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Journal of Chromatography A, 995 (2003) 153–160
JOURNAL OF CHROMATOGRAPHY A
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Simultaneous separation of inorganic anions and cations by using anion-exchange and cation-exchange columns connected in tandem in ion chromatography

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Received 4 November 2002; received in revised form 14 February 2003; accepted 18 March 2003

Abstract

Inorganic anions and cations in environmental waters were determined by ion chromatography. Stationary and mobile phases were examined for the simultaneous separation of both anions and cations. Cations detection by UV detection requires a mobile phase with a UV absorbing additive, which indirectly visualizes cations as negative peaks. Simultaneous separation of anions and cations were achieved when using an eluent that consists of inorganic acid with weak basic amino acid as additives. It was convenient to separate both anions and cations by coupling anion-exchange and cation-exchange columns in tandem. The order of the separation columns connected affected the elution profiles. When the eluent comprises of multiple anions and a single cation, the anion-exchange column should be connected in the upper stream, whereas when the eluent comprises multiple cations and a single anion, the cation-exchange column should be connected in the upper stream. Use of switching valves also allowed simultaneous separation of anions and cations in a single chromatographic run. In the present work, operating conditions were optimized for the simultaneous separation of anions and cations.

Keywords: Water analysis; Inorganic anions; Inorganic cations

1. Introduction

Ion chromatography is a useful technique for the determination of inorganic anions and cations that can be applied in environmental, food, industrial, pharmaceutical and clinical industries [1,2] since it offers a versatile, selective and sensitive analysis and is relatively low in cost. In many cases, anions and cations are usually determined separately using different separation systems. Improvements have been made for the simultaneous determination of both anions and cations in a single chromatographic run [3]. These approaches involve the use of a mixed bed of anion-exchange and cation-exchange materials [4,5] and anion-exchange and cation-exchange columns in tandem [6–9]. Other alternatives for simultaneously detecting anions and cations consist of a UV detector in series with a normal conductivity detector [10], two separating columns with two detectors [11], ion-exclusion and cation-exchange chromatography [12,13], chelation of cations with EDTA [10] and using an amphoteric stationary phase [14]. However, due to the different separating con-

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0021-9673/03/$ – see front matter
doi:10.1016/S0021-9673(03)00523-5

ditions, simultaneous separation of ions would have an effect on the shape of peaks and retention time of anions and cations, respectively.

In the present work, anion-exchange and cation-exchange columns were connected directly in series or via switching valves to allow simultaneous separation of anions and cations. UV and conductivity detectors were used for the detection. The effect of additives in the mobile phase was also examined. The operating system was optimized and was then applied to the determination of ions in river, pond and tap water.

2. Experimental

2.1. Apparatus

The chromatographic system used consists of a PU-980 high-performance liquid chromatography (HPLC) pump (Jasco, Tokyo, Japan), a Model-5095 loop injector with an injection volume of 20 μl (Rheodyne, Cotati, CA, USA), two Model-7000 six-port switching valves (Rheodyne), a UV-2070 ultraviolet detector (Jasco), a CM-8020 conductivity detector (Tosoh, Tokyo, Japan) and a Computer Aided Chromatography data processor (Nippon Filcon, Tokyo, Japan). The anion-exchange column used was a 50×4.6 mm TSK_{gel} IC-Anion-SW (Tosoh) and the cation-exchange column was a 150×4.6 mm TSK_{gel} Super IC-Cation (Tosoh). Fig. 1 shows two different systems used in this work: two ion-exchange columns are directly connected for A, whereas two ion-exchange columns are connected via each switching valve for B. The UV detector was operated at 210 nm and the flow-rate of the system was kept at 1.0 ml min^{-1}.

2.2. Reagents

In this study, all standard solutions and eluents were prepared from guaranteed-reagent-grade chemicals from Nacalai Tesque (Kyoto, Japan) using deionized water. The mobile phase solution consisted of 1.0 m*M* sulfuric acid with amino acid as the additive, and it was degassed in an ultrasonic bath before use. Environmental water samples used were taken from river, pond and tap water near and in the university, and they were filtered using 0.45-μm GL Chromatodisc filters (GL Science, Tokyo, Japan) before analysis.

