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Study of isotachophoretic separation behaviour of metal cations by means of particle-induced X-ray emission

II. Separation efficiency of lanthanoid model mixture

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ABSTRACT

The isotachophoretic (ITP) separation behaviour of fourteen lanthanoid cations coexisting with α-hydroxyisobutyric acid was studied in relation to the separation efficiency. It was confirmed that the stimulated separation efficiencies using approximate equations agreed well with the observed values, except for Eu–Gd. It was found that the separation efficiency of equimolar amounts of neighbouring lanthanoids varied discontinuously but periodically, forming four different groups ("tetrad effect"). The effect of the component ratio on the separation efficiency of minor lanthanoids from major components was studied by means of ITP fractionation and subsequent off-line analysis by particle-induced X-ray emission. It was confirmed that the separation efficiency of multi-component mixtures decreased with decrease in the abundance of the sample. For binary mixtures of neighbouring lanthanoids, however, the separability was almost independent of the abundance, as for strong electrolytes.

INTRODUCTION

The separation of lanthanoid cations is a typical application of isotachophoresis (ITP), where complex-forming equilibria with α-hydroxyisobutyric acid (HIB) were utilized [1]. The principle of the separation was clarified theoretically later [2] and the optimum separation conditions for equimolar amounts of fourteen lanthanoid cations were studied in relation to the pH of and the concentration of HIB in the leading electrolyte [1,2]. From the experimental results [1], the separation efficiency of Pr–Nd and Eu–Gd was calculated as *ca.* 30 nmol/C. They were the least separable pairs in the mixture of fourteen lanthanoid cations.

A knowledge of the separation efficiency is important not only from the preparative but also from the analytical viewpoint, because overloading of the sample is fatal for an ITP separation. As far as we know, there has been no further discussion concern-ing the separation efficiency of the lanthanoids even for equimolar binary mixtures.

The difference in effective mobility between the separands [3,4] is the most important factor affecting the separation efficiency. On the other hand, the composition of the sample solution is also an important factor, as discussed in previous papers [5,6]. A theoretical elucidation of the composition effect has been given, but it was for the strong electrolytes.

In contrast to the sample system treated previously [5,6], the complex-forming equilibria in isotachophoretic lanthanoid (Ln) zones were very complicated owing to the formation of multi-coordinated ion pairs such as $Ln(HIB)_n$ ($n = 1–4$). In addition, multi-coordinated ion pairs with acetic acid coexisted to a considerable extent [2]. Acetic acid was added to the leading electrolyte as a pH buffering agent.

For the study of the separation behaviour of metal cations, previous universal detectors such as the

potential gradient detector are no longer convenient, especially when the sample contains a considerable number of separands and they are not equimolar. A more specific detection method is necessary to distinguish the individual separands. For this purpose, we developed a coupled isotachophoresis–particle-induced X-ray emission (ITP–PIXE) method: The sample zones were fractionated using a preparative ITP analyser [7] and the fractions were analysed off-line by PIXE. In a previous paper [8], the recovery, migration order and separation efficiency of twenty metal cations were studied using ITP–PIXE. The recovery of the isotachophoretic separation of four lanthanoids (La, Ce, Gd and Lu) was 100% under the electrolyte conditions used.

In this work, first the separation efficiency of equimolar binary mixtures of lanthanoids were measured using a potential gradient detector. The separation behavior of minor lanthanoids from a major lanthanoid was then studied by means of ITP–PIXE in order to clarify the effect of composition on the separation efficiency in such a complex-forming system. The experimental results were compared with theoretical values.

