



Short communication

## Evaluation on the performance of four different column models mounted on the compact type-I coil planet centrifuge

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## ABSTRACT

Optimal positions of coiled separation columns on the type-I centrifuge were determined for four typical two-phase solvent systems to obtain the best separation efficiency (resolution and retention of stationary phase) for each with a suitable set of test samples. A set of short coiled columns is connected in series and mounted around the holder hub in four different ways: (model A) the tail of one unit with left-handedness was connected to the head of the next unit with right-handedness (TL-HR); (model B) the tail of one unit with left-handedness was connected to the tail of the next unit with right-handedness (TL-TR); (model C) the tail of one unit with left-handedness was connected to the tail of the next unit with left-handedness (TL-TL); (model D) the tail of one unit with left-handedness was connected to the head of the next unit with left-handedness (TL-HL). The results indicated that the performance of model D was the best among the four models. High revolution speed (800 rpm) is favorable to separation using the moderately hydrophobic solvent system of hexane–ethyl acetate–methanol–0.1 M HCl (1:1:1:1, v/v) (HEMW), while lower revolution speed (600 rpm) is beneficial to the separation with polar solvent system of 1-butanol–acetic acid–water (19:1:20, v/v) (BAW).

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### 1. Introduction

Since 1970s, several types of coil planet centrifuges have been developed and tested for their performance in counter-current chromatography [1–4]. Among them, type-I coil planet centrifuge yields a high efficiency for analytical separations [5–7]. However, the study was limited by its bulky centrifuge design.

Recently, we have developed the compact type-I coil planet centrifuge with a short column holder which can permit higher revolution speed than the original design providing wider application ranges [8] and optimized the column angle on the column holder [9]. The results of our studies suggested that the separation efficiency of type-I CCC was affected by various factors such as the shape and orientation of the column on the holder, elution mode and flow rate of the mobile phase. The separation efficiency of this centrifugal CCC was gradually improved by changing shape of the column [10–13], tubing geometries [14–16] and column angle against the centrifugal force [17].

In our present study, a set of short coiled columns with right or left handedness was arranged parallel to each other in four different

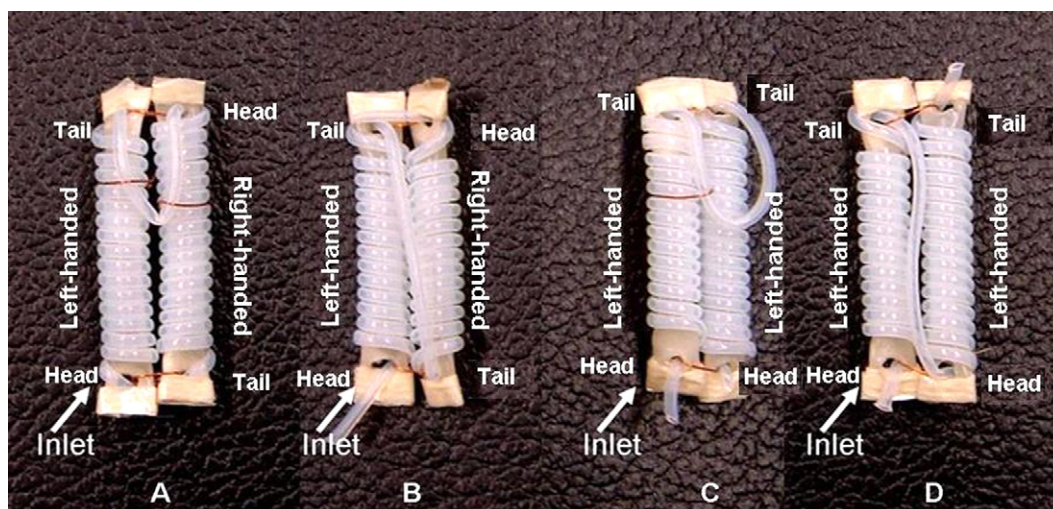
ways where the tail of one unit is connected to the head or tail of the next coil unit (Fig. 1). A series of experiments were performed two typical two-phase solvent systems, each with a set of test samples, to compare the performance of four column models in terms of retention of the stationary phase and peak resolution at various flow rates.

