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Significant reduction of the detection limit in ion chromatography by relative analyte enrichment with column switching☆

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Abstract

The use of the column-switching technique in ion chromatography for relative analyte enrichment is described. Two columns are combined in series via a switching valve and electrical conductivity detection with chemical ion suppression is used. It is shown that the contribution of the additional high-pressure multi-port switching valve to the peak width is negligible. In order to demonstrate the potential of the method the following examples are given: (1) determination of a low concentration of ammonium in the presence of a large excess of sodium; and (2) determination of a low concentration of bromide in the presence of a large excess of nitrate. In the former case the trace component is eluted after the main peak and in the latter in front of the main peak. The detection limit in trace analysis can be reduced 5000-fold with relative analyte enrichment by column switching. It was found that the same precision and accuracy can be obtained with column switching as in single-column operation without peak interference.

Keywords: Detection limit; Column switching; Anions; Cations

1. Introduction

In ion chromatographic applications, often a trace component has to be identified and quantified besides an interfering major ion present in a large excess. In order to solve this problem, a combination of a high-performance separation method with a high-performance detection method is required. Multi-column chromatography in the on-line mode, realized by column switching, offers ideal features for a separation method for this kind of analytical problem. Column switching is a chromatographic technique in which fractions of the mobile phase are selectively transferred from the outlet of one column to the inlet of another column.

In this paper, the improvement of the degree of separation by reducing the peak heights of interfering components relative to the peak height of the analyte is demonstrated. This is achieved by selecting the size of the fraction to

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be transferred from the preceding to the following column such that it contains the total amount of analyte and as little as possible of the overlapping components. By this two-stage fractionation the analyte is gradually enriched relative to the interfering components and therefore at a given resolution the degree of separation is improved compared with a single-stage operation. Column switching was first applied in liquid chromatography with low-performance columns [1–4] and later also in high-performance liquid chromatography [5–14], where it was used in a multidimensional mode [11,12,14] and also for relative analyte enrichment [13,14].

This paper reports on the development of the method for the determination of low concentrations of analyte in the presence of a large excess of an interfering component. In the first application the analyte is ammonium, which is eluted after sodium, the interfering component. In the second application, the separation of bromide, which is eluted in front of the main peak nitrate, is optimized by relative analyte enrichment with column switching.

2. Experimental

2.1. Chemicals

All chemicals were of analytical-reagent grade: sodium carbonate (water free), sodium hydrogencarbonate dihydrate, sodium nitrate, sodium nitrite, sodium sulfite, potassium bromide, ammonium chloride, sulfuric acid (96%, Suprapur), hydrochloric acid (30%, Suprapur) and 1000 mg/l fluoride, chloride, phosphate, sulfate and sodium Titrisol standards (Merck, Darmstadt, Germany). DL-2,3-Diaminopropionic acid monohydrochloride DAP) was obtained from Fluka (Buchs, Switzerland) and 0.1 *M* tetrabutylammonium hydroxide (TBAOH) from Dionex (Sunnyvale, CA, USA).

Calibration standards were prepared by appropriate dilutions of the stock solutions with ultrapure 18 MΩ cm HPLC grade water. Eluents and regenerants were prepared with the same type of water.

2.2. Apparatus

The instrument used was a Model 4010i ion chromatograph (Dionex), equipped with an electrical conductivity detector with integrated background suppression via an anion micro-membrane suppressor (Dionex). The instrument was modified by the plumbing of an additional high-pressure multiport valve (Dionex), allowing column switching. A schematic flow diagram of the system is shown in Fig. 1.

