Published in final edited form as:

J Chromatogr A. 2011 July 1; 1218(26): 4065–4070. doi:10.1016/j.chroma.2011.04.081.

IMPROVED PARTITION EFFICIENCY WITH THREADED CYLINDRICAL COLUMN IN VORTEX COUNTER-CURRENT CHROMATOGRAPHY

Yoichiro Ito^{1,*}, Zhiyong Ma^{1,4}, Robert Clary², Jimmie Powell², Martha Knight³, and Thomas M. Finn³

¹Bioseparation Technology Laboratory, Biochemistry and Biophysics Center, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA

²Machine Instrumentation, Designing and Fabrication, National Institutes of Health, Bethesda, MD, USA

³CCBiotech LLC, 9700 Great Seneca Highway, Rockville, MD, USA

⁴College of Pharmacy, Harbin Medical University, Harbin 150081, China

Abstract

Type-I coil planet centrifuge produces a uniformly circulating centrifugal force field to produce vortex motion of two immiscible solvent phases in a cylindrical cavity of the separation column to perform efficient countercurrent chromatography. The partition efficiency obtained from the original vortex column was substantially improved by threading the cylindrical cavity to increase the area of mass transfer between the two phases. Partition efficiency of the threaded column was evaluated by three different two-phase solvent systems with a broad range of hydrophobicity each with a set of suitable test samples. Overall results of the present studies indicated that the threaded cylindrical column substantially improves the partition efficiency in terms of theoretical plate number, peak resolution, and height equivalent of one theoretical plate. The results also indicated that higher peak resolution is produced by eluting either the upper phase in the head to tail direction or the lower phase in the reversed direction. When there is a choice in the mobile phase, a better separation is achieved by using the less viscous phase as the mobile phase. Since the present system gives extremely low column pressure, it may be a potential alternative to the conventional type-J HSCCC system for a large-scale preparative separation.

Keywords

vortex counter-current chromatography; type-I coil planet centrifuge; cylindrical vortex column; threaded cylindrical vortex column

1. Introduction

During the past few decades high-speed countercurrent chromatography (HSCCC) has been widely used for separation and purification of natural and synthetic products with a type-J coil planet centrifuge with a coiled separation column [1-5]. The method is based on the type-J planetary motion where the column holder revolves around the central axis of the centrifuge while it synchronously rotates about its own axis in the same direction. This motion creates an Archimedean Screw effect on the coiled separation column coaxially

*Fax: 301-402-0013, itoy2@mail.nih.gov .

mounted on the holder resulting in high retention of the stationary phase and efficient mixing of the two phases. Although the method yields efficient solute partitioning, the mixing of the two phases is carried out along the length of the coiled tube which tends to broaden the solute band [6]. In addition the column pressure generated by the Archimedean screw force increases with the enhanced revolution speed of the column.

As stated earlier [7], the vortex CCC system uses a type-I coil planet centrifuge in which the column holder synchronously rotates about its own axis in the reversed direction [8,9]. This planetary motion produces a uniformly circulating centrifugal force field of the same strength synchronously with the revolution at every point on the column holder. Consequently, the system creates a well-known vortex effect on the two immiscible solvent phases so that they undergo circular motion synchronously with the rotating centrifugal force within each cylindrical cavity of the separation column. This planetary motion produces efficient mixing of the two phases in a plane perpendicular to the column axis, hence sample band broadening becomes minimum to enhance the peak resolution. Another important feature of the present system is that the use of cylindrical separation column (instead of the coil) avoids the generation of the Archimedean screw force so that the separation can be performed under minimum column pressure. In fact the stopping the revolution does not alter the column pressure at the outlet of the pump! The preliminary experiment with this vortex CCC column has been performed using a series of cylindrical columns with different diameters (3 mm, 4 mm, 5 mm, 7.5 mm, 10 mm and 12.5 mm) and it was found that the smallest column (3 mm diameter) yielded the highest partition efficiency [7]. The partition efficiency of this 3 mm diameter column of 6 m in length has been tested and expressed in terms of theoretical plate number (N or TP), peak resolution (R_s) and height equivalent to a theoretical plate (HETP). As expected, the column yielded short HETP of only 2 cm compared with 15 cm needed for the conventional multilayer coil separation column in type-J HSCCC [7].

In the present study the above cylindrical vortex column is threaded to enhance partition efficiency. The partition efficiencies obtained from this modified column are compared with those from the original column.

2. Experimental

2.1. Apparatus

The type-I coil planet centrifuge and the vortex CCC column were fabricated at the NIH Machine Shop (Fig. 1). The centrifuge carries the vortex column and a counter-weight at a distance of 10 cm from the central axis of the apparatus. The designs of the apparatus and vortex column were described in detail elsewhere [7]. As mentioned earlier, the separation column undergoes type-I synchronous planetary motion, i.e., the column counter-rotates about its own axis once during every revolution cycle. This planetary motion is effected by coupling a pair of identical toothed pulleys, one (stationary pulley) is mounted at the bottom plate on the axis of the revolution and other other (planetary pulley) at the lower end of the column holder shaft. This planetary motion allows the flow tubes to rotate without twisting.