### 2. Experimental

#### 2.1. Apparatus

The apparatus used in the present study is a type-I coil planet centrifuge [8] (hydrodynamic equilibrium CCC system) fabricated at the NIH Machine Shop, Bethesda, MD, USA. 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 type-I 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 uni-

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**Fig. 1.** The coiled column is mounted on the holder hub in four different ways: (model A) the tail of one unit with left-handedness was connected to the head of the next unit with right-handedness (TL-HR); (model B) the tail of one unit with left-handedness was connected to the tail of the next unit with right-handedness (TL-TR); (model C) the tail of one unit with left-handedness was connected to the tail of the next unit with left-handedness (TL-TL); (model D) the tail of one unit with left-handedness was connected to the head of the next unit with left-handedness (TL-HL).

formly circulating around every point on the column holder. The separation column is made by winding a single piece of 0.76 mm I.D. tubing (PTFE SW No. 22, Zeus Industrial Products, Orangeburg, SC, USA) onto 43 pieces of 5 mm O.D. and 5 cm long nylon pipes in such a way that every other coil unit has opposite handedness (Fig. 1A and B) or all coil units are in the same handedness (Fig. 1C and D). In all models the length of the tubing is approximately 20 m with a total capacity of 7.8 ml. These coiled columns were mounted around the holder hub with all coil units parallel to the axis of the holder.

The revolution speed of the apparatus was 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. Test samples including tryptophyl-tyrosine (Trp-Tyr), valyl-tyrosine (Val-Tyr), 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) were all obtained from Sigma Chemicals (St. Louis, MO, USA).

## 2.3. Two-phase solvent systems and sample solutions

In the present study, the solvent system and test samples were chosen according to our previous study [8,9]. Two-phase solvent systems composed of 1-butanol–acetic acid–water (19:1:20, v/v) (BAW) and hexane–ethyl acetate–methanol–0.1 M HCl (1:1:1:1, v/v) (HEMW) were used to separate the dipeptide and DNP-amino acid test samples, respectively. Each solvent mixture was thoroughly equilibrated in a separatory funnel by repeated vigorous shaking and degassing several times, and the two phases separated shortly before using. 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 BAW. Sample solution 2 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 10 ml

of the upper phase of HEMW. 0.2 ml sample solution was charged in each run.

## 2.4. 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 pressurized 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.

## 2.5. Evaluation of partition efficiency

The partition efficiency of the separation column in each run was evaluated by computing the 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.

## 3. Results and discussion

A compact type-I coil planet centrifuge developed in our laboratory produced excellent CCC separation of DNP-amino acids and dipeptides using a suitable two-phase solvent system [8]. In order to further improve its performance, four different columns were tested as illustrated in Fig. 1: (model A) the tail of one unit with left-handedness was connected to the head of the next unit with right-handedness (TL-HR); (model B) the tail of one

**Table 1**  
Comparison of the performance of four different column models at the various flow rates by compact type-I counter-current chromatography.

