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Simultaneous separation of common mono- and divalent cations on a calcinated silica gel column by ion chromatography with indirect photometric detection and aromatic monoamines–oxalic acid, containing crown ethers, used as eluent

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Abstract

The application of unmodified silica gel (Super Micro Bead Silica Gel B-5, SMBSG B-5) as a cation-exchange stationary phase in ion chromatography with indirect photometric detection (IC–IPD) for the separation of common mono- and divalent cations (Li^+, Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}) was carried out using various aromatic monoamines {tyramine [4-(2-aminoethyl)phenol], benzylamine, phenylethylamine, 2-methylpyridine and 2,6-dimethylpyridine} as eluents. When using these amines as eluents, the peak resolution between these mono- and divalent cations was not quite satisfactory and the peak shapes of NH_4^+ and K^+ were largely destroyed on the SMBSG B-5 silica gel column. Hence, the application of SMBSG B-5 silica gel calcinated at 200, 400, 600, 800 and 1000 °C for 5 h in the IC–IPD was carried out. The peak shapes of the monovalent cations were greatly improved with increasing calcination temperature and, as a result, symmetrical peaks of these mono- and divalent cations were obtained on the SMBSG B-5 silica gel calcinated at 1000 °C as the stationary phase. In contrast, the peak resolution between these mono- and divalent cations was not improved. Therefore, crown ethers [18-crown-6 (1,4,7,10,13,15-hexaoxacyclooctadecane), 15-crown-5 (1,4,7,10,13-pentaoxacyclopentadecane)] were added to the eluent for the complete separation of these mono- and divalent cations. Excellent simultaneous separation and highly sensitive detection at 275 nm were achieved in 25 min on a column (150×4.6 mm I.D.) packed with SMBSG B-5 silica gel calcinated at 1000 °C by elution with 0.75 m*M* tyramine–0.25 m*M* oxalic acid at pH 5.0 containing either 1.0 m*M* 18-crown-6 or 10 m*M* 15-crown-5.

Keywords: Mobile phase composition; Indirect detection; Stationary phases, LC; Amines, aromatic; Crown ethers; Oxalic acid; Inorganic cations

1. Introduction

Ion chromatography with indirect photometric detection (IC–IPD), developed by Small and Miller [1], offers a simple and convenient method for the determination of inorganic and organic anions and cations [2]. Copper(II) ion is widely employed as the eluent ion in IC–IPD for cations [3]. The main reason is that the simultaneous separation of common mono- and divalent cations (Li^+, Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}) can be achieved on both sulfonated styrene–divinylbenzene copolymer (PS–

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DVB) resin and sulfonated silica columns. However, due to the low UV absorbance, the detection sensitivities of these cations are moderate. Furthermore, the determination of these cations is often affected by organic compounds co-existing in analytical samples, because of the detection wavelength at 220–240 nm.

One of the best ways to eliminate these drawbacks is to utilize protonated aromatic monoamines (PAMAs) with high UV absorbance as eluent ions. Haddad and Foley applied various PAMAs as eluent ions in IC–IPD with a lightly sulfonated PS–DVB resin column for the separation and detection of various cations [4]. Unfortunately, although highly sensitive detection of analyte cations was achieved, the simultaneous separation of common mono- and divalent cations could not be achieved. This was because (1) the affinity of the divalent cations for the sulfonic acid group as cation-exchanger is much stronger than that of the monovalent cations for the sulfonic acid group and (2) the eluent strength of PAMAs was weaker than that of copper ion.

Since the silanol group on the surface of silica gel behaves as a weak acid at $pK_a \approx 7.1$ [5], it is possible to apply unmodified silica gel as a cation-exchange stationary phase in IC with conductimetric detection (IC–CD) for cations. In IC–CD, Li^+ is mainly used as the eluent ion for the separation and detection of analyte cations [6–10]. The greatest advantage is that the simultaneous separation of major mono- and divalent cations (Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}) can be achieved [9,10]. This is because the affinities of these mono- and divalent cations for the dissociated silanol group as the cation exchanger are very similar. Therefore, it was expected that unmodified silica gel would be one of the most suitable cation-exchange stationary phases in IC–IPD using PAMAs as eluent ions for the simultaneous separation of common mono- and divalent cations (Li^+, Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}). However, the application has not been popular. This may be because commercially available silica gels, except for pure silica gel synthesized by the hydrolysis of pure tetraethoxysilane [$Si(OCH_2CH_3)_4$], contain various polyvalent metals as impurities in the silica matrix and, consequently, an undesirably strong interaction occurs between PAMAs and the polyvalent metals on the surface of the silica gel [11–14]. A modification of the silica gel was required before its successful application in IC–IPD. Thermal treatment (calcination) is an easy and effective way for the modification of silica gel [15]. However, calcinated silica gels have only been evaluated as stationary phases in normal-phase HPLC. The application of calcinated silica gel as the cation-exchange stationary phase in IC–IPD using PAMAs as the eluent ions for the separation of mono- and divalent cations has not yet been carried out.

The aim of this study was to expand the utility of silica gel as the cation-exchange stationary phase in IC for cations. The application of silica gel (Super Micro Bead Silica Gel B-5) calcinated at 200, 400, 600, 800 and 1000 °C for 5 h in IC–IPD using various PAMAs as eluent ions was carried out for the simultaneous separation of common mono- and divalent cations.

2. Experimental

2.1. Equipment

The ion chromatograph consisted of a Tosoh (Tokyo, Japan) LC-8020 chromatographic data processor, a Tosoh CCPM-II-R solvent delivery pump operated at a flow-rate of 1 ml min^{-1}, a Tosoh UV-8020 UV–Vis spectrophotometric detector, a Tosoh CM-8020 conductimetric detector, a Tosoh CO-8020 column oven operated at 35 °C, a Tosoh DS-8023 on-line degasser and a Rheodyne (Cotati, CA, USA) Model 9125 injector equipped with 20 or 50 μl sample loops.

A Perkin-Elmer (Shelton, CT, USA) Lambda 18NS double beam UV–Vis spectrometer was used for the measurements of the UV spectra of eluents.

A Toa Denpa (Tokyo, Japan) IM-40S ion meter with a glass electrode was used for the measurements of the pH of eluents and natural water samples.

2.2. Chemicals

A Fuji-Silysia Chemical (Kasugai, Japan) Super Micro Bead Silica Gel B-5 (SMBSG B-5, lot No. 902530) porous spherical silica gel for HPLC was used in this work. The SMBSG B-5 silica gel was calcinated at 200, 400, 600, 800 and 1000 °C for 5 h.