THEORETICAL

Efficiency of separation of binary mixtures

The efficiency of separation of component A in a binary mixture of ions A and B, *i.e.* the separable molar amount of ions A per unit amount of electricity, can be defined in a similar manner to that reported by Boček *et al.* [9] and Mikkers *et al.* [10] as follows:

$$
\begin{aligned}
\frac{dN_A}{dQ} &= \frac{N_A}{It_{res,AB}} = \frac{N_A(V_{ITP} - V_{A/AB})}{Il_A} \\
&= \frac{N_A(E_A m_A - E_{AB} m_{B,AB})}{Il_A} \\
&= C^t_{A,AB}(E_A m_A - E_{AB} m_{B,AB})/J \quad (1)
\end{aligned}
$$

where N_A is the molar amount of the first migrating sample ion A, Q is the amount of electricity, I the migration current, $t_{res,AB}$ the resolution time of the mixed zone AB, V_{ITP} the isotachophoretic velocity, $V_{A/AB}$ the boundary velocity between zone A and mixed zone AB, l_A the zone length of zone A, E_A and E_{AB} the potential gradient of zone A and mixed zone

AB, respectively, m_A the effective mobility of ion A at the steady state, $m_{B,AB}$ that of the second sample ion B at the transient state, $C^t_{A,AB}$ the concentration of ion A in the mixed zone AB and J the current density. A different expression of eqn. 1 can be derived for ion B:

$$
\frac{dN_B}{dQ} = C^t_{B,AB}(E_{AB} m_{A,AB} - E_B m_B)/J \quad (2)
$$

The efficiency of separation of a binary mixture, S_{eff}, can be calculated as

$$
S_{eff} = (N_A + N_B)/Q \quad (3)
$$

As the complex-forming equilibria in the lanthanoid zones are very complicated, simulation of the mixed zone to evaluate exactly E_{AB}, $m_{A,AB}$, etc., is not practical: the simulation program SIPSR [5,6] for transient-state analysis cannot be applied to complex-forming equilibria at present.

In order to permit numerical calculation, several assumptions were introduced. When the amount of ion B is negligibly small in comparison with that of ion A, $C^t_{A,AB}$ can be replaced with C^t_A and E_{AB} with E_A as a first approximation. Eqns. 1 and 3 can then be rewritten as

$$
\begin{aligned}
\frac{dN_A}{dQ} &= C^t_A E_A(m_A - m_{B,A})/J \\
&= C^t_A(m_A - m_{B,A})/k_A \quad (4)
\end{aligned}
$$

$$
S_{eff,A} \approx N_A/Q = C^t_A(m_A - m_{B,A})/k_A \quad (5)
$$

where $m_{B,A}$ is the effective mobility of ion B in zone A and k_A is the conductivity of zone A. A similar expression for ion B can be written as

$$
S_{eff,B} \approx N_B/Q = C^t_B(m_{A,B} - m_B)/k_B \quad (6)
$$

where $m_{A,B}$ is the effective mobility of ion A in zone B and k_B is the conductivity of zone B. The meaning of $m_{A,B}$ and $m_{B,A}$ has been discussed in detail in relation to the "zone stability" [11].

Numerical calculation of eqns. 5 and 6 is possible using our simulation program SIPS after minor modification and the simulation results are given later.

EXPERIMENTAL

Chemicals

Lanthanoid chlorides ($LnCl_3 \cdot 6H_2O$), α-hydroxy-isobutyric acid (HIB) and carnitine hydrochloride

were obtained from Katayama Kagaku (Osaka, Japan). Two cationic dyes, toluidine blue (TB) and astrazon pink (AP), and hydroxypropylcellulose (HPC) were obtained from Tokyo Kasei (Tokyo, Japan). The lanthanoid chlorides and HPC were of extra-pure grade and HIB and carnitine hydrochloride were of guaranteed-reagent grade. The viscosity of the 2% HPC aqueous solution was 1000–4000 cP at 20°C according to the specification.