In position 1 (single-column mode) of the switching valve, the effluent from the first column C1 was led directly to the conductivity detector, consisting of a micro-membrane suppressor, the detector cell and the electronics. In position 2 (switching mode), the switching valve directed the effluent from column C1 to column C2 and from there to the detector. The chromatograms were monitored and processed by a Model 4270 computing integrator (Spectra

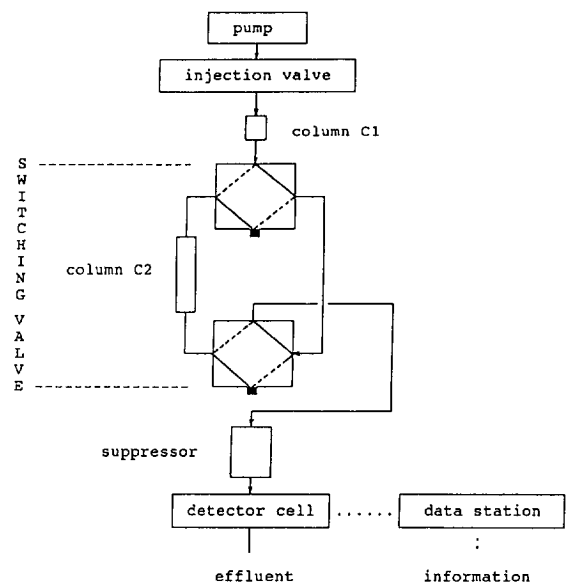

Fig. 1. Schematic flow diagram of an ion chromatograph equipped with an additional valve for column switching. Solid lines, flow path at switching position 1; dashed lines, flow path at switching position 2.

Physics, Santa Clara, CA, USA) or by a Model AI-450 data station (Dionex). The injector and the switching valve were activated by the pump control.

The working conditions for anion analysis were as follows: eluent, aqueous solution of 1.7 m*M* $NaHCO_3$–1.8 m*M* Na_2CO_3 (pH 10.7); flow-rate, 2 ml/min; column C1 = AG4A anion exchanger (Dionex) (50 × 4.6 mm I.D.) and column C2 = AS4A anion exchanger (Dionex) (250 × 4.6 mm I.D.); column switching, 0.5 and 1.3 min after injection; injection volume, 50 μl; detector, electrical conductivity with chemical ion suppression; micro-membrane suppressor continuously regenerated with 25 m*M* H_2SO_4 at 4 ml/min.

The working conditions for cation analysis were as follows: eluent, aqueous solution of 4 m*M* HCl–0.2 m*M* DAP; flow-rate, 1 ml/min; column C1 = CG3 cation exchanger (Dionex) (50 × 4.6 mm I.D.) and column C2 = CG3 cation exchanger (CG3, Dionex) (50 × 4.6 mm I.D.); column switching, 1.9 min after injection; injection volume, 50 μl; detector, electrical conductivity with chemical ion suppression; micro-membrane suppressor continuously regenerated with 70 m*M* TBAOH at 5 ml/min.

3. Results and discussion

3.1. Effect of the additional switching valve on the peak width

In order to evaluate the peak broadening caused by the switching valve, a system without an additional valve was compared with the total arrangement shown in Fig. 1. Table 1 shows the data obtained using standard conditions and injecting 50 μl of a mixed anion standard (eight species). The $t_{R_i}^2$ versus σ^2 curve shows that the measured points from the modified system have no significant deviation from the linear regression line, which is calculated using the data for the standard system without switching valve. The intersection of the regression line estimates the value of the extra-column contribution to the peak variance.

Table 1
Effect of the additional switching valve on the peak width

Ion (i)	Standard system		System with additional value	
	t_{R_i} (min)	σ_{t_i} (s)	t_{R_i} (min)	σ_{t_i} (s)
Fluoride	0.98	1.07	1.04	1.19
Chloride	1.63	1.53	1.68	1.53
Nitrite	2.02	1.79	2.06	1.87
Bromide	3.13	2.72	3.19	2.72
Nitrate	3.63	3.73	3.68	3.66
Phosphate	5.85	5.78	5.91	5.69
Sulfite	7.12	6.80	7.16	6.71
Sulfate	7.88	7.39	7.93	7.39
Regression data[a]: $\sigma_{t_i}^2 = \sigma_{ex}^2 + t_{R_i}^2/N$				
	$\sigma_{t_i}^2 = 0.000081 + t_{R_i}^2/4022$		$\sigma_{t_i}^2 = 0.000039 + t_{R_i}^2/4098$	
	$\sigma_{v_{ex}} = 18 \pm 30$ μl		$\sigma_{v_{ex}} = 12.5 \pm 25$ μl	
	$r = 0.9976$		$r = 0.9987$	

