Journal of Chromatography A, 1217 (2010) 1313–1319

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Compact type-I coil planet centrifuge for counter-current chromatography

Yi Yang[a,b,c], Dongyu Gu[a,b,c], Yongqiang Liu[b,c], 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, 10 Center Drive, Building 10, Room 8N230, Bethesda, MD 20892-1762, USA
[b] Xinjiang Key Laboratory of Plant Resources and Natural Products Chemistry, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumqi 830011, China
[c] Graduate University of the Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Article history:
Received 28 July 2009
Received in revised form
15 December 2009
Accepted 21 December 2009
Available online 4 January 2010

Keywords:
Compact type-I coil planet centrifuge
Counter-current chromatography
DNP-amino acids
Revolution radius
Retention of the stationary phase
Resolution
Retention time

ABSTRACT

A compact type-I coil planet centrifuge has been developed for performing counter-current chromatography. It has a revolution radius of 10 cm and a column holder height of 5 cm compared with 37 and 50 cm in the original prototype, respectively. The reduction in the revolution radius and column length permits application of higher revolution speed and more stable balancing of the rotor which leads us to learn more about its performance and the future potential of type-I coil planet centrifuge. The chromatographic performance of this apparatus was evaluated in terms of retention of the stationary phase (S_f), peak resolution (R_s), theoretical plate (N) and peak retention time (t_R). The results of the experiment indicated that increasing the revolution speed slightly improved both the retention of the stationary phase and the peak resolution while the separation time is remarkably shortened to yield an excellent peak resolution at a revolution speed of 800 rpm. With a 12 ml capacity coiled column, DNP-DL-glu, DNP-β-ala and DNP-L-ala were resolved at R_s of 2.75 and 2.16 within 90 min at a flow rate of 0.4 ml/min. We believe that the compact type-I coil planet centrifuge has a high analytical potential.

Published by Elsevier B.V.

1. Introduction

High-speed counter-current chromatography (HSCCC) has been widely used for the separation and purification of biological samples with the conventional two-phase solvent systems [1–3].

In the past many different types of seal-free planetary centrifuge systems have been introduced for performing counter-current chromatography including type-J, type-X, type-L, type-I and their hybrids [4]. Among those the type-I coil planet centrifuge developed in 1971 [5] produced higher separation efficiency [6], but only few studies have been reported on this apparatus. This original type-I coil planet centrifuge was built by modifying a large floor-model centrifuge with the revolution radius of 37 cm and column height of ca. 50 cm that limits the maximum revolution speed at 700 rpm [5,6]. In order to increase the revolution speed of the type-I coil planet centrifuge, we have constructed a compact model with the revolution radius of 10 cm and the column holder height at 5 cm, which permits higher revolution speed with stable balancing of the centrifuge system. In the present paper, the CCC performance of this new centrifuge is demonstrated by separations of dipeptide and DNP-amino acid samples each with the suitable two-phase solvent system.

2. Experimental

2.1. Apparatus

The apparatus used in the present study is a type-I coil planet centrifuge, hydrodynamic CCC equilibrium system, fabricated at the NIH Machine Shop, Bethesda, MD, USA. The cross-sectional view through the central axis of the apparatus is diagrammatically shown in Fig. 1. It holds a separation column on one side and a counterweight on the other side of the rotor symmetrically at 10 cm from the central axis of the apparatus. The column holder undergoes a synchronous planetary motion, i.e., the holder counter-rotates about its own axis once during one revolution around the central axis of the centrifuge. This unique planetary motion is produced by coupling a pair of identical toothed pulleys, one fixed on the bottom of the central axis of the centrifuge (stationary pulley) and the other around the lower end of the column holder shaft (planetary pulley) with a toothed belt. This planetary motion produces a centrifugal force field uniformly circulating around every point on the column holder. The separation column is made by winding a single piece of 0.85 mm I.D. tubing (PTFE SW No. 20, Zeus Industrial Products, Orangeburg, SC, USA) onto 47 pieces of 5 mm O.D. and 5 cm long

* Corresponding author. Tel.: +1 301 496 1210; fax: +1 301 402 0013.
E-mail address: itoy2@mail.nih.gov (Y. Ito).

