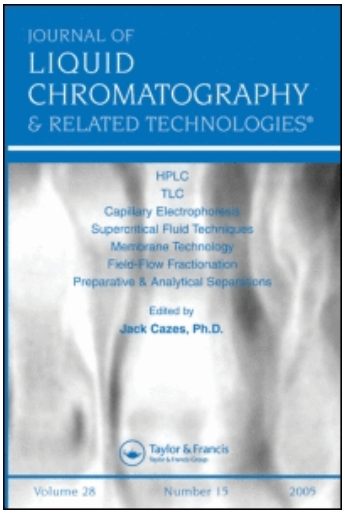

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Improved High-Speed Countercurrent Chromatograph with Three Multilayer Coils Connected in Series. III. Evaluation of Semianalytical Column with Various Biological Samples and Inorganic Elements

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IMPROVED HIGH-SPEED COUNTERCURRENT CHROMATOGRAPH WITH THREE MULTILAYER COILS CONNECTED IN SERIES. III. EVALUATION OF SEMIANALYTICAL COLUMN WITH VARIOUS BIOLOGICAL SAMPLES AND INORGANIC ELEMENTS

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ABSTRACT

The partition efficiency of the countercurrent chromatographic centrifuge was improved by mounting a set of three multilayer coils prepared from a 1.07 mm I.D. and ca 300 m length of PTFE (polytetrafluoroethylene) tube with a total capacity of 270 ml. The high performance of the present apparatus has been successfully demonstrated in separations of various testing samples which include DNP (dinitrophenyl) amino acids, indole auxins, tetracycline derivatives, flavonoids, bacitracin, triterpenoic acids, and lanthanoid chlorides.

INTRODUCTION

In the past, many natural and synthetic products have been efficiently separated by high-speed countercurrent chromatography

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(CCC) with various two-phase solvent systems (1-3). The original single column system has produced efficient preparative separations. Recently, we have introduced a high-speed CCC centrifuge equipped with a set of three multilayer coils connected in series. It has given the expected threefold increase in both partition efficiency and sample loading capacity as demonstrated in semipreparative separations with multilayer coils of 1.6 mm I.D. and a total capacity of 400 ml (4,5).

In the present paper, the capability of the apparatus was further extended by the use of a smaller-bore column (ca 1 mm I.D.) connected in series. Highly efficient chromatographic separations were demonstrated with a variety of testing samples ranging from submilligram to 100 mg, including DNP amino acids, indole auxins, tetracycline derivatives, bacitracin, flavonoids from Hippophae rhamnoides, triterpenoic acids from Boswellia carterii, and rare earth elements each with a suitable two-phase solvent system.

MATERIALS AND METHODS

Apparatus

The design of the apparatus employed in the present study has been described in detail elsewhere (4), and therefore only a brief statement is given here. The apparatus holds a set of three identical columns symmetrically on the rotary frame at a distance of 7.5 cm from the central axis of the centrifuge. Each column holder is equipped with two planetary gears, one of which engages

to an identical stationary sun gear mounted around the central stationary pipe of the centrifuge. This gear arrangement produces a desired 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 to an identical gear on the rotary tube support mounted between the column holders. This gear engagement 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, thus 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) by winding it directly onto the holder hub (7.5 cm diameter), making 13 layers of the coil between a pair of flanges spaced 5 cm apart. The beta value ranged from 0.5 at the internal terminal to 0.75 at the external terminal. (Beta is an important parameter used to determine the hydrodynamic distribution of the two solvent phases in the rotating coil (1) and $\beta = r/R$, where r is the distance from the column holder axis to the coil and R, the distance from the holder axis to the axis of the centrifuge.) Each multilayer coil consists of about 400 helical turns with an approximately 90 ml capacity. In order to prevent dislocation of the multilayer coil on the column holder, the innermost layer of the coil was glued onto the holder hub with an RTV silicone rubber adhesive sealant (General Electric Company,

Waterford, NY, U.S.A.) while the whole column and the peripheral portion of the flanges were wrapped with a heat shrinkable PVC tube.

Each terminal of the multilayer coil was connected to a flow tube, 0.55 mm I.D. and 0.45 mm wall thickness. The use of this thick-wall, small-bore tubing is required at the flexing portion of the flow tube to withstand the back pressure created from the long narrow-bore semianalytical columns. Serial connection between the three multilayer coils was made in such a way that the internal terminal of the first column is connected to the external terminal of the second column, and the internal terminal of the second column is attached to the external terminal of the third column. In this way, all three columns are subjected to the same head-tail elution mode. As previously described, each interconnection flow tube runs across the rotary frame along the rotary tube support where it is secured with nylon ties. Each flexing portion of the flow tubes is lubricated with grease and protected with a sheath of Tygon tubing to prevent direct contact with metal parts. Both inlet and outlet flow tubes are secured on the centrifuge wall each with a silicone-rubber-padded clamp.

