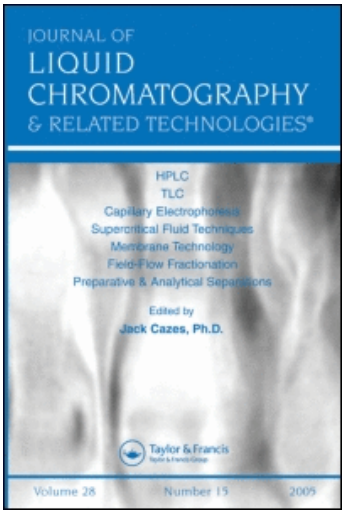

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NOVEL DESIGN FOR CENTRIFUGAL COUNTERCURRENT CHROMATOGRAPHY: II. STUDIES ON NOVEL GEOMETRIES OF ZIGZAG TOROIDAL TUBING

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□ *The toroidal column using a zigzag pattern has been improved in both retention of the stationary phase and peak resolution. To further improve the retention of stationary phase and peak resolution, a series of novel geometric designs of tubing (plain, mid clamping, flattened, and flat twisted tubing) was evaluated for their performance in CCC. The results showed that the tubing, which was flattened vertically against the centrifugal force (vert-flattened tubing) produced the best peak resolution among them. Using vert-flattened tubing a series of experiments was performed to study the effects of column capacity and sample size. The results indicated that a 0.25 mL capacity column is ideal for analysis of small amount samples.*

Keywords clamped tubing, countercurrent chromatography, flattened tubing, flat twisted tubing, resolution, zigzag toroidal column

INTRODUCTION

Column design is the heart of the chromatographic system that leads to the successful separation. Therefore, it is surprising to find that until now only few publications have been dedicated to the column design for countercurrent chromatography, a support free chromatographic technique, which has been widely used for the separation and purification of natural products.[1,2]

Geometries of tubing and its mounting patterns lead to different chromatographic behaviors and separation efficiency. In the previous

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CCC studies, there are only six papers involved in the modified tubing shape and its mounting patterns. Degenhardt et al. compared performance of custom made rectangular tubing with or without twisting and found that the twisted rectangular tubing yielded 15–30% higher efficiency in the separation of natural products than that of the conventional standard multilayer coil.[3] Cross pressed tubing has been introduced to improve the separation with the spiral tube assembly for HSCCC,[4] where resolution (Rs) has been remarkably increased compared with traditional plain tubing. Recently, flat twisted tubing mounted in the spiral tubing support in HSCCC produced efficient separation of protein samples.[5]

In our previous study, the toroidal column was designed as a triangular helical pattern and zigzag pattern to improve separation efficiency for the hydrostatic centrifugal CCC system. These tubing designs improved peak resolution (Rs) mainly due to the higher retention level of the stationary phase compared with the traditional toroidal coiled column.[6,7] Rs of the zigzag toroidal column was further improved by modifying the tubing geometry.

Recently, we have developed a novel toroidal zigzag column (Figure 1), which remarkably increased the retention of stationary phase, and by clumping a middle portion of each loop (mid clamping tubing) the separation was further improved. In the present study, a series of experiments

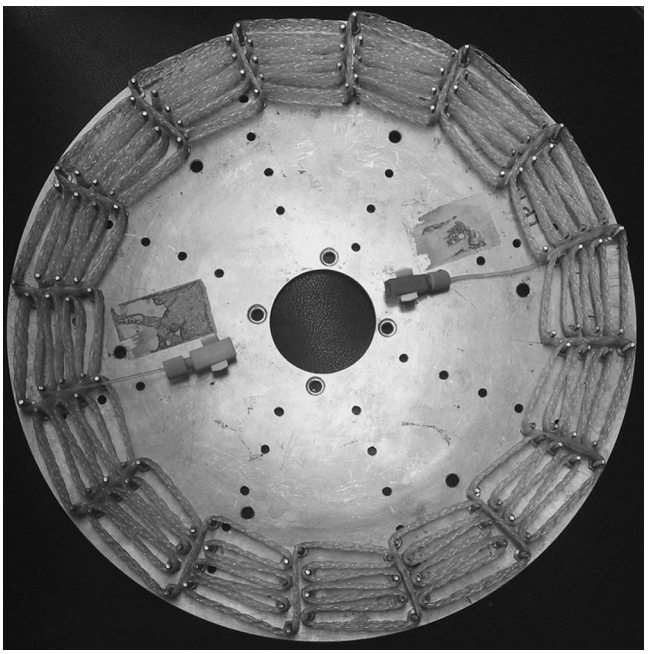


FIGURE 1 Photograph of the zigzag toroidal column for centrifugal countercurrent chromatography.