0021-9673/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.chroma.2009.12.055

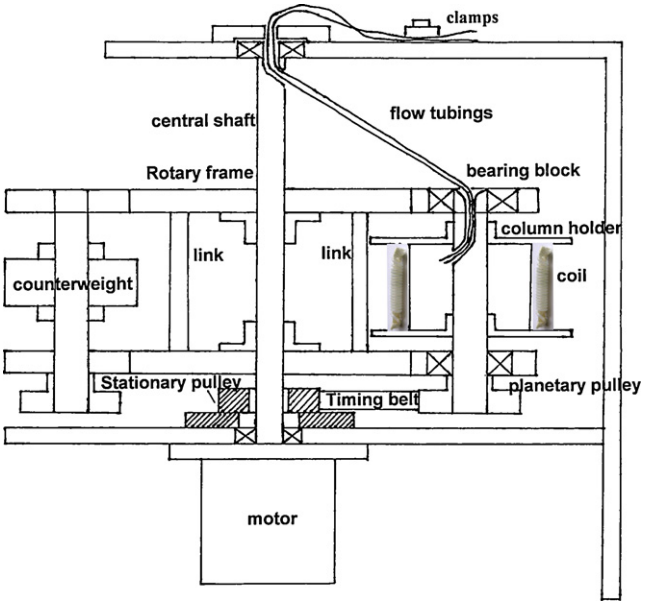

Fig. 1. The schematic drawing of the compact type-I coil planet centrifuge.

nylon pipes in such a way that every other coil unit has opposite handedness. The length of the tubing is approximately 20 m with a total capacity of 12 ml. The coiled column is mounted around the holder hub at various angles (against the horizontal plane) with all coil units arranged parallel to each other so that the head of one coil unit is connected to the tail of next coil unit (Fig. 2). The revolution speed of the apparatus is regulated from 600 to 1200 rpm with a speed controller (Bodine Electric, Chicago, IL, USA). A metering HPLC pump (Shimadzu LC-10ADVP, Columbia, MD, USA) was used for pumping the solvents, and the effluent was continuously monitored with a UV detector (LKB Instruments, Stockholm, Sweden).

2.2. 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-L-ala), N-2,4-dinitrophenyl-β-alanine (DNP-β-ala), N-2,4-dinitrophenyl-DL-glutamic acid (DNP-DL-glu), N-2,4-DNP-L-aspartic acid (DNP-L-asp), N,S-Di-(2,4-DNP)-L-cysteine (DNP-L-cys), N-2,4-DNP-L-isoleucine (DNP-L-ile), N-2,4-DNP-L-serine (DNP-L-ser), N-2,4-DNP-L-valine (DNP-L-val), N-2,4-DNP-L-leucine (DNP-L-leu), N-2,4-DNP-L-glutamine (DNP-L-gln), N-DNP-DL-methionine (DNP-DL-met) were all obtained from Sigma Chemicals (St. Louis, MO, USA).

2.3. Distribution coefficient measurement

The distribution coefficients (K_U) of each sample in the two-phase solvent system was determined using the conventional test tube method with a UV spectrophotometer (Genesis 10 UV, Thermo Spectronic, Rochester, NY, USA) at 280 nm. The absorbance of the upper phase was recorded as A_U and that of the lower phase was recorded as A_L. The K_U value was calculated according to the following equation: $K_U = A_U/A_L$.

2.4. Two-phase solvent systems and sample solutions

In the present study, a set of the two-phase solvent systems composed of 1-butanol–acetic acid–water at various volume ratios including 5:0:5 (solvent system I), 4.75:0.25:5 (solvent system II), 4.5:0.5:5 (solvent system III), 4.25:0.75:5 (solvent system IV), 4:1:5 (solvent system V) was used to separate the dipeptide test samples, while hexane–ethyl acetate–methanol–0.1 M HCl (1:1:1:1, v/v) (solvent system VI) was used to separate the DNP-amino acid test samples. Each solvent mixture was thoroughly equilibrated in a separatory funnel by repeating vigorous shaking and degassing several times, and the two phases separated shortly before use. The dipeptide sample solution was prepared by dissolving 25 mg of Trp-Tyr and 100 mg of Val-Tyr in 20 ml of the upper phase of solvent system, and 0.2 ml was charged in each run. The DNP-amino acid sample solution was prepared by dissolving 5.7 mg of DNP-L-ala, 5.1 mg of DNP-β-ala and 5.3 mg of DNP-DL-glu in 20 ml of the upper phase of solvent system VI, and 0.2 ml was charged in each run.