The apparatus can be operated at the maximum revolutional speed of 1,500 rpm with a speed control unit. A Beckman Accu Flo pump was used to pump the solvents, an LKB UV monitor (Uvicord S) to monitor the absorbance, and an LKB fraction collector (Ultrorac) to fractionate the effluent.

Reagents

Organic solvent, including n-heptane, n-hexane, chloroform, ethyl acetate, n-butanol, and methanol, were glass-distilled chromatographic grade and obtained from Burdick and Jackson Laboratories, Inc., Muskegon, MI, while USP 95% ethanol was obtained from Warner-Graham Company, Cockeysville, MD. Di-(2-ethylhexyl) phosphoric acid (DEHPA) was obtained from Sigma Chemical Co., St. Louis, MO, ammonium acetate and 1 N hydrochloric acid from Fisher Scientific Company, Fair Lawn, NJ, and arsenazo III from Aldrich Chemical Company, Milwaukee, WI.

Various testing samples used in the present studies are listed in Table 1. DNP amino acids, indole auxins, tetracycline derivatives and bacitracin were all obtained from Sigma Chemical Company. Lanthanum chloride anhydrous, praseodymium chloride heptahydrate, and neodymium chloride hexahydrate were obtained from Aldrich Chemical Company. The triterpenoic acid sample was obtained from the extract of Boswellia carterii (Burseraceae). The crude sea buckthorn (Hippophae rhamnoides) ethanol extract (dried powder) was obtained from China by the courtesy of Professor Tian-You Zhang of Beijing Institute of New Technology Application, Beijing, China.

Preparation of Two-Phase Solvent Systems and Sample Solutions

Two-phase solvent systems employed in the present studies are listed in Table 1. Each solvent mixture was thoroughly equilibrated in a separatory funnel by repeated vigorous shaking

TABLE 1. Summary of Experimental Conditions

Sample		Solvent System		Mobile Phase	Flow Rate (ml/h)	Revolution (rpm)	Pressure (psi)	Retention (%)
DNP amino acids								
DNP-L-valine (DNP-val)	2.4 mg	chloroform	2	lower	180	1250	255	57.7
DNP-L-alanine (DNP-ala)	2.4 mg	acetic acid	2	nonaqueous				
diDNP-L-cystine (diDNP-$(cys)_2$)	0.5 mg	0.1 M hydro-	1					
DNP-D,L-glutamic acid (DNP-glu)	4.8 mg	chloric acid						
DNP amino acids								
DNP-L-aspartic acid (DNP-asp)	2.4 mg	chloroform	2	upper	180	1250	90**	41.5
DNP-D,L-glutamic acid (DNP-glu)	2.4 mg	acetic acid	2	aqueous				
diDNP-L-cystine (diDNP-$(cys)_2$)	0.5 mg	0.1 M hydro-	1					
DNP-L-alanine (DNP-ala)	4.8 mg	chloric acid						
Indole auxins								
indole-3-acetamide (IA)	10 mg	n-hexane	1	lower	150	1250	220	53.3
indole-3-acetic acid (IAA)	30 mg	ethyl acetate	1	aqueous				
indole-3-butyric acid (IBA)	30 mg	methanol	1					
indole-3-acetonitrile (IAN)	30 mg	water	1					
Tetracycline derivatives								
oxytetracycline (OTC)	10 mg	ethyl acetate	1	lower	150	1250	220	54.0
chlortetracycline (CTC)	20 mg	n-butanol	1	aqueous				
doxycycline (DC)	20 mg	0.25 M ammonium acetate	2					
Rare earth elements								
lanthanum chloride ($LaCl_3$)	25 μg	0.02 M DEHPA*	1	lower	300	900	300	36.2
praseodymium chloride ($PrCl_3$)	25 μg	in n-heptane		aqueous				
neodymium chloride ($NdCl_3$)	25 μg	0.02 M hydro-	1					
		chloric acid						
Flavonoids	100 mg	chloroform	4	lower	180	1200	300	60.0
(sea buckthorn extract)		methanol	3	nonaqueous				
		water	2					
Bacitracin	100 mg	chloroform	5	lower	150	1200	220	50.0
		95% ethanol	4	nonaqueous				
		water	3					
Triterpenoic acids	100 mg	n-hexane	6	lower	150	1200	300	57.8
(*Boswellia carterii* extract)		95% ethanol	5	aqueous				
		water	1					

*di(2-ethylhexyl) phosphoric acid; **The tail-to-head elution mode resulted in a low back pressure.

and degassing several times at room temperature. For preparation of the two-phase solvent system for the rare earth element separation, DEHPA was first washed 5 times with 1 M hydrochloric acid and then washed twice with distilled water before being dissolved in heptane.