Sample	Solvent system	Revolution (rpm)	Flow rate (ml/min)	Column model A		Column model B		Column model C		Column model D	
				Sf (%)	Rs	Sf (%)	Rs	Sf (%)	Rs	Sf (%)	Rs
DNP-DL-glu DNP-β-ala DNP-L-ala	HEMW	800	0.1	39.1	2.57/1.95	25.6	1.65/1.49	20.5	0.86/1.26	44.3	2.70/2.10
			0.2	36.8	2.37/2.06	17.9	1.45/1.32	17.9	1.45/1.32	37.5	2.44/2.07
			0.4	23.7	1.52/1.46	12.8	1/0.85	13.2	0.83/0.67	15.4	1.83/1.55
			0.6	16.7	1.35/0.86	7.7	0.31/0.32	10.3	0.72/0.54	11.3	1.39/1.09
			0.8	9.3	0.99/0.73	3.8	–	8.9	–	7.7	1.32/1.07
			0.1	35.9	2.49/2.03	15.4	0.96/1.01	19.2	1.56/1.38	43.6	2.50/2.06
		600	0.2	33.3	1.91/1.88	16.7	1.26/1.26	17.9	1.39/1.36	34.6	2.19/1.92
			0.4	24.4	1.30/1.29	5.1	–	11.5	1.06/0.89	20.5	1.60/1.28
			0.6	21.8	1.26/1.13	4.9	–	10.3	0.56/0.53	17.9	1.32/1.16
			0.8	15.2	0.93/0.70	2.3	–	9.7	0.55/0.50	12.8	1.29/1.12
			0.1	25.3	1.44	15	1.03	19.9	1.58	21.8	0.77
			0.2	28.6	1.65	23.5	1.27	25.6	1.51	38.5	1.81
Val-Tyr Trp-Tyr	BAW	800	0.4	18.4	1.41	17.1	1.21	19.7	1.24	24.5	1.74
			0.6	15.8	1.23	16.7	1.08	18.3	1.35	19.2	1.69
			0.8	13.1	1.20	13.1	0.96	17.9	1.24	18.9	1.67
			0.1	36.1	2.73	30.8	2.22	28.2	1.06	44.6	2.80
		600	0.2	35.5	1.88	31.7	1.77	30.2	1.48	41.0	1.86
			0.4	25.6	1.61	25.6	1.74	24.4	1.29	37.2	1.79
			0.6	23.8	1.47	19.5	1.73	20.4	1.23	32.7	1.72
			0.8	19.9	1.28	17.9	1.70	19.8	0.97	26.4	1.54

unit with left-handedness was connected to the tail of the next unit with right-handedness (TL-TR); (model C) the tail of one unit with left-handedness was connected to the tail of the next unit with left-handedness (TL-TL); (model D) the tail of one unit with left-handedness was connected to the head of the next unit with left-handedness (TL-HL). Because the best separation was obtained using the lower mobile phase in our previous study [8], the present experiments were all performed using lower phase as the mobile phase which was introduced from the head of the first unit (inlet).

A series of experiments was carried out for studying on the performance of these models from A to D. Table 1 shows the comparison in retention of stationary phase (Sf) and resolution (Rs) between four models at various flow rates with the solvent systems of HEMW and BAW using DNP-amino acids and dipeptides as test samples, respectively. All models were investigated under the flow rates of 0.1–0.8 ml/min at revolution speeds of 600 and 800 rpm. Both Sf and Rs obtained from the models A and D were much better than those in the models B and C which showed very poor separation efficiency due to the loss of stationary phase. The performance of model D was slightly better than model A in all flow rates except for the dipeptides separation with 0.1 ml/min flow rate at the 800 rpm. At this point the model D showed very poor results, i.e., Rs was only 0.77 with 21.8% Sf.

In the HEMW separation system (Table 1), the Rs of the model D was always better than that of model A. But the Sf was somewhat different. At the rotational speed of 600 or 800 rpm, model D showed higher Sf than model A at the flow rate of 0.1–0.2 ml/min, but lower Sf than model A at the flow rates from 0.4 to 0.8 ml/min. In the BAW separation system (Table 1), both Sf and Rs in model D

were always better than those in model A except for that special data pointed out earlier. All in all, a low flow rate is in favor of Sf and Rs except for extremely low flow rates.