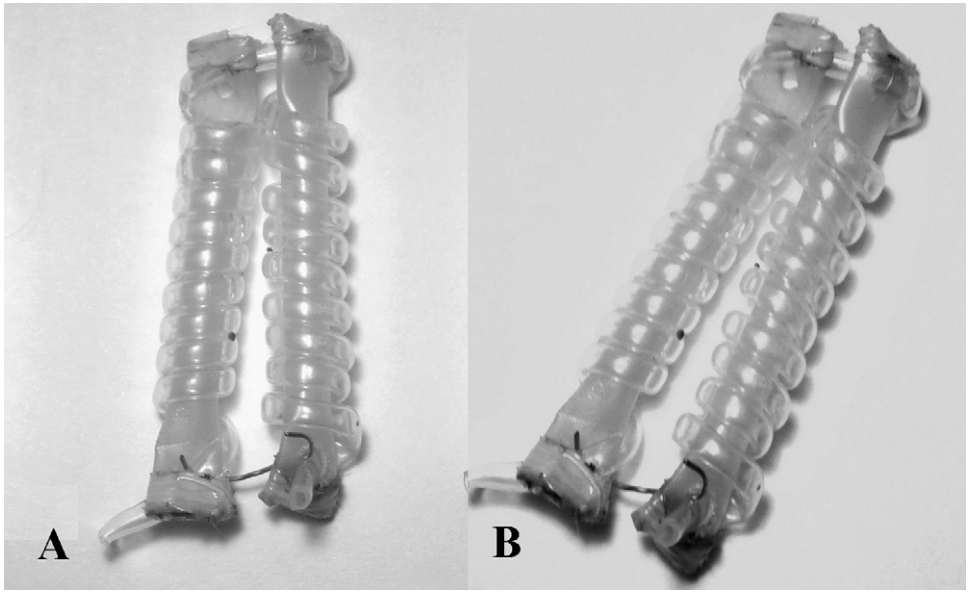

Fig. 2. The coiled column mounted on the holder in the vertical (A) and slanted (B) positions.

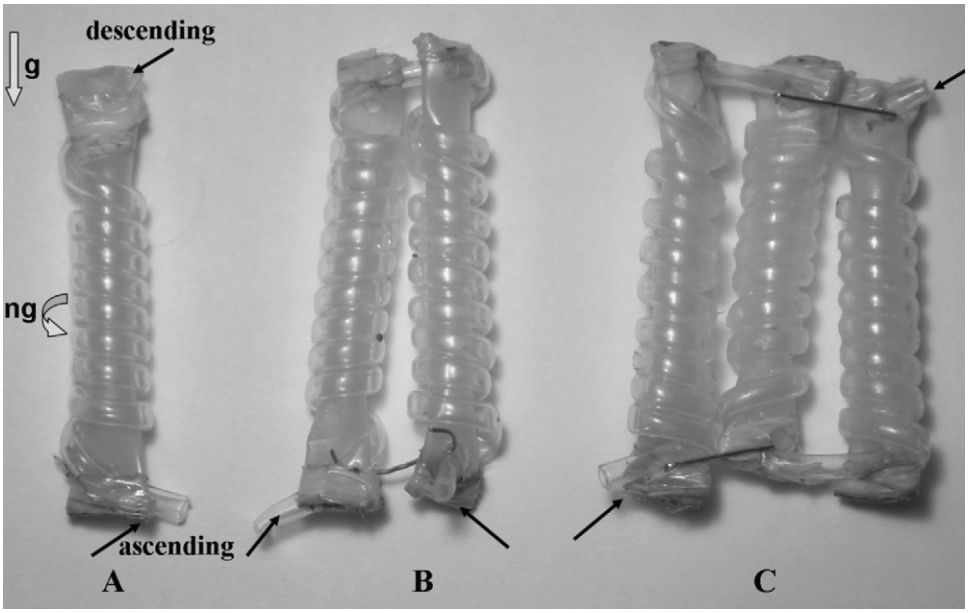

Fig. 3. The separation columns composed of one, two and three coil units.

2.5. Separation procedure

In each separation, the separation column was entirely filled with the stationary phase, followed by sample injection, and the column was rotated at a given revolution speed 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 strip-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 by the total column volume.

2.6. Evaluation of partition efficiency

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

$$N = \left(\frac{4t}{W}\right)^2 \quad (1)$$

$$R_s = \frac{2(t_2 - t_1)}{W_1 + W_2} \quad (2)$$

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

Table 1
Evaluation on retention of stationary phase at flow rate of 0.2 ml/min in the solvent system I using compact type-I counter-current chromatography.

