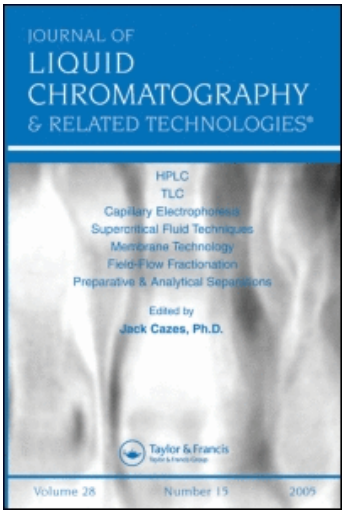

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Partition Efficiencies of an Eccentric Coiled Separation Column for Centrifugal Partition Chromatography

Jun-ichi Ikehata[a]; Kazufusa Shinomiya[a]; Yoichiro Ito[b]
[a] College of Pharmacy, Nihon University, Chiba, Japan [b] Center of Biochemistry and Biophysics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

To cite this Article Ikehata, Jun-ichi , Shinomiya, Kazufusa and Ito, Yoichiro(2005) 'Partition Efficiencies of an Eccentric Coiled Separation Column for Centrifugal Partition Chromatography', Journal of Liquid Chromatography & Related Technologies, 28: 17, 2807 — 2818
To link to this Article: DOI: 10.1080/10826070500225275
URL: http://dx.doi.org/10.1080/10826070500225275

Journal of Liquid Chromatography & Related Technologies®, 28: 2807–2818, 2005

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070500225275

Partition Efficiencies of an Eccentric Coiled Separation Column for Centrifugal Partition Chromatography

Jun-ichi Ikehata and Kazufusa Shinomiya
College of Pharmacy, Nihon University, Chiba, Japan

Yoichiro Ito
Center of Biochemistry and Biophysics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

Abstract: The partition efficiency of an eccentric coiled separation column, newly fabricated in our laboratory, was studied using centrifugal partition chromatography (CPC) with four different two-phase solvent systems including *n*-hexane-acetonitrile; *tert*-butyl methyl ether-aqueous 0.1% trifluoroacetic acid (1 : 1); 1-butanol-water (1 : 1); and 12.5% (w/w) polyethylene glycol 1000–12.5% (w/w) dibasic potassium phosphate. Each separation was performed by eluting either the upper phase in the ascending mode or the lower phase in the descending mode, each in clockwise and counterclockwise (CCW) column rotation. Better partition efficiencies were attained by the CCW rotation in both mobile phases in all the two-phase solvent systems examined.

The overall results demonstrated that the coiled separation column yielded substantially higher partition efficiencies than the commercial disk column. The eccentric coiled separation column is useful for the separation by CPC.

Keywords: Centrifugal partition chromatography, Efficiency, Eccentric coiled column

INTRODUCTION

Countercurrent chromatography (CCC) is a separation method based on the partition of solutes between two immiscible liquid phases. The absence of

Address correspondence to Dr. Kazufusa Shinomiya, College of Pharmacy, Nihon University, 7-7-1, Narashinodai, Funabashi-shi, Chiba, 274-8555, Japan. E-mail: kshino@pha.nihon-u.ac.jp

solid support eliminates various complications, such as loss of samples by adsorption and chemical degradation of compounds. Centrifugal partition chromatography (CPC) was introduced by Murayama et. al.[1] as one of the preparative CCC systems. In general, the existing CCC systems may be classified into two groups according to the mode of solute partitioning. One is called the hydrostatic equilibrium system (HSES) and the other is called the hydrodynamic equilibrium system (HDES). CPC belongs to HSES as does droplet CCC, whereas the high-speed CCC is the most advanced form of HDES, which has been widely used for the separation and purification of natural products. In HSES, the solute partitioning is carried out under a strong centrifugal force field where the mixing of the two phases is entirely relied on the flow pattern of the mobile phase in the separation column.