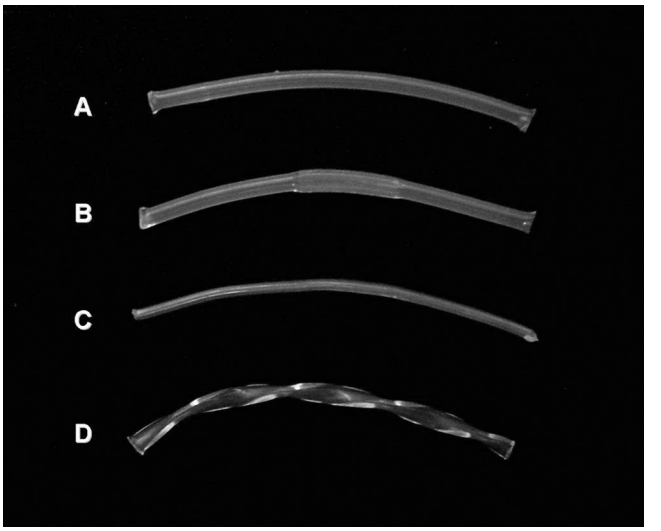


FIGURE 2 Different tubing geometries used for the zigzag toroidal column. (A) plain tubing; (B) mid-clamping tubing; (C) flattened tubing ; (D) flat-twisted tubing.

were performed using a set of modified zigzag toroidal tubing to compare their performance in terms of retention of the stationary phase and peak resolution at various flow rates (Figure 2).

EXPERIMENTAL

Apparatus

The present study uses a rotary seal free centrifuge fabricated by Pharma-Tech Research Corporation, Baltimore, Maryland, USA. It is equipped with an aluminum rotary plate measuring about 34 cm in diameter to hold a toroidal coil separation column on its periphery. The column is made by hooking a set of modified tubing (fluorinated ethylene propylene tubing from Zeus Industrial Products, Orangeburg, SC, USA) onto the screws upstanding over the rotary plate forming the same zigzag pattern (Figure 2). These tubing modifications include tubing clamped at the middle portion of the loop either parallel or perpendicular to centrifugal force (para-mid-clamping tube or vert-mid-clamped tubing, respectively), flattened tubing parallel or perpendicular to centrifugal force (para-flattened tube or vert-flattened tubing, respectively), and twisted flat tubing (flat-twisted tubing). Each terminal of the toroidal column is connected to flow tubes with a set of tubing connectors (Upchurch Scientific, Palm Spring, CA, USA) as shown in Figure 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, exits the centrifuge from the center of the upper plate where they are tightly held with a pair of clamps.

Reagents

1-Butanol of HPLC grade was purchased from Fisher Scientific, Fair Lawn, NJ, USA and acetic acid of analytical grade from Mallinckrodt Chemicals, Phillipsburg, NJ, USA. Dipeptide samples including tryptophyl-tyrosine (trp-tyr) and valyl-tyrosine (val-tyr) were obtained from Sigma Chemicals, St. Louis, MO, USA.

Two-Phase Solvent Systems and Sample Solutions

In the present study, a typical two-phase solvent system, 1-butanol-acetic acid-water (4:1:5, v/v) (BAW), was used to separate the dipeptide samples. The solvent mixture was thoroughly equilibrated in a separatory funnel by repeated vigorous shaking and degassing several times, and the two phases separated shortly before use. The sample solution was prepared by dissolving 25 mg of trp-tyr ($K_{up/lp} = 1.69$) and 100 mg of val-tyr ($K_{up/lp} = 0.53$) in 20 mL of the upper phase of BAW, and 50 μL was charged in each run.[5]

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. 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). 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 partition efficiency of the 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:

$$N = (4R/W)^2 \tag{1}$$

$$Rs = 2(R_2 - R_1)/(W_1 + W_2) \tag{2}$$

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

In order to make a fair comparison between the present results and the previous data obtained from the standard column (capacity V_2) with a larger capacity, the peak resolution (Rs) obtained from the present flat twisted spiral tube assembly (capacity V_1) was adjusted using the following equation:

$$Rs_{a_1} = Rs(V_2/V_1)^{1/2} \tag{3}$$

where Rs_{a1} is the adjusted peak resolution.