Table 1
Physical properties of Super Micro Bead Silica Gel B-5 silica gels calcinated at 200–1000 °C for 5 h

Calcination temp. (°C)	Particle size (μm)	Surface area ($m^2\ g^{-1}$)	Pore size (Å)	Pore volume ($ml\ g^{-1}$)	Packing density ($g\ ml^{-1}$)	Surface area per column[a] ($m^2\ column^{-1}$)
200	5.5	475	60	0.93	0.43	5.1×10^2
400	5.5	487	60	0.89	0.44	5.3×10^2
600	5.5	483	60	0.89	0.44	5.3×10^2
800	5.4	429	59	0.77	0.45	4.8×10^2
1000	5.0	336	57	0.59	0.55	4.6×10^2

[a] Column size: 150×4.6 mm I.D.

Table 1 shows the physical properties of the calcinated SMBSG B-5 silica gels. The determination of the surface area and pore volume of these silica gels was carried out using nitrogen adsorption isotherms for the gels at 77 K using a Beckman-Coulter (Fullerton, CA, USA) Omunisorp 360 gas sorption analyzer. The surface area was calculated from the BET equation. Determination of the particle size of the silica gels was carried out using a Horiba (Kyoto, Japan) LA-920 laser scattering particle size distribution analyzer.

The separation columns (150×4.6 mm I.D., stainless steel) were packed with the silica gels using the slurry-packing method.

All chemicals were of analytical reagent grade. Tyramine [4-(2-aminoethyl)phenol], benzylamine, phenylethylamine, 18-crown-6 (1,4,7,10,13,15-hexaoxacyclooctadecane) and 15-crown-5 (1,4,7,10,13-pentaoxacyclopentadecane) were purchased from Aldrich (Milwaukee, WI, USA) and other chemicals were purchased from Wako (Osaka, Japan). Distilled, deionized water was used for the preparation of the eluents and standard solutions.

3. Results and discussion

3.1. Chromatographic behavior of mono- and divalent cations on the SMBSG B-5 silica gel column using aromatic monoamines as eluents

In order to apply commercially available unmodified silica gel (Super Micro Bead Silica Gel B-5, SMBSG B-5) as a cation-exchange stationary phase in IC–IPD of common mono- and divalent cations (Li^+, Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}), the IC behavior of these cations on the SMBSG B-5 silica gel column (150×4.6 mm I.D.) was investigated using various aromatic monoamines as eluents. Fig. 1A–E show chromatograms obtained using (A) 0.75 m*M* tyramine [4-(2-aminoethyl)phenol]–0.25 m*M* oxalic acid at pH 5.0, (B) 0.5 m*M* benzylamine–0.25 m*M* oxalic acid at pH 5.0, (C) 0.5 m*M* phenylethylamine–0.25 m*M* oxalic acid at pH 5.0, (D) 0.25 m*M* 2-methylpyridine–0.1 m*M* oxalic acid at pH 5.5 and (E) 0.25 m*M* 2,6-dimethylpyridine–0.1 m*M* oxalic acid oxalic acid at pH 5.5 as eluents. Oxalic acid functioned as both a buffering agent and a complexing agent for the divalent cations. The pH of the eluent was adjusted using 0.1 *M* HNO_3.

As shown in Fig. 1A–E, when using these aromatic monoamines as eluents, it was very easy to elute the mono- and divalent cations in a reasonable period of time (20 min). This is because the affinities of these mono- and divalent cations for the dissociated silanol group on the surface of the silica gel as cation exchanger were very similar. Since the elution order of the monovalent cations was $Li^+ < Na^+ < NH_4^+ < K^+$, it was evident that the SMBSG B-5 silica gel functioned as a cation-exchange stationary phase under these IC–IPD conditions. Good separation of the divalent cations was achieved, because oxalic acid functioned as a complexing agent for the these cations. Unfortunately, the separation of the monovalent cations was not quite satisfactory and, in addition, the peak shapes of NH_4^+ and K^+ were largely destroyed. The degree of peak destruction was $Li^+ < Na^+ < NH_4^+ < K^+$ and the order was in good agreement with the elution order. In contrast, although the retention volumes of the divalent cations were larger than those of the monovalent