3. Results and discussion

3.1. Effect of additive in the mobile phase

In this research, amino acids were used as an additive in the mobile phase. Fig. 2 shows the structures (a) and the UV absorption spectra of the amino acids (b) that were examined. L-His, L-Arg, L-Trp and L-Phe absorb UV light, whereas L-Asn and L-Ala do not absorb UV light strongly. These amino acids were used to visualize analytes and to improve the shape of the cation peaks. L-His shows a good separation of five cations such as sodium, ammonium, potassium, magnesium and calcium, whereas the system peaks overlap with the analyte cations for L-Trp and L-Phe. Although L-Arg has a low UV absorbance and could visualize cations at lower wavelength, overlapping of magnesium ion with the system peak could not be avoided. L-Asn and L-Ala could not visualize cations since they do not absorb UV light. Thus, in the following experiments L-His was used since it could separate the five cations without overlapping with the system peak. L-His is a positively charged weakly basic amino acid under acidic conditions due to an aromatic nitrogen-heterocyclic imidazole side chain.

Fig. 3 illustrates effects of L-His on the retention and detection of cations for UV (A) and conductivity (CD) detection (B). When L-His was not added in the mobile phase, there was no detection of cations in the UV detection, as shown in the lower trace of Fig. 3A. On the contrary, the cations are detected by CD, independent of the presence or absence of L-His, as shown in Fig. 3B. In case L-His is included in the mobile phase, the cations can also be seen in by UV detection, as shown in the upper trace of Fig. 3A, indicating that L-His indirectly visualizes the analyte cations. This means that protonated L-His also competes with the analytes cations for the cation-exchange sites. It can also be seen that the retention of the analytes increases when L-His is added in the mobile phase. This may be because the elution

