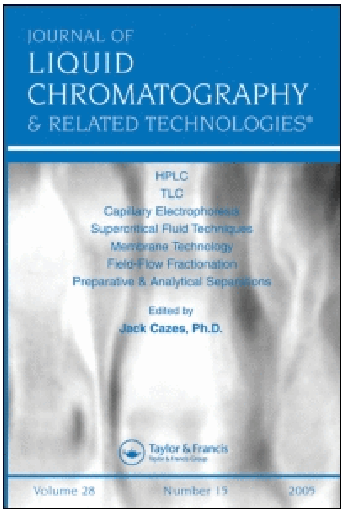

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HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY USING A SMALL COILED COLUMN

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ABSTRACT

Separation of selected lanthanoid elements was performed by high-speed countercurrent chromatography (CCC) equipped with a small coiled column of a 10 cm^3 capacity. A hexane solution of 2-ethylhexylphosphonic acid mono-2-ethylhexyl ester (EHPA) was retained in the tubing column as the stationary phase. The stationary phase retention increased with increasing rotational speed and with decreasing flow rate of the aqueous mobile phase. Mixtures of lanthanoids (Gd, Tb and Dy) were chromatographically eluted in the increasing order of extractability by introducing the buffered mobile phase, and excellent separation of lanthanoids was achieved.

Lanthanoids (Sm through Er) and yttrium were simultaneously enriched in the stationary phase from a large volume of a sample solution, and then separated from each other with reasonable resolution.

INTRODUCTION

Countercurrent chromatography (CCC) has been applied to separation of various kinds of materials, and several types of apparatus have been developed to achieve many separations.[1,2] Separation of rare earth elements (REEs) has been performed by employing some acidic organophosphorus compounds owing to their better selectivity in a series of REEs.[3-5] These extractants are of interest in an application for column components of high-speed countercurrent chromatography (HSCCC).[6,7]

The mutual separation of REEs has been achieved through a coiled column retaining an acidic organophsphorus extractant as a stationary phase without any solid support under the centrifugal field.[6,7] Moreover, a high loading capacity of the liquid stationary phase is convenient for preparative purification of chemical substances by the choice of an appropriate two-phase solvent system.[8-13]

The CCC method is also attractive for enrichment of chemical species by continuous-flow of a sample solution into a column,[14-18] because this is free from sample loss such as adsorption and contamination originated from support matrixes. Previously, an enrichment and subsequent chromatographic separation were performed through a CCC column of relatively large volume (ca. 270 cm^3).[19-21] Rare earths were completely collected in the stationary phase and then chromatographically eluted with good resolution by introducing the mobile phase of pH buffers. However, large amounts of stationary and mobile phases are required when a large column is used, and the speed of rotation is rather limited.

A small column could be conveniently used for an effective enrichment of desired species from a smaller volume of sample solution.[18] This offers a large saving in the stationary and the mobile phases compared with a middle column previously employed.

The present paper concerns the separation characteristics of a newly designed CCC provided with a small column (10 cm^3) retaining an acidic organophosphorus compound as a stationary phase in order to establish the separation of chemically analogous REEs.

EXPERIMENTAL

Reagents

2-Ethylhexylphosphonic acid mono-2-ethylhexyl ester (EHPA) was obtained from Daihachi Chemical Ind. Co., Ltd., and purified as described previously.[5] Arsenazo III (Dojindo Lab.) was used as a post-column reagent for the detection of lanthanoids.

CCC Apparatus

A prototype high-speed countercurrent chromatograph apparatus (HSCCC-R1; Hitachi Tokyo Electronics Corporation) is equipped with a single column holder on one side of the revolutionary frame and a counter weight on the opposite side to balance the column weight. The holder undergoes a synchronous planetary motion around the vertical axis and revolution around the central axis of the centrifuge with 10 cm orbital radius in the same direction. The ratio of rotation and revolution radii (β) is 0.5, and the maximum rotational speed is 1300 round per minute (rpm).

The coiled column was prepared by winding a 50 m PTFE tubing (i.d. 0.5 mm) in three layers of total 150 helical turns onto the holder with a 10 cm diameter. The capacity of the column was 10 cm^3.

