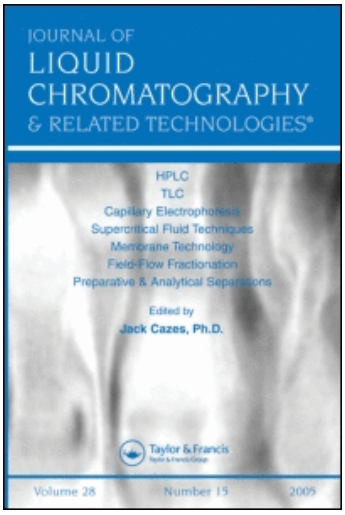


Journal of Liquid Chromatography & Related Technologies

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

Novel Design for Centrifugal Countercurrent Chromatography: I. Zigzag Toroidal Column

Yi Yang[abc]; Haji Akber Aisa[b]; Yoichiro Ito[a]

[a] Bioseparation Technology Laboratory, Biochemistry and Biophysics Center, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA [b] Xinjiang Key Laboratory of Plant Resources and Natural Products Chemistry, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumqi, P. R. China [c] Graduate University of the Chinese Academy of Sciences, Beijing, P. R. China

To cite this Article Yang, Yi , Aisa, Haji Akber and Ito, Yoichiro(2009) 'Novel Design for Centrifugal Countercurrent Chromatography: I. Zigzag Toroidal Column', Journal of Liquid Chromatography & Related Technologies, 32: 14, 2030 — 2042

To link to this Article: DOI: 10.1080/10826070903126856

URL: http://dx.doi.org/10.1080/10826070903126856

Journal of Liquid Chromatography & Related Technologies®, 32: 2030–2042, 2009

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070903126856

Novel Design for Centrifugal Countercurrent Chromatography: I. Zigzag Toroidal Column

Yi Yang,[1,2,3] Haji Akber Aisa,[2] and Yoichiro Ito[1]

[1]Bioseparation Technology Laboratory, Biochemistry and Biophysics Center, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA
[2]Xinjiang Key Laboratory of Plant Resources and Natural Products Chemistry, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumqi, P. R. China
[3]Graduate University of the Chinese Academy of Sciences, Beijing, P. R. China

Abstract: The toroidal coil using an equilateral triangular core has improved both retention of the stationary phase and peak resolution of the conventional toroidal coil in centrifugal countercurrent chromatography. To further improve the retention of stationary phase and peak resolution, a novel zigzag toroidal coil was designed and the performance of the system was evaluated at various flow rates. The results indicated that both retention of stationary phase and peak resolution were improved as the flow rate was decreased. Modification of the tubing by pressing at given intervals with a pair of pliers improved the peak resolution without increasing the column pressure. All these separations were performed under low column pressure indicating the separation can be further improved by increasing the column length and/or revolution speed without damaging the separation column.

Keywords: Countercurrent chromatography, Dipeptide, DNP-amino acid, Resolution, Retention of the stationary phase, Zigzag pattern column

Correspondence: Yoichiro Ito, Bioseparation Technology Laboratory, Biochemistry and Biophysics Center, National Heart, Lung, and Blood Institute, 10 Center Drive, Building 10, Room 8N230, Bethesda, MD 20892-1762, USA. E-mail: itoy@nhlbi.nih.gov

INTRODUCTION

High-speed countercurrent chromatography has been widely used for the separation and purification of natural products.[1–3] However, this hydrodynamic CCC system can not be efficiently applied to analytical separations, because the Archimedean screw effect is interfered with a strong cohesive force between liquid and the tube wall in a small diameter tubing resulting in loss of stationary phase from the column. This problem can be solved using a hydrostatic CCC system by arranging a narrow-bore coiled column around the periphery of the centrifuge bowl in a toroidal form in a seal-free centrifuge.[4] In this toroidal coil, the retention of the stationary phase is limited to considerably less than 50% of the total column capacity since the half a space of each helical turn is completely occupied with the stationary phase. In a typical separation, the retention of stationary phase is no more than 30% of the total column capacity and it sharply decreases with a higher flow rate of the mobile phase. In order to solve this problem, a triangular helical column has been introduced, where the dead space in each helical turn was reduced from 1/2 to 1/3 and the stationary phase retention was improved to

