

## Separation of rare earth elements by high-speed counter-current chromatography

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### ABSTRACT

Besides being widely used in electronic and glass industries, rare earth elements have recently been found to have important biological effects including the ability to stabilize and enhance interferon activity [J. J. Sedmak and S. E. Grossberg, *J. Gen. Virol.*, 52 (1981) 195]. In this paper, the rare earth elements have been separated using a high-speed counter-current chromatography (HSCCC) centrifuge equipped with three multilayer coils connected in series. Two-phase solvent systems were composed of *n*-heptane containing di-(2-ethylhexyl)phosphoric acid (stationary phase) and dilute hydrochloric acid (mobile phase) where the partition coefficient of each can be optimized by selecting the proper hydrochloric acid concentration. The mobile phase was eluted through the column at a flow-rate of 5 ml/min, while the apparatus was rotated at 900 rpm. Continuous detection of the rare earth elements was effected by means of a post-column reaction with arsenazo III and the elution curve was obtained by on-line monitoring at 650 nm. Excellent isocratic separations of closely related rare earth elements were achieved at high partition efficiencies up to several thousand theoretical plates. Versatility of the present method was demonstrated in an exponential gradient elution of hydrochloric acid concentration where fourteen rare earth elements were all resolved in about 4.5 h.

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### INTRODUCTION

High-speed counter-current chromatography (HSCCC) is the most advanced form of CCC which we have applied to the separations of various organic samples [1–3]. It provides advantages over other CCC methods such as high partition efficiency, rapid separation and the ability to perform dual CCC [1]. Recently, highly efficient chromatographic separations have been achieved using a set of three multilayer coils [4–6].

In the present study, the use of this device is demonstrated in the separation of rare earth elements. In addition to their many uses in the electronic industry, rare earth elements such as lanthanum, cerium, neodymium, samarium, gadolinium, dysprosium, erbium and ytterbium have recently been found to stabilize and enhance interferon activity [7]. The difficulty in separating the lanthanoid elements is mainly due to their formation of trivalent cations with almost equal diameters [8].

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Preparative-scale separation by means of conventional multistage extraction requires a very long time, while efficient analytical-scale separations can be made using high-performance liquid chromatography (HPLC) [9,10] and ion chromatography [11]. Recently, micograms to milligram quantities of the rare earth elements have been separated by centrifugal droplet CCC (centrifugal partition chromatography) [12,13]. The results of the present method are compared with those obtained from the centrifugal droplet CCC.

## EXPERIMENTAL

### *Apparatus*

The design of the CCC apparatus employed in the present study has been described in detail previously [4]. The apparatus holds a set of three identical columns symmetrically distributed on the rotary frame at a distance of 7.6 cm from the central axis of the centrifuge. Each column holder is equipped with two planetary gears, one of which is engaged with an identical stationary sun gear mounted around the central stationary axis of the centrifuge. This gear arrangement produces a planetary motion of each column holder, *i.e.*, one rotation about its own axis per one revolution around the central axis of the centrifuge in the same direction. The other gear on the column holder is engaged with an identical gear on the rotary tube support mounted between the column holders. This gear arrangement produces counterrotation of the tube support to prevent twisting of the flow tubes on the rotary frame.

All column holders can be removed from the rotary frame by loosening a pair of screws on each bearing block, facilitating the mounting of the coiled column on the holder. Each multilayer coil was prepared from a single piece of approximately 100 m long, 1.07 mm I.D. PTFE (polytetrafluoroethylene) tubing (Zeus Industrial Products, Raritan, NJ, U.S.A.) by winding it directly onto the holder hub (15 cm diameter), making 13 layers of the coil between a pair of flanges spaced 5 cm apart. The  $\beta$  values range from 0.5 at the internal terminal to 0.75 at the external terminal.  $\beta = r/R$ , where  $r$  is the distance from the column holder axis to the coil and  $R$  the distance from the holder axis to the centrifuge axis.  $\beta$  is a parameter determining the hydrodynamic distribution of the two solvent phases in the rotating coil. Each multilayer coil consists of about 400 helical turns with approximately 90 ml capacity. The apparatus can be operated at speeds up to 1200 rpm.