Chromatographic Procedures

The tubing column was filled with an organic solution of 0.02 M (M = mol dm^{-3}) $(EHPA)_2$, and then equilibrated with an aqueous mobile phase passing through the column under rotation. Equilibration holding a definite volume of the stationary phase had been established, and then a sample solution containing a mixture of lanthanoids was introduced into the column. After equilibration, carryover of the stationary phase from the tubing coil was negligibly small.

The pH values of the mobile phase were adjusted with $(H,Na)CHCl_2COO$ buffer solutions. Lanthanoids eluted were detected with UV-VIS absorbance at 650 nm by adding 0.014% (w/v) Arsenazo III in 90% (v/v) acetone to the effluent as previously described.[5]

RESULTS AND DISCUSSION

Retention of Stationary Phase

The stable retention of a suitable stationary phase in a tubing coil is one of the most significant factors in the CCC method.[22] The retention of the organic stationary phase containing 0.02 M $(EHPA)_2$ was examined by flowing the aqueous mobile phase at 0.3 cm^3 min^{-1} under the rotation of 1200 rpm at 35°C.

The ratio (V_s/V_c) of the stationary phase volume (V_s) over the column volume (V_c) is defined as the retention ratio. As given in Table 1, the retention ratio is very low (< 0.1) for toluene and kerosene solutions. The isooctane solution is retained around $V_s/V_c = 0.3$, but excess noise was produced in the detection system. Good retention was obtained for heptane and hexane solutions owing

Table 1

Retention of the Stationary Phase in the Tubing Column*

Solvent	ρ/g.cm^{-3} (25°C)	V_s/V_c
Toluene	0.86	< 0.10
Kerosene	0.79	< 0.10
Isooctane	0.69	0.30
Heptane	0.68	0.34
Hexane	0.66	0.39

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* Experimental conditions: 0.02 M $(EHPA)_2$; 0.1 M $(H,Na)CHCl_2COO$, 0.3 cm^{3}min^{-1}; 35°C, 1200 rpm.

to their lower densities; the stationary phase retention is promoted with a large difference in the densities between the organic stationary and the aqueous mobile phases.[23]

Figure 1 shows an example of CCC chromatogram obtained by using the stationary phase of 0.02 M $(EHPA)_2$ in hexane for a mixture of middle lanthanoids (Gd, Tb and Dy). The individual lanthanoids are eluted in the increasing order of their extractability, and their peaks are well separated from each other without an appreciable overlap and detectable noise.

Similar chromatograms were also obtained in the case of the stationary phase of a heptane solution. In subsequent experiments, the hexane solution was selected as a stationary phase owing to its higher retention in the tubing column, because this is desirable to enhance probably the mutual separation of metals.

Figure 2 shows the retention ratio of the hexane solution of 0.02 M $(EHPA)_2$ against the rotational speed at the flow rate of 0.3 cm^3 min^{-1} at 30°C. The retention ratio increases along with increasing rotational speed. The retention of the stationary phase is governed by many variables such as the physicochemical properties of two phases, apparatus and operational parameters.[2] However, a similar tendency of an increase in the retention ratio was observed in the comparable two phases of hexane and water.[2,24] The retention was further enhanced by the addition of 5% di(2-ethylhexyl)phosphoric acid which is an extractant similar to the present EHPA.[24]

Figure 3 shows the effect of the flow rate of the mobile phase on the retention ratio under the rotation of 1250 rpm. The V_s/V_c ratio was found to decrease with an increase in the flow rate, and higher retention of the stationary phase is attained at the low flow rate below 0.2 cm^3 min^{-1}.

