from 5 to 2 mM in eluent containing 1 mM TBAH resulted in a small increase in pH from 6.35 to 7.10, a small decrease in retention times of the sample anions and an increase in average plate number from 5260 to 6140 (Table 2). The chromatogram in Fig. 1 with an eluent containing 1 mM TBAH and 2 mM MOPS showed good resolution of all the sample ion

Table 2
Retention properties of fixed TBAH concentration and changed MOPS

| Anions | 1 mmol/l TBAH and 5 mmol/l MOPS (pH = 6.35) Conductivity = 51 μ S | | | 1 mmol/l TBAH and 2 mmol/l MOPS (pH = 7.10) Conductivity = 49 μ S | | |
|----------|---|--------|------------|---|--------|------------|
| | T_r (min) | Plates | Resolution | T_r (min) | Plates | Resolution |
| Fluoride | 9.88 | 800 | 1.23 | 9.47 | 4200 | 3.21 |
| Chloride | 11.9 | 6250 | 3.25 | 11.7 | 6400 | 3.59 |
| Nitrite | 14.6 | 5600 | 1.43 | 14.1 | 7000 | 1.83 |
| Bromide | 15.6 | 8900 | 1.21 | 15.3 | 8500 | 1.65 |
| Pyruvate | 17.8 | 1700 | – | 17.5 | 1600 | – |
| Nitrate | 19.4 | 8300 | – | 19.0 | 9200 | – |

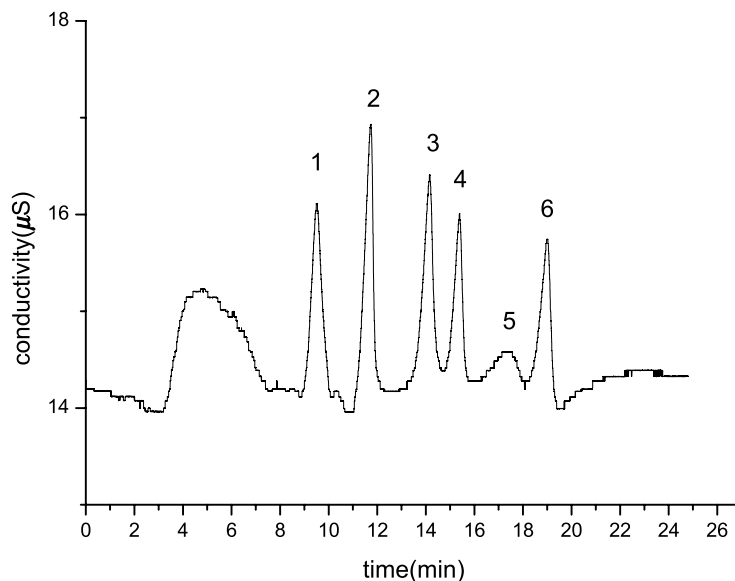


Fig. 1. Chromatogram obtained by using an aqueous eluent containing 1 mM TBAH and 2 mM MOPS, pH 7.1. Peaks: (1) 20 mg/l fluoride, (2) 20 mg/l chloride, (3) 30 mg/l nitrite, (4) 30 mg/l bromide, (5) 30 mg/l pyruvate, and (6) 30 mg/l nitrate.

peaks. The large, early system peak varied in area and was approximately proportional to the total ionic concentration of the sample. The broadness also varied with the concentration of MOPS and the eluent. This peak most likely resulted from cations (such as Na^+) from the sample which have a larger conductivity than the eluent ions. In separate experiments it was noted that cations are weakly retained by the column and also that the initial chromatographic peak disappeared when cation-suppressed detection was used. The broadness of this peak could be due to weak ion-exchange interaction of the sample cations

with $-\text{SO}_3^-$ from the MOPS anion or zwitterion in the adsorbed stationary phase.

The effect of adding a low percentage of methanol to the aqueous eluent was studied briefly. The data in Table 3 indicate that 10% methanol reduced the retention times of all the sample ions, but it also brought the retention times closer together so that peak resolution deteriorated. Addition of 10% methanol to the eluent reversed the elution order of nitrite and bromide and also that of nitrate and pyruvate.

The standard eluent containing 1.0 mM TBAH and 2 mM MOPS was modified by using 0.9 mM TBAH

Table 3
Retention properties of fixed TBAH and MOPS concentrations and varied methanol concentration

| Anions | 1 mmol/l TBAH and 5 mmol/l MOPS (pH = 6.35) TC = 51 μS | | | 1 mmol/l TBAH and 5 mmol/l MOPS and 10% Methanol (pH = 6.35) TC = 49 μS | | |
|----------|---|--------|------------|--|--------|------------|
| | T_r (min) | Plates | Resolution | T_r (min) | Plates | Resolution |
| Fluoride | 9.88 | 800 | 1.23 | 8.80 | 450 | – |
| Chloride | 11.9 | 6250 | 3.25 | 8.95 | 5350 | 0.97 |
| Nitrite | 14.6 | 5600 | 1.43 | 10.4 | 3150 | – |
| Bromide | 15.6 | 8900 | – | 10.1 | 7300 | 1.18 |
| Nitrate | 19.4 | 8300 | 1.09 | 11.0 | 6350 | – |
| Pyruvate | 17.8 | 1700 | – | 11.4 | 2900 | – |

