Published in final edited form as:

J Liq Chromatogr Relat Technol. 2010 January ; 33(16): 1542–1549. doi:10.1080/10826076.2010.503780.

Novel Designs for Centrifugal Countercurrent chromatography: V. Comparative Studies on Performance of Various Column Configurations

Yi Yang^{1,2}, Dongyu Gu², Haji Akber Aisa², and Yoichiro Ito^{1,*}

¹ 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, USA

² Xinjiang Key Laboratory of Plant Resources and Natural Products Chemistry, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumqi 830011, China

Abstract

The conventional toroidal coil in centrifugal countercurrent chromatography has a low level of stationary phase retention, since a half of each helical turn is entirely occupied by the mobile phase. In order to cope with this problem, several new column designs including zigzag, saw-tooth and figure-8 patterns have been introduced and their performance was compared in terms of retention of the stationary phase (Sf), peak resolution (Rs), theoretical plate number (N) and column pressures. Overall results of experiments indicate that the figure-8 column yields the highest Rs when the lower phase is used as the mobile phase. Since the column pressure of all these new columns are much lower than that in the traditional toroidal coil column, the separation efficiency can be improved using a long separation column without a risk of column damage by high back pressure.

Keywords

centrifugal countercurrent chromatography; toroidal coiled column; zigzag column; saw-tooth column; figure-8 column; stationary phase retention; peak resolution; dipeptide; DNP-amino acid

Introduction

Countercurrent chromatography (CCC), being a support-free liquid-liquid partition chromatographic technique, eliminates the risk of irreversible adsorption of sample components onto the solid support as often observed in conventional liquid chromatography [1]. A variety of existing CCC schemes can be classified into two forms, i.e., hydrostatic equilibrium system and hydrodynamic equilibrium system [2]. High-speed countercurrent chromatography that is the most advanced hydrodynamic equilibrium system has been widely used for the preparative separation and purification of natural products [3], while hydrostatic CCC system is efficiently applied to analytical separations using a narrow-bore coiled column arranged around the periphery of the centrifuge bowl in a toroidal form in a seal-free flow-through centrifuge [4]. In this toroidal coil CCC system, however, the retention of the stationary phase is limited to substantially less than 50% of the total column capacity, since the half of each helical turn is entirely occupied with the mobile phase. In order to cope with this problem,

* Corresponding author: Yoichiro Ito, Bioseparation Technology Laboratory, Biochemistry and Biophysics Center, National Heart, Lung, and Blood Institute, 10 Center Drive, Bldg.10, Room 8N230, Bethesda, MD 20892-1762, USA.

a triangular coiled column has been introduced which has improved the retention of the stationary phase to slightly over 40% [5]. Recently various column designs have been introduced to further improve the retention of the stationary phase, including zigzag column [6,7], saw tooth column [8] and figure-8 column [9]

In present study, the performance of these three different columns and the traditional coiled column was compared in separation of dipeptides and DNP-amino acid test samples each with a suitable two-phase solvent systems.

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 platform measuring about 34 cm in diameter to hold a separation column. The column is made by hooking approximately 17 m long, a 0.46 mm ID FEP (Fluorinated ethylene propylene) (Zeus Industrial Products, Orangeburg, SC, USA) tubing onto the screws upstanding on the rotary platform to form various column configurations (Fig. 1A) such as zigzag (Fig. 1B), saw tooth (Fig. 1C) and 8-figure (Fig. 1D) each with a total capacity of about 2.8 ml. Each terminal of the column is connected to flow tube (PTFE, Zeus Industrial Products) with a set of tubing connectors (Upchurch Scientific, Palm Spring, CA, USA). 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 of HPLC grade were purchased from Fisher Scientific (Fair Lawn, NJ, USA) and other solvents such as acetic acid and hydrochloric acid of analytical grade from Mallinckrodt Chemicals, Phillipsburg, NJ, USA. Test samples including tryptophyl-tyrosine (Trp-Tyr), valyl-tyrosine (Val-Tyr), N-2, 4-dinitrophenyl-L-alanine (DNP-ala), N-2, 4-dinitrophenyl- β -alanine (DNP- β -ala), and N-2, 4-dinitrophenyl-DL-glutamic acid (DNP-glu) were obtained from Sigma Chemicals (St. Louis, MO, USA).