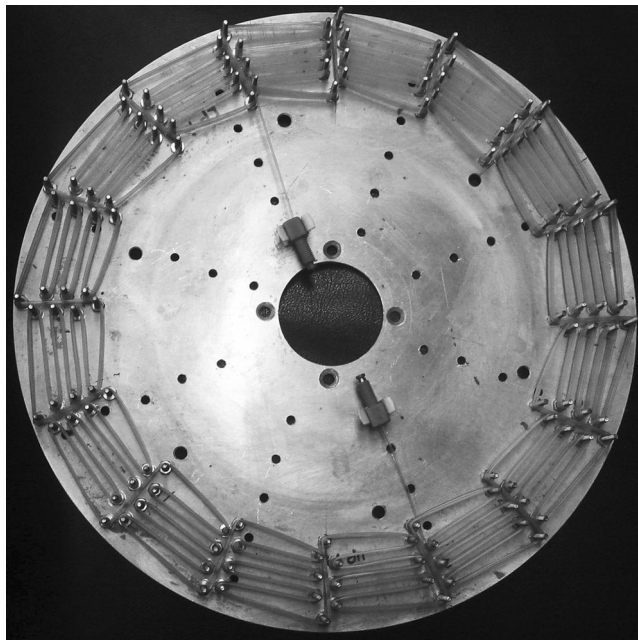


Figure 1. Design of the zigzag toroidal column for centrifugal countercurrent chromatography.

reach over 40%.[5] This experimental result clearly indicates that the retention of the stationary phase in the toroidal coil will be further improved by increasing the height of the triangular core and/or reducing the length of the side of triangle providing the dead space.

This paper introduces a novel configuration of the toroidal column with a zigzag pattern (Fig. 1). This design further improves the retention of stationary phase and peak resolution. The performance of this new configuration is demonstrated on the countercurrent chromatography separation of dipeptide and DNP-amino acid test samples using a rotary-seal-free continuous flow centrifuge system.

EXPERIMENTAL

Apparatus

The present study uses a rotary-seal-free centrifuge fabricated by Pharma-Tech Research Corporation, Baltimore, Maryland, USA. It holds an aluminum rotary plate measuring about 34 cm in diameter to hold a toroidal coil separation column. The column is made by hooking a 0.46 mm ID FEP (Fluorinated ethylene propylene, standard wall) (Zeus Industrial Products, Orangeburg, SC, USA) onto the screws fixed at the rotary plate making zigzag pattern with a total capacity of 7.8 mL. Each terminal of the toroidal column is connected with a set of tubing connectors (Upchurch Scientific, Palm Spring, CA, USA) as shown in Fig. 1. These flow tubes are put together and passed through the center of the central shaft downward and the hollow horizontal shaft of a miter gear, then led upward into the vertical hollow tube support, and finally exit the centrifuge from the center of the upper plate where they are tightly held with a pair of clamps.

Reagents

1-Butanol, hexane, ethyl acetate and methanol were purchased from Fisher Scientific, Fair Lawn, NJ, USA and other solvents such as acetic acid and hydrochloric acid from Mallinckrodt Chemicals, Phillipsburg, NJ, USA. Dipeptide samples including tryptophyl-tyrosine (trp-tyr), valyl-tyrosine (val-tyr) and N-2,4-dinitrophenyl-L-alanine (DNP-ala), N-2,4-dinitrophenyl-β-alanine (DNP-β-ala), N-2,4-dinitrophenyl-DL-Glutamic acid (DNP-glu) were obtained from Sigma Chemicals, St. Louis, MO, USA.

Two-Phase Solvent Systems and Sample Solutions

In the present study, two typical two-phase solvent systems including 1-butanol-acetic acid-water (4:1:5, v/v) and hexane-ethyl acetate-methanol-0.1 M HCl (1:1:1:1, v/v) were used to separate the dipeptide and DNP-amino acid test samples, respectively. Each solvent mixture was thoroughly equilibrated in a separatory funnel by vigorous shaking and degassing several times, and the phases separated shortly before use. The sample solution 1 was prepared by dissolving 25 mg of trp-tyr and 100 mg of val-tyr in 20 mL of the upper phase of 1-butanol-acetic acid-water, and 50 μL was charged in each run. And the sample solution 2 was prepared by dissolving 5.7 mg of DNP-ala, 7.1 mg of DNP-β-ala and 5.4 mg of DNP-glu in 10 mL of the upper phase of hexane-ethyl acetate-methanol-0.1 M HCl, and 50 μL was charged in each run.