Various testing samples selected for the present studies are also listed in Table 1 together with two-phase solvent systems used for separation. The sample solution for each separation was prepared by dissolving the sample mixture in 0.2 to 5 ml of the upper and/or the lower phases which were used for the separation.

Separation Procedure

Most of the separations were performed according to the standard method as follows: The separation column was first entirely filled with the stationary phase. This was followed by injection of the sample solution through the sample port. Then, the mobile phase was pumped into the column at a desired flow rate of 2.5 - 3 ml/min in the proper head-tail elution mode while the centrifuge was run at the desired revolutional speed. The effluent from the outlet of the column was continuously monitored with an LKB Uvicord S at 278 nm and fractionated with an LKB fraction collector.

In the separation of the rare earth elements, the column was first fully equilibrated with the mobile phase (0.02M HCl) before the sample charge. The on-line detection of the metal ions was effected by means of the post-column reaction with arsenazo III

(6). The effluent from the column was divided into two streams with a tee adapter and a low-dead-volume Shimadzu LC-6A pump. At the outlet of the pump, the arsenazo III ethanol solution (0.014%, w/v) was continuously added to the effluent stream at a rate of nearly two parts to one part of the effluent with a Rainin metering pump (Rainin Instrument Co., Woburn, MA). The colored effluent was first passed through a narrow mixing coil (0.55 mm I.D. x 1 m) which was immersed into 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 to monitor the absorbance at 650 nm. The other effluent stream through the tee adapter can be fractionated with a fraction collector or discarded.

Analysis of Fractions

Except for the rare earth elements, the fractions obtained from each separation were manually analyzed for determination of absorbance to draw an elution curve. An aliquot of collected fractions was mixed with a known volume of methanol or water (when the fractions contained salt), and the absorbance was measured at an optimum wavelength (430 nm for DNP-amino acids, 280 nm for indole auxins, 350 nm for tetracycline derivatives, 260 nm for flavonoids, 250 nm for bacitracin, and 210 nm for triterpenoic acids) with a Zeiss PM6 spectrophotometer.

Measurement of Partition Efficiency

From the obtained chromatograms, partition efficiency of the separation was computed and expressed in terms of theoretical plates (TP) according to the conventional gas chromatographic

equation

$$N = (4R/W)^2 \qquad (1)$$

where N is the number of theoretical plates, a measurement of partition efficiency; R, the retention time or volume of the peak maximum; and W, the peak width expressed in the same unit as R.

The partition efficiency can also be expressed in terms of peak resolution according to the following formula:

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

where R_s is the resolution of the adjacent two peaks expressed in the unit of 4δ in a Gaussian distribution; R_1 and R_2, the retention time (or volume) of two adjacent peaks ($R_1 > R_2$); and W_1 and W_2, the width (4δ) of the corresponding peaks. When $R_s = 1.5$, it represents a baseline separation (99.7% pure).

RESULTS AND DISCUSSION

The capability of the present apparatus was evidenced in semianalytical separations of various test samples with a variety of two-phase solvent systems. These testing samples may be divided into two categories, i.e., the synthetic mixtures for evaluation of the partition efficiency and for comparative studies; and the natural products samples with a wide range of polarities for evaluation of the system in natural product research. The successful separation of lanthanoids (La^{+3}, Pr^{+3}, and Nd^{+3}) further proved its capability in resolving inorganic elements.

Separation of Synthetic Mixtures

1) DNP amino acids: Since the introduction of CCC in 1970 (7), this group of compounds has been serving as the standard test

samples for all types of CCC instruments. The DNP amino acids offer a number of advantages, such as commercial availability, a wide range of hydrophobicity, and a sensitive detection at a single wavelength of 280 or 430 nm with high extinction coefficient values. In the present study, two sets of DNP amino acid mixtures were selected, one for the lower nonaqueous phase as the mobile phase and the other for the upper aqueous phase as the mobile phase. One component, diDNP-$(cys)_2$ contained in both sample mixtures, is a submilligram quantity and constitutes 5% of the total mass to serve as a reference compound for recovery rate of minor components.