[a] t_{R_i} = retention time of component i; $i = 0, 1, \ldots, 8$; σ_{t_i} = standard deviation of the peak i; N = average theoretical plate number (calculated by regression of σ_i^2 versus $t_{R_i}^2$).

For the system with a switching valve, a value of 12.5 ± 25 μl was found for the standard deviation for the extra-column band broadening. The corresponding value for the system without a switching valve was found to be 18 ± 25 μl. As can be seen from the regression data in Table 1, the difference is within the statistical error of the calculation and therefore not significant. In both cases the extra-column effect is negligible compared with the peak broadening effect of the column. Fig. 2 confirms that there is no significant visual peak-broadening effect on comparing the systems without and with the additional valve.

3.2. Determination of low concentrations of ammonium in the presence of a large excess of sodium by column switching

The determination of ammonium in growth media in fermentation broths is particularly important as it is often the primary source of nitrogen for the biosynthesis of amino acids and other nitrogen-containing molecules. Alkali and

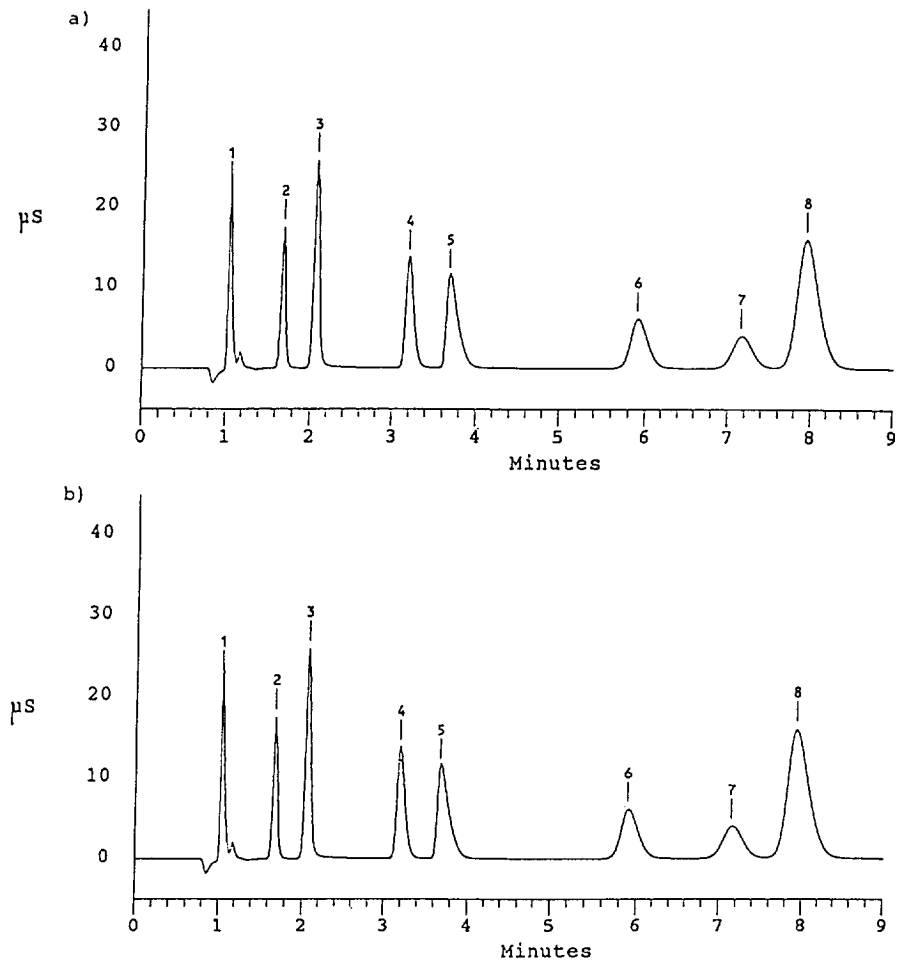

Fig. 2. Effect of the additional switching valve on the separation efficiency: (a) Chromatogram of the anion standard mixture on the system without the switching valve. Peaks: 1 = 2 mg/l fluoride; 2 = 3 mg/l chloride; 3 = 10 mg/l nitrite; 4 = 10 mg/l bromide: 5 = 10 mg/l nitrate; 6 = 10 mg/l phosphate; 7 = 15 mg/l sulfite; 8 = 15 mg/l sulfate. (b) Chromatogram of the same standard mixture on the system with the switching valve.

alkaline earth metals are generally present in such media. A problem in ion chromatographic analyses of ammonium is often the high concentration of sodium, which strongly overlaps the ammonium peak. The small peak is located at the rear of the large peak. In such a case, the column-switching technique offers the possibility of solving the problem by improving the peak-size ratio of ammonium relative to sodium.

On injecting a sample containing 0.2 mg/l of sodium and 2 mg/l of ammonium (sample size ratio, ammonium to sodium = 10) an almost baseline resolution is obtained on column C1 alone. The peak-area ratio a_{21} in this case is approximately 0.7. On operating columns C1 and C2 in series a significantly better resolution is obtained. On doubling of the column lengths, the resolution increases by $\sqrt{2}$.