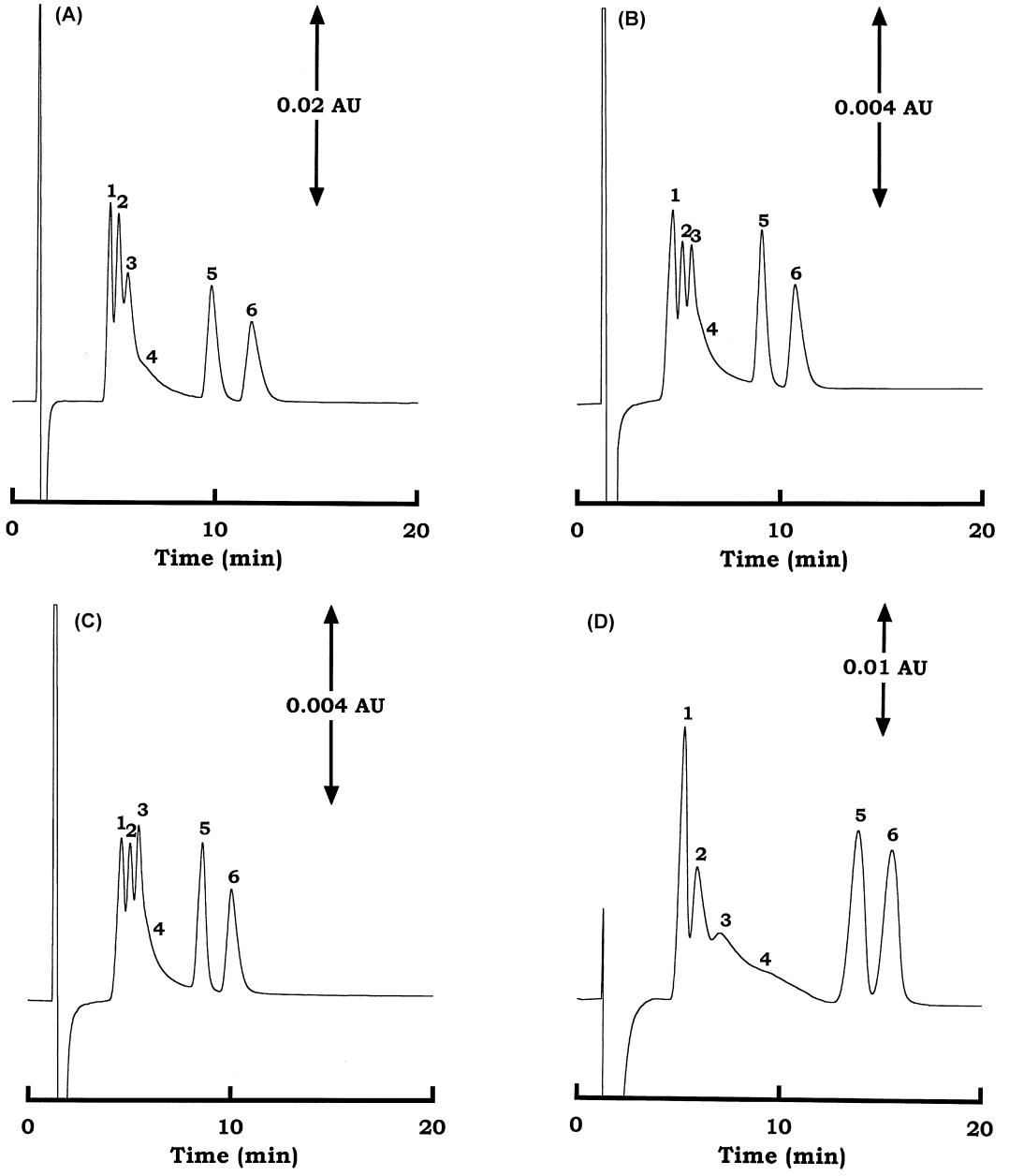

Fig. 1. Chromatograms of mono- and divalent cations on a Super Micro Bead Silica Gel B-5 silica gel column using various aromatic monoamines as eluents. Conditions: eluents, (A) 0.75 m*M* tyramine–0.25 m*M* oxalic acid at pH 5.0, (B) 0.5 m*M* benzylamine–0.25 m*M* oxalic acid at pH 5.0, (C) 0.5 m*M* phenylethylamine–0.25 m*M* oxalic acid at pH 5.0, (D) 0.25 m*M* 2-methylpyridine–0.1 m*M* oxalic acid at pH 5.5, (E) 0.25 m*M* 2,6-dimethylpyridine–0.1 m*M* oxalic acid at pH 5.5, (F) 0.25 m*M* oxalic acid, (G) 1 m*M* lithium hydroxide–0.5 m*M* oxalic acid at pH 5.0, the pH of eluents was adjusted using 0.1 *M* nitric acid; flow rate, 1 ml min^{-1}; column, Super Micro Bead Silica Gel B-5 (SMBSG B-5); column size, 150×4.6 mm I.D.; column temperature, 35 °C; detection, (A) indirect UV at 275 nm, (B) indirect UV at 256 nm, (C) indirect UV at 257 nm, (D) indirect UV at 253 nm, (E) indirect UV at 257 nm, (F) indirect conductivity, (G) conductivity; injection volume, 20 μl; sample concentration, 0.2 m*M* for monovalent cations and 0.1 m*M* for divalent cations. Peaks: 1 = Li^+, 2 = Na^+, 3 = NH_4^+, 4 = K^+, 5 = Mg^{2+}, 6 = Ca^{2+}.

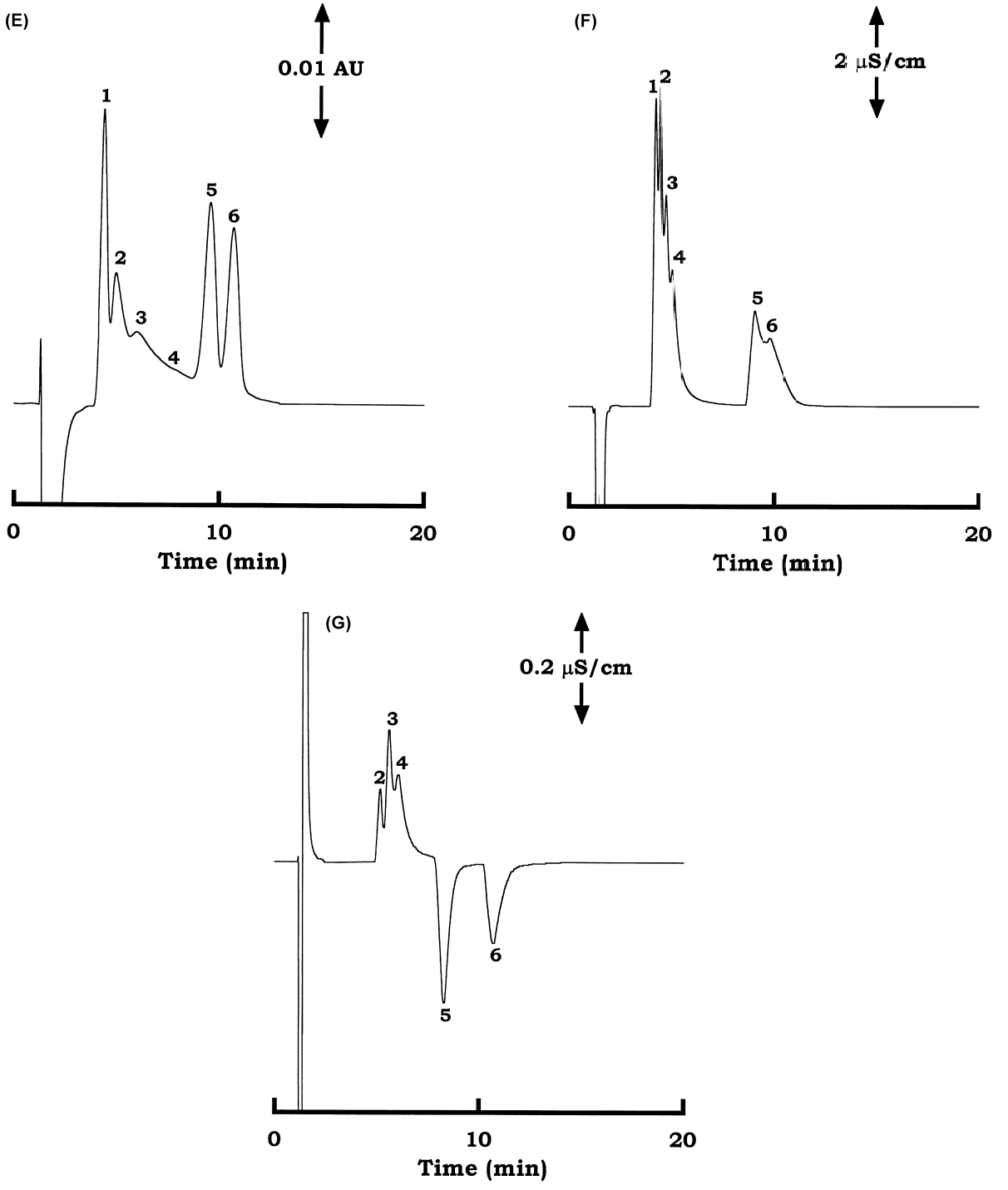

Fig. 1. (*continued*)