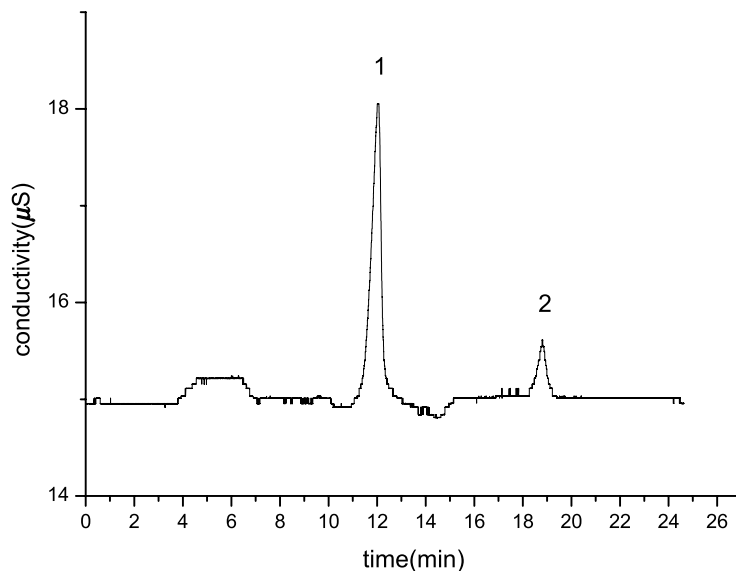


Fig. 2. Chromatogram of aerosol sample 7.5. Elution conditions as in Fig. 1. Peaks: (1) chloride, and (2) nitrate.

plus 0.1 mM tetramethylammonium hydroxide and also 0.8 mM TBAH plus 0.2 TMAH with a fixed MOPS concentration of 2.0 mM. The eluents containing TMAH gave slightly shorter retention times for the sample ions, but changes in the overall separation were minimal.

3.1. Calibration plots, detection limits, repeatability

A 25 µl sample containing six anions was separated repeatedly with a mobile phase containing 1 mM TBAH and 2 mM MOPS (see Fig. 1). Calibration plots of peak area versus concentration gave excellent linearities with r^2 values of 0.998 or 0.999 for each anion except pyruvate ($r^2 = 0.994$). The following limits of detection were obtained ($S/N = 3$): fluoride, 0.075 mg/l; chloride, 0.05 mg/l; nitrite, 0.07 mg/l; bromide, 0.15 mg/l; nitrate, 0.14 mg/l; and pyruvate, 0.62 mg/l. These compare well with those expected with conventional methods that use direct detection. Repeatability was also very good. The % R.S.D., $n = 10$, gave an average value of 1.8% for concentration of 0.9% for retention time of all anions except pyruvate, which had a R.S.D. of 4.2% for concentration and 1.7% for retention time. The ranges of linearity are from the detection limits to: -200 mg/l fluoride and chloride, and to

300 mg/l for nitrite, bromide, nitrate, and pyruvate. In each case, the intercept was reasonably close to zero.

3.2. Analysis of aerosol samples

Terrestrial aerosol samples from Hangzhou, China, and marine aerosol samples from the Pacific Ocean were collected as described in Section 2.2 and after dissolution were analyzed by the same chromatographic procedure used to prepare calibration plots. Fig. 2 was a representative of the terrestrial aerosol samples of the six anions studied in this paper. Only chloride and nitrate were found in the aerosol samples. In general, the anion content of terrestrial samples, which come mainly from crust dust and artificial emissions, was larger than that of sea area aerosols where the main source of anions is from sea salt. Over sea, colloidal nuclei are less than over land and the anion content of the sea aerosols is thus smaller.

Table 4 summarizes the results obtained for the analysis of aerosol samples. Samples 7.4, 7.5, and 7.6 were obtained on 4, 5, and 6 July from the top story of the Second Institute of Oceanography and sample 7.8 from the central story of Zhejiang University in China. Samples 7 and 9 were obtained during a cruise

Table 4
Analytical results of aerosol samples from Hangzhou, China, and the Pacific Ocean

| Sample no. | Anions content in aerosols (ng/l) | |
|------------|-----------------------------------|---------|
| | Chloride | Nitrate |
| 7.4 | 14.2 | 5.6 |
| 7.5 | 9.2 | 3.2 |
| 7.6 | 4.0 | 7.7 |
| 7.8 | 17.1 | 24.3 |
| 7 | 2.4 | 0.3 |
| 9 | 1.5 | 1.9 |

off the coast of China. Aerosols spiked with known amounts of anions gave 87% recovery of the added chloride and 85% recovery of the added nitrate.

4. Conclusions

The same columns widely used for HPLC can be adapted for ion chromatography simply by incorporating a suitable ion-pairing reagent in the mobile phase. Most ion chromatographic methods of this type

require the use of a suppressed conductivity detector. The present system consisting of an aqueous mobile phase containing a quaternary ammonium hydroxide and a zwitterionic reagent in conjunction with a reversed-phase silica column provides a simple, practical approach to ion chromatography. Sample anions are well resolved and can be detected at low mg/l (ppm) levels by direct conductivity.

Acknowledgements

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