Samples

Lanthanoid stock solutions were prepared by dissolving the chlorides in high-purity water obtained by treatment with an ion-exchange resin (Puric-R; Japan Organo, Tokyo, Japan). In order to study the efficiency of separation of lanthanoid, thirteen equimolar binary mixtures were prepared using the stock solutions. Each mixture contained only two neighbouring lanthanoids, e.g., La and Ce, and the concentration of each component was 5 mM. For La and Ce, binary mixtures with molar ratios of 5:1 (15.82 mM) and 10:1 (19.7 mM) were also prepared in order to study the effect of composition on the separation efficiency.

Multi-component mixtures containing fourteen lanthanoid cations [Z = 57–71 except Z = 61 (Pm)] were prepared for the same purpose, where La was the major component and the other thirteen cations of equimolar lanthanoids were minor components. The molar ratio of La to each of the individual minor components in the mixture was varied as 10:1, 20:1, 30:1, 40:1, 60:1, 80:1 and 500:1, and the total concentration of the model mixtures was 7.822, 9.948, 11.70, 13.14, 15.37, 17.01 and 25.00, respectively. Small amounts TB and AP were added to the mixtures to monitor the separation process and to determine the timing of fractionation.

Standard lanthanoid solutions (1000 ppm, for atomic absorption spectrometry) were used as analytical standards for PIXE analysis.

Electrolyte system

The electrolyte system used is summarized in Table I. The leading electrolyte was 20 mM ammonia solution containing 10 mM HIB and the pH of the electrolyte was adjusted to 4.80 by adding acetic acid. The pH measurements were carried out using a Horiba (Tokyo, Japan) Model F7ss expanded pH meter. The terminating electrolyte was a 10 mM

TABLE I

ELECTROLYTE SYSTEM USED IN ISOTACHOPHORETIC SEPARATION

HIB = α-Hydroxyisobutyric acid; CARH = carnitine hydrochloride; HPC = hydroxypropylcellulose.

Leading electrolyte	20 mM ammonia solution
Complexing agent	10 mM HIB
pH buffer	Acetic acid
pH of leading electrolyte	4.80
Terminating electrolyte	10 mM CARH
Additive	0.2% HPC

solution of carnitine hydrochloride. The actually migrating terminator was H^+. HPC was added to the leading and the terminating electrolytes (0.2%) in order to suppress electroendosmosis.

Preparative isotachophoretic analyser

The preparative analyser and the method of fractionation used were reported in a previous paper [7]. The sample migration in the apparatus can be divided into three stages (preseparation, separation and fractionation stages). The preseparation stage for large amounts of sample was not used in this work. The sample mixture was separated from the separation stage, which was designed for the complete separation of the preseparated samples or the separation of the relatively small amounts of samples of the order of 10^2 nmol or less. The separated zones were fractionated dropwise (5 μl) by a counter flow of leading electrolyte through a nozzle. The fractions were collected drop by drop on Nuclepore filters for PIXE analysis.

In order to obtain the amount of electricity in the electrophoretic process, the migration current was integrated using a microcomputer with an analog-to-digital conversion interface (sampling rate = 2.070 s per datum).

PIXE analysis

For the measurement of PIXE spectra, a Van der Graaff accelerator (Model AN-2500; Nisshin High Voltage, Tokyo, Japan) at the Faculty of Engineering, Hiroshima University, was used. The energy of the H^+ beam was 2.0 MeV and the beam current was 20 nA. The detector used was a high-purity Ge detector (ORTEC Model GLP-10180) and the multi-

channel analyser used was a Model AMS-1000 (Laboratory Equipment, Tokyo, Japan). A typical single run took 250 s to measure the PIXE spectrum of a fraction.

The Nuclepore filter used as the target backing was 5 μm thick and with a pore size of 0.1 μm, mounted on an aluminium flame. The impurities in the Nuclepore filter were mainly Cr and Fe and the abundance of Fe was one tenth of that of Cr, although the amounts were not the same from one unit to another. The PIXE spectrum was measured for each fraction after drying in a desiccator. The diameter of the fraction spot was *ca.* 3 mm. The sample amount was in the range 2.08–2.62 μg (29.4–37.1 μg cm^{-2}). As the volume of one fraction was *ca.* 5 μl, a few or more lanthanoids were contained in a fraction when the abundances were small. The PIXS data reduction software developed previously [12] was used to analyse the PIXE spectra. All the calculations were carried on an NEC (Tokyo, Japan) PC-9801RA microcomputer (CPU = 80 386, co-processor = 80 387, clock = 16 MHz).