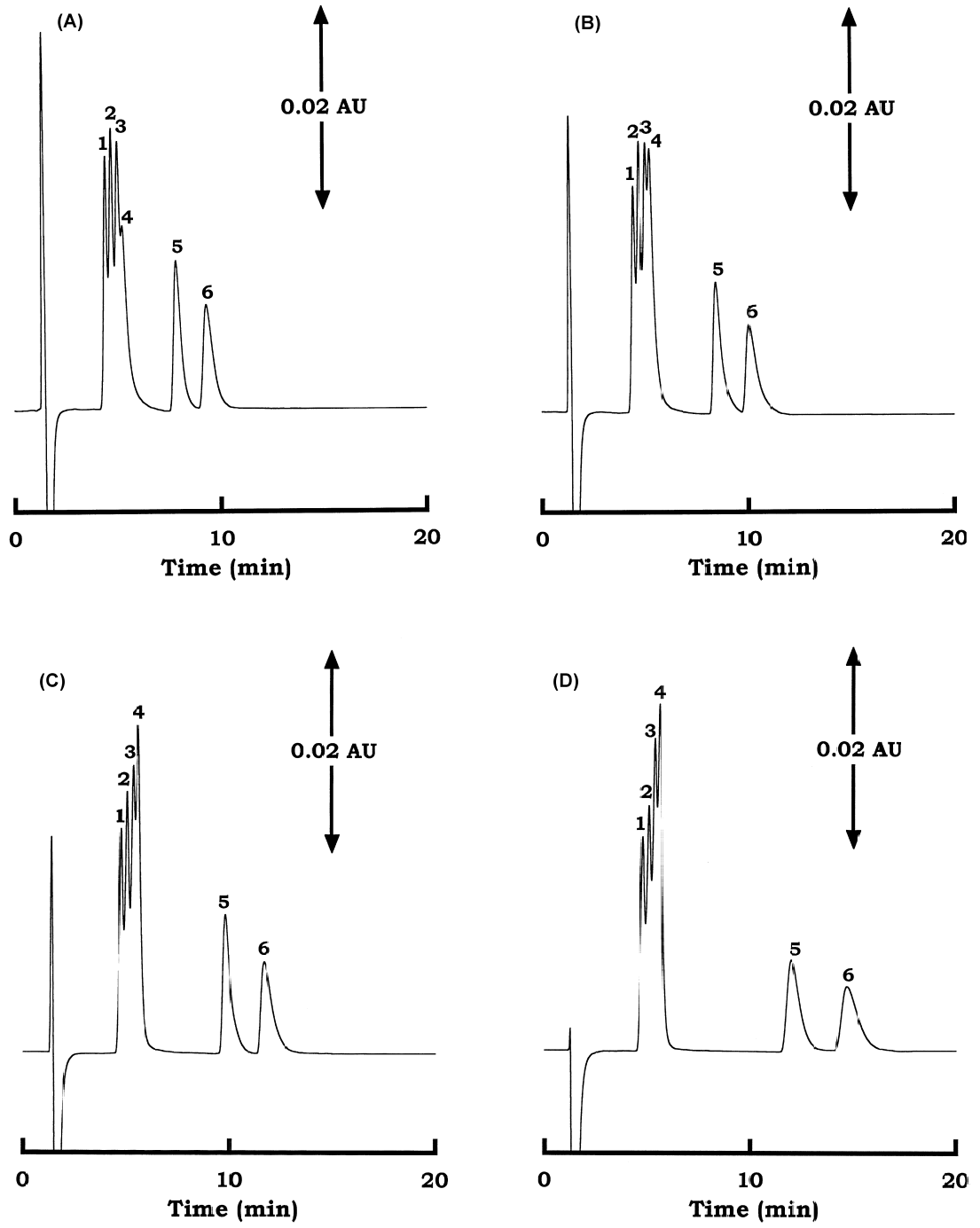

Fig. 2. Chromatograms of mono- and divalent cations on calcinated SMBSG B-5 silica gel columns using 0.75 m*M* tyramine–0.25 m*M* oxalic acid at pH 5.0 as eluent. Conditions: columns, (A) SMBSG B-5 silica gel calcinated at 400 °C for 5 h (SMBSG-400), (B) SMBSG B-5 silica gel calcinated at 600 °C for 5 h (SMBSG-600), (C) SMBSG B-5 silica gel calcinated at 800 °C for 5 h (SMBSG-800), (D) SMBSG B-5 silica gel calcinated at 1000 °C for 5 h (SMBSG-1000); eluent, 0.75 m*M* tyramine–0.25 m*M* oxalic acid at pH 5.0; detection, indirect UV at 275 nm. Other conditions as in Fig. 1.

cations, the peak shapes of the divalent cations were much better than those of NH_4^+ and K^+. However, although no conclusive explanation for the peak destruction could be found, it was suspected that the main cause could be attributed to the undesirably strong interaction between these PAMAs and the polyvalent metals present as impurities in the SMBSG B-5 silica gel. This is because this silica gel is not a pure silica gel synthesized by the hydrolysis of pure tetraethoxysilane [$Si(OCH_2CH_3)_4$]. Fig. 1F and G show chromatograms of these mono- and divalent cations on the SMBSG B-5 silica gel column obtained using (F) 0.25 m*M* oxalic acid and (G) 1 m*M* lithium hydroxide–0.5 m*M* oxalic acid at pH 5.0 as the eluents and conductimetric detection. As can be seen from Fig. 1F and G, although the peak resolution between the monovalent cations was also unsatisfactory, the peak shapes of NH_4^+ and K^+ were much better than those obtained using PAMAs as the eluent ions. Since these eluent ions (H^+ and Li^+) were retained on the SMBSG B-5 silica gel as the cation-exchange stationary phase by the cation-exchange mechanism, the above results were strongly supported by the fact that the main cause of peak destruction in IC–IPD was due to the strong interaction (adsorption) between PAMAs and the polyvalent metals in the SMBSG B-5 silica gel.