The revolution speed of the apparatus is regulated from 0 to 1200 rpm while 600 to 1000 rpm was applied. In the present studies a set of 3 mm diameter cylindrical column (5 cm deep and 120 units with total capacity of about 42 ml) tested in the previous studies [7] was modified in such a way that the upper cylinder was threaded with a 6-40 right-handed tap and the lower cylinder with a 6-40 left-handed tap (Fig. 2). This creates a weak Archimedean Screw effect so that the elution can be performed in either head toward tail or tail toward the head depending on the direction of revolution. As shown in the figure,

cylindrical partition units are connected in series with narrow ducts (ca. 1 mm I.D.) in such a way that the side opening (inlet) is joined to the center opening (outlet).

The solvent was eluted with an HPLC pump (Waters 510, Waters, Milford, MA, USA) and the effluent was monitored with an LKB Uvicord SII (LKB Instruments, Stockholm, Sweden) to record the elution curve with a strip-chart recorder (Pharmacia LKB REC102, Pharmacia, Stockholm, Sweden).

2.2. Reagents

All organic solvents including hexane, ethyl acetate, acetonitrile, methanol, 1-butanol were in chromatographic grade (Fisher Scientific, Fair Lawn, NJ, USA). Hydrochloric acid and acetic acid were reagent grade and purchased from Mallinckrodt Baker, inc., Phillipsburg, NJ, USA. Test samples including, Sudan I and II, N-2,4-DNP-DL-glutamic acid (DNP-glu), N-2,4-DNP-L-alanine (DNP-ala), valyl-tyrosine (Val-Tyr), were purchased from Sigma Chemicals, St. Louis, MO, USA while L-tryptophyl-L-tyrosine (Trp-Tyr) were from Bachem, Torrance, CA, USA.

2.3. Two-phase solvent systems and sample solutions

In the present studies, 3 sets of two-phase solvent systems with a broad range of hydrophobicity were selected as follows: hexane-acetonitrile for separation of Sudan I ($K = 0.39$) and Sudan II ($K = 0.77$); hexane-ethylacetate-methanol-0.1 M hydrochloric acid (1 : 1 : 1 : 1, v/v) for separation of DNP-glu ($K = 0.44$) and DNP-ala ($K = 2.36$); and 1-butanol-acetic acid-water (4 : 1 : 5, v/v) for separation of Trp-Tyr ($K = 0.53$) and Val-Tyr ($K = 1.69$). The partition coefficient values (K expressed as solute concentration in the upper phase divided by that in the lower phase) in each test sample are indicated in the parentheses. Each solvent mixture was thoroughly equilibrated in a separatory funnel by repeated shaking and degassing and the two phases separated shortly before use. The sample solutions for each set of solvent systems were made by a suitable amount of the sample mixture in a given volume of upper phase.

2.4. VCCC procedure

The separation was initiated by filling the column with the stationary phase, either upper or lower phase, followed by sample injection through the sample port. Then, the column was rotated at a given revolution rate while the column was eluted with the mobile phase at a desired rate. The effluent from the column was continuously monitored with an LKB Uvicord SII and collected in a graduated cylinder to measure the replaced volume of the stationary phase to determine the retention of the stationary phase (S_f). The chromatographic curve was traced with a strip-chart recorder. Separation was performed by eluting both upper and lower phases as the mobile phase in either head to tail or tail to head elution mode. After completing the separation, the column was flashed with the stationary phase used for the next run at a high flow rate of 9.9 ml/min. This process entirely replaces the aqueous phase in the column with the stationary phase while some amount of organic phase still remains in the column. The amount of the other phase still remained in the column was determined by flashing the aqueous phase from the middle opening to the side opening of the cylindrical unit (opposite direction from the run) at 800 rpm in the tail to head elution mode for 10 – 15 min. The volume of the organic stationary phase thus collected was used to correct the S_f values determined above.

2.5. Evaluation of partition efficiency

The partition efficiency of each run was determined from the chromatogram and expressed in terms of theoretical plate number of each peak (TP or N), peak resolution (Rs), and height equivalent of one theoretical plate (HETP) using the following conventional equations:

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

$$Rs=2(T_{R2}-T_{R1})/(W_1+W_2) \quad (2)$$

where T_R indicates the retention time and W , the peak width of each specified peak. When two peaks are only partially resolved, Rs is approximated by the following modified equation using W'_1 for the front half of the first peak and W'_2 for the rear half of the second peak as follows:

$$Rs=(T_{R2}-T_{R1})/(W'_1+W'_2) \quad (3)$$

Height equivalent of one theoretical plate (HETP) was computed by dividing the total column length (600 cm) by averaged N values between the first and second peaks.