Column angle	Rotational speed (rpm)	Elution mode	S_f (%)
45°	800	H-A	42.67
		T-A	12.51
		T-D	12.51
		H-D	45.83

3. Results and discussion

In the previous study using the original type-I coil planet centrifuge, a set of test samples was separated at high partition efficiency in 8–10 h [5,6]. It also showed that the coiled column mounted at 60° angle produced higher level of stationary phase retention for a polar butanol solvent system than that of straight coiled column [6]. Therefore, in the beginning of the present study, a coiled column with 45° angle was used for the studies on the compact type-I coil planet centrifuge as shown in Fig. 2B. A suitable elution mode is very important for a successful CCC experiment. Fig. 3 shows three short columns with one, two and three coil units. In the two and three coil units the handedness of neighboring units are opposite. In the single coil unit (Fig. 3A) there are four different elution modes for the lower mobile phase, i.e., pumping at the lower head end in the ascending mode (H-A), lower tail end in the ascending mode (T-A), upper head end in the descending mode (H-D), and upper tail end in the descending mode (T-D). Among them, one can expect that H-D will produce the highest stationary phase retention supported by two forces, Archimedean Screw force generated by the planetary motion of the column (ng) and the

Table 2
Evaluation on performance of dipeptides separation at flow rate of 0.2 ml/min in the different BAW solvent systems using compact type-I counter-current chromatography.

Solvent system	K_U		Elution mode	S_f (%)	R_s
	val-tyr	trp-tyr			
I	0.14	1.16	H-A	12.5	0.76
			H-D	15.5	1.03
II	0.28	1.15	H-A	42.67	1.56
			H-D	45.83	1.48
III	0.36	1.36	H-A	29.17	1.36
			H-D	33.33	1.06
IV	0.55	1.43	H-A	20.83	1.26
			H-D	24	1.02
V	0.53	1.69	H-A	4.17	–
			H-D	4.17	–

Note: Sample size: 0.2 ml; rotational speed: 800 rpm; column angle: 45°.

Table 3
Comparison of performance of the angle of 45 and 90° coiled column using compact type-I counter-current chromatography.

Sample	Solvent system	Column angle	Rotational speed (rpm)	S_f (%)	N	R_s
val-tyr trp-tyr	II	45°	600	30.13	400/400	1.51
			800	42.67	273/196	1.56
			1000	29.17	260/161	1.31
			1200	20.88	917/183	1.08
		90°	600	20.83	463/313	1.76
			800	32.17	1264/347	2.00
			1000	20.83	60/148	0.52
			1200	17.39	–	One peak
DNP-DL-glu DNP-β-ala DNP-L-ala	VI	45°	600	37.50	1547/799/409	2.37/1.72
			800	41.30	952/658/359	2.13/1.58
			1000	37.52	1366/547/298	2.03/1.35
			1200	36.67	561/405/207	1.43/1.02
		90°	600	36.67	339/992/542	1.89/1.94
			800	40.01	876/794/420	2.38/1.81
			1000	37.51	1079/635/357	2.18/1.57
			1200	33.33	227/250/163	1.02/0.82

Note: Flow rate: 0.2 ml/min; elution mode: H-A.

Table 4
Comparison of performance of DNP-amino acids separation in the solvent system VI at the different given flow rate using compact type-I counter-current chromatography.

Flow rate (ml/min)	S_f (%)	N	R_s	t_R (min)
0.1	46.90	992/635/331	1.53/1.25	102.4/127.9/162.9
0.2	40.87	1024/850/411	2.39/1.83	40.4/60.5/83.1
0.3	37.39	1665/1060/562	2.53/2.15	30.6/40.7/56.3
0.4	30.43	1771/1354/716	2.75/2.16	24.2/32.2/42.8
0.5	28.70	1820/1479/742	2.64/1.92	19.2/25.0/32.0
0.6	26.96	1452/1600/772	2.52/1.83	16.2/21.0/26.4
0.8	21.22	1438/1064/1103	1.88/1.51	12.8/15.9/19.1
1.0	16.24	1372/1165/997	1.62/1.29	10.7/12.8/15.0
1.5	12.55	767/1849/1101	1.19/1.15	7.5/8.6/9.7
2.0	10.83	748/2182/1267	0.93/0.85	5.9/6.5/7.1

Note: Sample: DNP-DL-glu, DNP-β-ala, DNP-L-ala; sample size: 200 μl; solvent system: VI; rotational speed: 800 rpm; elution mode: H-A; angle: 90°.