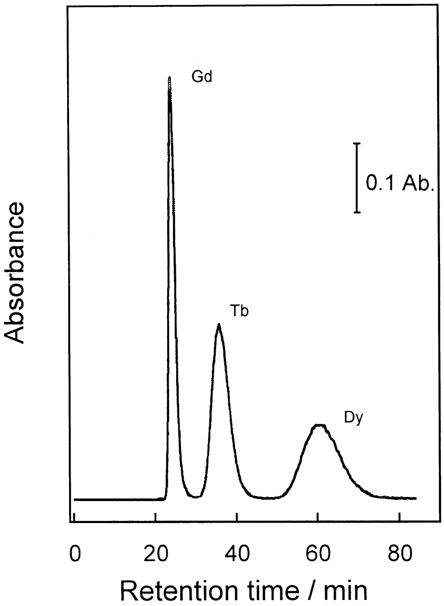

Figure 1. Chromatographic separation of middle lanthanoids. Stationary phase: 0.02 M $(EHPA)_2$ in hexane; sample: 5 x 10^{-5} M each Gd, Tb, Dy, 0.1 cm^3; mobile phase: 0.1 M $(H,Na)CHCl_2COO$ (pH 1.50), 0.3 cm^3 min^{-1}; 1200 rpm; 30°C.

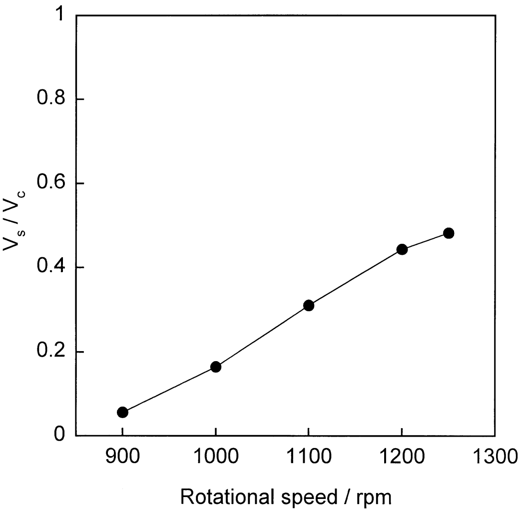

Figure 2. Effect of the rotational speed on the retention of the stationary phase. Stationary phase: 0.02 M $(EHPA)_2$ in hexane; mobile phase: 0.1 M $(H,Na)CHCl_2COO$ (pH 1.50), 0.3 cm^3 min^{-1}; 30°C.

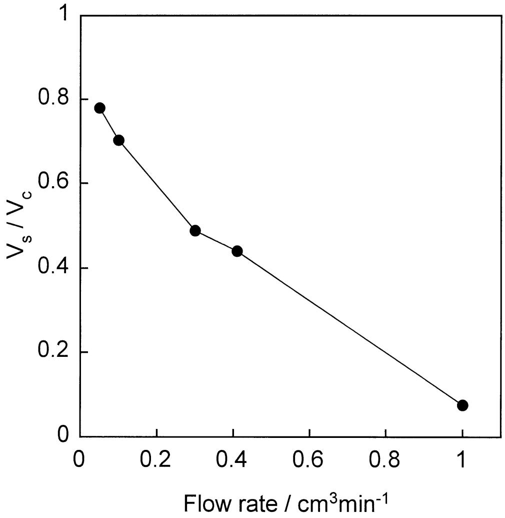

Figure 3. Effect of the flow rate on the retention of the stationary phase. 1250 rpm. Other conditions are same as those in Figure 2.

Chromatography with $(EHPA)_2$ Stationary Phase

The chromatographic retention of solutes can be expected from the liquid-liquid extraction data. The retention volume (V_R) of a desired element is related to the volumes of the mobile and stationary phases and the distribution ratio (D),[21]

$$V_R = V_m + D\, V_s$$

where V_m is the mobile phase volume. The chromatographic behavior of lanthanoid elements was tested through a coiled column containing 0.02 M $(EHPA)_2$ as the stationary phase.

Effect of Rotational Speed

Figure 4 presents CCC chromatograms obtained under different rotational speeds for the sample solution (0.1 cm^3) containing equimolar of gadolinium, terbium and dysprosium. Each lanthanoid element is eluted in the regular order by passing the mobile phase of pH 1.50.