The flow diagram of the present assembly is illustrated in Fig. 1. A Shimadzu LC-6A pump (pump I) was used to pump the mobile phase, another Shimadzu LC-6A pump (pump II) or stream splitter delivers a portion of the effluent to the spectrophotometer and a Rainin 1011/B-100-S pump (pump III) was used to add the post-column reagent to the effluent. A Shimadzu SPD-6AV spectrophotometer was used to monitor absorbance of the effluent and an LKB fraction collector (Ultrorac) to collect the effluent. At an early stage of development, it was found that the use of a peristaltic pump for pump II produced problems of excessive sample band broadening and damage to the tubing by exposure to *n*-heptane carried over from the stationary phase. These complications were all eliminated by replacing the peristaltic pump with the above Shimadzu LC-6A pump which provided a uniform flow with a small dead space. A Buchler Instrument gradient maker (Model KCI-23A2A1) was used for gradient elution of 14 lanthanoid elements.

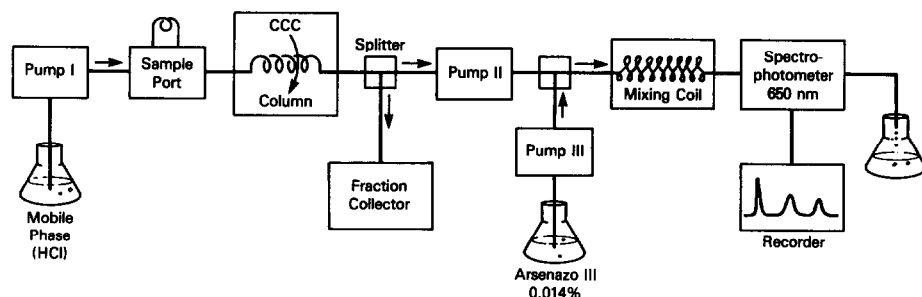


Fig. 1. Flow diagram of instrumentation assembly for separation of rare earth elements by high-speed CCC.

### Reagents

*n*-Heptane of a glass-distilled chromatographic grade was purchased from Burdick and Jackson Labs., Muskegon, MI, U.S.A. Di-(2-ethylhexyl) phosphoric acid (DEHPA) was obtained from Sigma, St. Louis, MO, U.S.A.; 1 *M* hydrochloric acid from Fisher, Fairlawn, NJ, U.S.A. and arsenazo III from Aldrich, Milwaukee, WI, U.S.A. Lanthanoid chlorides including  $\text{LaCl}_3$ ,  $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$ ,  $\text{PrCl}_3 \cdot 7\text{H}_2\text{O}$ ,  $\text{SmCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{TbCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{DyCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{HoCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{ErCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{TmCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{YbCl}_3 \cdot 6\text{H}_2\text{O}$  and  $\text{LuCl}_3 \cdot 6\text{H}_2\text{O}$  were obtained from Aldrich and  $\text{NdCl}_3 \cdot 6\text{H}_2\text{O}$  from Fluka, Buchs, Switzerland.

### Preparation of two-phase solvent and sample solutions

DEHPA was washed several times with 1 *M* hydrochloric acid followed by washing twice with de-ionized water and then dissolved in *n*-heptane (stationary phase). One molar hydrochloric acid was diluted with de-ionized water (mobile phase). Sample solutions were prepared by dissolving various lanthanoid chlorides in 0.02 *M* hydrochloric acid.

### Partition coefficient measurement

In order to determine the optimal composition of the solvent system, the partition coefficient ( $K$ ) for each rare earth element was measured by a simple test tube experiment. Rare earth elements were detected by means of the color reaction [8] with arsenazo III, a dye which forms a complex with rare earths shifting its absorbance maximum from 540 nm to 650 nm. Because quantitation with the color reaction is provided by the aqueous phase, the measurement was performed as follows: Equal volumes (about 3 ml) of the stationary non-aqueous phase (0.02 *M* DEHPA in *n*-heptane) and the mobile aqueous phase (dilute hydrochloric acid) were equilibrated in a separatory funnel at room temperature, the two phases were separated and the absorbance of the lower aqueous phase ( $A_0$ ) was measured. Then a given amount of each rare earth element was dissolved in the lower phase and the solutions thoroughly equilibrated with an equal volume of the upper non-aqueous phase. The absorbance of the aqueous phase was measured before ( $A_T$ ) and after ( $A_L$ ) equilibration with the upper phase. From these absorbance values, the partition coefficient ( $K_T$ ) obtained by the test tube measurement is given by:

$$K_T = (A_T - A_L)/(A_L - A_0) \quad (1)$$

The partition coefficient of each component was also calculated from the elution curve by:

$$K_E = (R - R_{SF}) / (V_C - R_{SF}) \quad (2)$$

where  $R$  is the retention volume of the peak maximum,  $R_{SF}$  the retention volume of the solvent front and  $V_C$  the total column capacity.