unit gravity (g) which is steadily acting downward. Among these two forces, the unit gravity is considered to produce much smaller effect. When two coil units are connected as shown in Fig. 3B, this unit gravity effect is completely canceled out by the second unit, and there are only two elution modes of head to tail (H) and tail to head (T). Increasing the number of coil units to three as shown in Fig. 3C, the unit gravity effect reappears but with less effect on the retention of the stationary phase compared with that on the single coil unit. As the number of column unit is further increased as in our separation column (47 units), the effect of the unit gravity on the ascending and descending elution modes on the retention of the stationary phase becomes negligible, and therefore we consider only two elution modes of head to tail (H) and tail to head (T) for the lower mobile phase (Table 1). Table 1 shows that among the above four elution modes H-A and H-D produced over 40% of stationary phase retention (S_f) which is much higher than those of other two reversed elution modes. Next, these two proper elution modes were further examined in the separation of dipeptides. Table 2 shows a set of data obtained from the compact type-I coil planet centrifuge with a coiled column consisting of about 25 m of

Table 5
Comparison of performance of DNP-amino acids separation in the solvent system VI at the different given rotational speed using compact type-I counter-current chromatography.

Revolution (rpm)	Flow rate (ml/min)	S_f (%)	N	R_s	t_R (min)
600	0.2	36.67	339/992/542	1.89/1.94	46/63/85
800		40.01	876/794/420	2.38/1.81	46/64/88
1000		37.51	1079/635/357	2.18/1.57	46/63/85
1200		33.33	227/250/163	1.02/0.82	49/64/81
600	0.4	30.41	1904/1530/841	2.7/2.11	24/31.3/40.6
800		30.43	1771/1354/716	2.75/2.16	24.2/32.2/42.8
1000		33.04	1156/1186/535	2.25/1.70	23.8/31/39.9
1200		32.48	982/759/411	1.84/1.37	23.5/30.3/38.5
600	1.0	14.78	1346/1093/913	1.39/1.15	10.6/12.4/14.4
800		16.24	1372/1165/997	1.62/1.29	10.7/12.8/15.0
1000		19.66	2178/2099/1508	2.1/1.85	10.5/12.6/15.1
1200		21.38	995/1361/1279	1.66/1.53	10.3/12.5/14.8
600	2.0	10.26	240/1195/503	0.45/0.43	6.2/6.7/7.2
800		10.83	748/2182/1267	0.93/0.85	5.9/6.5/7.1
1000		14.29	538/539/350	0.94/0.92	5.8/6.6/7.8
1200		17.65	1444/1011/797	0.77/0.81	5.7/6.4/7.2

Note: Sample: DNP-DL-glu, DNP-β-ala, DNP-L-ala; sample size: 200 μl; elution mode: H-A; angle: 90°.

