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Determination of inorganic anions by ion chromatography using a graphitized carbon column dynamically coated with cetyltrimethylammonium ions

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Abstract

The simultaneous determination of inorganic anions by ion chromatography using a dynamically coated graphitized carbon column with cetyltrimethylammonium (CTA) ions was investigated with suppressed conductivity detection. Column preparations with CTA and sodium carbonate–sodium hydrogencarbonate concentration in the eluent were examined to optimize the separation of seven common anions (F^- , Cl^- , NO_2^- , Br^- , NO_3^- , HPO_4^{2-} and SO_4^{2-}). Calibration curves were linear from 0.5 to 5 $\mu\text{g/ml}$ for F^- , from 1.0 to 10 $\mu\text{g/ml}$ for Cl^- , from 1.5 to 15 $\mu\text{g/ml}$ for NO_2^- , from 2.0 to 20 $\mu\text{g/ml}$ for Br^- and NO_3^- , from 5.0 to 50 $\mu\text{g/ml}$ for HPO_4^{2-} and from 3.0 to 30 $\mu\text{g/ml}$ for SO_4^{2-} with correlation coefficients (r) of 0.999 or better. The relative standard deviations of peak areas were between 0.3 and 0.9% for 10 repeated measurements. The application of this newly developed method was demonstrated by the determination of inorganic anions in the water for pharmaceutical purposes. Using CTA-Br as the coating agent, a permanently coated ion-exchange column was obtained, which allowed efficient separations of seven anions without adding any coating agent to the eluent. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Stationary phases, LC; Graphitized carbon; Mobile phase composition; Cetyltrimethylammonium coating; Inorganic anions

1. Introduction

Among the wide variety of methods available for determination of inorganic anions, ion chromatography (IC) based on an ion-exchange separation and conductivity detection has been extensively studied [1,2]. On the other hand, reversed-phase ion-interaction chromatography on the chemically bonded ODS (C_{18}) column has also been applied to inor-

ganic anions [3,4]. This method however, has the disadvantage of low chemical resistance, because the ODS layer tends to be exfoliated as the silica base is dissolved under alkaline conditions [1,3] which makes such columns incompatible with the eluents used in suppressed conductivity detection.

A graphitized carbon column is an alternative non-polar stationary phase for liquid chromatography [5,6]. As well as having excellent flexibility in terms of the pH at which the column can be used (pH 1–14), the carbon particles also have an almost

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homogeneous surface [7]. The authors have previously reported on the simultaneous determination of inorganic anions by a graphitized carbon column using tetrabutylammonium (TBA) as a ionic modifier and sodium carbonate eluent [8–12]. In the present study, the separation behavior of seven common inorganic anions, F^- , Cl^- , NO_2^- , Br^- , NO_3^- , HPO_4^{2-} and SO_4^{2-} were examined by a cetyltrimethylammonium (CTA)-coated graphitized carbon column with various exchange capacities, using sodium carbonate–sodium hydrogencarbonate solution as the eluent and suppressed conductivity detection. The permanent coating system with CTA was compared to the ion-interaction system with TBA [12]. The method developed was then applied to the determination of inorganic anions in water for pharmaceutical purposes.

2. Experimental

2.1. Apparatus

A Dionex Model 4500i ion chromatograph (Sunnyvale, CA, USA) was equipped with an anion micromembrane suppressor (AMMS). Sample injection was via a Rheodyne Model 7125 injector (Cotati, CA, USA) with a 50- μ l sample loop. After the separation, the chromatographic data were processed by a Hitachi Model D-2500 integrator (Tokyo, Japan).