RESULTS AND DISCUSSION

Binary equimolar mixture of lanthanoids

The efficiency of separation of binary mixtures of neighbouring lanthanoids was simulated and compared with the experimental results. Table II shows the theoretical separation efficiency of the binary mixture of neighbouring lanthanoids simulated by the use of eqns. 5 and 6, respectively. As discussed before, $S_{\text{eff,A}}$ (eqn. 5) gives the separation efficiency per unit amount of electricity for the binary mixture in which the first migrating component is the major component of the mixture. In contrast, $S_{\text{eff,B}}$ (eqn. 6) gives the necessary value where the second migrating component is the major component of the mixture. The electrolyte conditions used in the present simulation are given in Table I. Apparently from Table II, the separation efficiency of binary mixtures of neighbouring lanthanoids is almost independent of the ratio of the sample components, because the difference between the mobility differences $m_{\text{A}} - m_{\text{B,A}}$ and $m_{\text{A,B}} - m_{\text{B}}$ is small. For example, for La–Ce, $m_{\text{A}} - m_{\text{B,A}} = (31.7–28.7) \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} and $m_{\text{A,B}} - m_{\text{B}} = (32.2–29.2) \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}, *i.e.* the differences are both $3 \cdot 10^{-5}$ cm^2 V^{-1}

TABLE II

SIMULATIONAL AND EXPERIMENTAL SEPARATION EFFICIENCY OF A BINARY MIXTURE OF NEIGH-BOURING LANTHANOIDS

For electrolyte conditions, see Table I.

Lanthanoide pair	Separation efficiency (nmol C^{-1})				Difference (%)
	$S_{\text{eff,A}}$ (sim.)	$S_{\text{eff,B}}$ (sim.)	($S_{\text{eff,A}} + S_{\text{eff,B}}$)/2		
			Sim.	Obsd.	
La–Ce	261	298	280	262	+7
Ce–Pr	136	143	140	180	−22
Pr–Nd	100	104	102	123	−17
Nd–Sm	337	376	357	364	−2
Sm–Eu	216	216	216	161	+34
Eu–Gd	163	157	160	27	+493
Gd–Tb	337	359	348	309	+13
Tb–Dy	230	236	233	245	−5
Dy–Ho	169	176	173	197	−12
Ho–Er	255	261	258	279	−8
Er–Tm	177	186	182	234	−22
Tm–Yb	193	202	198	228	−13
Yb–Lu	212	213	213	198	+8

s^{-1}. For Yb–Lu, the corresponding values are $(17.1–15.8) \cdot 10^{-5}$ and $(17.7–16.5) \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}, *i.e.* the differences are almost identical at $1.3 \cdot 10^{-5}$ and $1.2 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}. Table II also shows the average values of the separation efficiencies, $(S_{\text{eff,A}} + S_{\text{eff,B}})/2$, which might represent the efficiency of separation of an equimolar binary mixture.

