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Studies on elution conditions for the determination of anions by supressed ion-interaction chromatography using a graphitized carbon column

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Abstract

A new method has been developed for ion-interaction chromatography with suppressed conductivity detection and a new graphitized carbon packing, which is sintered from carbonic material at a high temperature. Combinations of various eluting agents, tetrabutylammonium hydroxide (TBA) and acetonitrile have been investigated to optimize the separation of eight common anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-}, HPO_4^{2-} and I^-). Calibration curves were linear from 0.5 to 10 μg/ml for F^-, from 1.0 to 20 μg/ml for Cl^-, NO_2^- and NO_3^-, from 2.5 to 50 μg/ml for Br^- and SO_4^{2-} and from 5.0 to 100 μg/ml for HPO_4^{2-} and I^- with a correlation coefficient (*r*) of 0.999 or better. The relative standard deviations (R.S.D.s) of peak areas were between 0.2 and 0.9% for 10 repeated measurements. The application of this newly developed method was demonstrated by the determination of chloride, bromide and sulfate in pharmaceutical compounds using the direct injection method. The analytical results were within ±2% (relative) of the theoretical value, and thus in good agreement with the theoretical value for each sample.

Keywords: Stationary phases, LC; Graphitized carbon; Mobile phase composition; Anions; Tetrabutylammonium

1. Introduction

In general, styrene–divinylbenzene-based surface layer ion-exchangers or polyacrylate and silica-based porous ion-exchangers have been used as column packing for ion chromatography [1]. Sometimes factors such as ion-exchange capacity, the adjustment of resolution of various ions, organic solvent-resistance and pressure-resistance present problems in the optimization of separation conditions [1,2]. Alternatively, instead of using an ion-exchange column, an ODS (C_{18}) column may be used with an ion-interaction reagent to achieve separation of ions. It, 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 with suppressed conductivity detection.

Graphitized carbon packing, a newly developed

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material for high-performance liquid chromatography (HPLC), is a carbonic material prepared by treatment at a high temperature and, therefore, it has excellent chemical and physical resistance [4]. The authors have previously reported on the simultaneous determination of several inorganic anions by ion chromatography using a graphitized carbon column [5–8]. In these papers, basic studies of the elution conditions for several inorganic anions were described. A general-purpose column of 5 cm×4.6 mm I.D. was used. Furthermore, we employed an ion-interaction reagent, tetrabutylammonium hydroxide (TBA), as the eluent instead of an anion-exchange group, and sodium carbonate as the eluting agent. However, for the present study, in order to achieve a higher resolution for the determination of anions in pharmaceutical compounds, a 10 cm long column was used for precision analysis, with sodium carbonate, sodium hydrogen carbonate, sodium hydroxide and sodium tetraborate as eluting agents. Furthermore, a dynamic coating method was examined in detail and a direct injection method was applied to the determination of chloride, bromide and sulfate in pharmaceutical compounds.

2. Experimental

2.1. Apparatus

The ion chromatographic equipment consisted of a Model ICA-5120 pump (Toa, Tokyo, Japan), a Model ICA-5220 conductivity detector (Toa, Tokyo, Japan), an injector (Rheodyne, Cotati, CA, USA), a Model ICA-3052 column oven (Toa, Tokyo, Japan), a Model D-2500 integrator (Hitachi, Tokyo, Japan) and a Model AMMS-MPIC suppressor (Dionex, Sunnyvale, CA, USA). The separation column was a Carbon BI-01 (100×4.6 mm I.D., average particle size 3.5 μm, surface area 30~100 m^2/g, carbon content larger than 99.5%, Bio-Tech Research, Saitama, Japan).

2.2. Materials

Millipore (Milford, MA, USA) Milli-Q 18 MΩ water was used for all eluent and sample preparation. Sodium carbonate, sodium hydrogen carbonate, sodium hydroxide, sodium tetraborate (all analytical-reagent grade) and acetonitrile (HPLC grade) were purchased from Wako Pure Chemical (Osaka, Japan). TBA (ion chromatography grade) was purchased from Dionex. Other chemicals were of analytical reagent grade (Wako Pure Chemical). Pretreatment of the sample solution was performed with a Toyopak IC-SP cartridge (Tosoh, Tokyo, Japan).