Recently, Ito et. al. reported the effect of the Coriolis force on the partition efficiency in the toroidal coil CCC centrifuge, where the sharpness of the elution peak varied according to the direction of the coil rotation.[2–4] However, in their studies, this effect was observed only in the protein separation with a viscous aqueous-aqueous polymer phase system, while no measurable effect was detected in the separation of dipeptides with conventional organic-aqueous two-phase solvent systems. In our previous studies,[5] the effect of the Coriolis force on partition efficiency and stationary phase retention in CPC were further investigated by changing the direction of column rotation. The overall result of the experiments revealed that the Coriolis force clearly affects the CPC separation in four different two-phase solvent systems, including organic-aqueous and aqueous-aqueous two-phase solvent systems. During these studies, we found that the commercial disk column failed to retain the organic stationary phase of the 1-butanol-water solvent system, which is useful for separation of polar compounds such as sugars[6] and water-soluble carboxylic acids.[7] In the present paper, we describe the performance of our eccentric coiled separation column which solved the above problem.

EXPERIMENTAL

Apparatus

The CPC apparatus used in the present study was obtained from Senshu Scientifc Co. (Tokyo, Japan) as a commercial unit originally fabricated by Sanki Engineering Ltd. (Kyoto, Japan). The eccentric coiled column for CPC was prepared by winding a 1 mm ID PTFE (polytetrafluoroethylene) tubing (Flon Kogyo, Tokyo, Japan) onto 8 cm long, 5 mm OD nylon pipes, forming a series of tight left-handed coils. A set of 42 coil units was symmetrically arranged around the holder hub of 12 cm diameter, in such a way that the axis of each coil unit is parallel to the central axis of the centrifuge. Figure 1 illustrates the schematic drawing of the eccentric coiled column newly fabricated in our laboratory. The total column capacity is 29.0 mL.

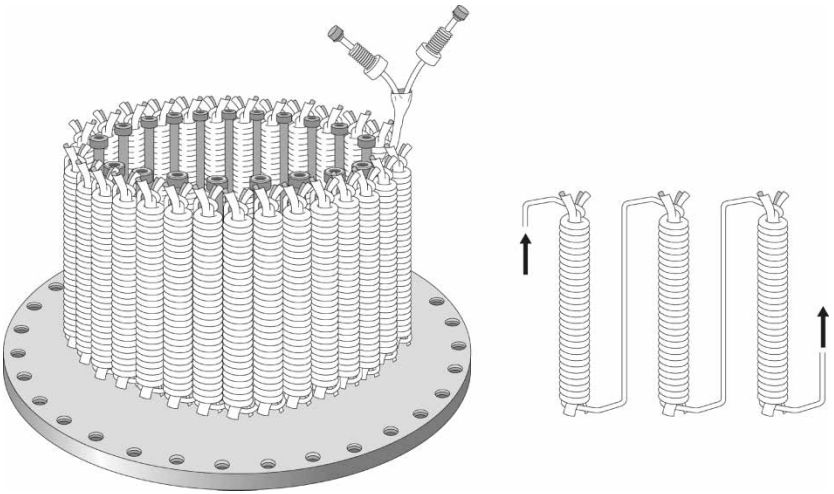

Figure 1. Schematic drawing of the eccentric coiled column for CPC newly fabricated in our laboratory.

Reagents

Hippuric acid (HA), *p*-amino-HA, *p*-methyl-HA, benzoic acid, tartaric acid, succinic acid, fumaric acid, and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) were purchased from Wako Pure Chemicals (Osaka, Japan). 2-Nitrophenylhydrazine hydrochloride (ONPH) was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Polyethylene glycol (PEG) 1000 (M.W. 1000), cytochrome C (horse heart), myoglobin (horse skeletal muscle), and lysozyme (chicken egg) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of reagent grade.

Preparation of Two-Phase Solvent Systems and Sample Solutions

We selected four two-phase solvent systems with a broad range in polarity. They are in increasing order of polarity: (I) *n*-hexane-acetonitrile (ACN); (II) *tert*-butyl methyl ether (MBE)-aqueous 0.1% trifluoroacetic acid (TFA);[8] (III) 1-butanol (1-BuOH)-water;[7] and (IV) 12.5% (w/w) PEG 1000–12.5% (w/w) dibasic potassium phosphate.