3. Results and discussion

3.1. Separation of Sudan dyes with hexane-acetonitrile system

Table I top panel summarizes the results of separation of Sudan I and Sudan II dyes with hexane-acetonitrile binary solvent system. The partition efficiencies are expressed by three parameters, i.e., theoretical plate number of each peak (TP), peak resolution between the two dyes (Rs), and height equivalent of one theoretical plate (HETP), as indicated at the top of the table. Separations were performed under various experimental conditions by changing the flow rates (0.5-3 ml/min), revolution speeds (600 – 1000 rpm), and elution mode (head to tail and tail to head directions) using both upper and lower phases as mobile phase. In order to ease the comparison of data, the best results are highlighted with bold numbers in each group (each row). In general, the partition was increased with higher revolution speed and lower flow rates of the mobile phase, producing the best separation at the flow rates of 0.5 and 1 ml/min. Under these conditions, the separations yielded HETP values of 1 – 2 cm compared with about 20 cm that is usually required for the conventional multilayer coil separation column. Comparison of Rs between upper and lower mobile phases can't be made properly due to relative K values of two compounds which favors the upper phase mobile. However, it is interesting to note that in the upper mobile phase in the head to tail elution mode yielded higher Rs values than the tail to head elution mode, while this relationship was reversed in lower phase mobile. This tendency was well correlated with the retention of stationary phase (S_f), i.e., higher retention was obtained by eluting the upper phase in the head to tail direction or lower phase in the reversed direction. This hydrodynamic effect may be explained as follows: The spiral grooves made on the internal wall of each cylindrical cavity forms a weak Archimedean Screw effect which drives both phases toward the head of each unit. This force, however, acts differently between the two phases. Since the upper hexane phase is strongly hydrophobic and has a high affinity to the wall, its movement along the spiral grooves is much more restricted than that of the lower acetonitrile phase that has less affinity to the wall. Therefore, when the upper phase is used as the mobile phase, the head to tail elution mode efficiently drives the lower phase back

toward the head (inlet) to improve the retention of the stationary phase. In contrast, when the upper phase is used as the stationary phase, the tail to head elution mode quickly drives the lower mobile phase toward the head (outlet) of each unit leaving a larger volume of the upper phase in the column.

3.2. Separation DNP-amino acids with hexane-ethyl acetate-methanol-0.1M HCl (1:1;1;1, v/v)

Table 1 middle panel similarly shows the partition results of DNP-glu and DNP-ala with the retention of the stationary phase. Here, the effects of the head – tail elution mode and retention of the stationary phase on R_s was much more clearly pronounced as shown in bold number: The better results were obtained using the upper mobile phase in the head to tail elution mode or the lower mobile phase in the tail to head elution mode. Since this two-phase solvent system gives nearly symmetrical K values around $K = 1$ for the two test samples (DNP-glu: $K = 0.44$ and DNP-ala: $K = 2.36$), one can compare the effects of the choice of the mobile phase on the R_s . As clearly shown, the upper phase mobile always yielded higher R_s values than those from lower phase mobile under otherwise identical experimental conditions, despite less retention of the stationary phase (S_f). Although the reason of this hydrodynamic effect is difficult to explain, it is more likely that viscosity of the two phases plays a major role in solute partitioning where higher R_s is produced using less viscous phase as the mobile phase, since in the butanol solvent system described below this effect was completely reversed, i.e., the less viscous lower aqueous mobile phase produced higher R_s than viscous upper butanol mobile phase.

Since this group of two-phase solvent systems is widely used for separation of a variety of natural products with a broad range of hydrophobicity, the results of the present VCCC system would provide a broad application to the separation of moderately hydrophobic compounds with a large preparative column with a high flow rate (5 ml/min) under a high revolution speed (1000 rpm).

3.3. Separation of dipeptides with 1-butanol-acetic acid-water (4:1:5, v/v)

A set of data for separation of Trp-Tyr ($K=0.53$) and Val-Tyr ($K=1.69$) is similarly summarized in Table 1 bottom panel. With a few exceptions, R_s and S_f values declined as the flow rate was increased from 0.5 – 3 ml/min while they were improved by increasing the revolution speed from 600 to 1000 rpm. Because the separation yielded several hundred theoretical plates (TP), the HETP values showed an excellent range mostly between 1 and 2 cm. As in other solvent systems, upper mobile phase yielded higher R_s and S_f in the head to tail elution mode while the lower mobile phase showed an opposite trend at high revolution speeds of 800 – 1000 rpm. As stated earlier, the lower phase mobile shows substantially higher R_s than the upper mobile phase under otherwise identical experimental conditions. Despite a lower level of S_f , the system could yield high R_s values even at a relatively high flow rate of 3 ml/min. Since this polar solvent system is not efficiently applied to the conventional multilayer coil, the present method will be an alternative approach for the separation of polar compounds.