The most suitable PAMA as eluent ion in IC–IPD was then investigated. As shown in Fig. 1A–C, when using tyramine, benzylamine and phenylethylamine as eluents, the peak resolution between these mono- and divalent cations was unsatisfactory. In contrast, the detection sensitivities of the cations obtained using protonated tyramine as the eluent ion were much higher than those obtained when using both protonated benzylamine and phenylethylamine as eluent ions. This is because the calculated molar extinction coefficient of tyramine [1.5×10^{-3} (mol cm)$^{-1}$ at 275 nm] is much larger than those calculated for benzylamine [2.6×10^{2} (mol cm)$^{-1}$ at 256 nm] and phenylethylamine [2.1×10^{2} (mol cm)$^{-1}$ at 257 nm]. As shown in Fig. 1D and E, when using protonated 2-methylpyridine and 2,6-dimethylpyridine as eluent ions, a more sensitive detection of these cations was achieved. This is because the calculated molar extinction coefficients of 2-methylpyridine [4.2×10^{3} (mol cm)$^{-1}$ at 253 nm] and 2,6-dimethylpyridine [4.3×10^{3} (mol cm)$^{-1}$ at 257 nm] are larger than that of tyramine. Unfortunately, the peak shapes of these mono- and divalent cations obtained using protonated 2-methylpyridine and 2,6-dimethylpyridine as eluent ions were broadened in comparison to those obtained using protonated tyramine as eluent ion. This might be because (a) the eluent concentration of 2-methylpyridine (0.25 m*M*) and 2,6-dimethylpyridine (0.25 m*M*) was considerably lower than that of tyramine (0.75 m*M*), and (b) protonated 2-methylpyridine and 2,6-dimethylpyridine would be adsorbed on the SMBSG B-5 silica gel strongly in comparison to protonated tyramine.

Considering the peak shape and detection sensitivity, it was concluded that protonated tyramine was the most suitable eluent ion for IC–IPD.

3.2. Chromatographic behavior of mono- and divalent cations on calcinated SMBSG B-5 silica gel columns using tyramine as eluent

In order to apply the SMBSG B-5 silica gel as the cation-exchange stationary phase in IC–IPD for the successful separation of these mono- and divalent cations, modification of the SMBSG B-5 silica gel was required. Thermal treatment (calcination) of the SMBSG B-5 was carried out and the IC behavior of the mono- and divalent cations on columns packed with the gels calcinated at 200, 400, 600, 800 and 1000 °C for 5 h (SMBSG-200, -400, -600, -800 and -1000) was investigated. Fig. 2A–D show chromatograms of the cations on (A) SMBSG-400, (B) SMBSG-600, (C) SMBSG-800 and (D) SMBSG-1000 columns obtained using 0.75 m*M* tyramine–0.25 m*M* oxalic acid at pH 5.0 as eluent. The chromatogram on the SMBSG-200 column has already been shown in Fig. 1A.

As shown in Fig. 1A and Fig. 2A–D, with increasing calcination temperature, the IC behavior of these cations changed dramatically. For the monovalent cations, the peak shapes were improved. When using the SMBSG-1000 column, symmetrical peaks were obtained for the monovalent cations. In contrast, the difference in retention volume between the monovalent cations decreased. For divalent cations, the retention volumes changed significantly. Since the change in the retention volumes was not directly dependent on the change of the total surface area of the calcinated SMBSG B-5 silica gel columns, as

shown in Table 1, it was suspected that the main cause could be attributed to changes of the chemical properties of the SMBSG B-5 silica gel by the calcination procedure. In order to investigate the changes in the chemical structure of the SMBSG B-5 silica gel, X-ray diffraction was performed on the calcinated gels. Unfortunately, the X-ray diffraction patterns of these gels showed almost no variation, because the silica substrates were amorphous. Details concerning the effect of the calcinating temperature will be the subject of future work.

Considering the IC behavior of these mono- and divalent cations, it was decided that SMBSG-1000 was the most suitable cation-exchange stationary phase for our IC–IPD studies.

3.3. Effect of crown ethers in the eluent on the chromatographic behavior of mono- and divalent cations on the SMBSG-1000 column

Calcination of the SMBSG B-5 silica gel was very effective in improving the peak shapes of the monovalent cations. Unfortunately, the peak resolution between the monovalent cations was not satisfactory on these calcinated columns. It is known that the addition of a crown ether [18-crown-6 (1,4,7,10,13,15-hexaoxacyclooctadecane)] to an acidic eluent in IC–CD using a weakly acidic cation-exchange stationary phase is very effective in improving the peak resolution between these mono- and divalent cations [16,17]. Therefore, the addition of crown ethers [18-crown-6 (1,4,7,10,13,15-hexaoxacyclooctadecane) and 15-crown-5 (1,4,7,10,13-pentaoxacyclopentadecane)] to the eluent (0.75 m*M* tyramine–0.25 m*M* oxalic acid at pH 5.0) was investigated for the complete separation of these mono- and divalent cations on the SMBSG-1000 column.

3.3.1. 18-Crown-6

Fig. 3 shows the relationship between the concentration of 18-crown-6 in the eluent and the retention volumes of these mono- and divalent cations.

For the monovalent cations, with increasing concentration of 18-crown-6 in the eluent, the retention volumes of Li^+ and Na^+ decreased slightly and the retention volume of NH_4^+ increased slightly. In contrast, the retention volume of K^+ increased drastically initially and then remained almost constant. The order of increase in the retention volumes was $Li^+ < Na^+ < NH_4^+ \ll K^+$. Since the order is in good agreement with the stability constants of the complexes formed between the monovalent cations and 18-crown-6 ($\log K_{Na^+} = 0.8$, $\log K_{NH_4^+} = 1.23$ and $\log K_{K^+} = 2.03$) [18], it was evident that 18-crown-6 was adsorbed on SMBSG-1000 and functioned as a selective cation exchanger for the monovalent cations. Complete separation of the monovalent cations was achieved at a concentration of 18-crown-6 ≥ 1.0 m*M*. For the divalent cations, with increasing concentration of 18-crown-6, the retention volumes