In order to evaluate this simulation, the separation efficiencies of binary mixtures were determined: the separable amount of sample was determined by repeated analyses of equimolar binary mixtures, increasing the amount of sample injected until the step of the mixed zone was found in the potential gradient detector trace. A coupled separation tube was used (16 cm × 1 mm I.D. × 2 mm O.D. and 30 cm × 0.5 mm I.D. × 1 mm O.D.). A typical single run took about 50 min. The migration current was 300 μA for the first 22 min, then it was decreased to 150 μA. The separation efficiency, S_{eff}, was evaluated using the equation

$$S_{\text{eff}} = \frac{\text{separable amount of the mixture (nmol)}}{\text{applied amount of electricity (C)}} \quad (7)$$
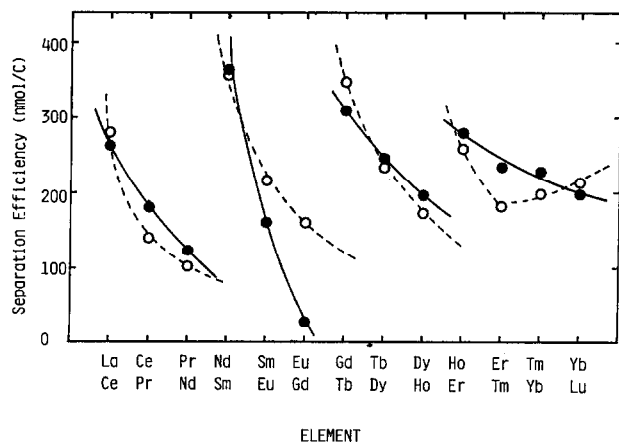
Fig. 1. Experimental (solid lines) and simulational (dashed lines) separation efficiencies of binary mixtures of neighbouring lanthanoids. For the electrolyte system used, see Table I. Migration current = 150 μA.

The observed separation efficiencies are shown in Fig. 1 and Table II. The agreement between the approximately evaluated values and the observed values was good except for the Eu–Gd mixture. If the Eu–Gd mixture was not considered, the average difference was 13%, which is good enough for the calculation of the sample load in the ITP separation. The minimum separation efficiency of 30 nmol C^{-1} for Eu and Gd was the same as in previous work,

although the electrolyte conditions used were different.

It was noticeable in Fig. 1 that the separation efficiency changed discontinuously but periodically with the atomic number, suggesting that the so-called "tetrad effect" of lanthanoids was also valid for the separation efficiency [13]. Such a periodic change of separation efficiency is apparently due to the periodic change of the difference in effective mobility, which was caused by similar changes in the stability constants of the ion pairs formed [14].

Table III shows the separation efficiencies simulated for all combinations of fourteen lanthanoid cations. Table III shows a square matrix where the rows and columns correspond to the components of binary mixtures. The diagonal values are zero, which means that the sample is not a binary mixture. The upper triangle of the matrix corresponds to $S_{eff,A}$ and the lower triangle to $S_{eff,B}$. Apparently from Table III, when the difference between the mobility differences $m_A - m_{B,A}$ and $m_{A,B} - m_B$ is large, e.g., for La–Lu, $S_{eff,A}$ and $S_{eff,B}$ differ significantly. In such a case, the separation efficiency depends on the molar ratio of the components.

Effect of composition on the efficiency of separation of lanthanoids

As discussed previously for multi-component

TABLE III

SIMULATED SEPARATION EFFICIENCY (nmol C^{-1}) FOR ALL COMBINATIONS OF FOURTEEN LANTHANOIDS

Assuming this table to be a matrix, the rows and columns correspond to the components of binary mixtures. The upper triangle matrix corresponds to $S_{eff,A}$ and the lower to $S_{eff,B}$. For the electrolyte conditions, see Table I.