3. 4. Comparison of partition efficiency between the original and threaded VCCC columns

A set of data obtained from the threaded cylindrical column is compared with that from the original column in Table 2 where the data obtained from the original column is indicated under “ref”. Throughout Table 2 higher numbers in each group (each row) between the original and threaded columns are highlighted with bold fonts. It clearly shows that the threaded cylindrical column yields substantially higher partition efficiency than the original non-threaded column in all the two-phase solvent systems examined.

The improved partition behavior of the threaded cylindrical column demonstrated above may be attributed to the increased surface area over the internal wall of the cylindrical cavity. As reported earlier [7], the interface areas between the two phases in the VCCC column may be divided into two parts: one is a vertical rectangular interface formed between the two phases and the other, the internal wall surface where one of the phases with higher wall affinity (hydrophobic upper phase) constantly forms a thin layer against the other phase to serve for mass transfer between the two phases. In the threaded column this second interface area is nearly doubled with minimum increase of the column capacity. In the original column, the vertical interface area formed within each cylindrical cavity is estimated as 1.5 cm^2 ($0.3 \text{ cm} \times 5 \text{ cm}$) while area of the internal wall surface is 4.7 cm^2 ($0.3 \text{ cm} \times \pi \times 5 \text{ cm}$). Since at any given moment the lower phase covers about one half of the inner surface to contribute the mass transfer, the second partition area (4.7 cm^2) should be divided in half to 2.35 cm^2 . Consequently, the total effective mass transfer area becomes 3.85 cm^2 . In the threaded column the wall surface area may be doubled to 9.4 cm^2 providing a net 4.7 cm^2 increase in the effective mass transfer area. Therefore, the total area of the effective mass transfer area becomes 6.2 cm^2 ($4.7 + 1.5 \text{ cm}^2$) or 1.6 times that of the original column. Assuming that the above two different interface areas have equivalent mass transfer rates and that the partition efficiency increases in proportion to the interface area, the threaded column should yield square root of 1.6 or 1.27 times R_s of the original column. Comparative data of R_s values between the original and threaded columns in Table 2 strongly support the above hypothesis. These results give some useful suggestions for further improvement of the VCCC column that the internal surface of the cylindrical cavity may be further increased by modifying the pitch and depth of spiral grooves in each cylindrical cavity of the separation column.

3.5. Prediction of preparative capability of VCCC

As mentioned earlier, the development of VCCC was only recently started, and all experiments have been performed with a short column of 6 m in length. Nevertheless, its low column pressure and a large effective column space (about 1/3 of the whole column volume) promise its preparative capability which may exceed that of the conventional type-J HSCCC multilayer coiled column with 320 ml capacity: If the present column (16 cm diameter and 5 cm in height) is entirely made with a 3 mm cylindrical unit (now under fabrication), it will have near 1000 cylindrical units with over 300 ml capacity which becomes comparable with the conventional multilayer coil in Pharma-Tech HSCCC instrument (semi-preparative column with 1.6 mm ID). Please note that if the theoretical plate number of the column proportionally increases with the column length, this VCCC column would produce a few thousand theoretical plates compared with several hundred theoretical plates in the conventional HSCCC column. This column capacity can be further increased by eliminating the central shaft between the pair of plates supporting the column and the counterweight to accommodate a larger column of 32 cm in diameter (4 times of the present column). Since about one third of the column volume is available for the column space, the column capacity should be increased to 1200 ml in a compact bench-top unit (58 cm wide and 32 cm high). Because of the light weight of the column made of polyethylene (density is less than 1) and only one pair of flying flow tubing, the revolution speed of the column may be increased to 1400 rpm at a higher flow rate as in HPCCC. In addition, its belt driven system is more stable and much quieter than the gear-driven type-J coil planet centrifuge.

4. Conclusions

Overall results of the present studies described above clearly demonstrate that the threaded cylindrical column yields higher partition efficiency than the original non-threaded column.

This improvement may be attributed to the increased interface area between the two phases over the internal wall surface. In this threaded cylindrical column, the better separation (R_s) can be achieved by eluting either the upper phase in the head to tail direction or the lower phase in the tail to head direction. When you can choose between the upper or the lower phase, the less viscous phase should be used as the mobile phase to achieve a higher peak resolution. The efficiency of the VCCC column may be further improved by modifying the pitch and depth of the spiral grooves.