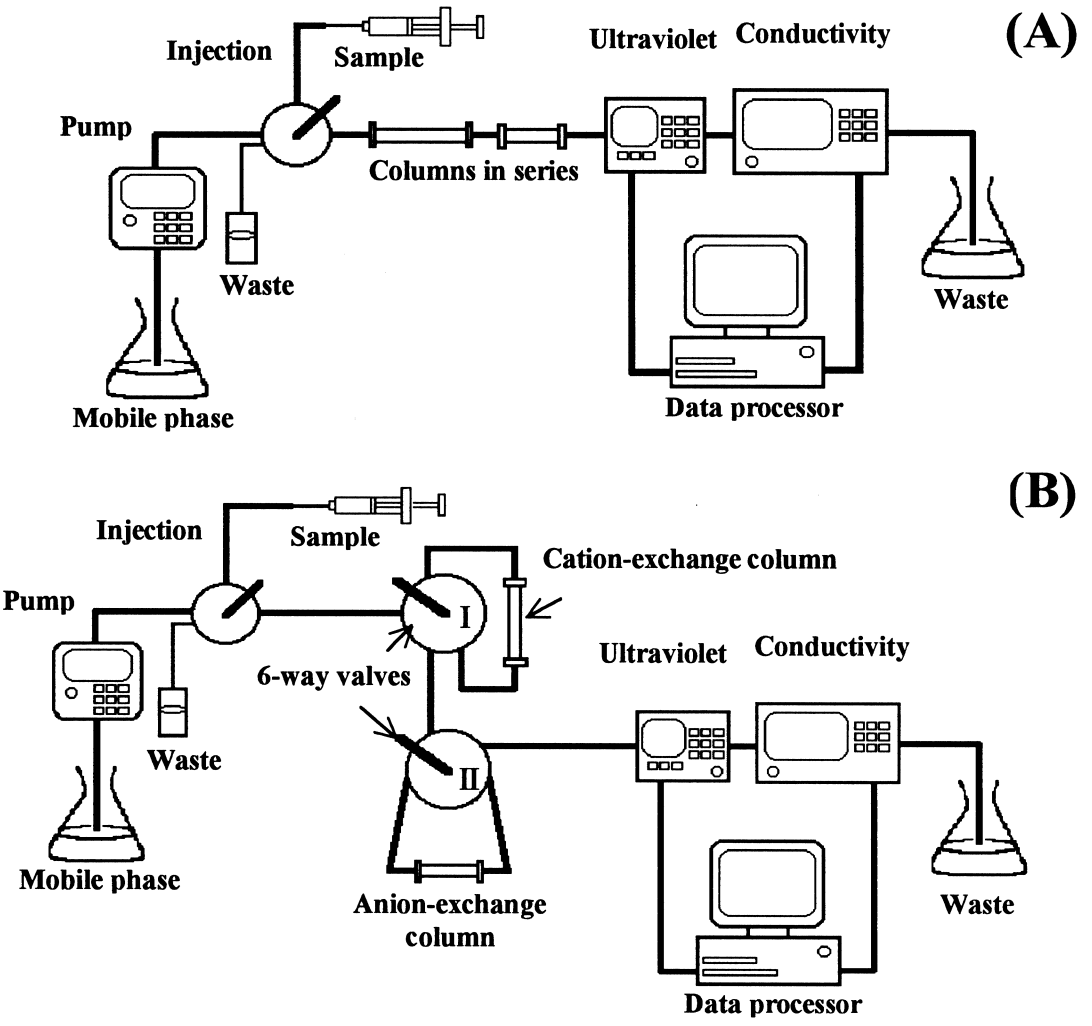


Fig. 1. Experimental apparatus. Direct coupling of two columns and two detectors in series (A) and coupling of two columns via two switching valves (B).

power of protonated L-His may be weaker than that of hydronium ion, resulting in the decrease in the elution strength. The pH and conductivity of 1.0 m*M* sulfuric acid containing 0.1 m*M* L-His were 2.82 and 0.66 mS cm^{-1}, respectively, whereas those of 1.0 m*M* sulfuric acid were 2.75 and 0.73 mS cm^{-1}, respectively. These data show that the former eluent contains less hydronium ion than the latter eluent, leading to the decrease in the elution strength for the former eluent compared to the latter. These results indicate that the use of L-His leads to less sensitivity for CD detection of cations. However, the use of L-His allows us to use a single detector; i.e., a UV detector, for the simultaneous determination of both anions and cations. It may have a possibility to improve the sensitivity of cations by using an additive with a higher molar extinction coefficient. The background of the eluent (1.0 m*M* sulfuric acid containing 0.1 m*M* L-His) at 210 nm was 0.48 absorbance. In addition, the use of L-His also slightly improved the resolution of sodium and ammonium ions.

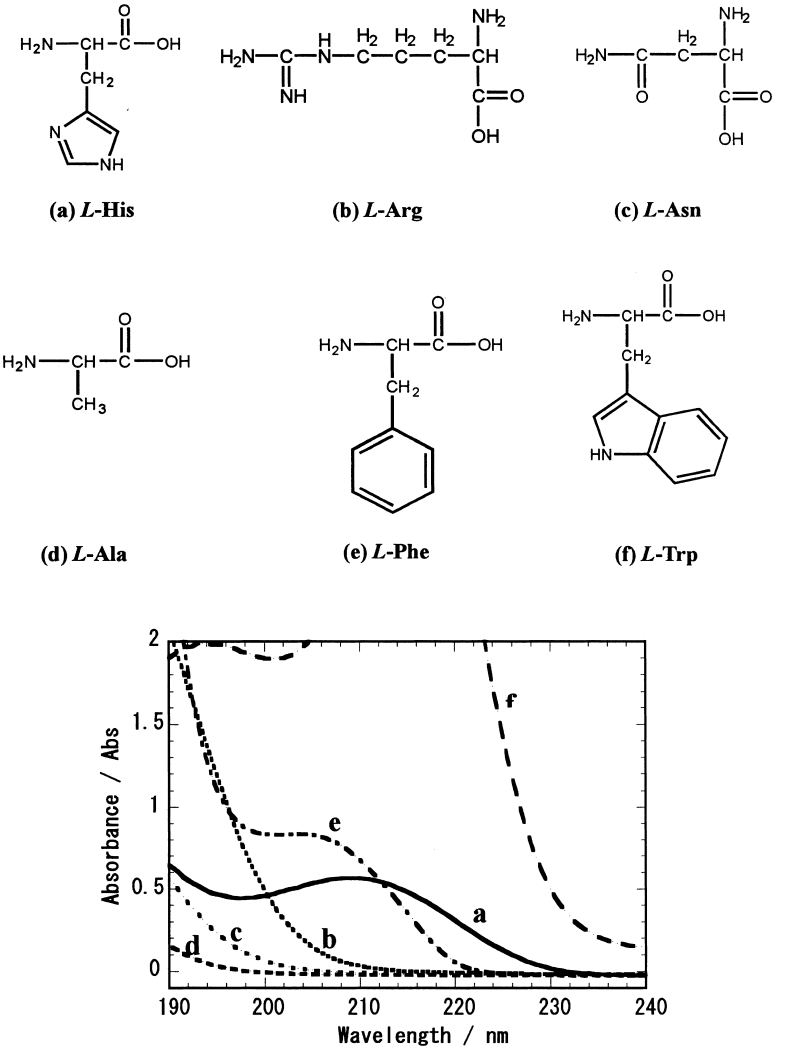
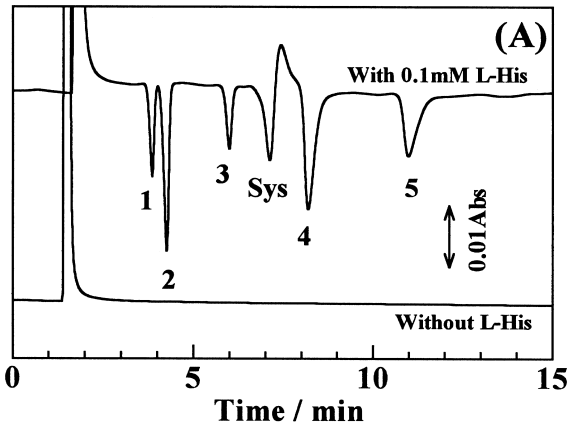