Figs. 1A and B show the chromatograms of DNP amino acids obtained with a two-phase solvent system composed of chloroform-acetic acid-0.1 M hydrochloric acid (2:2:1, v/v/v). In Fig. 1A, the lower phase was employed as the mobile phase, while in Fig. 1B the upper phase was used as the mobile phase. Both separations were performed at a flow rate of 180 ml/h at 1,250 rpm. In each experiment, a 10 mg quantity of sample mixture dissolved in 1 ml of the stationary phase was efficiently separated into four symmetrical peaks within 3 hours. The partition efficiencies estimated from Eq. 1 range from 3,000 to 5,000 TP, which are substantially greater than 1,200-3,500 TP obtained from the semipreparative column of 1.6 mm I.D. used in the previous studies (4). The multilayer coils used in the present studies consist of about 1,200 helical turns of a 300 m length of tubing, thus yielding an average of 3.5 TP per turn or a 7.5 cm length for

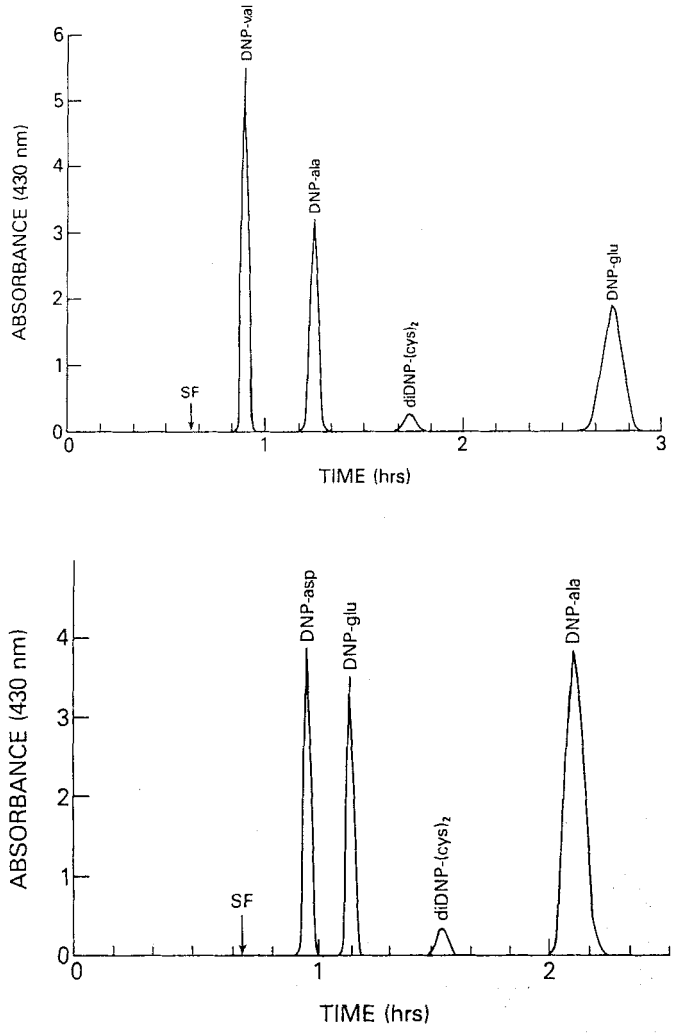

FIGURE 1. High-speed CCC separations of DNP amino acids with the semi-analytical multilayer coils. A: lower phase mobile; B: upper phase mobile. SF:solvent front. For experimental conditions, see Table 1.

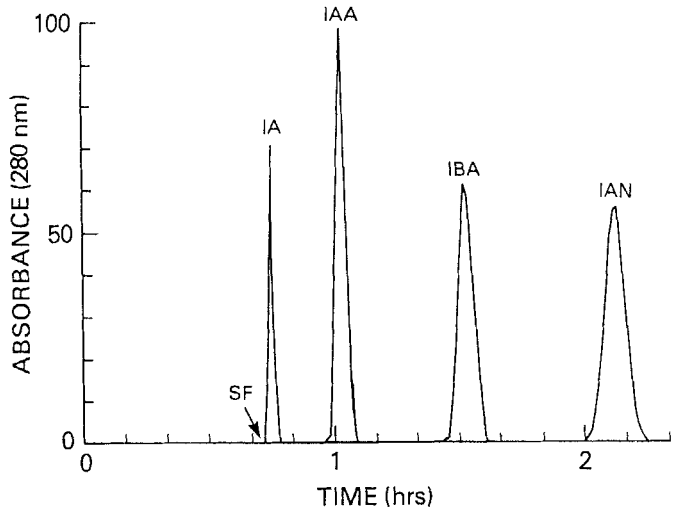

FIGURE 2. High-speed CCC separation of indole auxins with the semianalytical multilayer coils. SF:solvent front. For experimental conditions, see Table 1.