References

- [1]. Ito Y. CRC Crit. Rev. Anal Chem. 1986; 17:65.
- [2]. Conway, WD. Apparatus, Theory and Applications. VCH; 1990.
- [3]. Ito, Y.; Conway, WD., editors. High-Speed Countercurrent Chromatography. Wiley-Interscience; New York: 1996.
- [4]. Menet, J-M.; Thiébaud, D., editors. Countercurrent Chromatography. Vol. Volume 82. Marcel Dekker; New York: 1999. Chromatographic Science Series
- [5]. Berthod, A., editor. Countercurrent Chromatography: The Support-Free Liquid Stationary Phase, Comprehensive Analytical Chemistry. Vol. vol. XXXVIII. Elsevier; Amsterdam: 2002.
- [6]. Berthod A. J. Chromatogr. A. 2006; 1126:347. [PubMed: 16759661]
- [7]. Ito Y, Ma Z-Y, Clary R, Powell J, Knight K, Finn TM. J. Chromatogr. A. in press.
- [8]. Ito Y, Bowman RL. Science. 1971; 173:420–422. [PubMed: 5557320]
- [9]. Yang Y, Gu D-Y, Aisa HA, Ito Y. J. Chromatogr. A. 2010; 1217:3167–3170. [PubMed: 20346457]

Figure 1.
Diagram of the vortex counter-current chromatograph [7]

Figure 2. diagram of four of the 120 cells of the threaded cylindrical column for VCCC In this column design, with clockwise revolution (viewed from the top) the inlet of the column becomes the tail and the outlet of the column becomes the head, and vice versa when the direction of the revolution is reversed (counterclockwise).

Table 1

Summary of partition data of VCCC with three two-phase solvent systems
 Top panel: separation of Sudan dyes (Sudan I & II) with hexane-acetonitrile binary system; middle panel: separation of DNP-amino acids (DNP-glu & DNP-ala) with hexane-ethyl acetate methanol-0.1 M hydrochloric acid; bottom panel separation of dipeptides (Trp-Tyr & Val-Tyr) with 1-butanol-acetic acid-water (4:1:5, v/v).

Solvent System (volume ratio)	Mobile Phase	RPM	Flow Rate (ml/min)	TP Peaks I - II H→T / T→H	Rs H→T / T→H	HETP (cm) H→T / T→H	S _f (%) H→T / T→H
Hexane	upper	600	0.5	648 - 392 / 576 - 426	1.83 / 1.68	1.2 / 1.2	47 / 44
Acetonitrile			1.0	393 - 175 / 400-278	1.69 / 1.28	2.1 / 1.8	44 / 41
			2.0	238 - 207 / 266 - 162	1.06 / 0.97	2.7 / 2.8	37 / 39
Sample			3.0	156 - 127 / 122 - 115	0.74 / 0.76	4.2 / 5.1	37 / 34
Sudan I (K=0.39)		800	0.5	508 - 354 / 598 - 478	1.29 / 1.33	1.4 / 1.1	46 / 37
Sudan II (K=0.77)			1.0	449 - 362 / 534 - 438	1.31 / 1.38	1.5 / 1.2	44 / 32
each 5 mg in 0.5 ml up			2.0	316 - 286 / 555 - 395	1.36 / 1.05	2.0 / 1.3	37 / 34
			3.0	306 - 187 / 320 - 160	0.90 / 0.85	2.4 / 2.5	24 / 32
		1000	0.5	307 - 242 / 300 - 307	1.19 / 1.14	2.2 / 2.0	37 / 37
			1.0	392 - 313 / 470 - 419	1.39 / 1.33	1.7 / 1.3	41 / 32
			2.0	370 - 334 / 567 - 400	1.29 / 1.15	1.7 / 1.2	34 / 32
			3.0	331 - 207 / 434 - 259	0.97 / 0.92	2.2 / 1.7	36 / 30
	Lower	600	0.5	652 - 496 / 663 - 324	1.17 / 1.27	1.1 / 1.0	42 / 44
			1.0	788 - 511 / 744 - 506	0.98 / 1.11	0.9 / 1.0	33 / 37
			2.0	484 - 367 / 576 - 298	0.60 / 0.69	1.4 / 1.4	26 / 26
			3.0	-	-	-	-
		800	0.5	503 - 400 / 529 - 396	1.30 / 1.37	1.3 / 1.3	47 / 51
			1.0	423 - 367 / 563 - 344	1.10 / 1.28	1.5 / 1.3	42 / 44
			2.0	613 - 327 / 564 - 324	0.68 / 0.85	1.3 / 1.4	33 / 37
			3.0	-	-	-	-
		1000	0.5	334 - 262 / 383 - 281	1.01 / 1.11	2.0 / 1.8	51 / 51
			1.0	400 - 281 / 583 - 419	1.14 / 1.19	1.8 / 1.2	47 / 47
			2.0	544 - 340 / 467 - 331	0.85 / 0.98	1.4 / 1.5	33 / 42