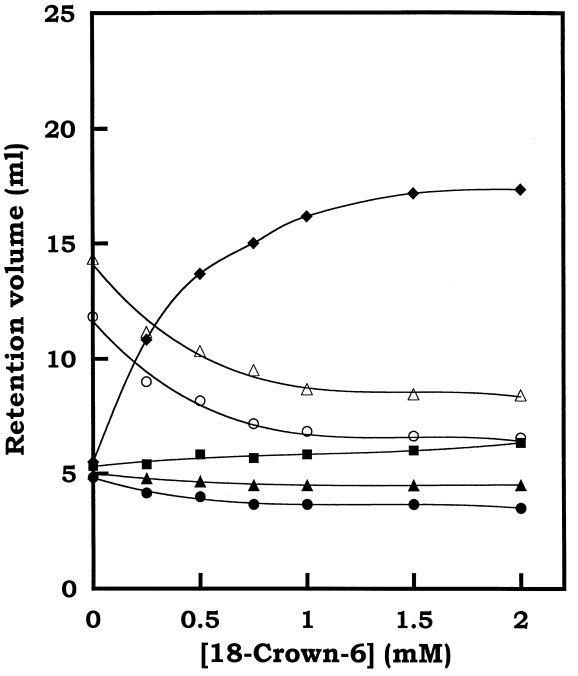

Fig. 3. Effect of the 18-crown-6 concentration in 0.75 m*M* tyramine–0.25 m*M* oxalic acid at pH 5.0 as eluent on the retention volumes of mono- and divalent cations on the SMBS-1000 column. Conditions: eluent, 0.75 m*M* tyramine–0.25 m*M* oxalic acid at pH 5.0 containing 0–2 m*M* 18-crown-6; column, SMBSG-1000. Symbols: (●) Li^+, (▲) Na^+, (■) NH_4^+, (♦) K^+, (○) Mg^{2+}, (△) Ca^{2+}. Other conditions as in Fig. 2.

decreased significantly initially and thereafter decreased gradually. Since the order of decrease in the retention volumes was $Ca^{2+} \leq Mg^{2+}$, the difference in retention behavior was also attributed to the stability of the complexes formed between the divalent cations ($\log K_{Ca^{2+}} < 0.8$) [16]. Details concerning the decrease in retention volumes will be the subject of future work. Complete group separation between these mono- and divalent cations was achieved in the concentration range of 18-crown-6 between 0.5 and 1.0 m*M*.

From the above results, it was concluded that the optimum concentration of 18-crown-6 was 1.0 m*M*. As shown in Fig. 4, excellent simultaneous separation of these mono- and divalent cations was achieved on the SMBSG-1000 column in 20 min by elution with 0.75 m*M* tyramine–0.25 m*M* oxalic acid–1.0 m*M* 18-crown-6 at pH 5.0 (eluent A).

3.3.2. 15-Crown-5

Fig. 5 shows the relationship between the concentration of 15-crown-5 in the eluent and the retention volumes of these mono- and divalent cations.

For the monovalent cations, with increasing concentration of 15-crown-5 in the eluent, the retention volumes increased. The order of increase in the retention volumes was $Li^+ < NH_4^+ < Na^+ < K^+$. Complete separation of the monovalent cations was achieved at a concentration of 15-crown-5 of ≥10 m*M*. The concentration of 15-crown-5 (10 m*M*) required for the complete separation of the mono-

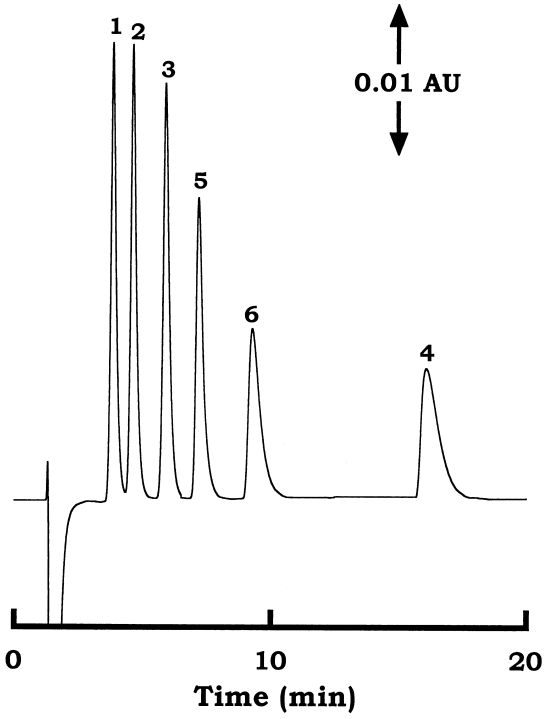

Fig. 4. Chromatogram of mono- and divalent cations on the SMBS-1000 column using 0.75 m*M* tyramine–0.25 m*M* oxalic acid–1.0 m*M* 18-crown-6 at pH 5.0 as eluent. Conditions: eluent, 0.75 m*M* tyramine–0.25 m*M* oxalic acid–1.0 m*M* 18-crown-6 at pH 5.0 (eluent A). Peaks: 1=Li^+, 2=Na^+, 3=NH_4^+, 4=K^+, 5=Mg^{2+}, 6=Ca^{2+}. Other conditions as in Fig. 3.

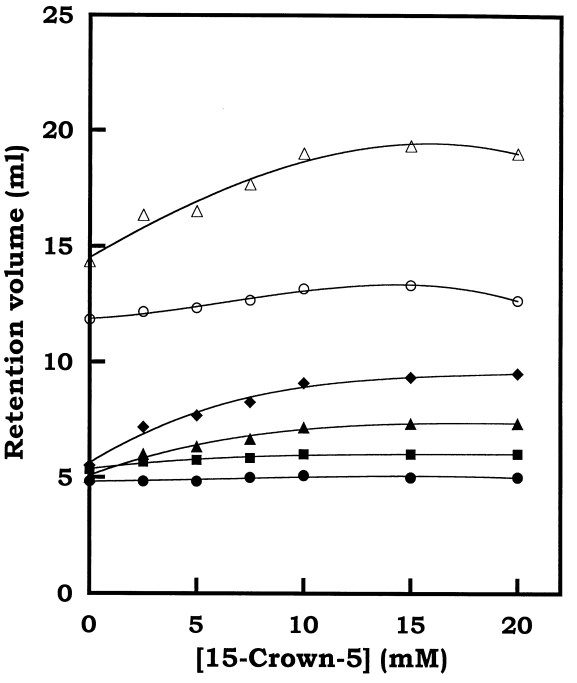

Fig. 5. Effect of the 15-crown-5 concentration in 0.75 m*M* tyramine–0.25 m*M* oxalic acid at pH 5.0 as eluent on the retention volumes of mono- and divalent cations on the SMBS-1000 column. Conditions: eluent, 0.75 m*M* tyramine–0.25 m*M* oxalic acid at pH 5.0 containing 0–20 m*M* 15-crown-5. Symbols: (●) Li^+, (▲) Na^+, (■) NH_4^+, (♦) K^+, (○) Mg^{2+}, (△) Ca^{2+}. Other conditions as in Fig. 4.

valent cations was 10 times that of 18-crown-6 (1 m*M*). This is because the stability of the complexes formed between 15-crown-5 and the monovalent cations ($\log K_{Na^+}=0.7$, $\log K_{NH_4^+}=1.71$ and $\log K_{K^+}=0.74$) is lower than that between 18-crown-6 and the monovalent cations [18]. For the divalent cations the retention volumes increased initially and then decreased slightly. The degree of increase in the retention volume was $Mg^{2+}<Ca^{2+}$ and was in good agreement with the stability of the complexes formed between the divalent cations and 15-crown-5 [18]. Complete group separation between these mono- and divalent cations was achieved in the 15-crown-5 concentration range between 0 and 20 m*M*.