one TP. These figures are quite comparable to those obtained from the semipreparative column.

2) Indole auxins: Fig. 2 shows a chromatogram of indole auxins obtained with a two-phase solvent system composed of n-hexane-ethyl acetate-methanol-water (1:1:1:1, v/v/v/v). The separation was performed by eluting the lower aqueous phase at a flow rate of 150 ml/h under a revolutional speed of 1,250 rpm. A 100 mg quantity of the sample mixture dissolved in 2 ml of the two-phase mixture was efficiently separated in slightly over 2 hours with partition efficiencies ranging from 2,500 to 4,500 TP which show a substantial improvement over 1,200-3,500 TP obtained from the 1.6 mm I.D. semipreparative column (5).

This solvent system has a special advantage--it can be applied to a variety of samples with a wide range of hydrophobicity simply by modifying the volume ratio between n-hexane and ethyl acetate. All these solvent systems provide a satisfactory retention of the stationary phase in the multilayer coil and permit a stable uv trace of the elution curve on the recorder.

3) Tetracycline derivatives: As described earlier (5), the separation of the tetracycline derivatives is greatly affected by the composition of the aqueous phase. The use of a salt-free solvent system such as ethyl acetate-n-butanol-water (3:2:5) produced extremely broad peaks for CTC (second peak) and DC (third peak). However, an addition of 0.25 M ammonium acetate or neutral sodium phosphate to the aqueous phase remarkably improved the partition efficiency.

Fig. 3 shows a chromatogram of tetracycline derivatives obtained with a two-phase solvent system composed of ethyl acetate-n-butanol-0.25 M ammonium acetate (1:1:2, v/v/v). The lower aqueous phase was eluted at a flow rate of 150 ml/h under a revolutional speed of 1,250 rpm. Three components were well separated in slightly over 3 hours at partition efficiencies ranging from 380 TP (first peak) to 1,400 TP (Third peak). The low partition efficiency of the first peak may be a result of an unresolved impurity hidden on the right side of the main peak as indicated by an asymmetry of the peak shape.

4) Rare earth elements: In order to demonstrate the versatility of the present high-speed CCC method, separation of

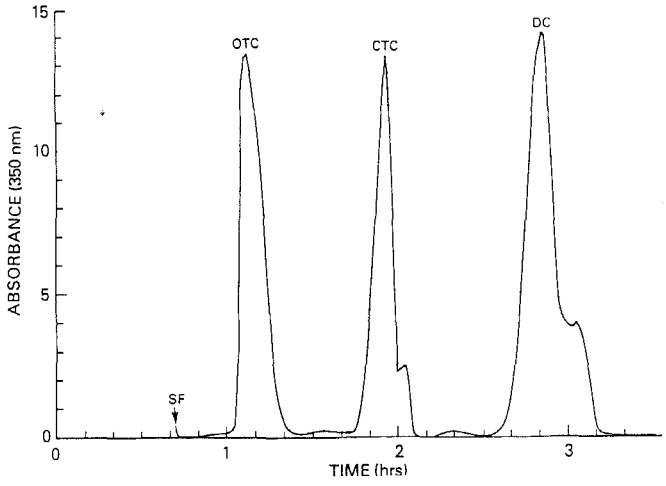

FIGURE 3. High-speed CCC separation of tetracycline derivatives with the semianalytical multilayer coils. SF:solvent front. For experimental conditions, see Table 1.

three rare earth elements, $LaCl_3$, $PrCl_3$ and $NdCl_3$, was performed on the basis of their affinity to the ligand DEHPA, which is totally retained in the stationary heptane phase.