Solvent System (volume ratio)	Mobile Phase	RPM	Flow Rate (ml/min)	TP Peaks I - II	Rs	HETP (cm)	S _f (%)
				H→T / T→H	H→T / T→H	H→T / T→H	H→T / T→H
			3.0	693 - 320 / 654 - 382	0.69 / 0.66	1.2 / 1.2	28 / 37
Hexane	upper	600	1.0	279 - 159 / 279 - 134	2.44 / 2.88	2.7 / 2.9	29 / 29
Ethyl acetate			2.0	159 - 92 / 134 - 87	1.96 / 1.57	2.9 / 5.4	31 / 37
Methanol			3.0	202 - 76 / 193 - 72	1.39 / 1.25	4.3 / 7.3	39 / 24
0.1 M HCl			5.0	153 - 64 / 153 - 53	1.20 / 1.05	3.2 / 5.8	24 / 21
(1:1:1)		800	1.0	311 - 179 / 355 - 192	2.72 / 2.72	2.4 / 2.2	29 / 27
			2.0	446 - 148 / 306 - 141	2.27 / 1.84	2.0 / 2.7	29 / 20
sample			3.0	259 - 113 / 188 - 96	1.79 / 1.54	3.2 / 4.2	27 / 20
DNP-DL-glu (K=0.44)			5.0	256 - 71 / 315 - 73	1.19 / 1.16	3.7 / 3.1	26 / 13
DNP-L-ala (K=2.26)		1000	1.0	270 - 127 / 273 - 138	2.48 / 2.55	3.0 / 2.9	36 / 31
Each 1 mg in 0.1 ml up			2.0	267 - 142 / 267 - 159	2.54 / 2.39	2.9 / 2.8	31 / 27
			3.0	242 - 115 / 207 - 131	2.12 / 1.97	3.4 / 3.6	29 / 17
			5.0	195 - 91 / 348 - 96	1.54 / 1.33	4.2 / 2.7	20 / 13
	Lower	600	1.0	476 - 149 / 520 - 169	1.72 / 1.80	1.9 / 1.6	35 / 40
			2.0	519 - 113 / 507 - 121	1.09 / 1.25	1.9 / 1.9	26 / 33
			3.0	433 - 101 / 433 - 120	0.89 / 0.98	2.2 / 2.2	23 / 26
			5.0	324 - 72 / 265 - 80	0.56 / 0.61	3.0 / 3.5	21 / 21
		800	1.0	329 - 155 / 364 - 138	2.18 / 2.16	2.5 / 2.4	42 / 49
			2.0	387 - 118 / 306 - 118	1.50 / 1.56	2.4 / 2.8	33 / 40
			3.0	322 - 172 / 311 - 112	1.38 / 1.28	2.4 / 2.8	28 / 33
			5.0	343 - 102 / 420 - 113	0.69 / 0.87	2.7 / 2.3	23 / 23
		1000	1.0	271 - 142 / 483 - 170	2.19 / 2.34	2.4 / 1.8	42 / 47
			2.0	377 - 201 / 400 - 157	1.49 / 1.62	2.1 / 2.1	33 / 42
			3.0	433 - 128 / 333 - 115	1.44 / 1.62	2.1 / 2.7	30 / 35
			5.0	477 - 155 / 442 - 139	0.95 / 1.05	1.9 / 2.1	25 / 28
1-butanol	upper	600	0.5	697 - 404 / 590 - 484	1.61 / 1.47	1.1 / 1.1	23 / 19
Acetic acid			1.0	453 - 268 / 595 - 306	1.08 / 1.03	1.7 / 1.3	20 / 18
water			2.0	289 - 263 / 381 - 170	0.77 / 0.70	2.2 / 2.5	19 / 11