2.3. Analytical conditions

The operating conditions for ion-interaction chromatography were as follows: eluent, 1 m*M* TBA–2 m*M* Na_2CO_3–5% CH_3CN; flow-rate, 0.8 ml/min; column temperature, 40°C; injection volume, 50 μl; detector, electrical conductivity; suppressor, chemical ion suppression; micro-membrane suppression continuously regenerated with 12.5 m*M* H_2SO_4 at 1 ml/min. A typical chromatogram of the standard mixture is shown in Fig. 1. The retention behaviors were observed with a fixed concentration of acetonitrile at 5%, while changing the concentrations of TBA and eluting agent. The determination of chloride, bromide and sulfate in pharmaceutical com-

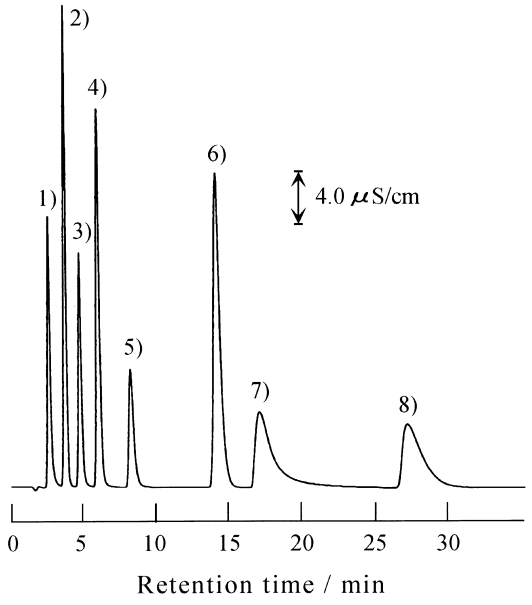

Fig. 1. Ion chromatogram of a standard mixture. Eluent: 1 m*M* TBA–2 m*M* Na_2CO_3–5% CH_3CN. Ions (μg/ml): 1=F^- (4); 2=Cl^- (8); 3=NO_2^- (8); 4=Br^- (20); 5=NO_3^- (8); 6=SO_4^{2-} (20); 7=HPO_4^{2-} (40); 8=I^- (40).

pounds was made by comparing the chromatographic peak areas to calibration curves obtained by the least-squares method.

2.4. Sample pretreatment

The direct injection method was utilized to qualitatively determine the presence of anions in pharmaceutical compounds. In this method, 10 to 40 mg of quinine·H_2SO_4, quinine·HCl and dextromethorphan·HBr were dissolved in 250 ml of water and this sample solution was passed through a Toyopak IC-SP cartridge to eliminate cations. After discarding the first 1 ml of eluate, the following eluate was manually injected into a 50-μl loop. The Toyopak IC-SP cartridge was washed with 10 ml of water.

3. Results and discussion

A graphitized carbon packing carries π-electrons on its surface, and thus it has not only an hydrophobic interaction but also a π–π interaction. However, it does not have an intrinsic ion-exchange capacity. Therefore, we used the technique of ion-interaction chromatography with an ion-interaction reagent and adjusted the retention time by changing the concentration of the ion-interaction reagent [1].

3.1. Effect of eluting agent on retention behavior

In general, the eluting agents used in suppressor anion chromatography are sodium carbonate, sodium hydrogen carbonate, sodium hydroxide and sodium tetraborate. Therefore, the effects of eluting agent on retention behavior were studied by examining the retention behavior of eight common anions with four kinds of eluting agents with fixed concentrations of TBA at 1 m*M* and acetonitrile at 5%. The relationships between the logarithm of the capacity factor (k') and the concentration of sodium carbonate or sodium hydrogen carbonate as an eluting agent are shown in Figs. 2 and 3.

The ion chromatograms on various eluting agent concentration ratios of sodium carbonate and sodium hydrogen carbonate are shown in Fig. 4. The slopes of the log k' for bivalent anions were similar to those of general ion-exchangers, being twice as large as for univalent anions when using sodium carbonate or sodium hydrogen carbonate. In the case of a mixed eluent of sodium carbonate and sodium hydrogen carbonate, the order of elution of sulfate and phosphate (HPO_4^{2-}) reversed when the sodium carbonate concentration was increased. The typical chromatogram with sodium tetraborate as the eluting agent is shown in Fig. 5. When sodium tetraborate or sodium

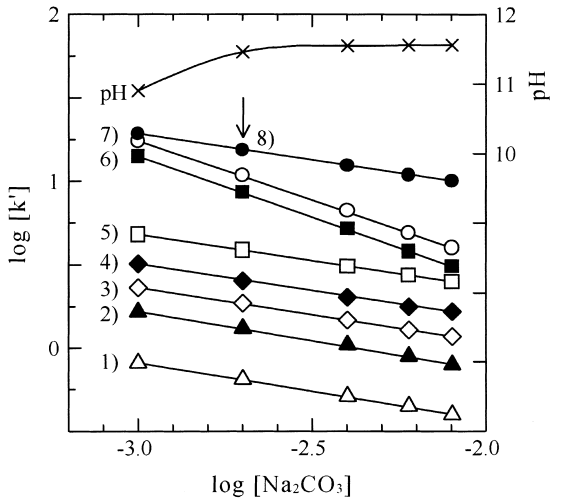

Fig. 2. Effect of Na_2CO_3 concentration on capacity factor (k'). Ions: 1=F^-; 2=Cl^-; 3=NO_2^-; 4=Br^-; 5=NO_3^-; 6=SO_4^{2-}; 7=HPO_4^{2-}; 8=I^-.