From the above results, it was concluded that the optimum concentration of 15-crown-5 was 10 m*M*. As shown in Fig. 6, excellent simultaneous separation of these mono- and divalent cations was achieved on the SMBSG-1000 column in 25 min by elution with 0.75 m*M* tyramine–0.25 m*M* oxalic acid–10 m*M* 15-crown-5 at pH 5.0 (eluent B).

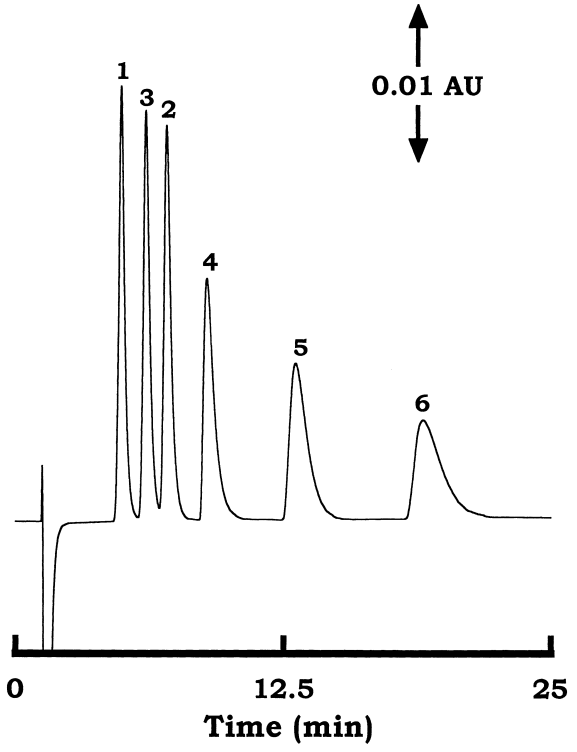

Fig. 6. Chromatogram of mono- and divalent cations on the SMBS-1000 column using 0.75 m*M* tyramine–0.25 m*M* oxalic acid–10 m*M* 15-crown-5 at pH 5.0 as eluent. Conditions: eluent, 0.75 m*M* tyramine–0.25 m*M* oxalic acid–10 m*M* 15-crown-5 at pH 5.0 (eluent B). Peaks: 1=Li^+, 2=Na^+, 3=NH_4^+, 4=K^+, 5=Mg^{2+}, 6=Ca^{2+}. Other conditions as in Fig. 5.

3.4. Analytical performance parameters

Table 2 shows the detection limits (signal-to-noise ratio of 3) of these mono- and divalent cations at an injection volume of 20 μl. Highly sensitive detection was achieved in the IC–IPD. The detection limits were much lower than those obtained by IC with conductimetric detection using both unmodified silica gel as the cation-exchange stationary phase and Li^+ as the eluent ion [10].

Calibration graphs were obtained by plotting the chromatographic peak area versus the concentration of the mono- and divalent cations. Linear calibration graphs ($r^2 \geq 0.99$) were obtained in the concentration range between 0.005 and 1.0 m*M* for these mono- and divalent cations.

The relative standard deviations of the chromatographic peak areas of these mono- and divalent cations, the concentrations of which were 0.2 m*M* for the monovalent cations and 0.1 m*M* for the divalent cations, were <0.9% ($n=10$). Reproducible chromatograms were obtained during repeated chromatographic runs.

Table 2
Detection limits of common mono- and divalent cations

Cation	Eluent A		Eluent B	
	μ*M*	ng ml^{-1}	μ*M*	ng ml^{-1}
Li^+	0.35	2.4	0.39	2.7
Na^+	0.32	7.4	0.49	11
NH_4^+	0.43	7.8	0.44	7.9
K^+	1.6	63	0.88	34
Mg^{2+}	0.25	6.1	0.66	16
Ca^{2+}	0.44	18	1.1	44

Signal-to-noise ratio 3. Injection volume 20 μl. Eluent A: 0.75 m*M* tyramine–0.25 m*M* oxalic acid–1 m*M* 18-crown-6 at pH 5.0. Eluent B: 0.75 m*M* tyramine–0.25 m*M* oxalic acid–10 m*M* 15-crown-5 at pH 5.0.

3.5. Application to the separation of major cations in natural water samples

The proposed IC–IPD method was applied to the determination of major mono- and divalent cations (Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}) in river and rain water samples. Samples were analyzed after filtration through a 0.45 μm membrane filter.

Fig. 7A and B show chromatograms of river water samples obtained using (A) eluent A and (B) eluent B. Determination of these mono- and divalent cations was achieved by IC–IPD at an injection volume of 20 μl. In contrast, accurate determination of these cations, especially K^+, in the rain water sample was very difficult using IC–IPD at an injection volume at 20 μl, because the concentration of the cation in the rain water sample was considerably lower than that in the river water sample. Fig. 8A and B show chromatograms of a rain water sample at an injection volume of 50 μl obtained using (A) eluent A and (B) eluent B. As shown in these figures, IC–IPD was successfully applied for the determination of these mono- and divalent cations in the rain water sample at an injection volume of 50 μl. From the above results, it is demonstrated that the proposed IC–IPD method is very effective for the determination of major mono- and divalent cations in natural water samples.

Thermally treated SMBSG B-5 silica gel (SMBSG-1000) was successfully applied as the cation-exchange stationary phase in IC–IPD for the simultaneous separation of common mono- and divalent cations using 0.75 m*M* tyramine–0.25 m*M* oxalic acid at pH 5.0 containing either 1 m*M* 18-crown-6 or 10 m*M* 15-crown-5 as eluent. The above results expand the utility of conventional silica gel as the cation-exchange stationary phase in IC for cations.