Fig. 2. Structures of amino acids employed and their UV spectra. Solutions: 1.0 m*M* sulfuric acid containing 0.1 m*M* L-His (a), 0.5 m*M* L-Arg (b), 0.1 m*M* L-Asn (c), 0.1 m*M* L-Ala (d), 0.1 m*M* L-Phe (e), and 0.1 m*M* L-Trp (f).

3.2. Effect of the order of ion-exchange columns in series

It is convenient to separate anions and cations simultaneously by using anion-exchange and cation-exchange columns connected in series. However, the order of the columns connected affected the elution profiles, as suggested in the literature [15]. The order of cation-exchange followed by anion-exchange columns showed a better baseline than vice versa because the mobile phase used contains multiple cations, i.e., hydronium and protonated L-His ions. Due to this situation, anions have a possibility of forming ion pairs with these two counter cations, leading to unidentified peaks. Thus, when using a mobile phase containing multiple cations, the cation-exchange column should be connected in series before the anion-exchange column.

Fig. 4 shows the chromatograms using 1.0 m*M* sulfuric acid containing 0.1 m*M* L-His as the eluent in case columns are connected in series in the order of cation-exchange followed by anion-exchange col-



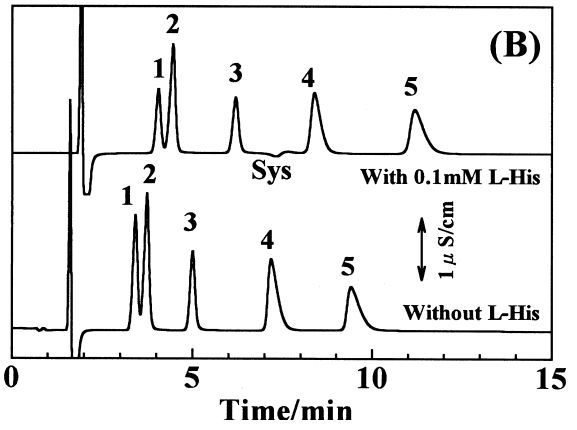
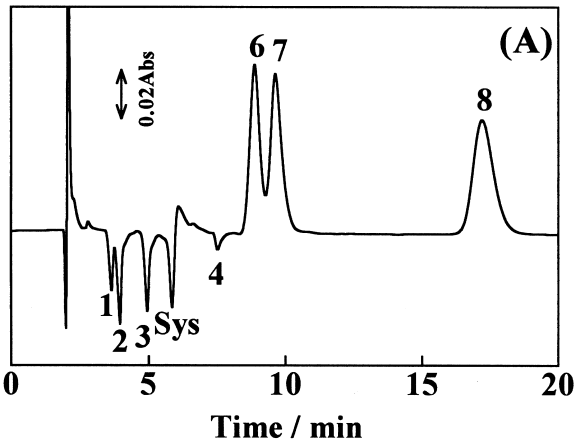


Fig. 3. Effects of L-His on the retention and detection of cations for UV (A) and CD detection (B). Eluent, 1.0 m*M* H_2SO_4 with (upper traces) or without 0.1 m*M* L-His (lower traces). Column: TSK_{gel} Super IC-Cation (150×4.6 mm I.D.). Flow-rate, 1.0 ml min^{-1}. Analytes: 1=0.75 m*M* Na^+; 2=1.0 m*M* NH_4^+; 3=0.5 m*M* K^+; 4=0.5 m*M* Mg^{2+}; and 5=0.5 m*M* Ca^{2+}. Injection volume: 20 μl. UV detection at 210 nm.