And the peak resolution was similarly compared at the same column length as follows:

$$Rs_{a_2} = Rs(L_2/L_1)^{1/2} \tag{4}$$

where Rs_{a2} is the adjusted peak resolution and L indicates the column length.

RESULTS AND DISCUSSION

In the past, plain tubing was mainly used as the separation coil for HSCCC. In this tubing geometry, the centrifugal force acting perpendicularly to the axis of tubing gives the identical effect in all directions. However, if the shape of the tubing was modified into a flat form, the tubing has maximum and minimum internal diameters and the centrifugal force produces different effects on the hydrodynamic motion of the two phases according to its acting direction, parallel (across the minimum diameter forming minimum interface area) and perpendicular (across the maximum diameter forming maximum interface area) to the flat surface. The mutual relationship between the cross sectional area and minimal and maximum internal diameters of flat tubing was studied by squashing a 5 mm I.D. O-ring between two parallel straightedges. The results of this simulating model study are shown in Figure 3, where both minimum and maximum internal diameters, internal area, and their ratio are plotted.

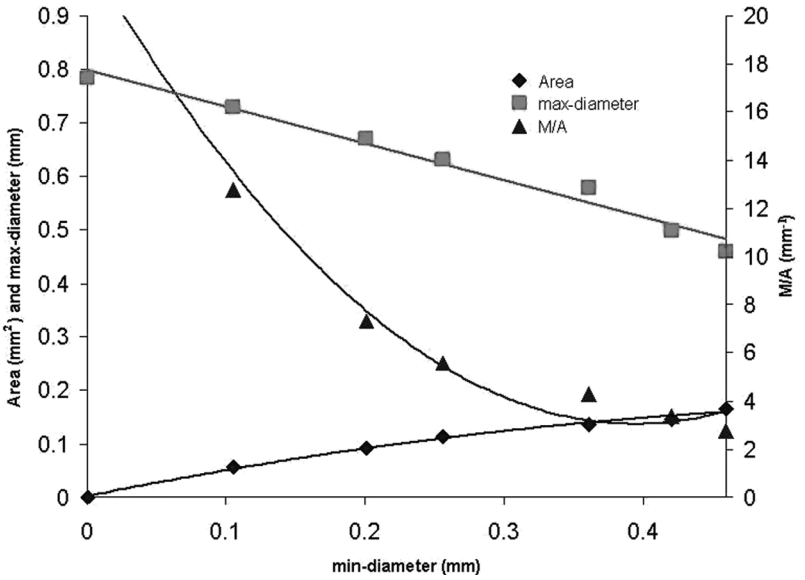


FIGURE 3 The relationship between the minimum and maximum diameters and the internal area of the squashed O-ring. M/A represents maximum internal diameter divided by the area.

As the O-ring is squashed, the area of the internal area is decreased and the ratio between the maximum internal diameter and the area (M/A) is sharply increased. In the actual separation with the two phase solvent system in the flat tubing, M represents the interfacial area when the centrifugal force is acting perpendicular to the surface of the flat tubing and the A, the volume of the two phases forming the interface. Since the partition efficiency increases with the interface area per volume of the two phases in the tubing, the best result is expected by applying the centrifugal force perpendicularly to the flat surface of the tubing provided that the retention of the stationary phase is adequately maintained under a low flow rate.

A series of experiments was performed on the separation of dipeptides with the polar BAW solvent system in centrifugal countercurrent chromatography. Table 1 summarizes the retention of stationary phase, peak resolution, and N in 6 sets of zigzag toroidal tubing, each with different geometry and orientation including plain tubing, tubing clamped at the middle of each loop in two different directions, flat tubing mounted in different directions, and flat twisted tubing. In both clamped and flattened tubing, vert- indicate the centrifugal force acting perpendicular to the flatted surface, and para- indicates that acting parallel to the flattened surface. When the plain tubing is modified to mid-clamped or flattened tubing, the capacity decreases while the length increases appreciably.