#### *Separation procedure*

Each separation was initiated by filling the entire column with the stationary non-aqueous phase followed by injection of 100  $\mu$ l sample solution containing 25  $\mu$ g of each component through the sample port. Then the mobile aqueous phase was eluted through the column at a rate of 5 ml/min in the proper elution mode while the apparatus was rotated at 900 rpm. Continuous detection of the rare earth elements was effected by means of a post-column reaction with arsenazo III [9] and the elution curve determined by monitoring the effluent at 650 nm using a Shimadzu SPD-6AV spectrophotometer equipped with an analytical flow cell. The effluent was divided into two streams with a tee adapter and a low-dead-volume Shimadzu LC-6A pump (pump II) used to deliver a portion of the effluent at a flow-rate of 1.4 ml/min to the spectrophotometer (see Fig. 1). At the outlet of this pump, the arsenazo III-ethanol solution (0.014%, w/v) was continuously added to the effluent stream at a flow-rate of 2.7 ml/min with a Rainin metering pump (pump III). The resulting effluent was first passed through a narrow mixing coil (PTFE tube, 1 m  $\times$  0.55 mm I.D.) immersed in a water bath heated to *ca.* 40°C and then led through an analytical flow cell (1 cm light path) of a Shimadzu SPD-6AV spectrophotometer set to monitor the absorbance at 650 nm. The other effluent stream through the tee adapter was either collected or discarded (see Fig. 1).

#### *Measurement of partition efficiencies*

From each chromatogram the partition efficiency of the separation was calculated and expressed in terms of theoretical plates (TP) according to the equation:

$$N = (4R/W)^2 \quad (3)$$

where  $N$  is the partition efficiency in TP,  $R$  the retention time or volume of the peak maximum and  $W$  the peak width expressed in the same units as  $R$ .

The partition efficiency can also be expressed in terms of peak resolution using the formula:

$$R_s = 2(R_1 - R_2) / (W_1 + W_2) \quad (4)$$

where  $R_s$  is the resolution of two adjacent peaks measured in units of  $4\delta$  in a Gaussian distribution,  $R_1$  and  $R_2$  are the retention times or volumes of two adjacent peaks ( $R_1 > R_2$ ) and  $W_1$  and  $W_2$  are the widths ( $4\delta$ ) of the corresponding peaks. When  $R_s = 1.5$ , baseline separation (99.7% pure) is indicated.

## RESULTS AND DISCUSSION

The optimum range of the partition coefficient values ( $K_T$ ) for neodymium, praseodymium, cerium and lanthanum was obtained with a solvent pair composed of 0.02 M DEHPA in *n*-heptane (stationary phase) and 0.02 M hydrochloric acid (mobile phase). Fig. 2 shows  $K_T$  values of these elements each plotted against the sample concentration from 0.005 to 0.025 mM in the mobile phase. As indicated in the diagram, the left chart was obtained from the ligand (DEHPA) treated with 1 M hydrochloric acid before being dissolved in *n*-heptane and the right chart was obtained from the ligand without the hydrochloric acid treatment. Using the above solvent systems, lanthanum, praseodymium and neodymium were separated at a flow-rate of 5 ml/min at a revolution speed of 900 rpm. Fig. 3A shows a chromatogram obtained from the solvent without the hydrochloric acid pretreatment. The three lanthanoid elements were well resolved in 2.5 h but each peak displays marked skewness as expected since  $K_T$  increases with decreased sample concentration (Fig. 2, right). This effect was largely eliminated by using the hydrochloric acid-treated ligand as illustrated in Fig. 3B. The maximum column pressure during these separations was about 300 p.s.i.

From these chromatograms, the partition coefficients ( $K_E$ ) were computed for each peak using eqn. 2 and compared with the  $K_T$  values obtained from the test tube measurements (Fig. 2). As shown in Table I, the two partition coefficient values,  $K_E$  and  $K_T$ , are generally in a close agreement in both groups. With both methods the non-treated groups shows substantially higher  $K$  values than the hydrochloric acid-treated group, especially with lanthanum and praseodymium.