Fig. 4 shows a chromatogram of the three lanthanides obtained with a two-phase solvent system composed of 0.02 M DEHPA in n-heptane and 0.02 M hydrochloric acid as recently reported by Kitazume et al. (8). The separation was performed by eluting the lower aqueous phase at 300 ml/h under a revolutional speed of 900 rpm. Three components were well resolved within 1 1/2 hours with partition efficiencies of 6,300 TP for the first peak (La), 1,100 TP for the second peak (Pr) and 780 TP for the third peak (Nd). These figures represent over one order of magnitude greater

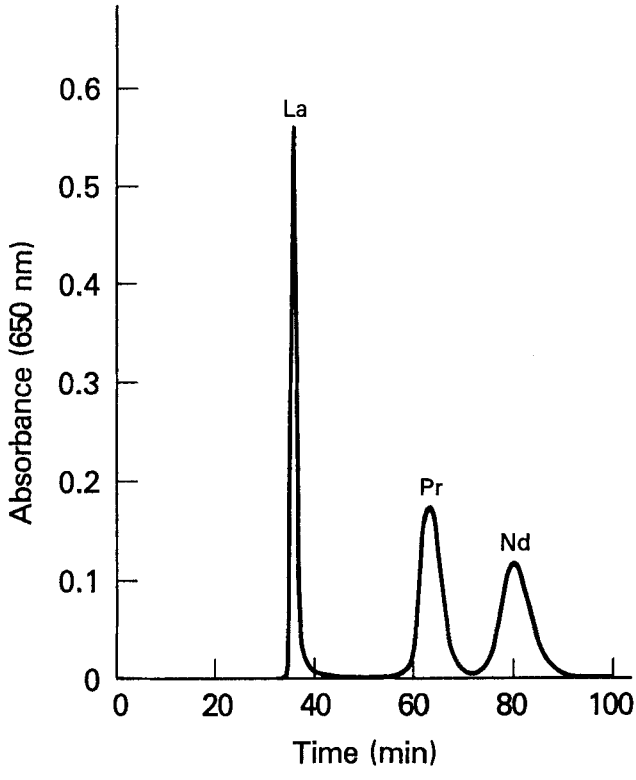

FIGURE 4. High-speed CCC separation of rare earth elements with semianalytical multilayer coils. SF:solvent front. For experimental conditions, see Table 1.

than those obtained by the centrifugal partition chromatograph with the same solvent system (9). The peak resolutions, R_S, computed from Eq 2 are 5.83 between the first and the second peaks and 1.82 between the second and the third peaks. The complete separation ($R_S \geq 1.5$) between Pr and Nd, which was difficult even at the raised ambient temperature of 55°C with a modified ligand (2-ethylhexyl phosphonic acid mono-2-ethylhexyl ester) (10), was

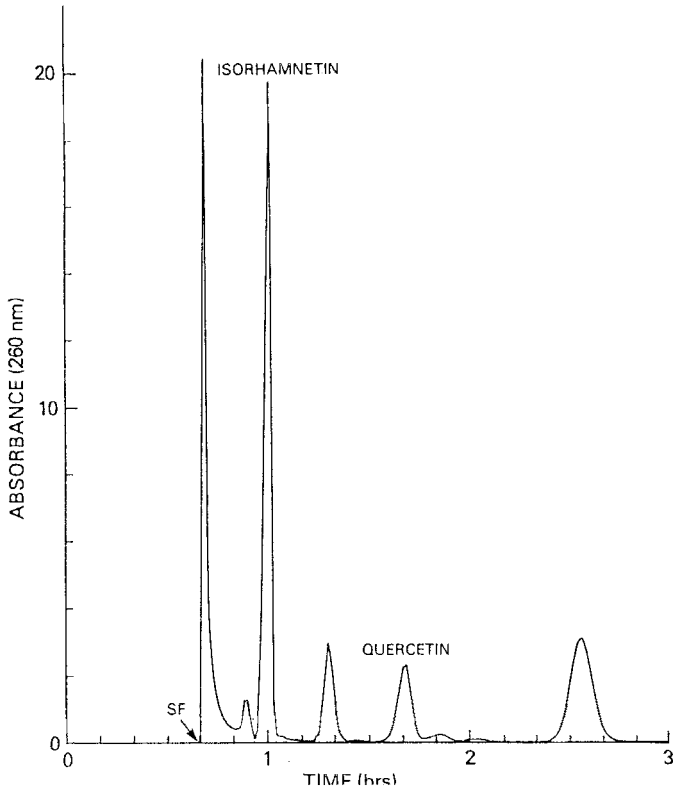

FIGURE 5. High-speed CCC separation of flavonoids with semianalytical multilayer coils. SF:solvent front. For experimental conditions, see Table 1.

achieved at room temperature in a much shorter elution time. A more detailed description for the procedure and the separations of other lanthanoid elements with the present apparatus will be reported elsewhere (8,11).