If a sample containing 100 mg/l of sodium and 2 mg/l of ammonium (sample size ratio, ammonium to sodium = 0.02) is injected, column C1 alone gives only a shoulder of ammonium on the rear of the main sodium peak. In this case, the peak-size ratio can be enhanced by column switching. The switching cycle starts with column C1 (switching valve position 1) and the chromatogram from the first column effluent is monitored. After 1.9 min the effluent from column C1 is directed to column C2, transferring the rest of the sample containing the total ammonium to column C2, where the separation is continued.

After the cut-off of most of the sodium peak, some disturbances due to the back-pressure change occur and after that two peaks corresponding to the rest of the sodium and the ammonium are detected. The effect of the improvement of the peak-size ratio of peak 2 to peak 1 is large, but the improvement in the isolation of peak 2 is not dramatic but significant. This is the result of the low sample-size ratio (0.02) and the relatively low resolution of these components, $R_{21} = 5.6$, where $R_{21} = (t_{R_2} - t_{R_1})/\sigma_{t_1}$, t_{R_2} and t_{R_1} are the retention times of the eluting components 1 and 2 and σ_{t_1} is the standard deviation of the peak of the first-eluting component. In addition, at the base a chromatographic peak is usually wider at the rear than at the front.

The effect of the accuracy of the switching time was tested using different switching points. On activating the switching valve 2.3 min after the injection, nearly all of the sodium was removed but also some part of the ammonium. If the switching point was set 1.8 min after the injection, the improvement in the peak-size ratio was reduced. The second peak was quantitatively transferred but at the same time a large fraction of the first peak reached column C2. In this case the systematic error for quantification by peak height was calculated to be more than 10%. The optimum switching point was 1.9 min after the injection, which gave a significant improvement in the peak-size ratio, resulting in a moderate improvement in the isolation of ammonium.

This example shows the relative analyte enrichment effect in the case when the trace component elutes after the main component.

3.3. Determination of low concentrations of bromide in the presence of a large excess of nitrate

The determination of bromide in the presence of a very large excess of nitrate becomes possible by using the column-switching technique under the standard conditions optimized for the anion separation. The same hardware configuration is used as shown in Fig. 1. In this case an anion-exchange column (length 50 mm) is used as C1 and a column with the same packing (but 250 mm in length) as C2, resulting in a total column length of 300 mm and a length ratio of 1:5.