Partition Coefficient Measurement

Partition coefficients ($K_{UP/LP}$) of each sample in the two-phase solvent system were determined using the conventional test tube method with a UV spectrophotometer (Genesis 10 UV, Thermo Spectronic, Rochester, NY, USA) at 280 nm as described elsewhere.[6] All K values are given in Table 1.

Separation Procedure

In each separation, the separation column was entirely filled with the stationary phase, either upper or lower phase, followed by sample injection, and the column was rotated at 1000 rpm while the mobile phase was pumped into the coiled column at a given flow rate (10 μL/min,

Table 1. Partition coefficient (K) of test samples in the two-phase solvent system

Two-phase solvent system (volume ratio)	Test sample	$(K_{UP/LP})^*$
1-buthanol-acetic acid-water (4:1:5)	Trp-Tyr	1.69
	Val-Tyr	0.53
Hexane-ethyl acetate-methanol-0.1 M HCl (1:1:1:1)	DNP-L-ala	2.36
	DNP-β-ala	1.18
	DNP-DL-glu	0.45

*K: Partition coefficient; UP: Upper phase; LP: Lower phase.

20 μL/min, 30 μL/min, 40 μL/min, 50 μL/min or 100 μL/min). The effluent from the outlet of the coiled column was continuously monitored with a Uvicord IIS (LKB, Stockholm, Sweden) at 280 nm and the elution curve was traced using a stripped-chart recorder (Pharmacia, Stockholm , Sweden). In order to improve the tracing, ethanol was mixed to the effluent at the inlet of the detector using a tee connector and a fine mixing tube (PTFE 0.4 mm ID × ca 1 m). After the desired peaks were eluted, the run was stopped and the column contents were collected into a graduated cylinder by pressured air to determine the volume of the stationary phase retained in the column. The retention of the stationary phase was computed by dividing the volume of the retained stationary phase with the total column volume.

Evaluation of Partition Efficiency

The parathion efficiency of separation column in each run was evaluated by computing theoretical plate number (N) for each peak and peak resolution (Rs) between the peaks using the following conventional equations:

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

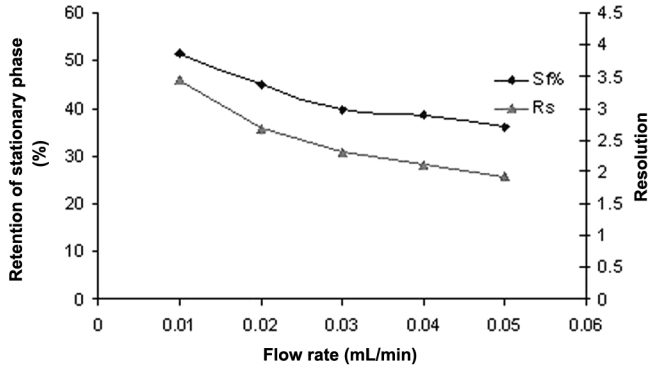
$$Rs = 2(R2 - R1)/(W1 + W2) \quad (2)$$

where R and W indicate the retention time and the peak width in Eq. (1) and those for the specified peaks in Eq. (2), respectively.