The partition efficiencies of the separation were determined from the chromatogram (Fig. 3B) using eqns. 3 and 4: These range from 5900 theoretical plates (TP) for the first peak (lanthanum) to 520 TP for the third peak (neodymium), while the peak resolution between the first and the second peak is 6.94 and that between the second and the third 1.74. Another set of lanthanoid elements (thulium, ytterbium and lutetium), which are most difficult to resolve [13], was also separated with the present method using a modified solvent composition of 0.003 M DEHPA in *n*-heptane as

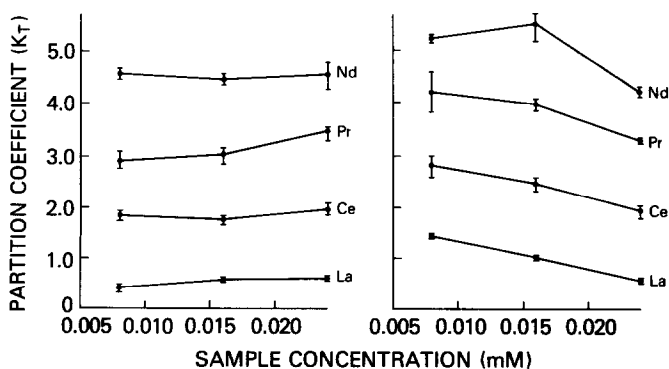


Fig. 2. Effects of hydrochloric acid treatment of ligand on the partition coefficients of light rare earth elements. (A) After washing with 1 M hydrochloric acid; (B) without pretreatment.

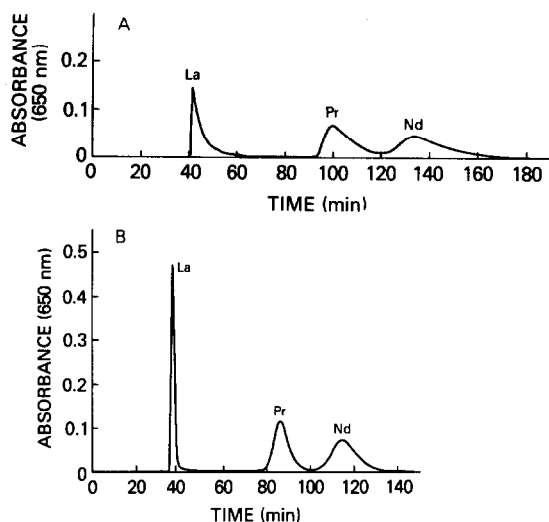


Fig. 3. Isocratic separation of lanthanum, praseodymium and neodymium with non-treated (A) and hydrochloric acid-treated (B) ligands by high-speed CCC. The experimental conditions were as follows: apparatus = HSCCC centrifuge with 7.6 cm revolution radius; column = three multilayer coils connected in series, 300 m  $\times$  1.07 mm I.D., 270 ml capacity; stationary phase = 0.02 *M* DEHPA in *n*-heptane; mobile phase = 0.02 *M* hydrochloric acid; sample = LaCl<sub>3</sub>, PrCl<sub>3</sub> and NdCl<sub>3</sub> each 0.001 *M* in 100  $\mu$ l of 0.02 *M* hydrochloric acid; revolution = 900 rpm; flow-rate = 5 ml/min; pressure = 300 p.s.i.

stationary phase and 0.1 *M* hydrochloric acid as mobile phase. The results showed much reduced partition efficiencies ranging from 170 TP for the first peak (thulium) to 60 TP for the third peak (lutetium).

In Table II these results are compared with those obtained by other CCC methods where  $N$  is the partition efficiency in TP,  $R_s$  is the peak resolution between the indicated two peaks and CDCCC stands for centrifugal droplet CCC (centrifugal partition chromatography). In both  $N$  and  $R_s$  groups, the middle column (CDCCC<sup>b</sup>) [12] indicates the efficiencies obtained using the same solvent pairs used in the present HSCCC method and the right column (CDCCC<sup>c</sup>) improved efficiencies with

TABLE I  
PARTITION COEFFICIENT VALUES

Upper phase: 0.02 *M* DEHPA in *n*-heptane; lower phase: 0.02 *M* hydrochloric acid.

Element	$K_E$ (average)		$K_T$	
	No washing <sup>a</sup>	Washing <sup>b</sup>	No washing <sup>a</sup>	Washing <sup>b</sup>
Lanthanum	0.5	0.3	0.48–1.40	0.33–0.59
Praseodymium	3.2	2.8	3.25–4.23	2.75–3.51
Neodymium	4.7	4.5	4.15–5.72	4.25–4.78

<sup>a</sup> DEHPA was dissolved in *n*-heptane without pretreatment.

<sup>b</sup> DEHPA was washed several times with 1 *M* hydrochloric acid before dissolving in *n*-heptane.