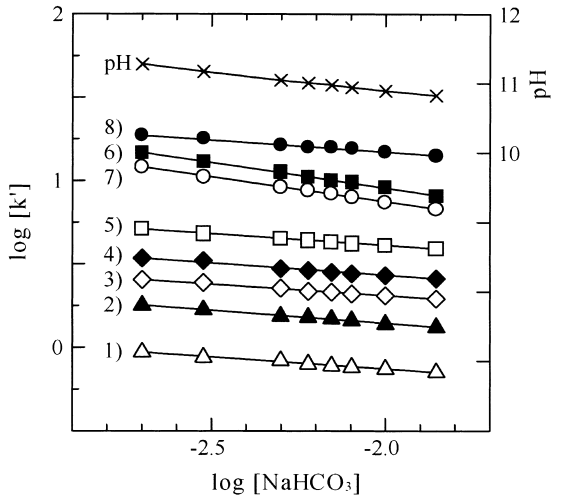

Fig. 3. Effect of $NaHCO_3$ concentration of capacity factor (k'). Ions: 1=F^-; 2=Cl^-; 3=NO_2^-; 4=Br^-; 5=NO_3^-; 6=SO_4^{2-}; 7=HPO_4^{2-}; 8=I^-.

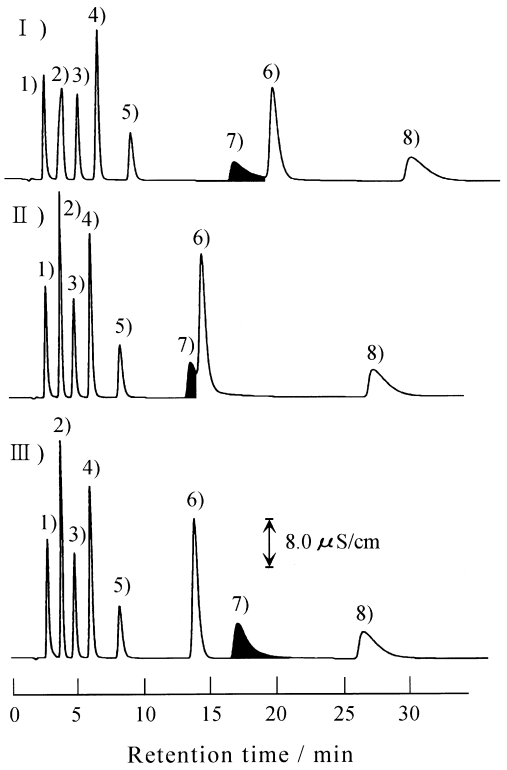

Fig. 4. Ion chromatograms of standard mixture. Eluent: (I) 1 m*M* TBA–4 m*M* $NaHCO_3$–5% CH_3CN; (II) 1 m*M* TBA–1 m*M* Na_2CO_3–2 m*M* $NaHCO_3$–5% CH_3CN; (III) 1 m*M* TBA–2 m*M* Na_2CO_3–5% CH_3CN. Ions: 1=F^-; 2=Cl^-; 3=NO_2^-; 4=Br^-; 5=NO_3^-; 6=SO_4^{2-}; 7=HPO_4^{2-}; 8=I^-.

hydroxide is used as an eluting agent, the baseline was not steady. A 2 m*M* sodium carbonate solution was chosen as the eluting agent, because good resolution of anions was achieved within 30 min.