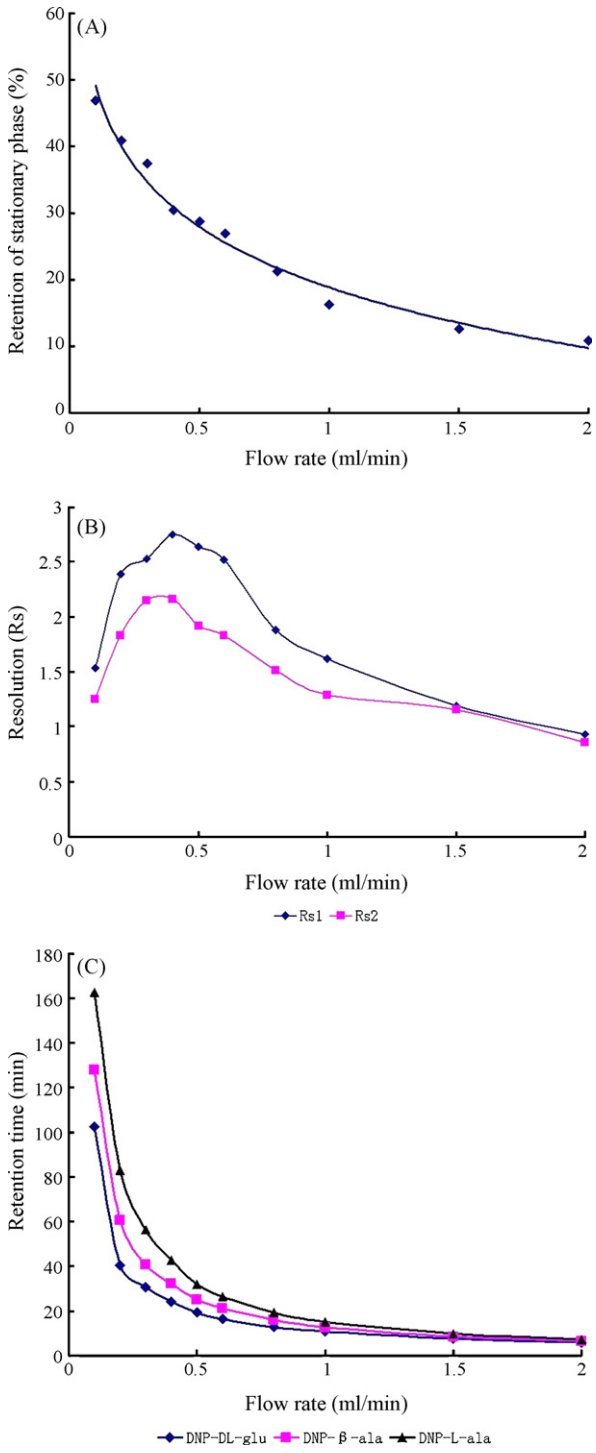

Fig. 4. Comparison of performance of DNP-amino acid separation at different flow rates using the compact type-I coil planet centrifuge. Sample: DNP-DL-glu, DNP-β-ala, DNP-L-ala; sample size: 0.2 ml; elution mode: H-A; solvent system: VI; revolution speed: 800 rpm; capacity: 12 ml. (A) Relationship between retention of stationary phase and flow rate; elution mode: H-A with lower mobile phase; (B) relationship between R_s and flow rate; (C) relationship between retention time and flow rate.

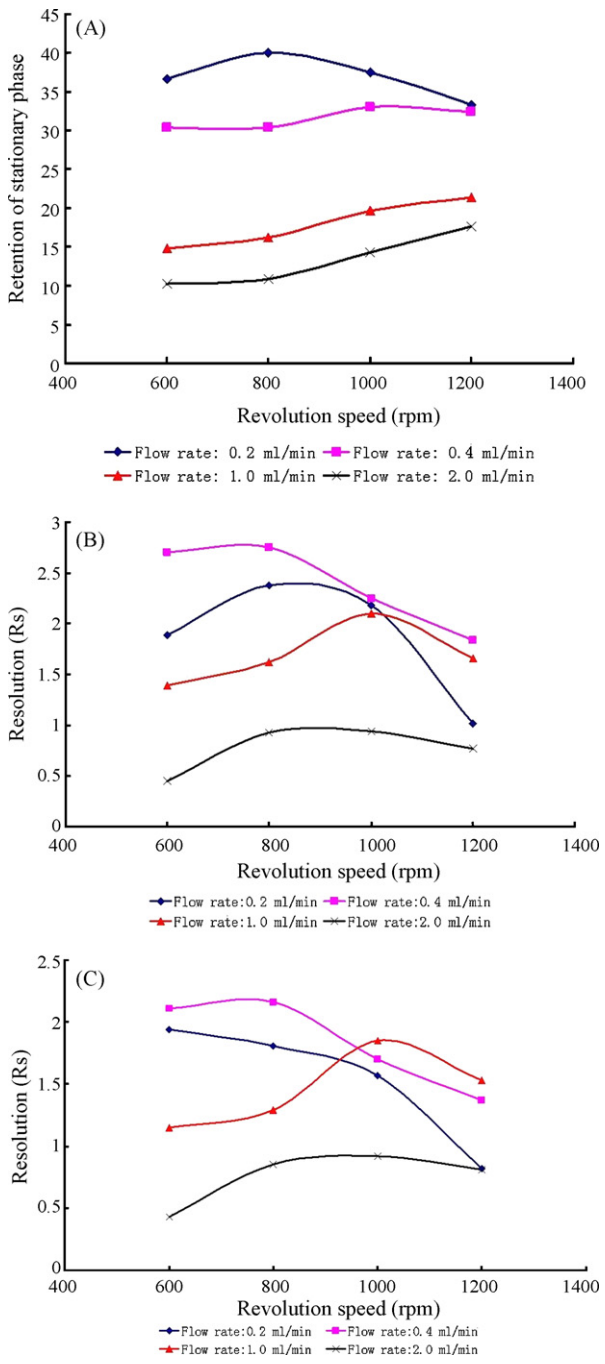

Fig. 5. DNP-amino acid separation at different revolution speeds using the compact type-I coil planet centrifuge. Sample: DNP-DL-glu, DNP-β-ala, DNP-L-ala; sample size: 0.2 ml; elution mode: H-A; solvent system: VI; capacity: 12 ml. (A) Relationship between retention of the stationary phase and revolution speed; elution mode: H-A with lower mobile phase; (B) relationship between R_{s1} (DNP-DL-glu and DNP-β-ala) and revolution speed; (C) relationship between R_{s2} (DNP-β-ala and DNP-L-ala) and revolution speed.