And then, a series of experiments was performed to examine the effects of revolution speed on Sf and Rs of four models. The results are summarized in Table 2 where the revolution speed was tested at the flow rate of 0.2 ml/min. Models A and D showed much better performance than models B and C. In HEMW system, model D showed the best Sf and Rs values among all models at the revolution speed of 800 rpm yielding the maximum Rs values at 2.44 (DNP-β-ala and DNP-DL-glu) and 2.07 (DNP-L-ala and DNP-β-ala) with 37.5% Sf. In the BAW system, the performance of both Sf and Rs at revolution speed at 600 rpm was better than the others. Model A at a revolution speed at 600 rpm yielded the best Rs at 1.88 with the Sf of 35.5% which is very similar to the Rs at 1.86 of model D at the same separation conditions. The performance at revolution speed of 800 rpm is slightly worse than that with 600 rpm. The results indicated that higher revolution speed over 800 does not improve separation of the type-I CCC, which is quite different from the performance of type-J CCC.

The overall results of the present studies clearly show that the performance of 4 different column models is divided into two groups: models A and D are much more efficient than models B and C in both Sf and Rs. Since the first column unit is identical in all models, this difference in performance must be derived from configurations of the second column unit and the flow tube between the units. In the first unit which is common to all models, the two phases establish hydrodynamic equilibrium from the inlet (head) of the unit where each coil unit is occupied nearly equal volume of

**Table 2**  
Comparison of the performance of four different column models at various revolution speeds by compact type-I counter-current chromatography.

Sample	Solvent system	Revolution (rpm)	Column model A		Column model B		Column model C		Column model D	
			Sf (%)	Rs	Sf (%)	Rs	Sf (%)	Rs	Sf (%)	Rs
DNP-DL-glu DNP-β-ala DNP-L-ala	HEMW	400	21.2	1.12/1.15	10.5	0.63/0.52	7.1	–	15.4	1.24/1.15
		600	33.3	1.91/1.88	16.7	1.26/1.26	17.9	1.39/1.36	34.6	2.19/1.92
		800	36.8	2.37/2.06	17.9	1.45/1.32	17.9	1.45/1.32	37.5	2.44/2.07
		1000	27.7	1.46/1.65	15.4	1.01/0.98	12.8	0.77/0.73	30.7	1.84/1.81
Val-Tyr Trp-Tyr	BAW	400	16.1	1.68	21.9	1.18	23.1	1.06	29.5	1.79
		600	35.5	1.88	31.7	1.77	30.2	1.48	41.0	1.86
		800	28.6	1.65	23.5	1.27	25.6	1.51	38.5	1.81
		1000	18.3	1.33	22.1	1.25	23.1	1.72	33.3	1.74

Note: Flow rate: 0.2 ml/min; mobile phase: lower phase.

each phase and any excess of either phase (in this case the lower mobile phase) occupies the tail (outlet). In this situation, the retention of the stationary phase is stable. Since the second units of models A and D have the similar geometry to the first column in the head–tail relationship, they can maintain stable retention of the stationary phase. In contrast, the second units of models B and C have the reversed head–tail relationship and the two phases establish the hydrodynamic equilibrium at the outlet of the unit. This causes steady carryover of the stationary phase from the head (outlet) that results in lower retention of the stationary phase and reduced  $R_s$  values.

On the other hand, the difference in performance within each group is not well understood. One possibility is the different geometries of flow tubes connecting the two coil units against the unit gravity. For example the flow tube of model A runs from the top of the first unit toward the bottom of the second unit. This tube configuration may facilitate the two phase exchange by the unit gravity to improve the separation whereas in model A only the half of the flow tube provides the effective partition space. The effect of the unit gravity in the main coil unit may be negligible due to the small pitch of the coiled column.

#### 4. Conclusions

Among four column models, model D showed the best separation except at the very low flow rate. And model A showed slightly

lower performance than model D. The result also indicated that a high revolution speed of 800 rpm is favorable to separation using the moderately hydrophobic solvent system (HEMW), but a lower revolution speed of 600 rpm is beneficial for separation with the polar solvent system (BAW).

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