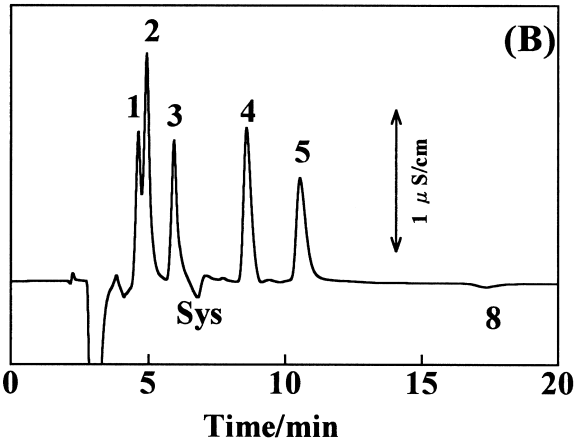


Fig. 4. Simultaneous separation of both anions and cations on cation-exchange and anion-exchange columns in series. Flow system as in Fig. 1A. Eluent: 1.0 m*M* sulfuric acid containing 0.1 m*M* L-His. Flow-rate: 1.0 ml min^{-1}. Columns: TSK_{gel} Super IC-Cation (150×4.6 mm I.D.) and TSK_{gel} IC-Anion-SW (50×4.6 mm I.D.), connected in series. Analytes: 1=0.75 m*M* Na^+; 2=1.0 m*M* NH_4^+; 3=0.5 m*M* K^+; 4=0.5 m*M* Mg^{2+}; 5=0.5 m*M* Ca^{2+}; 6=0.5 m*M* Br^-; 7=0.25 m*M* NO_3^-; and 8=0.5 m*M* I^-. Injection volume: 20 μl. UV detection at 210 nm.

umns. Fig. 4A and B demonstrate UV and CD detection of analytes, respectively. As can be seen in Fig. 4A, only four cations can be detected since calcium ion was overlapped with bromide and nitrate ions. Other anions such as iodate, bromate and nitrite were not included because they were detected in the first 10 min and overlapped with the cations.

It can be seen that the peak shapes and the resolution of cations were deteriorated in Fig. 4 compared to those in Fig. 3. This indicates that the deterioration of the cation peaks occurs when passing through the anion-exchange column placed in the down stream although the cations are not retained on the anion-exchange column. On the other hand, the peak shapes of the anions were not deteriorated even if they passed thorough the cation-exchange column. The reason for these results has not been clarified,

yet. Therefore, it is necessary to assemble the flow system using switching valves so that sample cations separated on the cation-exchange column do not pass through the anion-exchange column, as shown in the following section.

3.3. Flow system with switching valves

Saari-Nordhaus and Anderson, Jr. [16] have demonstrated simultaneous separation of anions and cations using a switching valve. They used one injector, one pump, an anion-exchange column, a cation-exchange column, a switching valve and a conductivity detector. The anion-exchange column was located in the upper stream, and the cation-exchange column was connected to the switching valve in the down stream, where the cation-exchange column could be bypassed. The cation-exchange column trapped the sample cations at the inlet of the cation-exchange column, while the sample anions were separated on the anion-exchange column.

We also examined the separation of ions via switching valves as shown in Fig. 1B. The present system comprised one injector, one pump, a cation-exchange column, an anion-exchange column, two switching valves and two detectors. The both columns were connected to each switching valve, and the both columns could be bypassed independently. Prior to injection of samples, the both cation-exchange and anion-exchange columns were equilibrated with the eluent and stabilized. When sample was injected, cations were retained on the cation-exchange column, whereas anions were passed through the cation-exchange column and trapped on the anion-exchange column. It was found that it took 1.45 min to completely trap anions on the anion-exchange column. In 1.45 min after the injection the six-way valve II was switched to the bypass. Under this condition the cations are being separated on the cation-exchange column, whereas the anions are trapped on the anion-exchange column. It took 10 min to separate the five cations. In 10 min after the injection the six-way valve II was switched back to the anion-exchange column and at the same time the six-way valve I was switched to the bypass. Fig. 5 demonstrates UV (A) and CD detection (B) of five cations and six anions in a single chromatographic run. In addition, the system shown in Fig. 1B is more useful for real samples than that shown in Fig. 1A because samples contain multiple cations and multiple anions.