3.2. Effect of TBA concentration on retention behavior

The effect of the concentration of the ion-interaction reagent on the retention behavior was examined with the concentration of sodium carbonate fixed at 2 m*M*, and that of acetonitrile at 5%, and based on measurements of retention time, theoretical plate number and resolution. The effect of TBA concentration on the capacity factor and the effect of TBA concentration on the number of theoretical plates are shown in Figs. 6 and 7, respectively. The

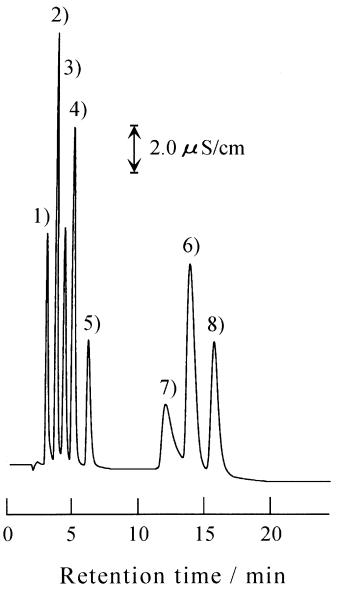

Fig. 5. Ion chromatogram of standard mixture. Eluent: 1 m*M* TBA–10 m*M* $Na_2B_4O_7$–10% CH_3CN. Ions: 1=F^-; 2=Cl^-; 3=NO_2^-; 4=Br^-; 5=NO_3^-; 6=SO_4^{2-}; 7=HPO_4^{2-}; 8=I^-.

retention time of each anion increased with increasing concentration of the ion-interaction reagent. However, the increase of retention time of sulfate was abnormally small in comparison with the other

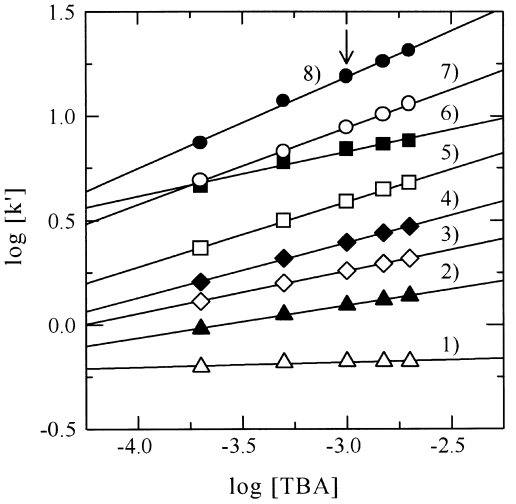

Fig. 6. Effect of TBA concentration on capacity factor (*k'*). Ions: 1=F^-; 2=Cl^-; 3=NO_2^-; 4=Br^-; 5=NO_3^-; 6=SO_4^{2-}; 7=HPO_4^{2-}; 8=I^-.

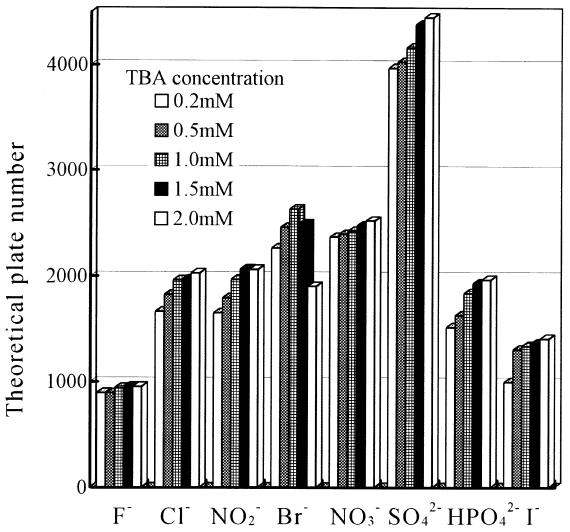

Fig. 7. Effect of TBA concentration on theoretical plate number of each ion.

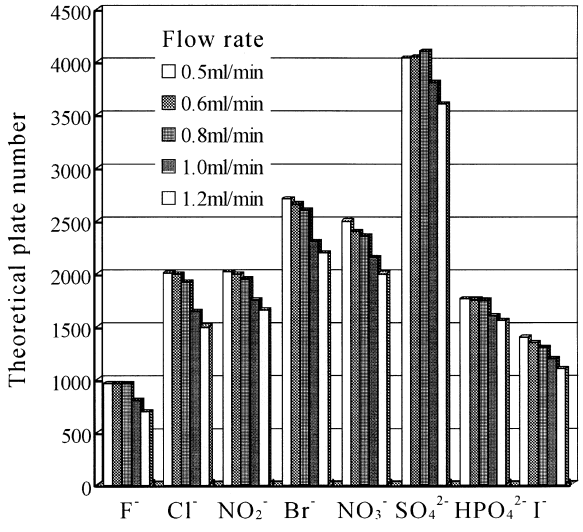

Fig. 8. Effect of flow-rate on theoretical plate number of each ion.

anions. The number of theoretical plates for almost all anions increased with an increase in the TBA concentration.