Solvent System (volume ratio)	Mobile Phase	RPM	Flow Rate (ml/min)	TP Peaks I - II	Rs	HETP (cm)	S _f (%)
(4:1:5)			3.0	-	-	-	-
		800	0.5	684 - 428 / 850 - 544	2.00 / 1.69	1.1 / 0.9	34 / 24
Sample			1.0	784 - 520 / 900 - 504	1.88 / 1.27	0.9 / 1.3	20 / 18
Trp-Tyr (K=0.53) .25mg			2.0	439 - 308 / 576 - 358	1.21 / 0.99	1.6 / 1.4	20 / 13
Val-Tyr (K=1.69) 1.0mg			3.0	571 - 221 / 462 - 111	0.85 / 0.52	1.5 / 2.1	17 / 11
In 0.1 ml up		1000	0.5	347 - 357 / 615 - 505	1.47 / 1.56	1.7 / 1.1	26 / 20
			1.0	538 - 505 / 589 - 653	1.72 / 1.45	1.2 / 1.0	30 / 20
			2.0	1089-557 / 981 - 809	1.46 / 1.37	0.7 / 0.7	20 / 13
			3.0	668 - 332 / 841 - 378	1.07 / 0.96	1.2 / 1.0	11 / 11
	Lower	600	0.5	448 - 289 / 598 - 317	2.33 / 2.66	1.6 / 1.3	35 / 37
			1.0	511 - 212 / 493 - 247	2.20 / 1.99	1.7 / 1.6	30 / 28
			2.0	420 - 147 / 332 - 134	1.50 / 1.29	2.1 / 2.6	21 / 22
			3.0	226 - 85 / 211 - 68	1.03 / 0.86	3.9 / 4.3	18 / 15
		800	0.5	590 - 389 / 485 - 303	2.88 / 2.86	1.2 / 1.5	37 / 44
			1.0	354 - 196 / 444 - 295	1.96 / 2.35	2.2 / 1.6	30 / 32
			2.0	314 - 199 / 442 - 271	1.42 / 1.66	2.3 / 1.7	23 / 23
			3.0	256 - 144 / 305 - 182	1.03 / 1.24	3.0 / 2.5	21 / 18
		1000	0.5	320 - 330 / 488 - 288	2.76 / 2.84	1.8 / 1.5	41 / 44
			1.0	354 - 356 / 676 - 286	2.41 / 2.81	1.7 / 1.2	30 / 38
			2.0	331 - 184 / 522 - 265	1.57 / 1.91	2.3 / 1.5	26 / 26
			3.0	278 - 173 / 502 - 236	1.31 / 1.55	2.7 / 1.6	21 / 18

TP: theoretical Plate number; Rs: peak resolution; HETP: height equivalent of one theoretical plate computed from the total column length (600 cm) divided by the average TP between the two peaks; Sf: retention of stationary phase; H: head; T: tail. Bold numbers indicate the highest efficiency in each group.

Table 2

Comparison of partition efficiency between the original and threaded cylindrical column
 Top panel: separation of Sudan dyes with hexane-acetonitrile binary system; middle panel: separation of DNP-amino acids with hexane-ethyl acetate-methanol-0.1 M hydrochloric acid; bottom panel: separation of dipeptides with 1-butanol-acetic acid-water (4:1:5, v/v).

Solvent System (volume ratio)	Mobile Phase	RPM	Flow Rate (ml/min)	TP Peaks I - II		Rs		HETP (cm)	
				H→T / T→H	ref	H→T / T→H	ref	H→T / T→H	ref
Hexane	upper	600	0.5	648 - 392 / 576 - 426	294-230	1.83 / 1.68	1.33	1.2 / 1.2	2.3
Acetonitrile			1.0	393 - 175 / 400-278	354-237	1.69 / 1.28	1.21	2.1 / 1.8	2.1
			2.0	238 - 207 / 266 - 162	119-93	1.06 / 0.97	0.81	2.7 / 2.8	5.7
Sample			3.0	156 - 127 / 122 - 115	100-64	0.74 / 0.76	0.53	4.2 / 5.1	7.3
Sudan I (K=39)		800	0.5	508 - 354 / 598 - 478	406-296	1.29 / 1.33	1.39	1.4 / 1.1	1.7
Sudan II (K=77)			1.0	449 - 362 / 534 - 438	467-256	1.31 / 1.38	1.28	1.5 / 1.2	1.6
each 0.5 mg in 0.1 ml up			2.0	316 - 286 / 555 - 395	225-201	1.36 / 1.05	0.94	2.0 / 1.3	2.8
			3.0	306 - 187 / 320 - 160	182-104	0.90 / 0.85	0.64	2.4 / 2.5	4.5
		1000	0.5	307 - 242 / 300 - 307	361-250	1.19 / 1.14	1.19	2.2 / 2.0	1.9
			1.0	392 - 313 / 470 - 419	380-275	1.39 / 1.33	1.08	1.7 / 1.3	1.8
			2.0	370 - 334 / 567 - 400	291-224	1.29 / 1.15	0.91	1.7 / 1.2	2.3
			3.0	331 - 207 / 434 - 259	254-155	0.97 / 0.92	0.75	2.2 / 1.7	2.9
Lower		600	0.5	652 - 496 / 663 - 324	626-443	1.17 / 1.27	0.98	1.1 / 1.0	1.1
			1.0	788 - 511 / 744 - 506	486-320	0.98 / 1.11	0.83	0.9 / 1.0	1.5
			2.0	484 - 367 / 576 - 298	-	0.60 / 0.69	-	1.4 / 1.4	-
			3.0	-	-	-	-	-	-
		800	0.5	503 - 400 / 529 - 396	581-359	1.30 / 1.37	1.21	1.3 / 1.3	1.3
			1.0	423 - 367 / 563 - 344	519-359	1.10 / 1.28	0.61	1.5 / 1.3	1.4
			2.0	613 - 327 / 564 - 324	384-321	0.68 / 0.85	0.53	1.3 / 1.4	1.7
			3.0	-	-	-	-	-	-
		1000	0.5	334 - 262 / 383 - 281	469-428	1.01 / 1.11	0.86	2.0 / 1.8	1.3
			1.0	400 - 281 / 583 - 419	566-389	1.14 / 1.19	1.03	1.8 / 1.2	1.3
			2.0	544 - 340 / 467 - 331	492-341	0.85 / 0.98	0.71	1.4 / 1.5	1.5
			3.0	693 - 320 / 654 - 382	388-328	0.69 / 0.66	0.52	1.2 / 1.2	4.7