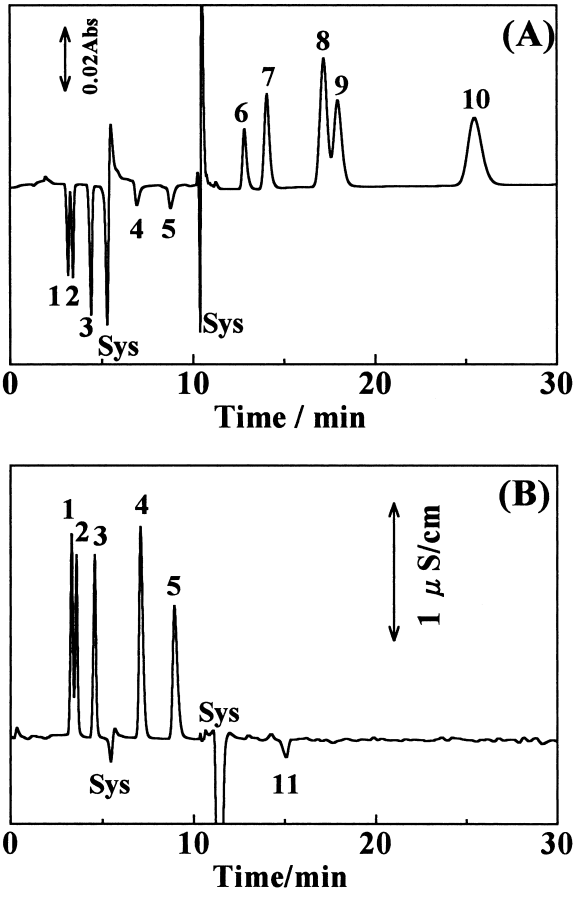


Fig. 5. Simultaneous separation of inorganic anions and cations via column switching. Flow system as in Fig. 1B. Detection: UV (A) and CD (B). Eluent: 1.0 m*M* H_2SO_4+0.1 m*M* L-His. Flow-rate: 1.0 ml min^{-1}. Columns: TSK_{gel} Super IC-Cation (150×4.6 mm I.D.) and TSK_{gel} IC-Anion-SW (50×4.6 mm I.D.), connected in series via six-way switching valves. Analytes: 1=0.6 m*M* Na^+; 2=0.5 m*M* NH_4^+; 3=0.6 m*M* K^+; 4=0.5 m*M* Mg^{2+}; 5=0.5 m*M* Ca^{2+}; 6=0.1 m*M* IO_3^-; 7=0.25 m*M* BrO_3^-; 8=0.25 m*M* Br^-; 9=0.1 m*M* NO_3^-; 10=0.25 m*M* I^- and 11=1.0 m*M* Cl^-. Injection volume: 20 μl. UV detection at 210 nm.

Tables 1 and 2 show the limits of detection (LODs) based on the signal-to-noise ratio of 3 and relative standard deviations (RSDs) for the method using switching valves, respectively. In addition, nitrite ion was not included in the separation since the retention is too small under acidic condition.

Table 1
Limits of detection (LODs) and retention times (t_R) of ions by the method with six-way switching valves

Ion	UV detection		CD detection	
	LOD (μM)	t_R (min)	LOD (μM)	t_R (min)
Na^+	4.0 (92 ppb)	3.32	5.0 (0.1 ppm)	3.48
NH_4^+	3.3 (59 ppb)	3.61	5.0 (0.09 ppm)	3.78
K^+	3.7 (0.14 ppm)	4.84	5.7 (0.2 ppm)	5.01
Mg^{2+}	5.8 (0.14 ppm)	7.62	5.0 (0.1 ppm)	7.81
Ca^{2+}	9.6 (0.4 ppm)	9.93	8.3 (0.3 ppm)	10.14
IO_3^-	0.86 (0.2 ppm)	14.57	–	–
BrO_3^-	1.5 (0.2 ppm)	15.87	–	–
Br^-	1.3 (0.1 ppm)	19.20	–	–
NO_3^-	0.86 (42 ppb)	20.04	–	–
Cl^-	–	–	204 (7.1 ppm)	16.96

Operating conditions as in Fig. 5.