The optimum TBA concentration was determined to be 1 m*M*, based on the number of theoretical plates, resolution and retention time for each of the eight common anions. Furthermore, the effect of acetonitrile concentration (3.0, 5.0 and 7.5%) on the retention behavior was examined. The optimum concentration of acetonitrile was found to be 5% for the same reasons as for TBA.

3.3. Effect of column temperature and flow-rate

The optimal column temperature (30, 35, 40 or 45°C) was obtained from the experimental results of the number of theoretical plates, resolution and the retention time of the eight common anions. The optimum temperature for the carbon column was determined as 40°C.

The effect of flow-rate on theoretical plate number of each ion is shown in Fig. 8. The effect of flow-rate on the retention behavior was investigated and the optimum flow-rate was established as 0.8 ml/min, based on the number of theoretical plates, resolution and retention time of the eight common anions.

3.4. Retention behavior of a dynamic coating

We used highly hydrophobic TBA as the ion-interaction reagent. Therefore, the carbon surface was coated completely with the ion-interaction reagent in equilibration [9]. This should make the surface an ion-exchanger, which led us to postulate that TBA would no longer be needed after coating. To investigate such a possibility, the TBA coating was applied following the procedure described in Section 2.3 with a flow-rate of 0.8 ml/min for 1 h. A mixture of 2 m*M* sodium carbonate and 5% TBA-free acetonitrile, was passed as eluent at a flow-rate of 0.8 ml/min for 20 min to attain equilibrium. The relationship between the retention time and the running time after dynamic coating was examined. The retention became weaker and the resolution became poorer with time. Satisfactory precision and stability during this determination could not be achieved, and consequently it is necessary to add TBA to the eluent for every run.

3.5. Linearity, reproducibility and detection limits

The linearity, reproducibility of the calibration curves and detection limits are shown in Table 1. The calibration curves obtained from the peak areas for the eight anions were linear, with high correlation

Table 1
Precision of ion-interaction chromatography based on peak area measurements

Anion	Linearity		Reproducibility		Limits of detection ng/ml ($S/N=3$)
	Range (μg/ml)	r ($n=5$)	μg/ml	R.S.D. (%) ($n=10$)	
F^-	0.5~10.0	0.9996	4.0	0.36	0.2
Cl^-	1.0~20.0	0.9994	8.0	0.26	0.3
NO_2^-	1.0~20.0	0.9995	8.0	0.35	0.5
Br^-	2.5~50.0	0.9993	20.0	0.76	1
NO_3^-	1.0~20.0	0.9991	8.0	0.64	1
SO_4^{2-}	2.5~50.0	0.9997	20.0	0.24	1
HPO_4^{2-}	5.0~100.0	0.9987	40.0	0.86	10
I^-	5.0~100.0	0.9985	40.0	0.94	10

R.S.D.=Relative standard deviation.

coefficients of 0.999. The relative standard deviations of peak areas were between 0.2 and 0.9% for 10 repeated measurements. The detection limits, reported in Table 1, were calculated as the amount injected that gave a signal that was three times the background noise. The detection limits of eight anions were between 0.5 and 10 ng/ml. It is similar detection limits compared with typical detection limits for ion chromatography.

3.6. Application to several samples

Attempts were made to determine anions in pharmaceutical compounds using the direct injection method. The results are shown in Table 2. The results obtained from the quantitative analysis were in good agreement, within ±1.9%, compared to the theoretical value for each sample. The number of theoretical plates showed agreement between the standard solutions and the sample solutions for each anion; the peak heights also agreed in this analysis. Consequently, this method can be applied to the determination of inorganic anions in various organic compounds, while avoiding the matrix effect.

The graphitized carbon column has been used for three years in our laboratories, and we used pure acetonitrile for the column and various eluents over a wide pH range. However, during this time the column did not show any change in the overall elution behavior, the retention time, the theoretical plate number nor any other property, and the excellent durability of this column was confirmed in all our experiments.

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Table 2
Determination of anions in organic compounds by the direct injection method

Sample	Anion	Calculated (%)	Found (%)	Deviation (%)
Quinine·H_2SO_4[a]	SO_4^{2-}	12.27	12.50	+0.23
Quinine·HCl[b]	Cl^-	8.93	9.00	+0.07
Dextromethorphan·HBr[c]	Br^-	21.58	21.63	+0.05

Each sample solution of drugs was prepared by passing through a Toyopak IC-SP cartridge, as a column to remove drugs.
[a] $(C_{20}H_{24}N_2O_2)\cdot H_2SO_4\cdot 2H_2O$.
[b] $(C_{20}H_{24}N_2O_2)\cdot HCl\cdot 2H_2O$.
[c] $C_{18}H_{25}NO\cdot HBr\cdot H_2O$.

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