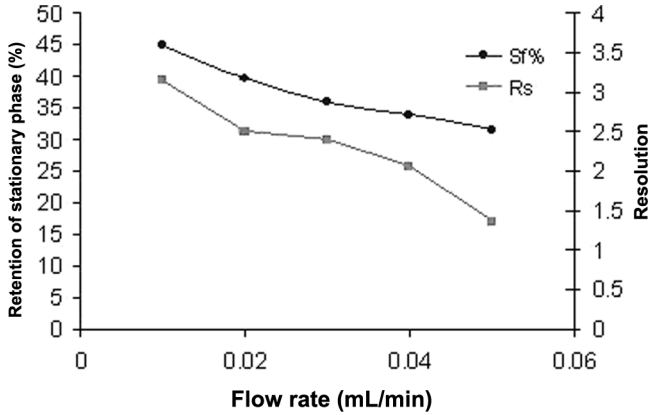
RESULTS AND DISCUSSION

As mentioned earlier, the traditional toroidal coil system has a problem of low retention of the stationary phase because one side of each helical turn is completely occupied by the mobile phase while the solute partition process is carried out in the other half. When the separation coil is wound onto a triangular core, the dead space is deceased and the efficient column space was increased from 1/2 to 2/3 of the total column space. Consequently, the retention of the stationary phase is much improved.[5] In order to further decrease the dead space and increase the peak resolution, a novel pattern of the column design, a zigzag toroidal column, was design as shown in Fig. 1. The photograph shows an aluminum rotary plate holding a zigzag toroidal coil separation column.

Figure 2 schematically illustrates the relationship between flow rate, retention of stationary phase and peak resolution in the zigzag column



(a)



(b)

Figure 2. Comparison of performance of various flow rates in dipeptide separation of zigzag toroidal column. (a) Lower mobile phase; (b) Upper mobile phase.

separation. When the lower phase was the mobile phase, the retention of stationary phase and peak resolution were decreased slowly with the increasing of flow rate (Fig. 2a), and similar results were obtained when the upper phase was the mobile phase (Fig. 2b). The experiments were performed on separation of dipeptides with high-polarity two-phase solvent system composed of 1-butanol-acetic acid-water at a volume ratio of 4:1:5. All of the stationary phase retention was over the 30%. The results of the separation of test samples in the zigzag column were summarized in Table 2. It should be noted that lower flow rate can yield higher retention of the stationary phase (Sf) and higher peak resolution (Rs). The results clearly showed that the lower mobile phase yielded higher retention of stationary phase and better peak resolution at the given flow rate than the upper mobile phase.

Table 2. Partition efficiency and stationary phase retention of zigzag toroidal column

No	Sample*	Solvent system**	Mobile phase	Flow rate (mL/min)	Retention of stationary phase (%)	Resolution	N***	Pressure (psi)
1	(1) TRP-TYR	BAW	Lower phase	0.05	35.89	1.93	$N_1 = 158.04$ $N_2 = 317.07$	17 to 92
2	(2) VAL-TYR			0.04	38.46	2.11	$N_1 = 175.49$ $N_2 = 320.22$	10 to 89
3				0.03	39.74	2.31	$N_1 = 187.45$ $N_2 = 366.68$	11 to 93
4				0.02	44.87	2.68	$N_1 = 249.87$ $N_2 = 473.06$	9 to 94
5				0.01	51.28	3.44	$N_1 = 318.95$ $N_2 = 532.54$	7 to 92
6			Upper phase	0.05	31.51	1.35	$N_1 = 324.00$ $N_2 = 170.63$	11 to 94
7				0.04	33.97	2.05	$N_1 = 700.21$ $N_2 = 316.59$	7 to 94
8				0.03	35.90	2.40	$N_1 = 667.12$ $N_2 = 303.17$	11 to 96
9				0.02	39.74	2.50	$N_1 = 707.56$ $N_2 = 330.03$	11 to 96
10				0.01	44.87	3.14	$N_1 = 1038.27$ $N_2 = 365.23$	7 to 96
11	(3) ALAN		Lower phase	0.10	38.46	$Rs_{3,4} = 1.71$ $Rs_{4,5} = 1.92$	$N_3 = 369.82$ $N_4 = 576.00$ $N_5 = 696.96$	18 to 158

(*Continued*)

Table 2. Continued

No	Sample*	Solvent system**	Mobile phase	Flow rate (mL/min)	Retention of stationary phase (%)	Resolution	N***	Pressure (psi)
12	(4) β-ALAN	HEMH		0.05	39.74	$Rs_{3,4} = 1.70$ $Rs_{4,5} = 1.96$	$N_3 = 393.50$ $N_4 = 509.89$ $N_5 = 557.86$	18 to 148
13	(5) GLU		Upper phase	0.10	43.59	$Rs_{3,4} = 1.04$ $Rs_{4,5} = 1.82$	$N_3 = 973.44$ $N_4 = 468.60$ $N_5 = 207.36$	18 to 166
14				0.05	46.15	$Rs_{3,4} = 1.09$ $Rs_{4,5} = 1.86$	$N_3 = 660.25$ $N_4 = 320.88$ $N_5 = 140.87$	18 to 163

Note. *TRP-TYR (K value = 1.69, UP/UL), VAL-TYR (K value = 0.53, UP/UL), ALAN: N-2,4-DNP-L-ALANINE (K value = 2.01, UP/UL), β-ALAN: N-2,4-DNP-β-ALANINE (K value = 1.18, UP/UL), GLU: N-2,4-DNP-DL-GLUTAMIC ACID (K value = 0.45, UP/UL).