	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
La	0	298	454	580	1041	1317	1472	1934	2212	2483	2883	3171	3477	3871
Ce	261	0	143	254	668	918	1072	1499	1765	2013	2382	2652	2937	3302
Pr	381	136	0	104	495	732	882	1287	1543	1777	2124	2380	2651	2991
Nd	466	232	100	0	377	609	758	1152	1404	1430	1967	2217	2480	2809
Sm	745	543	425	337	0	216	367	733	975	1183	1493	1725	1972	2272
Eu	916	731	620	538	216	0	157	517	758	962	1247	1473	1736	2030
Gd	1051	878	771	693	381	163	0	359	602	805	1111	1335	1578	1871
Tb	1287	1145	1048	982	706	503	337	0	236	425	713	923	1155	1429
Dy	1426	1303	1214	1156	906	715	550	230	0	177	450	650	872	1131
Ho	1510	1403	1320	1268	1041	862	699	394	169	0	261	453	669	917
Er	1709	1621	1544	1499	1290	1113	942	644	418	255	0	186	397	638
Tm	1775	1701	1629	1590	1402	1238	1071	790	571	418	177	0	202	430
Yb	1851	1790	1723	1690	1523	1371	1207	942	729	588	361	193	0	213
Lu	2030	1987	1927	1899	1747	1594	1420	1157	941	800	537	407	212	0

sample mixtures [5], the efficiency of separation of a pair of separands of interest is affected not only by the difference in the mobilities of the separands but also by the amount of the coexisting component. Further, the difference among the mobilities of components of interest and other coexisting components also affects the separation efficiency. Such an effect was called the composition effect [5,6].

The composition effect was studied for the mixture of lanthanoids, where La was assumed to be the major component and the other thirteen cations of equimolar lanthanoids were the minor components. In this instance, the separation of Ce, which was adjacent to the La zone, will be very difficult, although the separation efficiency between Eu and Gd is the poorest in the equimolar case. Hence La and Ce were separated under the conditions used (Table I) and the separation efficiency was evaluated by eqn. 7.

As examplified in ref. 6, the potential gradient detector signal of the mixed zone between the minor and the major component resembles that of the steady-state zone of the major component. In such a case, the separable amount could not be determined from mixed zone formation as in the equimolar case, but it should be determinable from the calibration line for the minor component. When the component ratio of the sample was 10:1, 20:1 or 30:1, potential gradient detection was still useful: Fig. 2 shows the observed zone length of Ce based on time. In Fig. 2, a bend was found in each calibration line, suggesting that the injected amount at the bend was a separable amount. In the case of the 10:1 mixture, two bends were found. The reason is unclear but it might be related to mixed zone formation among the matrix La and the minor components Ce and Pr.

When the component ratio was increased, the amount of minor components (Ce–Lu) decreased and it became difficult to detect the zone lengths accurately by means of a potential gradient detector. Therefore, the separated minor zone was fractionated with the preparative isotachophoretic analyser [8] and the fractions were analysed by PIXE.

Fig. 3 shows the PIXE results when the component ratio was varied as 40:1, 60:1 and 80:1. Bends were also found in calibration lines and the separation efficiency was evaluated similarly.

Fig. 4 summarizes the dependence of the component ratio on the efficiency of separation of
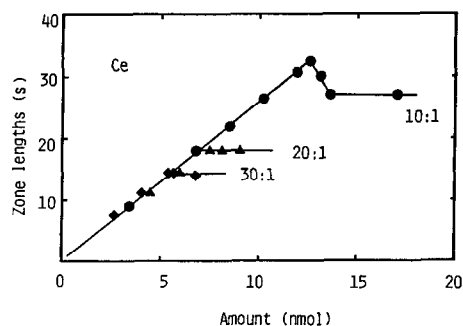

Fig. 2. Observed time-based zone length *vs.* injected amount of Ce with the use of a potential gradient detector. The samples were mixtures of fourteen lanthanoid cations where La was the major component and the other thirteen equimolar lanthanoids were the minor components. The ratio of La to the individual minor components was varied as 10:1, 20:1 and 30:1. For operational conditions, see Fig. 1.
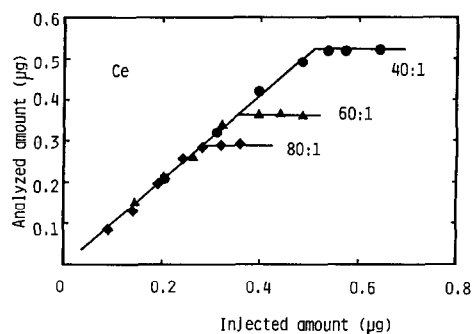