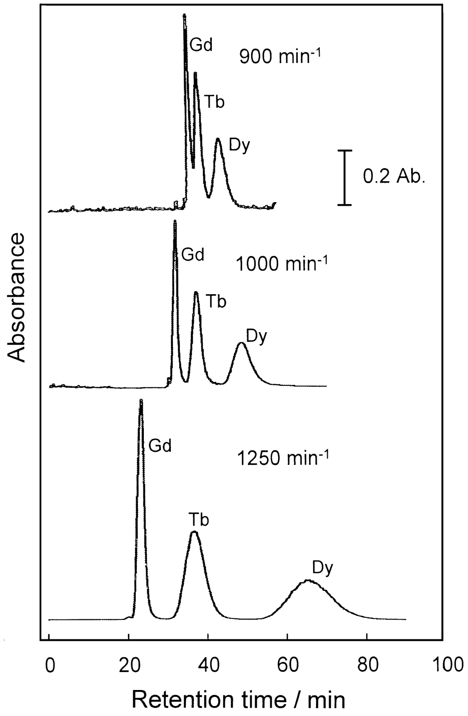

Figure 4. Chromatographic separation of middle lanthanoids at different rotational speeds. Stationary phase: 0.02 M $(EHPA)_2$ in hexane; sample: 5 x 10^{-5} M each ion at 900 and 1000 rpm, 1 x 10^{-4} M each ion at 1250 rpm, 0.1 cm^3; mobile phase: 0.1 M $(H,Na)CHCl_2COO$ (pH 1.50), 0.3 cm^3 min^{-1}; 30°C.

At a low rotational speed of 900 rpm, the volume of the mobile phase is rather large, and the chromatographic peaks are close to each other. The higher rotational speed leads to a decrease in mobile phase volume, and then resolution of adjacent peaks is enhanced owing to an increase in the stationary phase volume. These elution peaks are mutually separated under a high rotational speed of 1250 rpm.

The chromatographic parameters, the retention volume, the chromatographic distribution ratio (D_C), the chromatographic separation factor (α_C) of adjacent peaks and their resolution (R_S), were evaluated for the sample solution containing a mixture of lanthanoid elements. They are illustrated as a function of the rotational speed in Figure 5. The V_R value of the first peak for Gd decreases due to a decrease in the mobile phase with increasing rotational

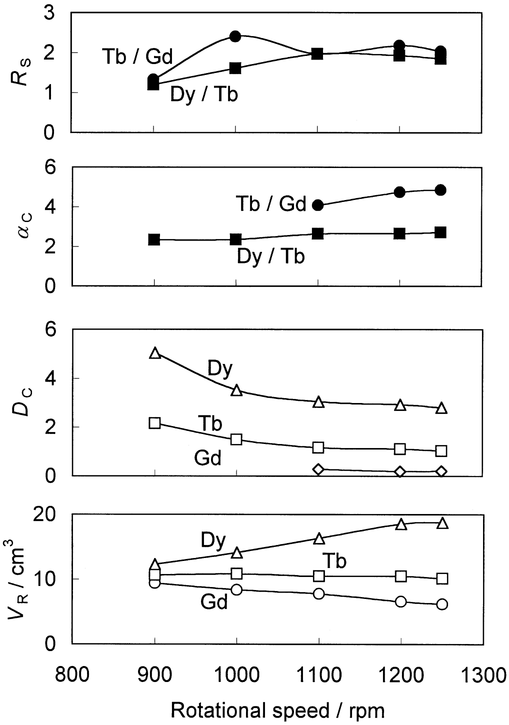

Figure 5. Effect of the rotational speed on chromatographic parameters. Stationary phase: 0.02 M $(EHPA)_2$ in hexane; sample: 5 x 10^{-4} M each ion, 0.1 cm^3; mobile phase: 0.1 M $(H,Na)CHCl_2COO$ (pH 1.50), 0.3 cm^3 min^{-1}; 30°C.

speed, and that of the last peak for Dy increases owing to a large retention of the stationary phase. The D_C values calculated, $D_C = (V_R - V_m) / V_s$, are nearly constant except in the region of 900-1000 rpm, where errors in parameters are rather large due to low chromatographic retention of lanthanoids. The α_C values, the ratio of D_C, are almost independent of the rotational speed, and they are found to be larger than 2 for Dy/Tb and lager than 4 for Tb/Gd. The resolution is appreciably improved along with rotational speed, and the high resolution is attained under rotation above 1000 rpm.