2.2. Reagents

Cetyltrimethylammonium bromide (CTA-Br), sodium carbonate, sodium hydrogencarbonate, acetonitrile and other reagents were purchased from Wako Pure Chemical (Osaka, Japan). The standard solutions of anions (F^- , Cl^- , NO_2^- , Br^- , NO_3^- , HPO_4^{2-} and SO_4^{2-}) were prepared by dissolving analytical grade sodium or potassium salt (Iwai Chemical, Tokyo, Japan) which had been dried overnight under vacuum at 100°C. Deionized water, further purified with a Millipore Milli-Q system (Bedford, MA, USA) with a specific resistance of 18.2 M Ω , was filtered through a 0.2- μ m membrane filter. Each standard solution for injection was prepared by

diluting the 1000 μ g/ml certified standards of seven common anions.

2.3. Column preparation and procedure

The graphitized carbon column employed was a Carbon IC BI-02 (Bio Tech Research, Saitama, Japan; 100 mm \times 4.6 mm I.D., average particle size 3.5 μ m, surface area 30~100 m²/g; carbon content greater than 99.5%). The column was at first washed with deionized water for 2 h at 1.0 ml/min. To coat the column, 0.5 mM CTA-Br in a water–acetonitrile (75:25) mixture was passed through the column at 1.0 ml/min for 1.0 h. After dynamically coating, the column was washed with deionized water for 2 h. The operating conditions for IC were as follows: eluent, 2.0 mM Na_2CO_3 –1.0 mM $NaHCO_3$; flow-rate, 1.0 ml/min; column temperature, 40°C; injection volume, 50 μ l; detector, electrical conductivity; suppressor, chemical ion suppression; micromembrane suppression continuously regenerated with 15 mM H_2SO_4 at 1 ml/min. A typical chromatogram of the standard mixture is shown in Fig. 1.

3. Results and discussion

3.1. Effect of CTA-Br concentration in coating solution on retention behavior

A graphitized carbon packing has π -electrons on its surface, so it is expected to exhibit not only hydrophobic interactions but also π – π interactions. Therefore, it does not have an inherently ion-exchange capacity. We previously used the technique of suppressed ion-interaction chromatography with 2 mM sodium carbonate containing 1 mM TBA ion as the eluent, however, it was necessary to add TBA to the eluent every run [8–12].

In the present paper, the effects of CTA-Br concentration at 0.25~1.0 mM in permanent coating solutions on the retention behavior was examined. After coating, the relative anion-exchange capacities of the columns coated with various concentrations of CTA-Br were determined from the retention of seven common anions with 2.0 mM sodium carbonate–1.0 mM sodium hydrogencarbonate eluent. The relationship between the retention time of the anions and

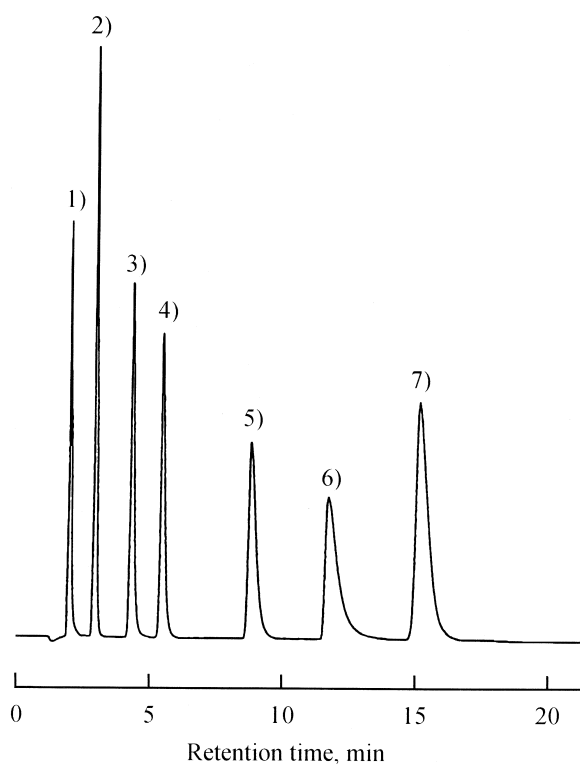


Fig. 1. Separation of inorganic anions on graphitized carbon column coated with CTA. Eluent: 2.0 mM Na_2CO_3 –1.0 mM NaHCO_3 . Ions ($\mu\text{g}/\text{ml}$): 1= F^- (5); 2= Cl^- (10); 3= NO_2^- (15); 4= Br^- (20); 5= NO_3^- (20); 6= HPO_4^{2-} (50); 7= SO_4^{2-} (30).