A set of test samples was selected for each two-phase solvent system according to their polarity. Each solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases separated after two clear layers formed. Sample solutions were prepared by dissolving each sample mixture in aliquots consisting of equal volumes of each phase of the two-phase solvent system used for separation. Test samples used for each two-phase solvent system are given in Table 1.

Table 1. Experimental conditions for separation by centrifugal partition chromatography with coiled column using four different kinds of two-phase solvent systems

Solvent system	Compound	Sample (mg)		Revolution speed (rpm)	Flow rate (mL/min)	Fractionate (mL/tube)	Detection (nm)
		LP mobile	UP mobile				
I. *n*-Hexane/ACN	2-Naphthalenesulfonic acid	0.5		1200	0.2	0.4	254
	Anthrone	0.5					
	Naphthalene	0.6	1.0				
	Acenaphthene	2.0	2.0				
	n-Dodecylbenzene		0.05 (mL)				
II. MBE/aqueous 0.1% TFA (1 : 1)	*p*-Amino hippuric acid	1.0		700	0.2	0.4	254
	Hippuric acid	1.5	1.5				
	p-Methyl hippuric acid	1.5	1.0				
	Benzoic acid		1.0				
III. 1-BuOH/H_2O	Tartaric acid	2.0		800	0.2	0.4	530 (EDC-ONPH method)
	Succinic acid	3.0					
	Fumaric acid	5.0					
IV. 12.5% (w/w) PEG 1000/12.5%(w/w) K_2HPO_4	Cytochrome C	2.0		1500	0.3	0.6	280
	Myoglobin	8.0	8.0				
	Lysozyme	10.0	3.0				

Abbreviations: LP = Lower phase; UP = Upper phase; ACN = Acetonitrile; MBE = t-Butyl methyl ether; TFA = Trifluoroacetic acid; PEG = Polyethylene glycol.

Separation Procedure

Each separation was initiated by completely filling the column with the stationary phase, followed by injection of the sample solution into the column inlet. Then, the mobile phase was pumped into the column using a reciprocating pump (Model LC-10ADVP, Shimadzu Corporation, Kyoto, Japan), while the coiled separation column was rotated at a desirable rotation speed. The effluent from the outlet was collected into test tubes using a fraction collector (Model SF-160, Advantec, Tokyo, Japan). The direction of the rotation was defined from the overview of the apparatus.

Analysis of Fractions

Each collected fraction was diluted with aliquots of desirable solvent and the absorbance was measured using a spectrophotometer (Model UV-1600, Shimadzu Corporation).

For the detection of polar carboxylic acid, a 100 μL of each fraction was separated and evaporated by a centrifugal evaporator (Model CVE-3100, Tokyo Rikakikai, Tokyo, Japan), and then redissolved in 2 mL of water. This solution was subjected to the specific color reaction (EDC-ONPH method) as follows:[9] Two milliliters of the sample solution was mixed with 1 mL each of 0.01 M ONPH in 0.4 M HCl and 0.15 M EDC in 4% (v/v) pyridine. The solution was incubated at 40°C for 30 min, mixed with 1 mL of 1.5 M sodium hydroxide solution, and again incubated at 60°C for 15 min to maximize color development. The absorbance was measured at 530 nm using a spectrophotometer.

RESULTS AND DISCUSSION

Figure 2 illustrates a set of CCC chromatograms obtained by CPC, using four different two-phase solvent systems by eluting the lower phase in a descending mode. The two-phase solvent systems and experimental conditions applied in these separations are summarized in Table 1. For each solvent system, the separations were performed by rotating the coiled separation column in both clockwise (CW; top row) and counterclockwise (CCW; bottom row) directions, under otherwise identical conditions. In these chromatograms, it was found that the separation between tartaric acid, succinic acid, and fumaric acid was successfully achieved using the 1-butanol-water system, which did not retain in the commercial disk column.

Table 2 summarizes the analytical data computed from the chromatograms. In the peak resolution, theoretical plate numbers, and stationary phase retention, the CCW rotation yielded somewhat higher values compared with the CW rotation in polar solvent systems.