Effect of Temperature

Change in temperature may affect not only kinetic factors but also hydrodynamic parameters in the CCC column. The chromatographic parameters

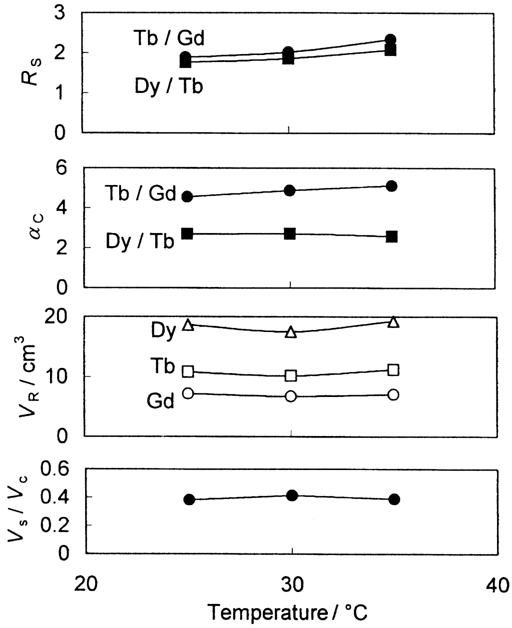

Figure 6. Effect of temperature on chromatographic parameters. Stationary phase: 0.02 M $(EHPA)_2$ in hexane; sample: 5 x 10^{-4} M each ion, 0.1 cm^3; mobile phase: 0.1 M $(H,Na)CHCl_2COO$ (pH 1.50), 0.3 cm^3 min^{-1}; 1200 rpm.

obtained at different temperatures (25-35°C) are illustrated in Figure 6. Stationary phase retention is not seriously affected in the temperature region tested. The retention volumes of lanthanoids are practically unchanged, and then the α_C values also remain unaltered irrespective of temperature change.

An appreciable improvement in resolution is attained with rising temperature. This is probably attributable to the kinetics factors, which appreciably enhance not only the rates of extraction and stripping reactions but also diffusion in the stationary and mobile phases.

Separation Ability of Small Column

Sufficient separation characteristics have been demonstrated by CCC equipped with the small column for the samples of the mixture of Gd, Tb, and Dy. For comparison, chromatographic conditions and some parameters obtained for two typical CCC apparatus employing a small column (10 cm^3) and a middle column (ca. 270 cm^3)[19] are summarized in Table 2. For the small col-

Table 2

Comparison of Chromatographic Parameters by CCC Apparatus Equipped with a Small Column and a Middle Column*

		Small Column	**Middle Column**
Column volume		10 cm^3	268 cm^3
Tube diameter		0.5 mm	1.5 mm
Tube length		50 m	150 m
Number of turns		150	300
Revolutionary speed		1200 rpm	800 rpm
Stationary phase		0.02 M $(EHPA)_2$ in hexane	0.02 M $(EHPA)_2$ in toluene
Mobile phase		0.1 M (Na,H)$CHCl_2COO$ pH 1.5	0.1M (Na,H)$CHCl_2COO$ pH 2.1
Flow rate		0.3 $cm^3 min^{-1}$	5.0 $cm^3 min^{-1}$
R_s	Tb/Gd	2.34	1.83
	Tb/Dy	2.07	1.80
N	Gd	1058	333
	Tb	347	235
	Dy	234	163
α_c	Tb/Gd	5.12	5.94
	Tb/Dy	2.58	2.64

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* 35°C, data for middle column are cited from Ref. 19.

umn, the hexane solution of 0.02 M $(EHPA)_2$ was used as the stationary phase in order to achieve suitable retention in a tubing coil with a short diameter, while the flow rate was rather low at 0.3 cm^3 min^{-1} and a rotational speed was high at 1200 rpm. No noticeable trouble, such as, column break occurred even at high rotational speed around 1200 rpm. The tubing of the small column was rather durable compared with the middle column.

The separation parameters are found to be good enough to separate from each other by CCC with a small column and they are comparable to those by a middle column. Although, the tubing coil of the small column (50 m) with turns of 150 is much shorter than that of the middle column (150 m) with turns of 300, the higher rotational speed for the small column would contribute to enhancing the separation, that is, chromatographic parameters such as N and R_s for small columns are higher than those for middle columns.