**BMW: n-Butanol/Acetic acid/Water (4:1:5, v/v); HEMH: Hexane/EtOAc/MeOH/0.1 M HCl (1:1:1:1, v/v).

***N: Theoretical plate number.

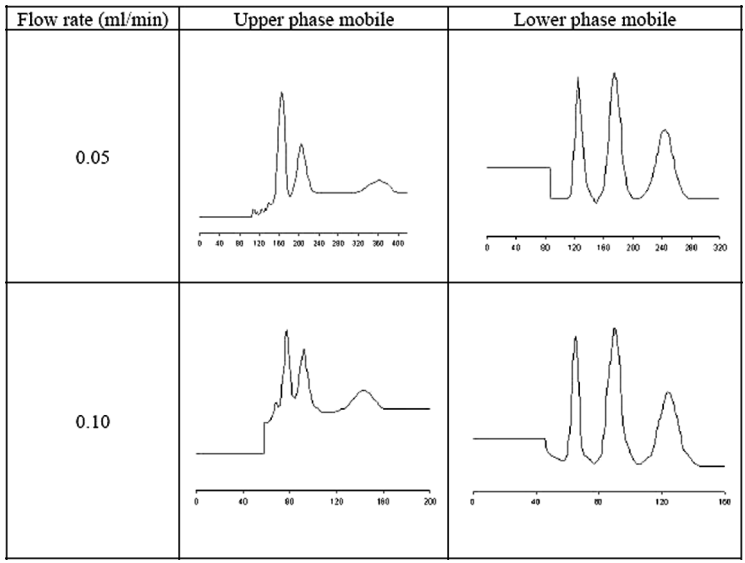


Figure 3. The performance of DNP-amino acids separation by zigzag toroidal column. (a) Lowe mobile phase; (b) Upper mobile phase.

Similar results were obtained in the moderately hydrophobic solvent system composed of hexane/ethyl acetate/methanol/0.1 M HCl (1:1:1:1, v/v) (Fig. 3 and Table 2). In this study on separation of three test samples, DNP-L-ala, DNP-β-ala, DNP-DL-glu, the lower mobile phase always yielded lower retention of stationary phase, but better peak resolution at all range of applied flow rates than those obtained by the upper mobile phase (Table 2).

In Fig. 4, chromatograms obtained from the right direction elution mode were compared with those obtained from the wrong direction elution modes in which the ratio between the effective column space and the dead space was reversed. When experiments were performed by eluting the upper or the lower phase in the right direction, the retention of stationary phase was at 31.51% and 35.89% with peak resolution at 1.35, 1.93, respectively. But when experiments were performed in the wrong direction elution mode, the retention stationary phase was reduced to 17.95% and 23.07% but with similar peak resolution of 1.29 and 1.94, respectively. The fact that the peak resolution in the wrong direction is very similar to the resolution in the right direction in spite of increasing of the stationary phase retention in the right direction may indicate that the long flow path between the stationary segments in the efficient column space may produce laminar flow which tends to spread the solute band.