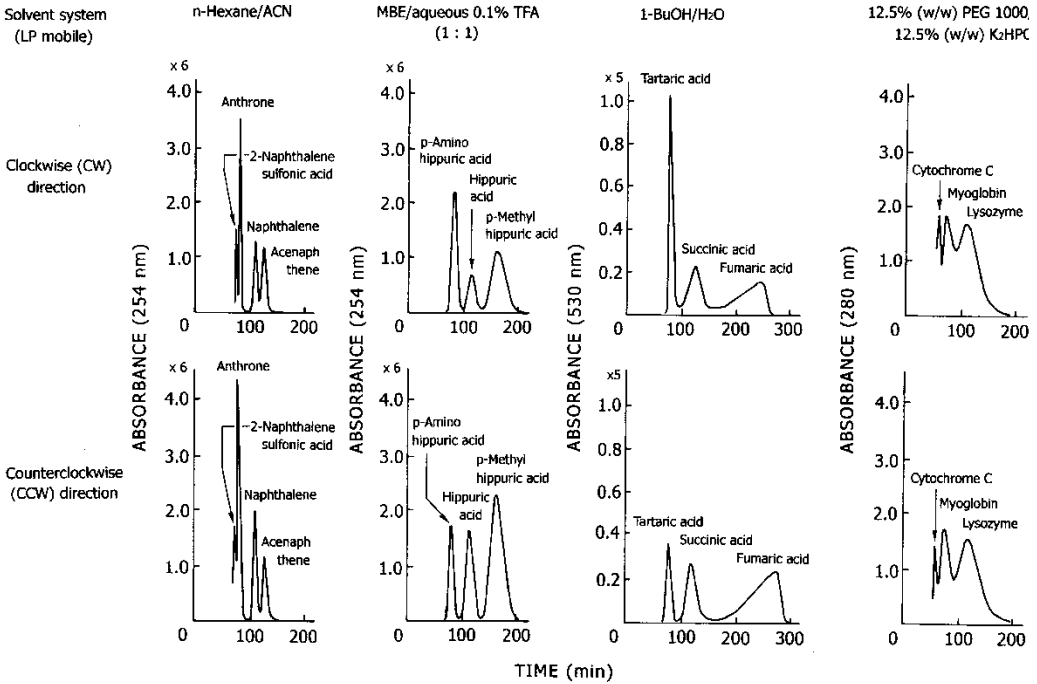

Figure 2. Chromatograms obtained by CPC using four different two-phase solvent systems by eluting the lower phase in the descending mode. Experimental conditions are summarized in Table 1.

Table 2. Analytical values obtained by CPC using four different two-phase solvent systems with lower phase mobile of descending mode

Solvent system	Compound	Retention volume (mL) (CW/CCW)	Partition coefficient (K)[a] (CW/CCW)	Resolution factor (Rs) (CW/CCW)	Theoretical plate number (N) (CW/CCW)	Retention of stationary phase (%) (CW/CCW)
I. *n*-Hexane/ACN	2-Naphthalenesulfonic acid	15.2/14.8	0.10/0.12	0.5/0.7	924/389	
	Anthrone	16.2/16.6	0.17/0.24	2.2/2.1	1296/765	38.6/40.0
	Naphthalene	21.8/22.4	0.53/0.59	0.9/1.5	742/784	
	Acenaphthene	25.0/27.6	0.74/0.91		625/940	
II. MBE/aqueous 0.1%	*p*-Amino hippuric acid	17.2/16.6	0.17/0.20	1.1/1.3	296/340	
TFA (1: 1)	Hippuric acid	22.6/23.0	0.55/0.61	1.2/1.2	227/220	39.6/39.6
	p-Methyl hippuric acid	32.6/33.0	1.25/1.26		146/144	
III. 1-BuOH/H_2O	Tartaric acid	16.0/15.4	0.20/0.18	1.4/1.3	455/196	
	Succinic acid	25.2/23.6	0.77/0.68	1.6/2.1	102/139	45.2/43.0
	Fumaric acid	50.0/55.6	2.30/2.60		89/99	
IV. 12.5% (w/w) PEG	Cytochrome C	18.3/17.1	0.20/0.11	0.3/0.7	165/203	
1000/12.5%(w/w)	Myoglobin	22.2/22.2	0.49/0.49	0.5/0.7	24/50	30.8/31.0
K_2HPO_4	Lysozyme	33.3/35.4	1.32/1.48		22/31	