Enrichment Separation of Selected Rare Earths

In the previous studies,[19,21] REEs, including yttrium, were simultaneously enriched into the middle column retaining $(EHPA)_2$ from a sample solution of a large volume (1000 cm^3), and then successively separated by flowing a large quantity of the mobile phase of pH buffers. If we use the small column for these procedures, effective enrichment will be accomplished from rather small volumes of the sample solution.

In order to further confirm the enrichment and separation, chromatographic procedures have been tested for a sample solution (100 cm^3) containing the pair of Eu and Gd which are one of the most difficult elements to be separated among all lanthanoids. Their enrichment separation was accomplished with reasonable resolution as illustrated in Figure 7. The D_C values evaluated from each peak are 3.33 for Sm, 7.78 for Eu, and 12.5 for Gd. These findings imply the possibility of the mutual separation of a series of REEs.

Figure 8 illustrates the chromatogram of REEs containing samarium through erbium and yttrium enriched from a 100 cm^3 solution containing related metal ions of 5 x 10^{-8} M each. Here, lanthanum was included as a marker of the starting point of the chromatogram. The pH of the mobile phase was controlled by mixing the buffer solutions of 0.2 M $(H,Na)CHCl_2COO$

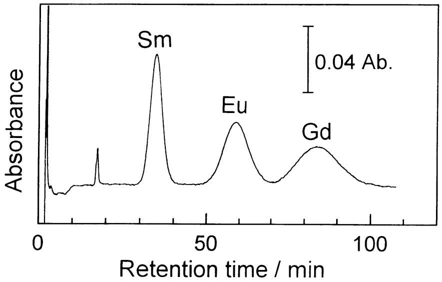

Figure 7. Chromatographic separation of Sm, Eu and Gd enriched from a sample solution (pH 4.05, 100 cm^3) containing 5 x 10^{-8} M each ion. Stationary phase of 0.02 M $(EHPA)_2$ in hexane; mobile phase: 0.2 M $(H,Na)CHCl_2COO$ (pH 2.13), 0.5 cm^3 min^{-1}; 1250 rpm; 35°C.

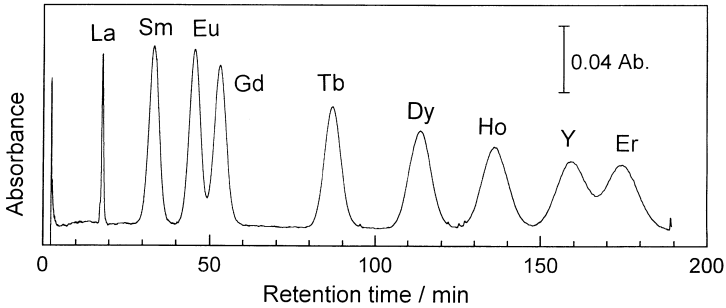

Figure 8. Chromatographic separation of selected REEs enriched from a sample solution (pH 4.07, 100 cm^3) containing 5 x 10^{-8} M each ion. Stationary phase of 0.02 M $(EHPA)_2$ in hexane; mobile phase: gradient elution from 0.2 M $(H,Na)CHCl_2COO$ (pH 2.16) to 0.2 M $CHCl_2COOH$ (pH 1.14) over 240 min, 0.5 cm^3 min^{-1}; 1250 rpm; 35°C.

(pH 2.16) and 0.02 M $CHCl_2COOH$ (pH 1.12). A series of REEs are successively separated with good resolution by gradient elution, and only the resolution of Y and Er is rather low due to its low separation factor, e.g. $\alpha_D = 1.5$ with EHPA.[7]

The enrichment and separation of selected REEs were successfully performed, and this CCC method will be applicable to samples containing a variety of metal ions. Such a small column will be advantageous not only by saving amounts of related solutions, but also because of its desirable mechanical points such as a compact revolutionary frame with higher rotational speed.

CONCLUSIONS

The CCC technique, employing the stationary phase of $(EHPA)_2$ having excellent separation properties, afforded a convenient method for separation of lanthanoid elements. High retention of the stationary phase in the small coiled column was attained at low flow rates of the mobile phase under high rotational speed, and then sufficient separation of lanthanoids was accomplished. The mixture of selected lanthanoids was effectively enriched into the organic stationary phase, and then successively separated into individual elements with good resolution.

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