Fig. 3. Analysed amount by IP–PIXE *vs.* injected amount of Ce. The ratio of the sample components (see caption of Fig. 2 for definition) was varied as 40:1, 60:1 and 80:1. For the operational conditions and the composition of the sample see Figs. 1 and 2, respectively.
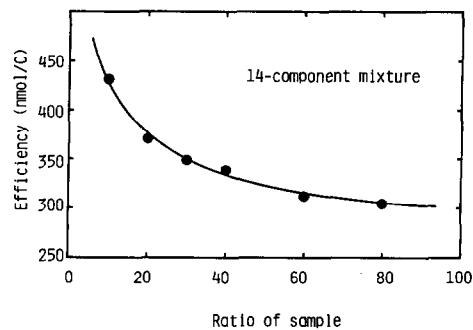

Fig. 4. Dependence of separation efficiency on the ratio of the sample components (see caption of Fig. 2 for definition). For the operational conditions and the composition of the sample see Figs. 1 and 2, respectively.

lanthanoid mixtures. The separation efficiency decreased with increase in the ratio and gradually the effect of the component ratio became smaller and the separation efficiency approached the limiting value of $S_{eff,La}$.

The efficiency of separation of La and Ce contained in the fourteen-component mixture can be calculated from the observed separation efficiency $(S_{eff,Ln})$ shown in Fig. 4 considering the composition of the mixture as follows:

$$S_{eff} = \frac{R+1}{R+13} \cdot S_{eff,Ln} \tag{8}$$

where R is the molar ratio of the major component (La) to the individual minor component.

Evaluated S_{eff} values for La and Ce are shown in Fig. 5 (curve 2). It is interesting that the efficiency of separation of La and Ce contained in the fourteen-component mixture increased with increase in the component ratio, which is just opposite of the tendency observed for the total amounts in Fig. 4. In fact, the phenomenon has been explained by the effect of coexisting components on the separation efficiency of isotachophoresis [5]. The total amount of twelve coexisting lanthanoids except for La and Ce is also shown in Fig. 5. Apparently the increase in

the efficiency of separation of La and Ce with increase in the component ratio depends on the decrease in the coexisting amount.

On the other hand, Fig. 5 also shows the observed efficiency of separation of binary mixtures of La and Ce, where La was selected as the major component. Apparently the separation efficiency is independent of the component ratio, which is in agreement with the simulational results in Table II. As the component ratio increases, the efficiency of separation of the fourteen-component mixture agreed with that of the binary equimolar mixture of La and Ce.

In conclusion, the separation of minor components is not difficult if the amount of electricity is appropriate. However, if it is not, it causes a major error in the determination. In a subsequent paper, we shall present the application of the present basic study to the separation of minor heavy lanthanoids from crude rare earth chlorides [15].

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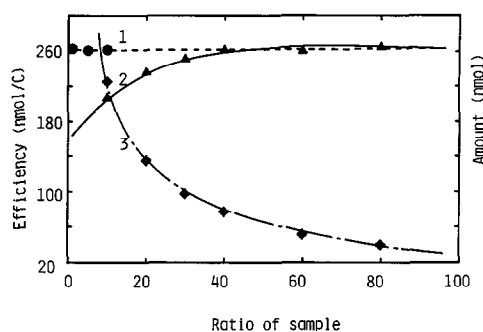15 J.-Y. Hu, T. Hirokawa, F. Nishiyama, Y. Kiso, K. Ito and E. Shoto, J. Chromatogr., 594 (1992) in press.

Fig. 5. Dependence of the efficiency of separation of La and Ce on the amount of coexisting component. (1) Two-component mixture of La and Ce; (2) La and Ce in a fourteen-component mixture (for the composition of the sample, see Fig. 2); (3) coexisting composition amount. For the operational conditions, see Fig. 1.