TABLE 1 Evaluation on Resolution Using Different Geometries of Zigzag Toroidal Tubing by Centrifugal Countercurrent Chromatography

Test Samples	Tubing	Layer	Capacity (mL)	Mobile Phase	Flow Rate (mL/min)	Sf (%)	Rs_{a1}*	Rs_{a2}**	N_{1a}	N_{2a}
Trp-tyr (1)	Plain	6.5	7.8	Lower phase	0.05	35.89	1.93	1.93	158	317
					0.02	44.87	2.68	2.68	249	473
					0.01	46.28	3.44	3.44	319	533
					0.005	48.22	4.05	4.05	606	1668
Val-tyr (2)				Upper phase	0.05	31.51	1.35	1.35	324	171
					0.02	39.74	2.5	2.5	707	330
					0.01	44.87	2.77	2.77	734	432
					0.005	46.67	3.02	3.02	1058	642
	Mid-para-clamping	2.5	3	Lower phase	0.05	30.10	1.76	1.74	226	580
					0.02	40.09	2.71	2.69	291	801
					0.01	41.23	3.48	3.46	390	892
					0.005	44.33	3.86	3.84	465	1461
				Upper phase	0.05	30.01	1.19	1.18	585	101
					0.02	40.13	1.26	1.25	764	138
					0.01	41.32	1.63	1.62	796	153
					0.005	43.33	2.82	2.80	725	374
	Mid-vert-clamping	2.5	3	Lower phase	0.05	28.95	2.18	2.18	218	949
					0.02	36.84	3.02	3.02	260	988
					0.01	42.11	3.78	3.78	364	1071
					0.005	44.74	4.46	4.46	510	1035
				Upper phase	0.05	28.91	1.71	1.71	660	263
					0.02	36.27	2.40	2.40	881	416
					0.01	42.03	2.59	2.59	1219	520
					0.005	44.56	3.58	3.58	1456	689

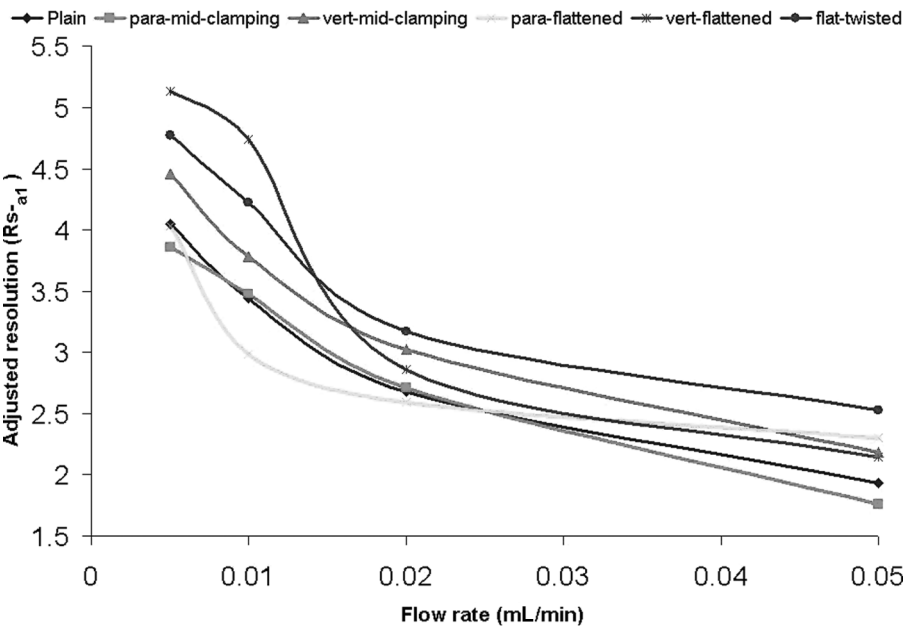
Para-flattened	3.5	4.0	Lower phase	0.05	31.12	2.30	2.23	245	239
				0.02	34.10	2.59	2.52	263	623
				0.01	35.23	2.98	2.90	326	864
				0.005	40.36	4.03	3.92	644	688
			Upper phase	0.05	30.82	1.12	1.09	324	198
				0.02	39.13	1.92	1.86	508	279
				0.01	42.45	2.18	2.16	549	373
				0.005	45.18	3.04	2.95	900	383
Vert-flattened	6.5	3.9	Lower phase	0.05	10.35	2.14	1.53	109	312
				0.02	20.23	2.86	2.04	228	551
				0.01	27.55	4.74	3.39	488	565
				0.005	39.23	5.13	3.63	442	494
			Upper phase	0.05	12.03	–	–	–	–
				0.02	22.17	1.02	0.73	139	124
				0.01	35.87	2.60	1.86	439	279
				0.005	43.49	3.50	2.19	525	291
Flat-twisted	2.5	1.5	Lower phase	0.05	19.05	2.53	1.79	200	616
				0.02	28.54	3.17	2.22	400	647
				0.01	31.00	4.22	2.96	374	671
				0.005	41.34	4.77	3.34	473	694
			Upper phase	0.05	20.13	1.60	1.13	116	175
				0.02	32.69	1.96	1.37	181	264
				0.01	38.24	2.99	2.11	248	378
				0.005	41.14	3.67	2.59	255	431