CTA-Br concentrations are shown in Fig. 2. An efficient separation of the seven anions was achieved with the column with 0.5 mM CTA-Br in the coating solution.

As the CTA ion was strongly adsorbed on the surface of the stationary phase, it is not necessary to add CTA to the eluent. Once coated, CTA was not removed during at least two months of use under an aqueous solution of sodium carbonate–sodium hydrogencarbonate as the eluent, and the coating can be removed upon treatment with 100% acetonitrile.

3.2. Effect of sodium hydrogencarbonate concentration in sodium carbonate eluent

The effect of sodium hydrogencarbonate concentration on the retention behavior was examined with the concentration of sodium carbonate fixed at 2.0 mM. The relationship between the retention time of

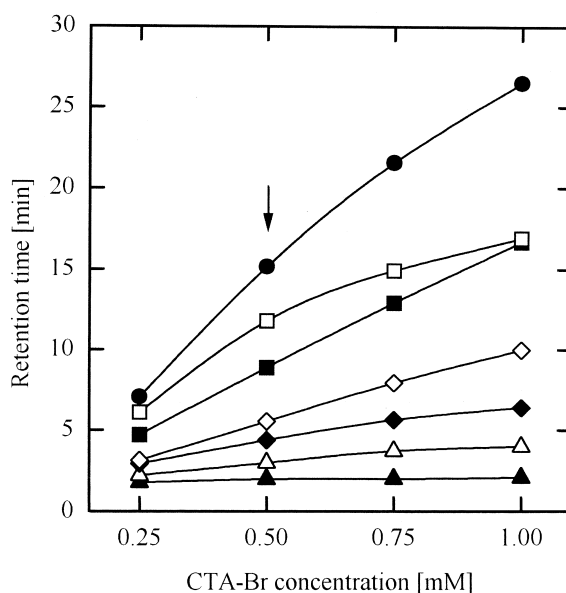


Fig. 2. Effect of CTA-Br concentration in column coating solution. Coating solution: CTA-Br containing acetonitrile–water (25:75), 1.0 ml/min for 1.0 h. Eluent: 2.0 mM Na_2CO_3 –1.0 mM NaHCO_3 . Ions ($\mu\text{g}/\text{ml}$): F^- \blacktriangle (5); Cl^- \triangle (10); NO_2^- \blacklozenge (15); Br^- \diamond (20); NO_3^- \blacksquare (20); HPO_4^{2-} \square (50); SO_4^{2-} \bullet (30).

anions and the concentration of sodium hydrogencarbonate is shown in Fig. 3. The retention of all the anions decreased with increasing sodium hydrogencarbonate concentration, as seen in the proposed general ion exchangers for IC [13]. In addition, a more significant decrease was observed in the retention of the divalent ions SO_4^{2-} and HPO_4^{2-} than in that of monovalent ions. A 2.0 mM sodium carbonate–1.0 mM sodium hydrogencarbonate solution was chosen as the eluting agent, because good resolution of anions was achieved within 18 min.

3.3. Effect of column temperature

The variation of retention factor as a function of temperature was studied from 25 to 40°C. The relationship between the retention of anions and column temperature is shown in Fig. 4. A more significant decrease was observed in the retention of NO_3^- than in that of other six anions. The overall changes in the retention of the other six anions studied were low over the temperature range studied. The optimum resolution was achieved at 40°C. The