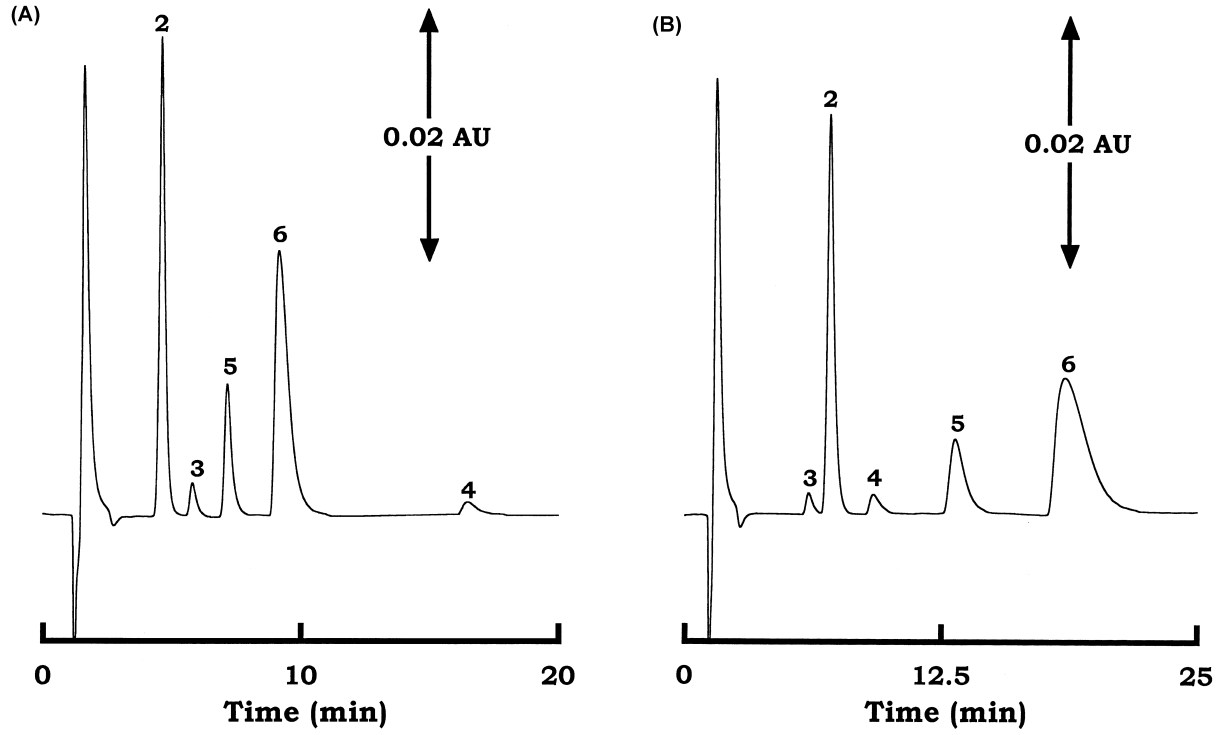

Fig. 7. Chromatograms of a river water sample obtained using (A) eluent A and (B) eluent B at an injection volume of 20 μl. Peaks (concentration, m*M*): 2 = Na^+ (0.26), 3 = NH_4^+ (0.016), 4 = K^+ (0.021), 5 = Mg^{2+} (0.055), 6 = Ca^{2+} (0.21).

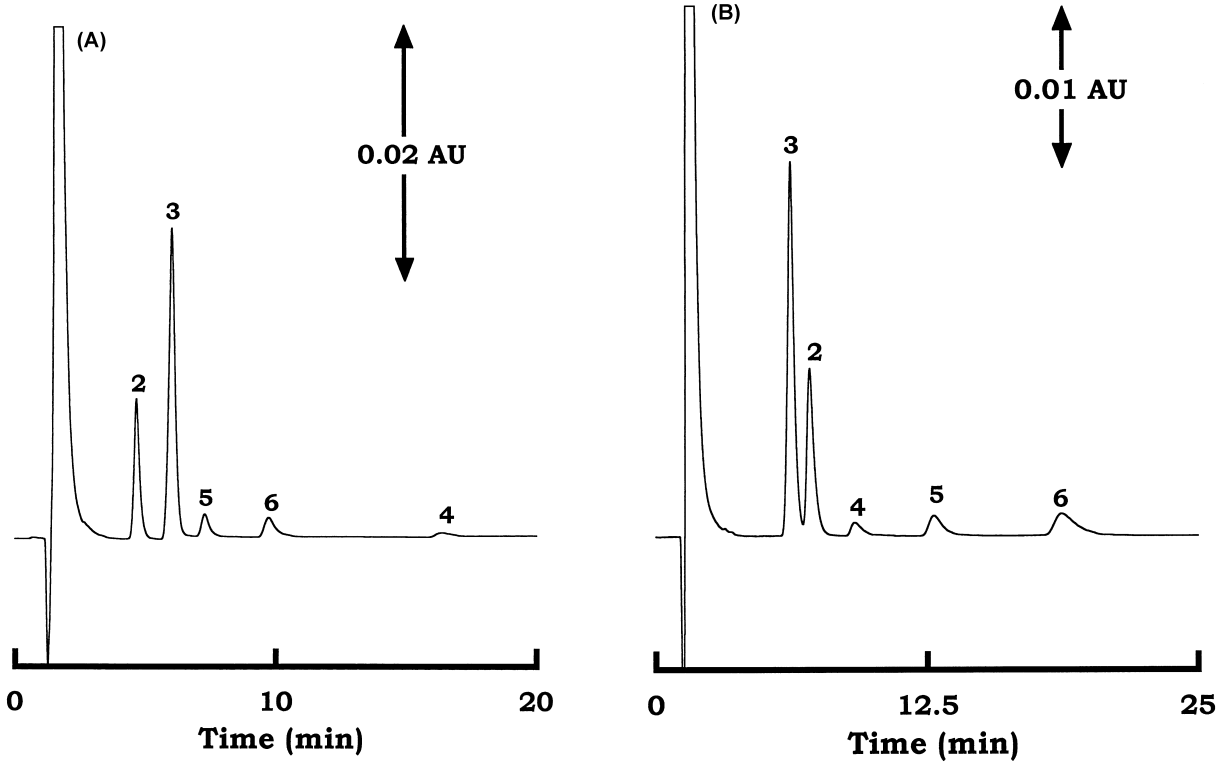

Fig. 8. Chromatogram of a rain water sample obtained using (A) eluent A and (B) eluent B at an injection volume of 50 μl. Peaks (concentration, m*M*): 2 = Na^+ (0.025), 3 = NH_4^+ (0.043), 4 = K^+ (0.003), 5 = Mg^{2+} (0.007), 6 = Ca^{2+} (0.014).

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