Note: *Resolution was adjusted at the same capacity. **Resolution was adjuested at the same length.

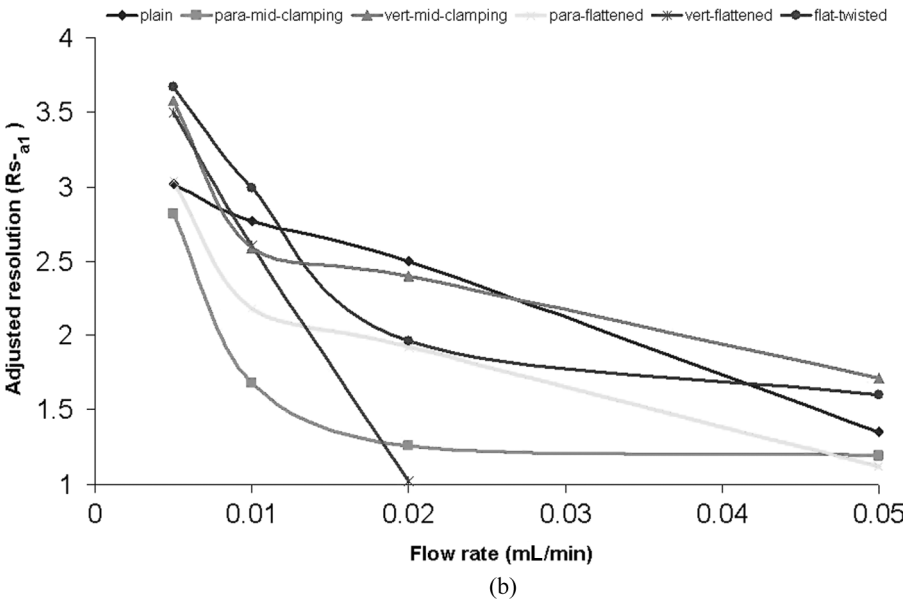
In all groups, the retention of stationary phase increases as the flow rate is decreased (Table 1). In the plain tubing, the retention of stationary phase with lower phase mobile was higher than that with upper phase mobile. And, when mid clamping tubing was tested, the retention of stationary phase with both lower and upper mobile phases was very similar. However, in the flattened tubing the retention of stationary phase with upper phase mobile was better than that with lower phase mobile and, as expected, the retention of stationary phase remarkably decreased. The stationary phase retention of para-mid-clamping and para-flattened tubing was better than that of vert-mid-clamping and vert-flattened tubing, respectively. When lower phase was the mobile phase, retention of stationary phase using plain tubing is the best at a given flow rate. When upper phase was the mobile phase, however, both plain and para-flattened tubing designs can produce good retention of the stationary phase, even at the high flow rate of 0.05 mL/min, while the stationary phase retention of vert-flattened tubing was only 12.03% at the same flow rate. The retention of stationary phase with the flat twisted tubing was slightly better than that with the vert-flattened tubing.