In order to test the resolution on column C1 and columns C1 and C2 coupled in series, an aqueous solution containing 0.2 mg/l of bromide and 0.2 mg/l of nitrate was injected. The chromatograms are shown in Fig. 3. The peak-size ratio in this case was approximately 1 as the detector selectivities for these substances were almost equal.

On injecting a sample containing 0.2 mg/l of bromide and 250 mg/l of nitrate, which corresponds to a ratio of 1:1250, into the coupled system (C1 and C2 in series), not even a shoulder can be seen (Fig. 4a). Fig. 4b shows that a baseline resolution of this sample is achieved

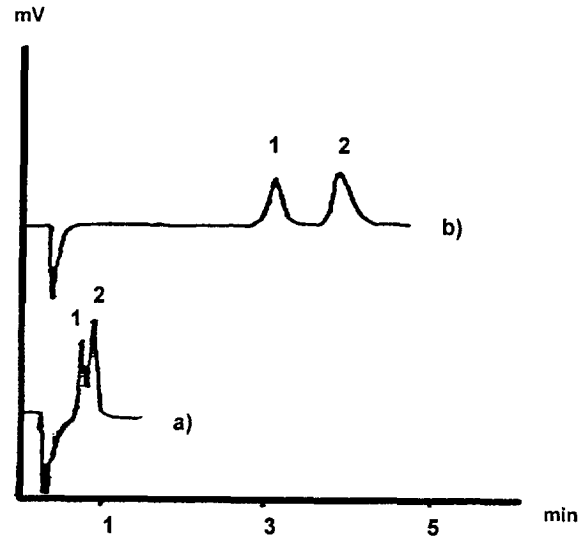

Fig. 3. Resolution of bromide and nitrate on column C1 and columns C1 and C2 in series. Chromatograms of a sample containing (1) 0.2 mg/l of bromide and (2) 0.2 mg/l of nitrate (a) on a single column C1 and (b) on two columns C1 and C2 in series. Column length, (a) 50 and (b) 300 mm. Resolution, defined as $R_{21} = (t_{R_2} - t_{R_1})/\sigma_{t_1}$, (a) 3.0 and (b) 7.4.

under the same conditions by applying column switching. In this case the minor peak is the first-eluting peak. Starting with columns C1 and C2 coupled in series (switching valve position 2), the sample was injected and the first part of the peak combination eluting from column C1 was transferred to column C2. Column C2 was then isolated from the flow (first switching action: position 2 to 1) and the remaining part of the peak (most of the excess of nitrate) was directed through the detector to waste. Then columns C1 and C2 were connected in series again (second switching action: position 1 to 2) and the separation was completed, eluting the analytes with a peak-size ratio that permitted a separation with baseline resolution (Fig. 4b).

The effect of switching time is demonstrated in Fig. 5. The first switching action was carried out at 0.5 and 0.6 min after injection. The second switching had to be carried out after complete elution of the interfering peak. Switching action 2 was performed at a constant time of 1.3 min after injection. It can be seen that the choice of the appropriate switching period is critical in this case. Fig. 5b shows the separation obtained with