[a]Partition coefficents were calculated according to the conventional formula: K = (Vr−Vsf)/(Vc−Vsf), where Vr, Vc and Vsf indicate the retention volume of the solute peak, the column capacity and the retention volume of the solvent front, respectively. Abbreviations: CW = Clockwise direction of rotation; CCW = Counterclockwise direction.

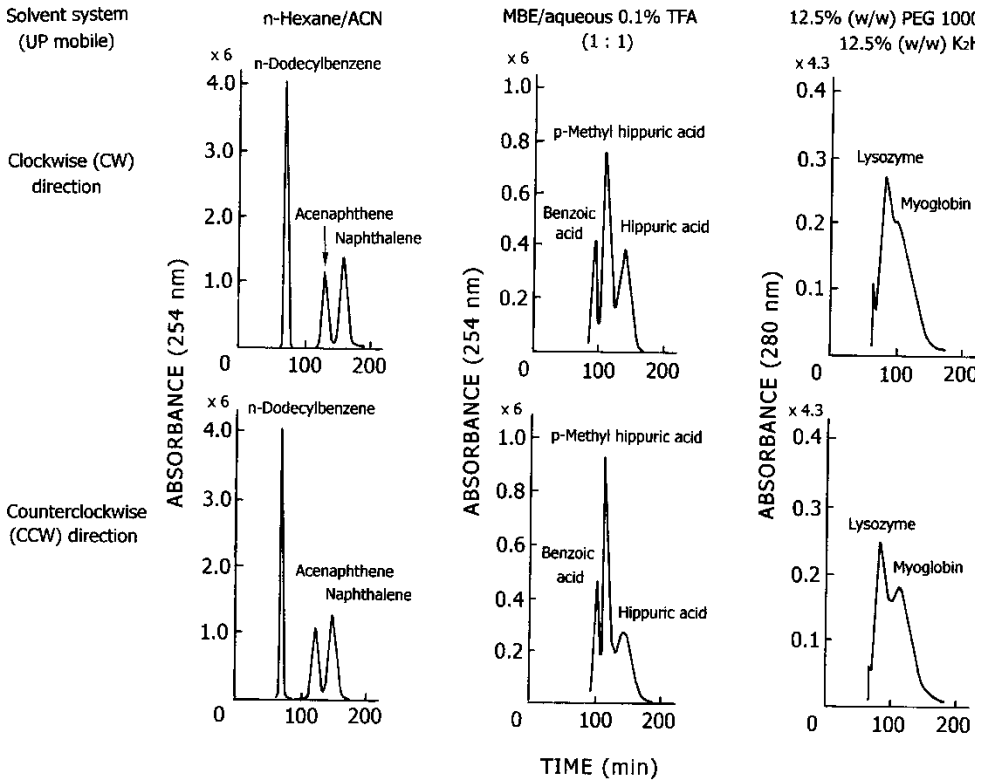

Figure 3. Chromatograms obtained by CPC using three different two-phase solvent systems by eluting the upper phase in the ascending mode. Experimental conditions are summarized in Table 1.

Table 3. Analytical values obtained by CPC using three different two-phase solvent systems with upper phase mobile of ascending mode