TABLE II  
COMPARISON IN PARTITION EFFICIENCIES BETWEEN HSCCC AND PREVIOUS METHODS

Element	$N^a$			$R_s^b$		
	HSCCC <sup>c</sup>	CDCCC <sup>d</sup>	CDCCC <sup>e</sup>	HSCCC <sup>c</sup>	CDCCC <sup>d</sup>	CDCCC <sup>e</sup>
Lanthanum	5900	34	169.0	6.94	1.4	2.70
Praseodymium	770	54	85.8	1.74	0.44	0.77
Neodymium	520	41	86.2			
Thulium	170	—	19.5	1.24	—	—
Ytterbium	74	—	—	0.79	—	—
Lutetium	61	—	—			

<sup>a</sup>  $N$ : Theoretical plate numbers.

<sup>b</sup>  $R_s$ : Peak resolution.

<sup>c</sup> 0.02 *M* DEHPA in *n*-heptane (stationary phase) and 0.02 *M* hydrochloric acid (mobile phase) were used for the separation of lanthanum, praseodymium and neodymium. 0.003 *M* DEHPA in *n*-heptane and 0.1 *M* hydrochloric acid were used for the separation of thulium, ytterbium and lutetium.

<sup>d</sup> The values were obtained from centrifugal droplet CCC or centrifugal partition chromatography with the same solvent system [12].

<sup>e</sup> The values were obtained from centrifugal droplet CCC with 2-ethylhexyl-phosphonic acid mono-2-ethylhexyl ester (EHPA) at 55°C [13].

a modified stationary phase containing 2-ethylhexyl-phosphonic acid mono-2-ethylhexyl ester (EHPA) at a higher temperature (55°C) [13]. As seen from the table, the present method yields much higher efficiencies than those produced by the other CCC methods. The separation time was also much shorter in HSCCC (2 h) than in the other two methods (3–30 h).

To further demonstrate the present method, a one-step separation of all 14 lanthanoid elements was performed by applying an exponential gradient of hydrochloric acid concentration in the mobile phase. The main problem in gradient elution is that the optimum range of the ligand concentration in the stationary phase is substantially different between the lighter and heavier groups of the rare earth elements. Because the separation of the heavy elements including thulium, ytterbium and lutetium is more difficult, the ligand concentration in the present experiment was selected at 0.003 *M* for best resolution. Consequently, the resolution of the light lanthanoid elements such as lanthanum, cerium, praseodymium and neodymium became less efficient compared with that observed in the isocratic separation shown in Fig. 3B. Fig. 4 shows the chromatogram of all 14 lanthanoid elements resolved in less than 5 h.

As indicated in Table II, the HSCCC method radically improved the results compared to the other CCC methods. The higher performance of the HSCCC over the CDCCC may be explained on the basis of the hydrodynamics inherent to each system. In the hydrostatic equilibrium system such as the CDCCC, mixing of the two solvent phases relies entirely on the flow of the mobile phase [14]. Due to the high interfacial tension between the two solvent phases used in the present separation, lack of a mixing force tends to form large droplets of the mobile phase, limiting the partition efficiency. On the other hand, HSCCC is a typical hydrodynamic equilibrium system where two solvent phases are vigorously mixed by the effect of planetary motion of the coil [1].

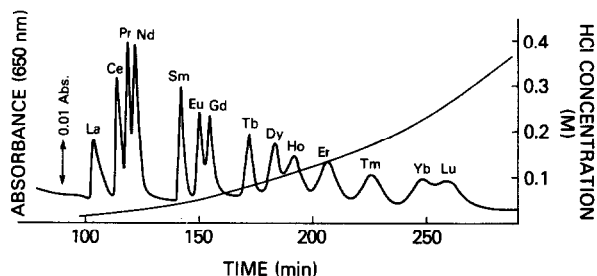


Fig. 4. Gradient separation of 14 rare earth elements obtained by high-speed CCC. The experimental conditions were as follows: apparatus = HSCCC centrifuge with 7.6 cm revolution radius; column = three multilayer coils connected in series, 300 m  $\times$  1.07 mm I.D., 270 ml capacity; stationary phase = 0.003 M DEHPA in *n*-heptane; mobile phase = exponential gradient of hydrochloric acid concentration from 0 to 0.3 M as indicated in the chromatogram; sample = 14 lanthanoid chlorides each 0.001 M in 100  $\mu$ l water; revolution = 900 rpm; flow-rate = 5 ml/min; pressure = 300 p.s.i.

Small droplets of the mobile phase produced by the vigorous mixing reduce the mass transfer resistance between the two phases resulting in high partition efficiency. Compared with HPLC and ion chromatography, the present method will provide a higher capacity for preparative-scale separations.

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