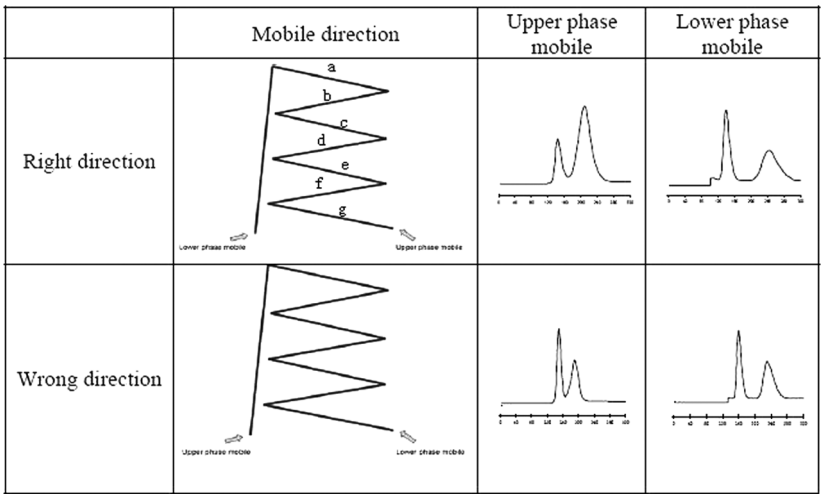


Figure 4. Comparison in the stationary phase retention and peak resolution in the zigzag toroidal column between four elution modes.

In our previous study, the retention of stationary phase and peak resolution was increased by changing the shape of the tube in the previous study.[7,8] In order to improve the peak resolution in the present study, we have modified the tubing in two different ways, i.e., compressing the tubing at given intervals and twisting the tubing after folding it into two to make a rope-like structure. Figure 5 shows the results of the experiments obtained from 1-butanol/acetic acid/water (4:1:5, v/v) using the four different types of zigzag columns (plain tube, middle-pressed tube, three-pressed tube and twisted tube). When the lower phase was the mobile phase, the retentions of stationary phase and resolutions of four typical tubes were very similar. But when the upper phase was used as the mobile phase, the retentions of stationary phase were similar but the peak resolutions were improved by the modified columns. The results clearly show that middle-pressed tube yields higher peak resolution than their respective counterparts (Table 3). The retention of stationary phase (Sf) and peak resolution (Rs) obtained from these experiments are listed in Table 3. The Rs value between Trp-Tyr and Val-Tyr obtained by the middle-pressed tube ($Rs = 2.01$, lower phase mobile; $Rs = 2.00$, upper phase mobile) exceeded those obtained from the three-pressed tube ($Rs = 1.96$, lower phase mobile; $Rs = 1.90$, upper phase mobile), plain tube ($Rs = 1.93$, lower phase mobile; $Rs = 1.35$, upper phase mobile) and of twisted tube ($Rs = 1.65$, lower phase mobile; $Rs = 1.61$, upper phase mobile). These results indicate that pressing the tube prevents laminar flow and enhances mixing of the two phases to

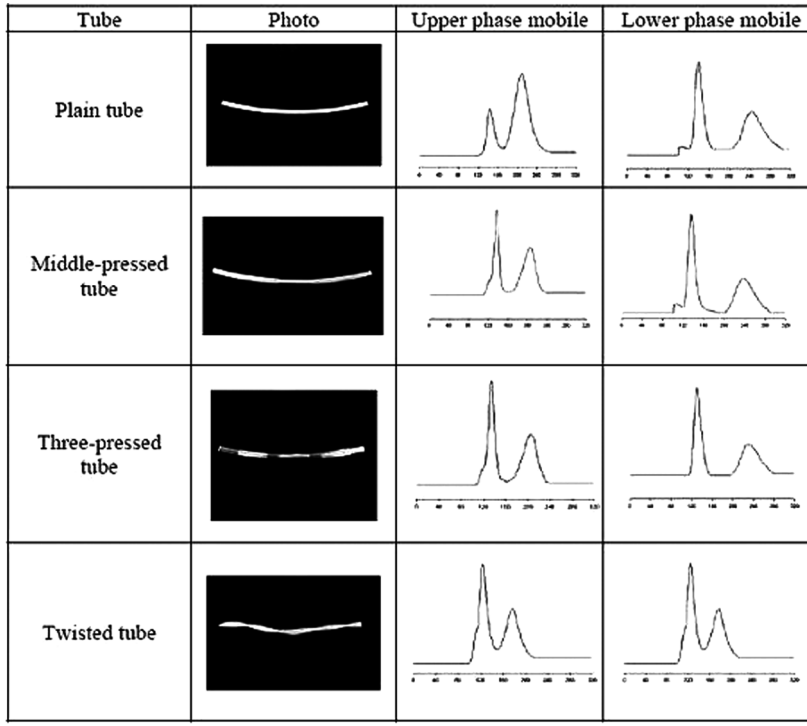


Figure 5. Comparison of performance of four zigzag tubes in separation of dipeptides.