Solvent system (%)	Compound	Retention volume (mL) (CW/CCW)	Partition coefficient (K)[a] (CW/CCW)	Resolution factor (Rs) (CW/CCW)	Theoretical plate number (N) (CW/CCW)	Retention of stationary phase (CW/CCW)
I. *n*-Hexane/ACN	*n*-Dodecylbenzene	14.8/13.6	0.08/0.03	3.3/3.3	447/913	
	Acenaphthene	26.0/24.0	0.81/0.68	1.3/1.0	676/435	43.4/39.6
	Naphthalene	32.0/29.2	1.20/1.01		522/468	
II. MBE/aqueous 0.1% TFA (1 : 1)	Benzoic acid	18.8/20.8	0.16/0.20	0.7/0.6	489/358	
	p-Methyl hippuric acid	22.0/23.2	0.43/0.43	0.8/1.0	230/538	23.0/35.2
	Hippuric acid	28.0/28.6	0.92/0.96		142/97	
III. 12.5% (w/w) PEG 1000/12.5%(w/w) K_2HPO_4	Lysozyme	25.2/25.2	0.67/0.64	0.2/0.5	25/43	24.6/27.9
	Myoglobin	30.3/34.2	1.11/1.50		24/36	

[a]Partition coefficents were calculated according to the conventional formula: $K = (Vr - Vsf)/(Vc - Vsf)$, where Vr, Vc and Vsf indicate the retention volume of the solute peak, the column capacity and the retention volume of the solvent front, respectively. Abbreviations: CW = Clockwise direction of rotation; CCW = Counterclockwise direction.

Table 4. Comparison of partition efficiencies between coiled column and commercial disk column on CPC with lower phase mobile

Two-phase solvent system	Sample	Theoretical plate number per 1 mL of column capacity (N/mL)	
		Coiled column (CW/CCW)	Commercial disk column (CW/CCW)[a]
I. *n*-Hexane/ACN	2-Naphthalenesulfonic acid	31.9/13.4	2.3/4.2
	Anthrone	44.7/26.4	3.6/1.4
	Naphthalene	25.6/27.0	3.8/5.7
	Acenaphthene	21.6/32.4	2.6/0.9
II. MBE/aqueous 0.1% TFA (1 : 1)	*p*-Amino hippuric acid	10.2/11.7	3.5/0.4
	Hippuric acid	7.8/7.6	0.6/0.4
	p-Methyl hippuric acid	5.0/5.0	0.4/0.4
III. 12.5% (w/w) PEG1000/12.5% (w/w) K_2HPO_4	Cytochrome C	5.7/7.0	2.3/26.9
	Myoglobin	0.8/1.7	0.6/0.3
	Lysozyme	0.8/1.1	0.3/0.2

[a]The values of theoretical plate number of commercial disk column were calculated from our previous experimental data (J.-I. Ikehata et al., J. Chromatogr. A, 1025, 169,2004). Abbreviations: CW = Clockwise direction; CCW = Counterclockwise direction.

Table 5. Comparison of partition efficiencies between coiled column and commercial disk column on CPC with upper phase mobile

Two-phase solvent system	Sample	Theoretical plate number per 1 mL of column capacity (N/mL)	
		Coiled column (CW/CCW)	Commercial disk column (CW/CCW)[a]
I. *n*-Hexane/AcN	*n*-Dodecyl benzene	15.4/31.5	0.9/0.8
	Acenaphthene	23.3/15.0	2.7/2.4
	Naphthalene	18.0/16.1	2.5/2.9
II. MBE/aqueous 0.1% TFA (1 : 1)	Benzoic acid	16.9/12.3	2.0/1.0
	p-Methyl hippuric acid	7.9/18.6	0.9/0.2
	Hippuric acid	4.9/3.3	0.7/0.3
III. 12.5% (w/w) PEG1000/12.5% (w/w) K_2HPO_4	Lysozyme	0.9/1.5	0.7/0.1
	Myoglobin	0.8/1.2	0.2/0.1

[a]The values of theoretical plate number of commercial disk column were calculated from our previous experimental data (J.-I. Ikehata et al., J. Chromatogr. A, 1025, 169, 2004). Abbreviations: CW = Clockwise direction; CCW = Counterclockwise direction.

Figure 3 similarly illustrates a set of CCC chromatograms obtained from three different solvent systems by eluting the upper phase in an ascending mode. As shown in Table 3, most of data obtained by the CCW column rotation show slightly higher efficiency than that obtained from the CW rotation for polar solvent systems, as in the case of the lower phase mobile. Higher stationary phase retention was also obtained in the CCW rotation than in the CW rotation. These results suggest that the Coriolis force affects the partition efficiency and retention of stationary phase for polar solvent systems using the eccentric coiled column by CPC.