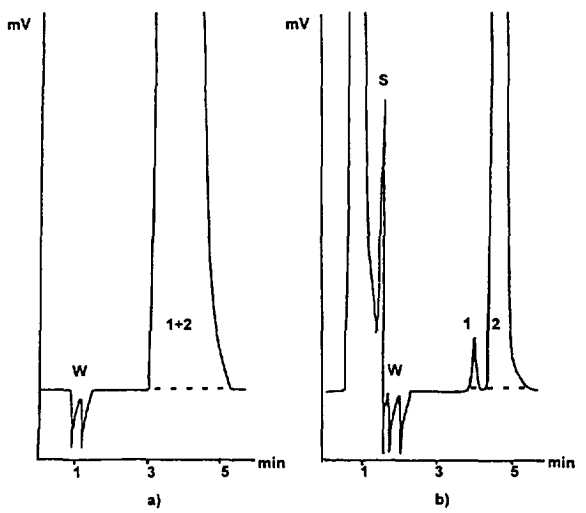

Fig. 4. Enhancement of the peak-size ratio by column switching. Chromatogram of a sample containing (1) 0.2 mg/l of bromide and (2) 250 mg/l of nitrate (a) on two columns C1 and C2 in series and (b) on two columns in the switching mode. Switching actions 0.5 and 1.3 min after injection. Dashed lines, estimated baseline; S, switching disturbance; W, water dip.

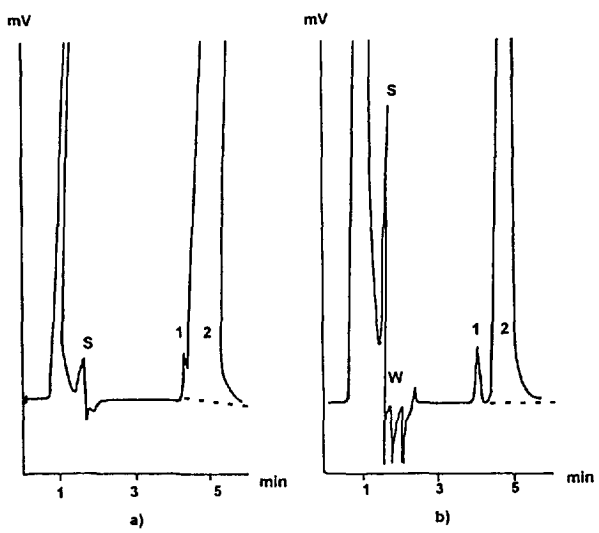

Fig. 5. Effect of switching time. Chromatogram of a sample containing (1) 0.2 mg/l of bromide and (2) 250 mg/l of nitrate obtained in the switching mode. Switching action (a) 0.6 and 1.3 min and (b) 0.5 and 1.3 min after injection. Dashed line, estimated baseline; S, switching disturbance; W, water dip.

Table 2
Precision data for the determination of bromide and ammonium by column switching and ion chromatography ($n = 5$)

Bromide				Ammonium[a]			
Concentration injected (mg/l)	Parameter	Retention time, t_R (min)	Peak height (mV)	Concentration injected (mg/l)	Parameter	Retention time, t_R (min)	Peak height (mV)
0.1	Average	4.28	100	2.0	Average	4.98	97.6
	S.D.	<0.01	1.3		S.D.	<0.01	1.7
	R.S.D. (%)	<0.2	1.3		R.S.D. (%)	<0.2	1.7
0.2	Average	4.25	158	10.0	Average	4.86	405
	S.D.	<0.01	2.1		S.D.	<0.01	2.9
	R.S.D. (%)	<0.2	1.3		R.S.D. (%)	<0.2	0.7
1.0	Average	4.25	628	20.0	Average	4.76	688
	S.D.	<0.01	4.4		S.D.	<0.01	4.6
	R.S.D. (%)	<0.2	0.7		R.S.D. (%)	<0.2	0.7

[a] In general the calibration graph is non-linear for ammonium and follows a quadratic equation.

the optimized switching period (switching actions: 0.5 and 1.3 min after injection). This results in a ratio below 1:5000 for the peak interference for these components.

Table 2 shows that the same precision data can be obtained with column switching as in single-column operation.

4. Conclusion

The applications presented demonstrate the potential of the column-switching technique, which permits one to adjust independently the parameters which affect the chromatographic separation. Especially the second example demonstrates the extreme usefulness of column switching for the relative analyte enrichment in ion chromatography. No significant negative effects on the analytical data are caused by the additional switching device. The easy set-up, optimization and automation make this method very practical.

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