Partition Coefficient Measurement [6]

The partition coefficient (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 as A_L . The K_U value was calculated according to the following equation: $K_U = A_U/A_L$.

Two-phase Solvent Systems and Sample Solutions

Two typical two-phase solvent systems including 1-butanol-acetic acid-water (4:1:5, 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, and the two phases separated shortly before use. 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 40 μ l of this stock solution was used for each separation. 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 hexane-ethyl acetate-methanol-0.1 M HCl (1:1:1:1, v/v), and 40 μ l of this solution was used for each separation.

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 strip-chart recorder (Pharmacia, Stockholm, Sweden). In order to improve the tracing, ethanol was added to the effluent at the inlet of the detector using a tee connector and a fine mixing tubing (PTFE 0.4 mm ID $\times$ ca 1 m) at a flow rate of 30 % that of the mobile phase. After the desired peaks were eluted, the run was stopped and the column contents were forced by pressurized air into a graduated cylinder to determine the volume of the stationary phase retained in the column. The stationary phase retention (Sf) was computed by dividing the volume of the retained stationary phase by the column volume and expressed as %.

Evaluation of Partition Efficiency

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

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

$$R_s = 2(t_2 - t_1)/(W_1 + W_2) \quad (2)$$

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

Results and Discussion

As shown in Fig. 1, the performance in terms of stationary phase retention (Sf), peak resolution (Rs), theoretical plate number (N) and column pressure (P) of the conventional toroidal coil, zigzag, saw tooth and figure-8 (fig-8) columns was investigated in present study. The results were summarized in table 1.

Fig. 2 shows retention of the stationary phase of four different columns in two typical solvent systems, HEMW and BAW, at a flow rate of 0.05 ml/min using the revolution speed of 1000 rpm. DNP-DL-glu, DNP- β -ala and DNP-L-ala were used as test samples for HEMW, and Val-Tyr and Trp-Tyr, for BAW. In general, retention of the stationary phase of the HEMW system was better than that in the BAW except that the retention (Sf) in the fig-8 column using the lower mobile phase was slightly lower than that in the BAW. When lower phase was mobile phase in the HEMW, the saw tooth column yielded the best Sf at 43.7 % followed by the fig-8 column with Sf at 43.1 % while Sf in the traditional coiled column was worst at 35.7 %. When the upper phase was mobile phase in the HEMW, the fig-8 column gave the best Sf at 55.6 % followed by the saw tooth, zigzag and coiled columns in this order. The Sf in the BAW system showed a similar tendency in both mobile phase groups, i.e., fig-8, saw tooth, zigzag and coiled column in this order (Table 1).

Fig. 3 shows peak resolution of test samples in the two typical two-phase solvent systems. When the lower phase was used as the mobile phase in the HEMW, fig-8 with Rs at 1.69 (1st peak/2nd peak) - 1.79 (2nd peak/3rd peak) was best followed by saw tooth with Rs at 1.54 -

1.55, zigzag at 1.36 - 1.29, and coiled column at 1.25 -1.34 (Fig. 3A and Table 1). But when the upper phase was as the mobile phase in HEMW, Rs of the coiled column at 1.60 - 0.88 was better than that of zigzag at 1.35 - 0.73, saw tooth at 1.27 -0.85, and fig-8 at 1.21 -0.63 (Fig. 3A and Table 1). In the BAW system, the results of Rs were also similar. When the lower phase was used as the mobile phase, Rs of fig-8 at 1.31 was best among others, whereas in the upper phase mobile the coiled column yielded the best Rs at 1.12 (Fig. 3B and Table 1). Among all columns tested, the fig-8 column showed the best Rs values in the lower phase mobile.

Table 1 shows that the theoretical plate number of the fig-8 column was also best when the lower phase is mobile phase indicating that the fig-8 column is highly efficient. The column pressure of all three new columns including zigzag, saw-tooth and fig-8 is much lower than that of the conventional coiled column indicating that a much longer column can be used to further improve the separation.

Conclusions

The overall results of our experiments indicate that among all columns tested the fig-8 column yielded the best performance when the lower phase was used as the mobile phase. Since the column pressure of all new columns is much lower than that in the conventional coiled column, the peak resolution can be further improved by increasing the length of the separation column.