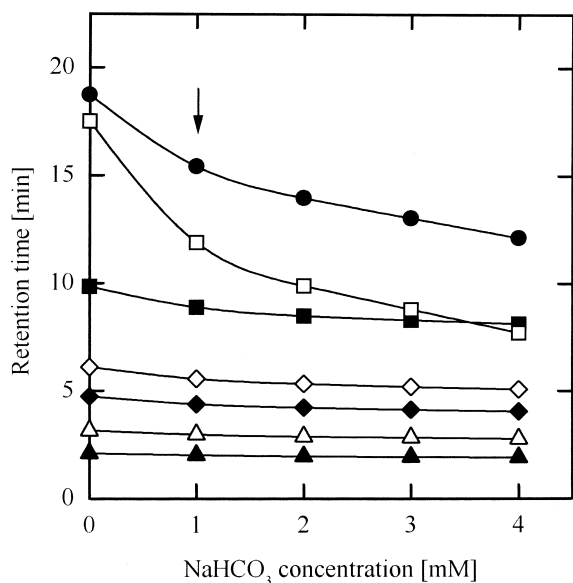


Fig. 3. Effect of NaHCO_3 concentration in 2 mM Na_2CO_3 eluent. Ions ($\mu\text{g}/\text{ml}$): F^- \blacktriangle (5); Cl^- \triangle (10); NO_2^- \blacklozenge (15); Br^- \diamond (20); NO_3^- \blacksquare (20); HPO_4^{2-} \square (50); SO_4^{2-} \bullet (30).

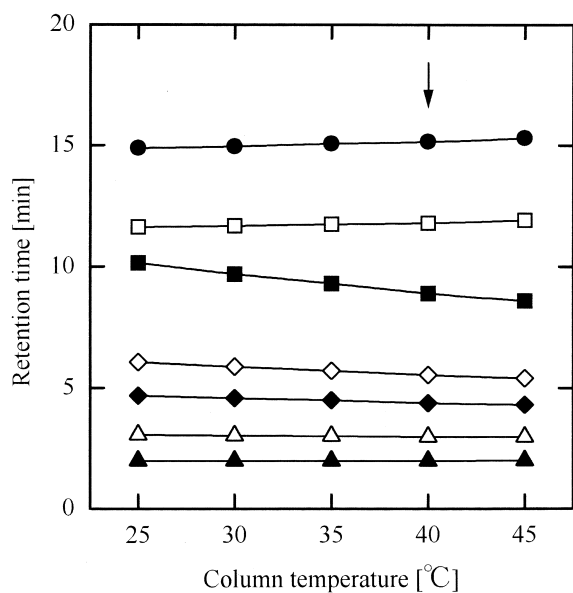


Fig. 4. Effect of column temperature on retention time. Ions ($\mu\text{g}/\text{ml}$): F^- \blacktriangle (5); Cl^- \triangle (10); NO_2^- \blacklozenge (15); Br^- \diamond (20); NO_3^- \blacksquare (20); HPO_4^{2-} \square (50); SO_4^{2-} \bullet (30).

observed temperature effects are identical to those observed with a commonly employed anion-exchange column using similar eluent systems [14].

3.4. Effect of organic solvent in the eluent

Non-ionic eluent modifiers, such as methanol or acetonitrile can be added to the eluent as a means of varying selectivity in suppressed IC [14]. Fig. 5 shows the effect of acetonitrile added to the eluent when a graphitized carbon column was used for separation of common anions. As the concentration of acetonitrile was increased, the retention of NO_2^- and NO_3^- decreased substantially more than that of F^- and Cl^- . However, the retention of SO_4^{2-} and HPO_4^{2-} increased with increasing concentration of acetonitrile. Fig. 6 shows the effect of the addition of acetonitrile on the trace analysis of common anions. The addition of acetonitrile was effective to retain F^- well out of the water dip (system dip) and to isocratically separate common anions at the ng/ml level. In this case, the separation between the F^- peak and system dip was improved compared to what has been reported for a common anion-exchange column. Using acetonitrile-carbonate as the