The performance of each group of tubing in Table 1 is compared in terms of peak resolution (Rs) and theoretical plate number (N) by adjusting the data at the same capacity (Rs_{a1}) and the same length (Rs_{a2}) of tubing, respectively, according to Eqs. (3) and (4) described earlier. All groups were tested at several flow rates ranging from 0.005 to 0.05 mL/min. Figure 4, illustrates the variations of adjusted resolution at the same column capacity at the flow rates from 0.005 to 0.05 mL/min in the dipeptide separation using 5 different types of the zigzag toroidal tubing. All the results were adjusted based on the Eq. (3). With increased flow rate, the adjusted resolution (Rs_{a1}) sharply decreased. When the lower phase is the mobile phase, the Rs_{a1} using flat twisted was the best at 2.53 and the para-mid-clamping tubing was the worst at the high flow rate of 0.05 mL/min. When the flow rate for para-mid-clamping tubing was decreased to 0.005 mL/min, the Rs_{a1} was sharply increased reaching 5.13, the highest level among all groups, apparently due to the improved retention of stationary phase to 39.23% and its broad interface. When upper phase was mobile, the Rs_{a1} was lower than that of lower phase mobile due to the low retention of stationary phase, and at a flow rate of 0.05 mL/min, the retention dropped down to 12.03%, showing a single peak in the chromatogram. But, when the flow rate was decreased to 0.005 mL/min, the retention of stationary phase was sharply increased to 43.5% with a high peak resolution at 3.50 (Table 1). Flat twisted, vert-flattened and vert-mid-clamping tubing all showed excellent separation (Table 1).

When the data were adjusted and compared at the same tubing length, the results was very different from the results adjusted at the tubing



(a)



(b)

FIGURE 4 Comparison in performance between six different types of zigzag tubing for separation of dipeptides. (a) lower phase mobile; (b) upper phase mobile. Revolution: 1000 rpm.

capacities. All the results were adjusted based on the Eq. (4). Though adjusted resolution ($Rs-_{a2}$ in Table 1) still increased with decreased flow rate, the $Rs-_{a2}$ using vert-flattened tubing was not the best at the lower flow rate. When the lower phase is mobile phase, the para-flattened tubing had a good performance of $Rs-_{a2} = 2.23$ at 0.05 mL/min of flow rate. But, when flow rate was decreased to 0.005 mL/min, the $Rs-_{a2}$ of vert-mid-clamping was the best. Vert-mid-clamping tubing also showed very good performance, and $Rs-_{a2}$ was 1.71 at higher flow rate and 3.58 at lower flow rate.

Since two different results were obtained in the above comparative method, a series of experiments were designed to compare the Rs at the similar peak retention time between the plain, vert-flattened and flat twisted tubing (Figure 5). The peak retention time was adjusted based on the column capacity by applying the different flow rates according to the column capacity. Figure 5A to Figure 5C, show the results of plain tubing, vert-flattened tubing and flat twisted tubing, respectively. The result revealed that the vert-flattened tubing at a lower flow rate yields slightly better peak resolution than those of plain and flat twisted tubing at a higher flow rate.

Finally, partition efficiency of the various column lengths of flattened zigzag tubing was compared. The capacity of each zigzag tubing layer is about 0.5 mL. The results show that Rs linearly increases with increased column capacity or column length (Figure 6). When the capacity is about

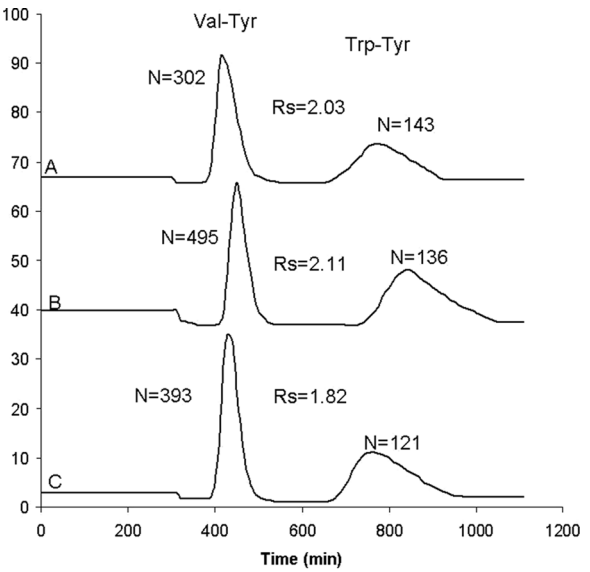


FIGURE 5 Comparison of performance of plain, flattened and flat-twisted tubing in separation of dipeptides at the same peak retention time.