Table 3. Stationary phase retention and peak resolution of 1-Butanol/Acetic acid/Water (4:1:5, v/v) in four typical column tubes

	Upper phase mobile		Lower phase mobile	
Tube	Sf (%)	Rs	Sf (%)	Rs
Plain tube	31.51	1.35	35.89	1.93
Middle-pressed tube	32.05	2.00	32.89	2.01
Three-pressed tube	32.05	1.90	35.80	1.96
Twisted tube	30.00	1.61	31.88	1.65

Note. Test samples: tryptophyl-tyrosine (trp-tyr), valyl-tyrosine (val-tyr); flow rate: 0.05 mL/min; revolution: 1000 rpm; solvent system: 1-Butanol/Acetic acid/Water (4:1:5, v/v); Sf: retention of stationary phase; Rs: peak resolution.

improve the solute partitioning process. But when the number of pressed points was increased, the peak resolution was not increased substantially. The retention of stationary phase (Sf) in all these groups is similar and range from 30% to 35%.

It is important to note that compared with the toroidal coil experiments in all separations with the zigzag columns showed low column pressure below 100 psi in dipeptide separation and considerably lower than 200 psi for DNP-amino acid separation indicating that peak resolution can be further improved simply by increasing the column length and/or revolution speed without damaging the separation column.

CONCLUSIONS

In the zigzag toroidal column, retention of the stationary phase and peak resolution is increased with decreased flow rate of the mobile phase. In the dipeptide separation with the polar solvent system of 1-Butanol/Acetic acid/Water (4:1:5, v/v), the lower mobile phase yielded higher retention of stationary phase and peak resolution at the given flow rate than those obtained from the upper mobile phase. But in the separation of DNP-amino acids with the moderately hydrophobic solvent system composed of hexane/ethyl acetate/methanol/0.1 M HCl (1:1:1:1, v/v), the results are quite opposite, and the upper mobile phase yielded better separation. The retention of stationary phase and peak resolution is considerably improved by changing the shape of the tube, and compressing the tube at the middle portion of each segment is yielded the best separation in zigzag toroidal column.

REFERENCES

1. Ito, Y. High-speed countercurrent chromatography. CRC Rev. Anal. Chem. **1986**, *17*, 65–143.
2. Yang, Y.; Gu, D.; Wu, H.; Aisa, H.A.; Zhang, T.; Ito, Y. Application of preparative high-speed countercurrent chromatography for separation of Elatine from Delphinium shawurense. J. Liq. Chromatogr. Rel. Technol. **2008**, *31*, 3012–3019.
3. Yang, Y.; Wu, T.; Yang, W.; Aisa, H.A.; Zhang, T.; Ito, Y. Preparative isolation and purification of four flavonoids from Flos Gossypii by high-speed countercurrent chromatography. J. Liq. Chromatogr. Rel. Technol. **2008**, *31*, 1523–1531.
4. Ito, Y.; Bowman, R.L. Countercurrent Chromatography with flow-through centrifuge without rotating seals, Anal. Biochem. **1978**, *85*, 614–617.
5. Ito, Y.; Yu, H. Triangular helical column for centrifugal countercurrent chromatography. J. Liq. Chromatogr. Rel. Technol. **2009**, *32*, 560–566.

6. Ito, Y.; Clary, R.; Powell, J.; Knight, M.; Finn, T.M. Spiral tube support for high-speed countercurrent chromatography. J. Liq. Chromatogr. Rel. Technol. **2008**, *31*, 1346–1357.
7. Ito, Y.; Clary, R.; Sharpnak, F.; Metger, H.; Powell, J. Mixer-settler counter-current chromatography with multiple spiral disk assembly. J. Chromatogr. A **2007**, *1172*, 151–159.
8. Ito, Y.; Clary, R.; Powell, J.; Knight, M.; Finn, T.M. J. Improved spiral tube assembly for high-speed counter-current chromatography. J. Chromatogr. A **2009**, *1214*, 4193–4200.

Received February 7, 2009
Accepted March 4, 2009
Manuscript 6476