Separation of Natural Products

1) Flavonoids from sea buckthorn extract: Fig. 5 shows a chromatogram of flavonoids from a crude ethanol extract of sea

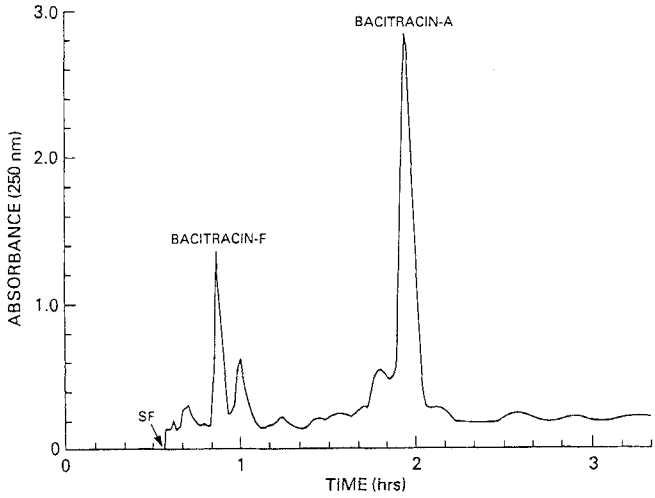

FIGURE 6. High-speed CCC separation of bacitracin with semianalytical multilayer coils. SF:solvent front. For experimental conditions, see Table 1.

buckthorn (Hippophae rhamnoides) obtained with a two-phase solvent system composed of chloroform-methanol-water (4:3:2, v/v/v). Separation was performed with the lower nonaqueous phase eluted at 180 ml/h under a revolutional speed of 1,200 rpm. A 100 mg quantity of the sample dissolved in 4.8 ml of the solvent mixture was separated into multiple peaks within 3 hours. The partition efficiency of the second peak (isorhamnetin) is 4,000 TP and that of the fourth peak (quercetin), 2,800 TP. This result shows a considerable improvement in peak resolution over those obtained from the 1.6 mm I.D. column (5) and also with other CCC instruments (12).

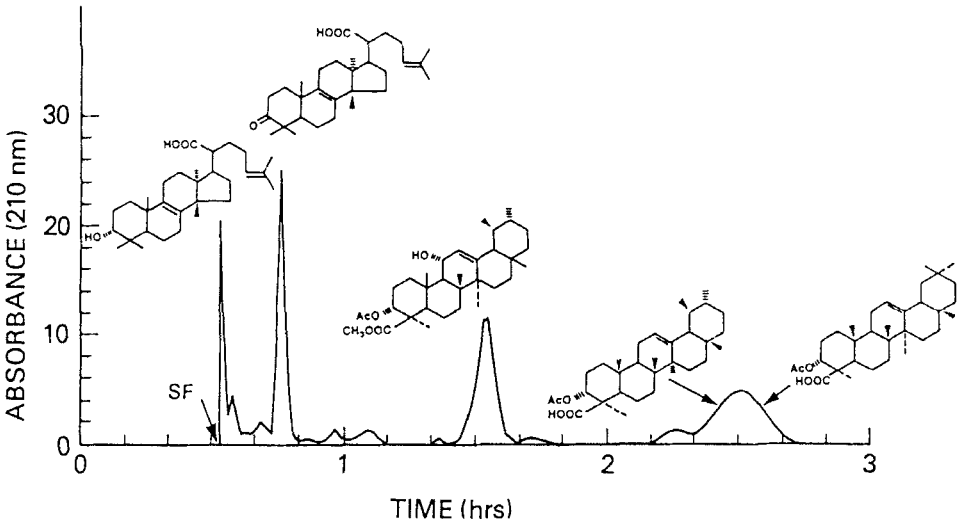

FIGURE 7. High-speed CCC separation of triterpenoic acids with semianalytical multilayer coils. SF:solvent front. For experimental conditions, see Table 1.

2) Bacitracin (BC): Commercial BC consists of a group of peptides with a bacteriocidic activity and is currently used as feed additives for livestock throughout the world. It contains the major active component, BC-A, its oxidation product, BC-F and over 20 other minor components with unknown nature, as shown by a reversed-phase HPLC analysis (13).