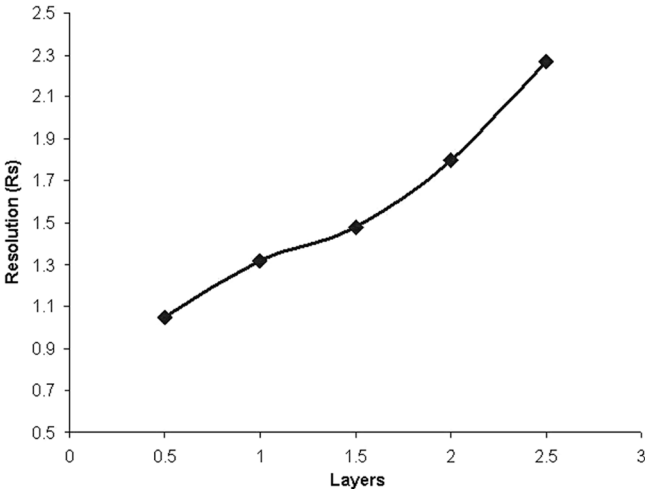


FIGURE 6 Relationship between tubing layers and resolution at the 0.005 mL/min of flow rate.

0.25 mL (half a layer), two test samples still can be resolved at Rs = 1.05. Using the 1.5 layers of tubing with 0.8 mL capacity the Rs can reach to 1.48, which achieved complete separation of two test samples.

With lower column capacity the sample size can be reduced to obtain the excellent resolution. A series of experiments was performed to determine the optimum sample size using flattened tubing (Figure 7). With increased sample size, peak resolution was sharply decreased. The 5 μL, sample size yielded the best separation at Rs = 1.17, whereas a large sample size of 40μL produced a single peak at Rs < 0.55.

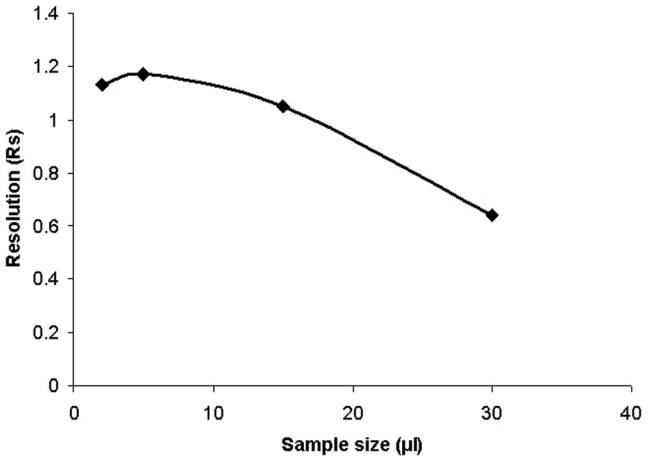


FIGURE 7 Relationship between resolution and sample size obtained from the small column capacity (0.25 mL). flow rate: 0.005 mL/min. revolution: 1000 rpm.

CONCLUSIONS

In the zigzag toroidal column, plain and flattened tubing both have very good performance. The flattened tubing produces slightly higher peak resolution than the plain tubing and, even with the decreased capacity at 0.25 mL, it yields the peak resolution of over Rs = 1. Especially, the vert-flattened tubing can yield a high peak resolution with a short column in lower aqueous phase mobile, which can be efficiently used for analytical separation of a minute amount of test samples for CCC/MS.

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.-Y.; 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. Degenhardt, A.; Schwarz, M.; Winterhalter, P.; Ito, Y. Evaluation of different tubing geometries for high-speed countercurrent chromatography. J. Chromatogr. A. **2001**, *922*, 355–358.
4. 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**, *1216*, 4193–4200.
5. Yang, Y.; Aisa, H.A.; Ito, Y. Flat-twisted tubing: Novel column design for spiral high-speed counter-current chromatography. J. Chromatogr. A. **2009**, *1216*, 5265–5271.
6. Ito, Y.; Yu, H. Triangular helical column for centrifugal countercurrent chromatography. J. Liq. Chromatogr. & Rel. Technol. **2009**, *32*, 560–566.
7. Yang, Y.; Aisa, H.A.; Ito, Y. Novel design for centrifugal countercurrent chromatography: I. zigzag toroidal column. J. Liq. Chromatogr. & Rel. Technol. *in press*.