The partition efficiencies were also compared between the coiled column and the commercial disk column shown in the previous study.[5] As summarized in Tables 4 and 5, each value of theoretical plate number per 1 mL of column capacity obtained by the coiled column, was much higher than that obtained by the commercial disk column. The overall results demonstrated that the eccentric coiled column can be useful for the separation by CPC with all two-phase solvent systems examined, including the 1-butanol-water system.

CONCLUSION

The partition efficiency of an eccentric coiled column newly fabricated in our laboratory was investigated using the CPC with four different types of two-phase solvent systems. By changing the direction of column rotation, the CCW rotation yielded somewhat higher partition efficiency and stationary phase retention, especially in the polar solvent systems. The hydrophilic 1-butanol-water system was also applicable to the separation for the coiled separation column, while the commercial disk column fails to retain this solvent system. The overall results demonstrated that the eccentric coiled column yields substantially high partition efficiencies than the original disk column, when compared in theoretical plate numbers per unit capacity.

ACKNOWLEDGMENTS

The authors would like to thank Mr. Haruo Sasaki (Machining Technology Center, College of Science and Technology, Nihon University, Chiba, Japan) for the electric adjustment of CPC apparatus, and Mr. Kazuyoshi Yasuno (Senshu Scientific Co., Tokyo, Japan) for his helpful advice.

This work was supported in part by grants from both of the Ministry of Education, Culture, Sports, Science, and Technology of Japan and College of Pharmacy, Nihon University, Chiba, Japan.

REFERENCES

1. Murayama, W.; Kobayashi, T.; Kosuge, Y.; Yano, H.; Nunogaki, Y.; Nunogaki, K. A new centrifugal counter-current chromatograph and its application. J. Chromatogr. **1982**, *239*, 643.
2. Ito, Y.; Ma, Y. Effects of Coriolis force on countercurrent chromatography. J. Liq. Chromatogr. & Rel. Technol. **1998**, *21*, 1.
3. Matsuda, K.; Matsuda, S.; Ito, Y. Toroidal coil counter-current chromatography. Achievement of high resolution by optimizing flow-rate, rotation speed, sample volume and tube length. J. Chromatogr. A **1998**, *808*, 95.
4. Ito, Y.; Matsuda, K.; Ma, Y.; Qi, L. Toroidal coil counter-current chromatography study of the mass transfer rate of proteins in aqueous-aqueous polymer phase system. J. Chromatogr. A **1998**, *802*, 277.
5. Ikehata, J.-I.; Shinomiya, K.; Kobayashi, K.; Ohshima, H.; Kitanaka, S.; Ito, Y. Effect of Coriolis force on counter-current chromatographic separation by centrifugal partition chromatography. J. Chromatogr. A **2004**, *1025*, 169.
6. Shinomiya, K.; Kabasawa, Y.; Ito, Y. Countercurrent chromatographic separation of sugars and their *p*-nitrophenyl derivatives by cross-axis coil planet centrifuge. J. Liq. Chromatogr. & Rel. Technol. **1999**, *22*, 579.
7. Shinomiya, K.; Kabasawa, Y.; Ito, Y. Countercurrent chromatographic separation of biotic dicarboxylic acids with polar two-phase solvent systems using cross-axis coil planet centrifuge. J. Liq. Chromatogr. & Rel. Technol. **2001**, *24*, 2625.
8. Shinomiya, K.; Sasaki, Y.; Shibusawa, Y.; Kishinami, K.; Kabasawa, Y.; Ito, Y. Countercurrent chromatographic separation of hippuric acid and related compounds using cross-axis coil planet centrifuge with eccentric coil assemblies. J. Liq. Chromatogr. & Rel. Technol. **2000**, *23*, 1575.
9. Shinomiya, K.; Ochiai, H.; Suzuki, H.; Koshiishi, I.; Imanari, T. Simple method for determination of urinary mucopolysaccharides. Bunseki Kagaku **1986**, *35*, T29.

Received February 15, 2005
Accepted March 16, 2005
Manuscript 6612