0.76 mm I.D. PTFE tubing with a total capacity of about 12 ml. The results indicate that the retention of stationary phase increased with the decreased concentration of acetic acid in the solvent system. But when the solvent system contained no acetic acid, both S_f and R_s were reduced. The elution mode of H-A for solvent system II produced the best R_s value at 1.56 for dipeptide separation.

A series of experiments was performed to evaluate effects of the column angle on R_s, N and S_f. Table 3 shows a set of results obtained from 90° and 45° column angles (against the horizontal plane) using two selected two-phase solvent systems including solvent systems II and VI at the various revolution speeds ranging from 600 to 1200 rpm. All in all, S_f of 45° coiled column is better than the 90° one, but R_s is quite opposite. In both solvent groups the R_s increased with S_f which becomes maximum at 800 rpm. Generally retention of the stationary phase increases with increased

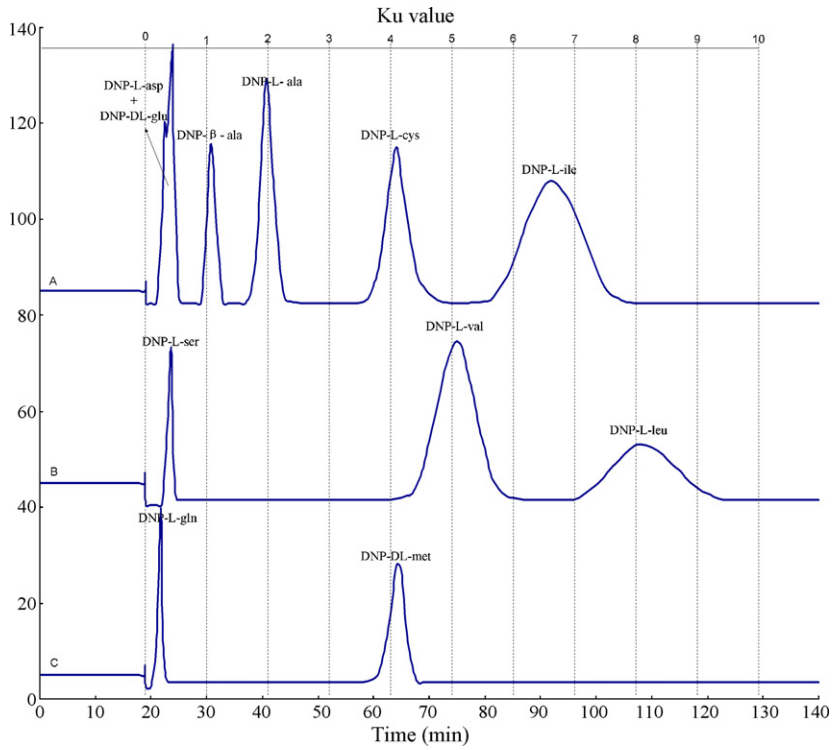

Fig. 6. Chromatograms for examination of K_U values of 11 DNP-amino acids. Sample: (A) DNP-L-asp, DNP-DL-glu, DNP-β-ala, DNP-L-ala, DNP-L-cys and DNP-L-ile; elution mode: H-A with lower mobile phase; (B) DNP-L-ser, DNP-L-val and DNP-L-leu; (C) DNP-L-gln, DNP-DL-met. Sample size: 0.2 ml; flow rate: 0.4 ml/min; elution mode: H-A; solvent system: VI; revolution speed: 800 rpm; capacity: 12 ml.

revolution speed in the conventional type-J coil planet centrifuge whereas in this compact type-I coil planet centrifuge a high revolution speed at 1000 or 1200 rpm causes too violent mixing between the two phases in the coil leading the loss of stationary phase from the column resulting in lower R_s values.

Next, a series of studies was performed to examine effects of flow rates on S_f, R_s, and t_R (Table 4). The results show that, as expected, Sf was decreased with increased flow rate (Fig. 4A). But R_s was improved at first and then decreased reaching the maximum value at 2.75 and 2.16 at the flow rate of 0.4 ml/min (Fig. 4B). The retention time, t_R, was remarkably shortened to 7 min at the highest flow rate of 2 ml/min (Fig. 4C) where R_s still remains moderate values of 0.93 and 0.85. These results indicate that the compact type-I coil planet centrifuge has a great potential for component analysis because of its small column capacity and very short separation time.