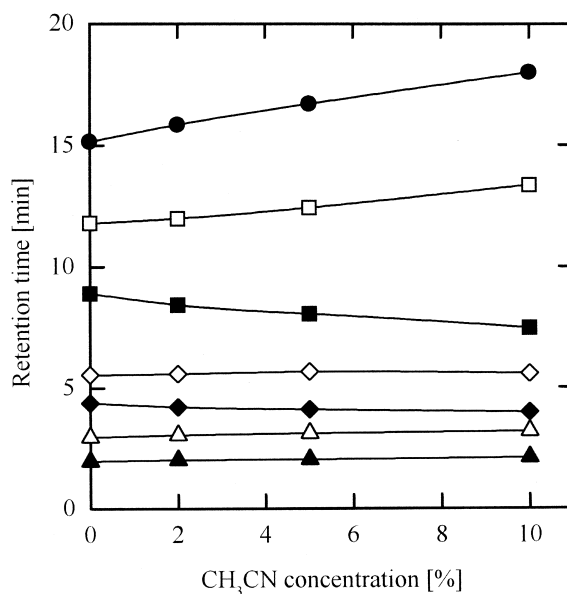


Fig. 5. Effect of acetonitrile concentration in the eluent. Ions ($\mu\text{g}/\text{ml}$): F^- \blacktriangle (5); Cl^- \triangle (10); NO_2^- \blacklozenge (15); Br^- \diamond (20); NO_3^- \blacksquare (20); HPO_4^{2-} \square (50); SO_4^{2-} \bullet (30).

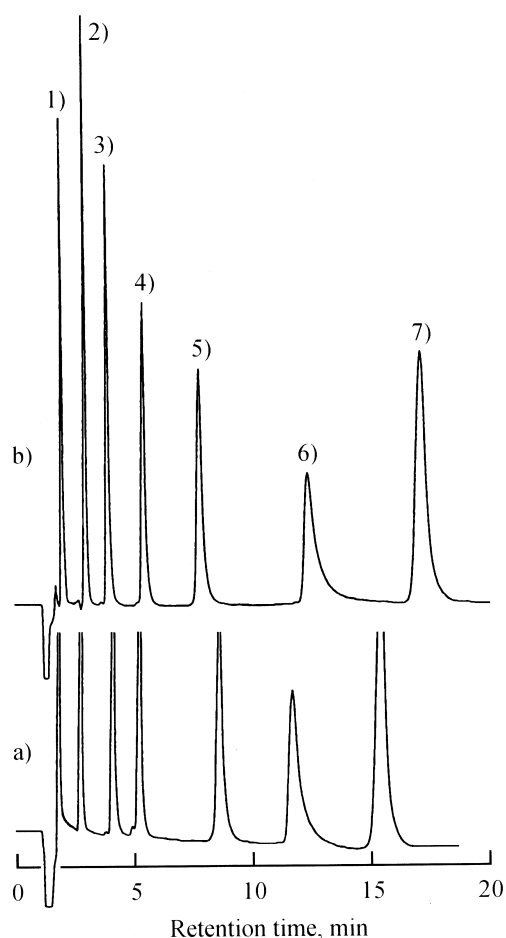


Fig. 6. Effect of acetonitrile content in the eluent on ion chromatograms. Eluent: (a) 2.0 mM Na_2CO_3 -1.0 mM NaHCO_3 ; (b) 2.0 mM Na_2CO_3 -1.0 mM NaHCO_3 -5% CH_3CN . Ions ($\mu\text{g}/\text{ml}$): 1= F^- (0.5); 2= Cl^- (1.0); 3= NO_2^- (1.5); 4= Br^- (2.0); 5= NO_3^- (2.0); 6= HPO_4^{2-} (5.0); 7= SO_4^{2-} (3.0).

eluent, the lifetime of the coated column was decreased under these conditions. This problem was overcome by the column recoating with 0.5 mM CTA-Br solution.