Although chloride could be detected by CD detection, the sensitivity was poor. This system was applicable to the simultaneous determination of common anions and cations except for chloride, nitrite and sulfate.

Although the present system achieved nearly the same detection limits (0.1–0.5 ppm) as those achieved by Saari-Nordhaus and Anderson, Jr. [16], the former system achieved much better resolution for cations than the latter.

3.4. Application to environmental waters

Since the system using switching valves showed a good separation, the system was optimized and applied for the determination of both anions and cations in environmental waters. The environmental water samples were taken from river, pond and tap water near and in the university and were filtered before injection. Fig. 6 illustrates the chromatogram obtained from the simultaneous separation of ions and Table 3 shows the concentration of each ion determined in ppm.

It should be noted that the system depicted in Fig. 1B allowed single-column ion-exchange separation

Table 2
Relative standard deviation (RSD) range for the retention time and peak signals

Detection	RSD (%)		
	Retention time	Peak area	Peak height
UV	0.02–0.51	1.8–9.7	1.2–8.0
CD	0.12–1.02	0.6–12.9	0.5–2.5

Operating conditions as in Fig. 5.

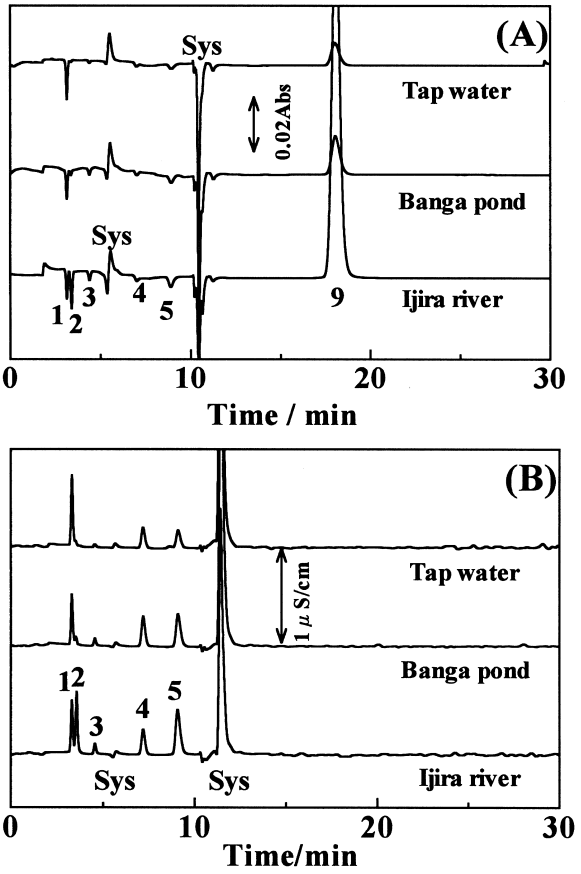


Fig. 6. Separation of inorganic ions in environmental waters. Operating conditions as in Fig. 5 except for the samples. Samples: tap water, Ijira river water and Banga Pond water.

Table 3
Concentration of ions detected in the environmental waters

	Concentration of ions (ppm)		
	Tap water	Banga pond	Ijira river
Na^+	3.22	2.76	2.76
NH_4^+	0.14	0.36	1.44
K^+	0.39	1.56	1.95
Mg^{2+}	1.20	1.44	1.68
Ca^{2+}	3.60	4.00	4.80
NO_3^-	1.86	3.10	9.92

Operating conditions as in Fig. 6.

as well as simultaneous separation of both anions and cations.

4. Conclusion

Flow systems with two ion-exchange columns, two switching valves and two detectors were assembled for ion chromatographic determination of both inorganic anions and cations. The system allowed single-column separation as well as tandem-column separation. The present flow system using switching valves was successfully applied to the simultaneous determination of anions and cations in river, pond and tap water. L-His added to the eluent allowed indirect UV detection of cations. The sensitivity of cations may be improved by using an additive with a higher molar extinction coefficient.

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