Table 5 shows the results of DNP-amino acid separation with the compact type-I coil planet centrifuge at various revolution speeds. Four different flow rates were tested for each revolution speed. Revolution speed at 800 rpm yielded the best R_s at low flow rates up to 0.4 ml/min, while S_f increased with increased revolution speed at higher flow rates of 1–2 ml/min (Fig. 5A) and a higher revolution speed of 1000 rpm yields the best R_s. A flow rate of 1 ml/min produced the best S_f of 40.01% at 800 rpm, while a flow rate of 0.4 ml/min gave the best S_f of 33.04% at 1000 rpm. When the flow rate was 0.4 ml/min, R_s was best at the revolution speed of 800 rpm among all other groups (Fig. 5B and C) and the t_R was only 1 h.

The K_U values of 11 DNP-amino acids including DNP-L-asp, DNP-DL-glu, DNP-β-ala, DNP-L-ala, DNP-L-cys, DNP-L-ile, DNP-L-ser, DNP-L-val, DNP-L-leu, DNP-L-gln and DNP-DL-met were measured using the compact type-I coil planet centrifuge at a flow rate of 0.4 ml/min by three single runs (Fig. 6). In this way the K_U value of a target compound is easily determined according to the value of secondary *X*-axis using the compact type-I coil planet centrifuge

Table 6
Results of the separation of eleven DNP-amino acids in solvent system VI using the compact type-I counter-current chromatography.

Test samples (weight)	K_1	K_2	N	t_R
DNP-L-gln	0.20	0.12	1936	20.5
DNP-L-asp	0.31–0.43	0.30	–	22.5–23.8
DNP-DL-glu	0.31–0.43	0.44	–	23.8
DNP-DL-ser	0.41	0.34	2209	23.5
DNP-β-ala	1.07	1.18	2460	31
DNP-L-ala	1.98	2.20	1060	40.7
DNP-L-cys	4.09	4.32	1024	64
DNP-DL-met	4.12	4.15	592	65.7
DNP-L-val	5.07	4.78	459	75
DNP-L-ile	6.61	6.98	280	92
DNP-L-leu	8.09	7.97	318	108.3

Note: K_1 was tested by compact type-I CCC; K_2 was tested by traditional UV method.

than other traditional methods. The distribution coefficient (K_U) of each sample in the two-phase solvent system was determined using the traditional shake-flask method at 280 nm [7–9] and the results summarized in Table 6. The data of K_U values using present method is similar to those using the traditional method.

4. Conclusions

In the compact type-I coil planet centrifuge with a short revolution radius the high revolution speed up to 1200 rpm can be applied with high stability of the centrifuge system. This compact type-I coil planet centrifuge can provide excellent separation of DNP-amino acids and dipeptides using a suitable two-phase solvent system. At a flow rate of 0.4 ml/min, 11 different DNP-amino acids were resolved at 800 rpm in 1 h except for DNP-glu and DNP-asp with a small separation factor of 1.4. The compact type-I coil planet centrifuge with a small column capacity, short separation time and high separation efficiency is ideal for component analysis.

References

[1] Y. Ito, CRC Rev. Anal. Chem. 17 (1986) 65.
[2] Y. Yang, D. Gu, H. Wu, H.A. Aisa, T. Zhang, Y. Ito, J. Liq. Chromatogr. Relat. Technol. 31 (2008) 3012.
[3] Y. Ito, J. Sandlin, W.G. Bowers, J. Chromatogr. 244 (1982) 247.
[4] Y. Ito, W.D. Conway, High-Speed Countercurrent Chromatography, Wiley-Interscience, New York, 1984.
[5] Y. Ito, R.L. Bowman, Science 173 (1971) 420.
[6] Y. Ito, R.L. Bowman, J. Chromatogr. Sci. 11 (1973) 284.
[7] Y. Ito, R. Clary, J. Powell, M. Knight, T.M. Finn, J. Liq. Chromatogr. Relat. Technol. 31 (2008) 1346.
[8] Y. Yang, H.A. Asia, Y. Ito, J. Liq. Chromatogr. Relat. Technol. 32 (2009) 2030.
[9] Y. Yang, H.A. Asia, Y. Ito, J. Chromatogr. A 1216 (2009) 5265.