Fig. 6 shows a CCC separation of BC with a two-phase solvent system composed of chloroform-95% ethanol-water (5:4:3, v/v/v). The lower nonaqueous phase was used as the mobile phase at a flow rate of 150 ml/h at 1,200 rpm. A 100 mg sample quantity dissolved in 4.8 ml of the phase mixture was separated into multiple peaks.

Partition efficiency of the major peak (BC-A) is 2,900 TP and that of the second peak (BC-F), 2,700 TP.

3) Triterpenoic acids: Fig. 7 shows a high-speed countercurrent chromatogram of triterpenoic acids, extracted from Boswellia carterii, with a two-phase solvent system composed of n-hexane-95% ethanol-water (6:5:1, v/v/v). Separation was performed with the lower aqueous mobile phase being eluted at a flow rate of 150 ml/h and at a revolutional speed of 1,200 rpm. A crude extract (100 mg) dissolved in 2 ml of the upper nonaqueous phase was separated into multiple peaks. A similar chromatogram was obtained previously with a 1.6 mm I.D. column (5). Partition efficiencies of the second and third peaks were both 2,100 TP. As revealed by HPLC analysis of the fractions, the fourth peak contains a mixture of two isomers which were only partially resolved under the present CCC condition.

The overall results of the present studies clearly demonstrate the high performance capabilities of the present system. Sample quantities ranging from submilligram to 100 mg were successfully separated within a few hours. The new system regularly provides partition efficiencies up to several thousand theoretical plates. We believe that the present high-speed CCC apparatus can be a convenient tool for separations of natural and synthetic products and inorganic elements in research laboratories.

REFERENCES

1. Ito, Y, High-speed countercurrent chromatography, CRC Crit. Rev. Anal. Chem., 17, 65, 1986.

2. Mandava, N. B. and Ito, Y., Editors, Countercurrent Chromatography, Application of Countercurrent Chromatography, Marcel Dekker, New York, 1988, p. 781.

3. Ito, Y., Sandlin, J., and Bowers, W. G., High-speed preparative countercurrent chromatography (CCC) with a coil planet centrifuge, J. Chromatogr., 244, 247, 1982.

4. Ito, Y., Oka, H. and Slemp, J. L., Improved high-speed countercurrent chromatograph with three multilayer coils connected in series. Part I. Design of the apparatus and performance of semipreparative columns in DNP amino acid separation, J. Chromatogr., 475, 229, 1989.

5. Ito, Y., Oka, H. and Lee, Y.-W., Improved high-speed countercurrent chromatograph with three multilayer coils connected in series. Part II. Separation of various biological samples with a semipreparative column, J. Chromatogr., in press.

6. Barkley, D. J., Blanchette, M., Cassidy, R. M. and Elchuk, S., Dynamic chromatographic systems for the determination of rare earths and thorium in samples from uranium ore refining processes, Anal. Chem., 58, 2222, 1986.

7. Ito, Y. and Bowman, R. L., Countercurrent chromatography: Liquid-liquid partition chromatography without solid support, Science, 167, 281, 1970.

8. Kitazume, E., Bhatnagar, M. and Ito, Y., Separation of rare earth elements by high-speed countercurrent chromatography, Abstract submitted for 1990 Pittsburgh Conference and Exposition on Analytical Chemistry and Applied Spectroscopy at New York, 1990.

9. Araki, Y., Okazawa, T., Kubo, Y., Ando, H. and Asai, H., Separation of lighter rare earth metal ions by centrifugal countercurrent type chromatography with di-(2-ethylhexyl) phosphoric acid, J. Liq. Chromatogr., 11, 267, 1988.

10. Akiba, K., Sawai, S., Nakamura, S. and Murayama, W., Mutual separation of lanthanoid elements by centrifugal partition chromatography, J. Liq. Chromatogr., 11, 2517, 1988.

11. Kitazume, E., Bhatnagar, M. and Ito, Y., Separation of rare earth elements by high-speed countercurrent chromatography, J. Chromatogr., in preparation.

12. Zhang, T. Y., Xiao, R., Xiao, Z. Y., Pannell, L. K. and Ito, Y., Rapid separation of flavonoids by analytical high-speed countercurrent chromatography, J. Chromatogr., 445, 119 1988.

13. Oka, H., Ikai, Y., Kawamura, N., Yamada, M., Harada, K., Yamazaki, Y. and Suzuki, M., Improvement of chemical analysis of antibiotics XV. Isocratic high-performance liquid chromatographic methods for the analysis and preparative separation of the components of bacitracin, J. Chromatogr., 462, 315, 1989.