3.5. Validation of the developed method

The result of linearity, reproducibility of calibration curves and detection limits are shown in Table 1. The calibration curves obtained from the peak areas for the seven anions were linear, with good correlation coefficients of 0.999. The relative standard deviations (RSDs) of peak areas were between 0.2 and 0.9% for 10 repeated measurements. The detection limits, summarized in Table 1, were calculated at the S/N ratio of 3. The detection limits of the seven anions were between 0.2 and 3 ng/ml for a 50- μl injection. The detection limit was improved for HPO_4^{2-} compared with the TBA ion-interaction system, whereas the other anions had similar detection limits.

The capacity factor (k') and the number of theoretical plates (N) from the peaks are shown in Table 2. The values of N obtained were 1500–5800 for the seven anions.

Table 2
Theoretical plate number for inorganic anions

Anion	Capacity factor (k')	Efficiency (N)
F^-	0.64	1870
Cl^-	1.48	4510
NO_2^-	3.03	4520
Br^-	3.61	5780
NO_3^-	6.41	4320
HPO_4^{2-}	8.83	2550
SO_4^{2-}	11.63	4790

Table 1
Precision of ion chromatography based on peak area measurements

Anion	Linearity		Reproducibility		Limits of detection, ng ml^{-1} ($S/N=3$)
	Range ($\mu\text{g ml}^{-1}$)	r ($n=6$)	$\mu\text{g ml}^{-1}$	RSD (% , $n=10$)	
F^-	0.5~5.0	0.9998	5.0	0.78	0.2
Cl^-	1.0~10.0	0.9995	10.0	0.35	0.3
NO_2^-	1.5~15.0	0.9999	15.0	0.38	0.5
Br^-	2.0~20.0	0.9996	20.0	0.37	1
NO_3^-	2.0~20.0	0.9993	20.0	0.51	1
HPO_4^{2-}	5.0~50.0	0.9994	50.0	0.87	3
SO_4^{2-}	3.0~30.0	0.9998	30.0	0.32	1

Table 3
Determination of inorganic anions in pharmacopoeial water

Anion	Usual water (tap water) ($\mu\text{g ml}^{-1}$)	Water for injection ($\mu\text{g ml}^{-1}$)
F^-	0.3	n.d.
Cl^-	321	0.02
$\text{N-NO}_2^-^{\text{a}}$	n.d.	n.d.
Br^-	n.d.	n.d.
$\text{N-NO}_3^-^{\text{a}}$	1.6	n.d.
HPO_4^{2-}	n.d.	n.d.
SO_4^{2-}	43.2	n.d.

n.d.=Not detected.

^a N-NO_2^- and N-NO_3^- =Nitrogen from nitrite and nitrate ion, respectively.

3.6. Application to water samples

As practical examples of the application of the present method, inorganic anions in water for pharmaceutical purposes were analyzed. Table 3 shows the analytical results of usual water (tap water) and the water for injection in the pharmacopoeia of Japan (JP XIII). Concentrations of $32 \mu\text{g/ml}$ of Cl^- , $43 \mu\text{g/ml}$ of SO_4^{2-} and $1.6 \mu\text{g/ml}$ of nitrogen from nitrate (N-NO_3^-) were detected in the tap water. In the water for injection, purified by reverse osmosis–ultrafiltration only ng/l levels of Cl^- were observed. Concentrations of the other six anions were all below the detection limit.

4. Conclusion

A sensitive and selective ion chromatographic procedure for the determination of inorganic anions was demonstrated. This method employed an aqueous solution mixture of sodium carbonate–sodium hydrogencarbonate as the eluent, used in conjunction with a graphitized carbon column coated with CTA ions. The retention behavior of common anions was proposed to be due to a ion-exchange mechanism

with the coating amounts of CTA ion on the surface of the stationary phase and the sodium carbonate–sodium hydrogencarbonate concentration in the eluent which governed the elution order of common anions. This method showed excellent reproducibility, linearity, lower detection limits and fewer interferences among the ions tested. Therefore, it was applied satisfactorily to the various water samples. The graphitized carbon column has been used for three years, and the excellent durability of the column was confirmed in all our experiments. The method permits the preparation of columns with a wide range of ion-exchange capacities.

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