References

1. Ito Y. Golden rules and pitfalls in selecting optimum conditions for high-speed counter-current chromatography. *Journal of Chromatography A* 2005;1065:145–168. [PubMed: 15782961]
2. Ito Y. High-speed countercurrent chromatography. *CRC Rev Anal Chem* 1986;17:65–143.
3. Yang Y, Gu D, Wu H, Aisa HA, Zhang T, Ito Y. Application of preparative high-speed countercurrent chromatography for separation of Elatine from Delphinium shawurens. *J Liq Chromatogr Rel Technol* 2008;31:3012–3019.
4. Ito Y, Bowman RL. Countercurrent Chromatography with flow-through centrifuge without rotating seals. *Anal Biochem* 1978;85:614–617. [PubMed: 646117]
5. Ito Y, Yu H. Triangular helix column for centrifugal countercurrent chromatography. *J Liq Chrom & Rel Technol* 2009;32:560–566.
6. Yang Y, Aisa HA, Ito Y. Novel Design for Centrifugal Countercurrent Chromatography: I. Zigzag Toroidal Column. *J Liq Chromatogr Rel Technol* 2009;32:2030–2042.
7. Yang Y, Aisa HA, Ito Y. Novel Design for Centrifugal Countercurrent Chromatography: II. Studies on Novel Geometries of Zigzag Toroidal Tubing. in press.
8. Yang Y, Aisa HA, Ito Y. Novel design for centrifugal countercurrent chromatography: III. saw tooth column. *J Liq Chromatogr Rel Technol*. Submitted.
9. Gu D, Yang Y, Aisa HA, Ito Y. Novel design for centrifugal countercurrent chromatography: IV. Figure-8 column. *J Liq Chromatogr Rel Technol*. Submitted.

Figure 1.
The unit of four different columns for centrifugal countercurrent chromatography. (A) Toroidal coil; (B) Zigzag; (C) Saw-tooth; (D) Figure-8.

Figure 2.

Comparison in stationary phase retention between four different columns for centrifugal countercurrent chromatography. Solvent system: HEMW and BAW; Samples: DNP-DL-glu, DNP- β -ala, & DNP-L-ala for HEMW and Val-Tyr & Trp-Tyr for BAW; Sample size: 40 μ l; Flow rate: 0.05 ml/min; Revolution speed: 1000 rpm.

Figure 3.

Comparison in the peak resolution between four different columns for centrifugal countercurrent chromatography. Sample size: 40 μ l; Flow rate: 0.05 ml/min; Revolution speed: 1000 rpm. (A) Solvent system: HEMW; Samples: DNP-DL-glu, DNP- β -ala, DNP-L-ala, (B) Solvent system: BAW; Samples: Val-Tyr, Trp-Tyr.

Table 1

Comparison in performance between 4 different columns at a flow rate of 0.05 ml/min for centrifugal countercurrent chromatography

Column	Samples	Solvent	Mobile phase	Sf (%)	Rs	N	P (psi)
Coiled	DNP-DL-glu		Lower phase	35.7	1.25/1.34	138/323/273	200
	DNP-β-ala	HEMH	Upper phase	42.8	1.60/0.88	265/236/252	214
	DNP-L-ala						
	Val-Tyr	BAW	Lower phase	35.3	1.18	136/49	90
Zigzag	Trp-Tyr		Upper phase	38.2	1.12	107/64	92
	DNP-DL-glu		Lower phase	39.8	1.36/1.29	171/267/266	87
	DNP-β-ala	HEMH	Upper phase	46.2	1.35/0.73	100/155/209	95
	DNP-L-ala						
Saw tooth	Val-Tyr	BAW	Lower phase	35.7	1.22	143/52	55
	Trp-Tyr		Upper phase	39.5	0.77	32/120	63
	DNP-DL-glu		Lower phase	43.7	1.54/1.55	176/284/333	109
	DNP-β-ala	HEMH	Upper phase	51.9	1.27/0.85	89/140/216	121
Fig-8	DNP-L-ala						
	Val-Tyr	BAW	Lower phase	43.1	1.26	102/39	63
	Trp-Tyr		Upper phase	46.8	0.92	40/46	69
	DNP-DL-glu		Lower phase	43.1	1.69/1.79	273/486/301	98
Fig-8	DNP-β-ala	HEMH	Upper phase	55.6	1.21/0.63	110/94/189	111
	DNP-L-ala						
	Val-Tyr	BAW	Lower phase	45.5	1.31	155/66	71
	Trp-Tyr		Upper phase	47.0	0.99	55/37	87