Solvent System (volume ratio)	Mobile Phase	RPM	Flow Rate (ml/min)	TP Peaks I - II	Rs	HETP (cm)
				H → T / T → H	ref	H → T / T → H
Hexane	upper	600	1.0	279 – 159 / 279 – 134	86-70	1.86
Ethyl acetate			2.0	159 – 92 / 134 – 87	76-40	1.45
Methanol			3.0	202 -- 76 / 193 – 72	71-35	1.19
0.1 M HCl			5.0	153 – 64 / 153 – 53	-	3.2 / 5.8
(1:1:1:1)		800	1.0	311–179 / 355–192	196-110	1.65
Sample			2.0	446 – 148 / 306 – 141	152-79	1.32
DNP-DL-glu (K=0.44)			3.0	259 – 113 / 188 – 96	141-71	1.31
DNP-zL-ala (K=2.36)			5.0	256 – 71 / 315 – 73	-	1.19 / 1.16
each 1 mg in 0.1 ml up		1000	1.0	270 – 127 / 273 – 138	144-110	2.23
			2.0	267 – 142 / 267 – 159	118-52	1.33
			3.0	242 – 115 / 207 – 131	144-61	1.52
			5.0	195 – 91 / 348 – 96	-	1.54 / 1.33
Lower		600	1.0	476–149 / 520 – 169	377-95	1.01
			2.0	519 – 113 / 507 – 121	171-18	0.63
			3.0	433 – 101 / 433 – 120	-	0.89 / 0.98
			5.0	324 – 72 / 265 – 80	-	0.56 / 0.61
		800	1.0	329 – 155 / 364 – 138	400-144	1.15
			2.0	387 – 118 / 306 – 118	358-82	0.96
			3.0	322 – 172 / 311 – 112	-	1.38 / 1.28
			5.0	343 – 102 / 420 – 113	-	0.69 / 0.87
		1000	1.0	271 – 142 / 483 – 170	358-146	1.28
			2.0	377 – 201 / 400 – 157	376-119	1.03
			3.0	433 – 128 / 333 – 115	502-130	0.65
			5.0	477 – 155 / 442 – 139	-	0.95 / 1.05
1-butanol	upper	600	0.5	697-404 / 590 – 484	441-301	1.38
Acetic acid			1.0	453–268 / 595 – 306	186-139	0.62
water			2.0	289 – 263 / 381 – 170	400-100	0.67
(4:1:5)			3.0	-	-	-

Solvent System (volume ratio)	Mobile Phase	RPM	Flow Rate (ml/min)	TP Peaks I - II		Rs		HETP (cm)	
				H→T / T→H	ref	H→T / T→H	ref	H→T / T→H	ref
		800	0.5	684 - 428 / 850 - 544	287-294	2.00 / 1.69	1.42	1.1 / 0.9	2.1
Sample			1.0	784 - 520 / 900 - 504	344-272	1.88 / 1.27	1.21	0.9 / 1.3	1.9
Trp-Tyr (K=0.53) 0.25 mg			2.0	439 - 308 / 576 - 358	228-125	1.21 / 0.99	0.97	1.6 / 1.4	2.7
Val-Tyr (K=1.69) 1.0 mg			3.0	571 - 221 / 462 - 111	202-/53	0.85 / 0.52	0.75	1.5 / 2.1	3.4
each in 0.1 ml up		1000	0.5	347 - 357 / 615 - 505	210-261	1.47 / 1.56	1.48	1.7 / 1.1	2.5
			1.0	538 - 505 / 589 - 653	352-296	1.72 / 1.45	1.49	1.2 / 1.0	1.9
			2.0	1089-557 / 981 - 809	243-212	1.46 / 1.37	1.00	0.7 / 0.7	2.4
			3.0	668 - 332 / 841 - 378	252-208	1.07 / 0.96	0.78	1.2 / 1.0	2.6
Lower		600	0.5	448 - 289 / 598 - 317	221-111	2.33 / 2.66	1.69	1.6 / 1.3	3.6
			1.0	511 - 212 / 493 - 247	170-118	2.20 / 1.99	1.31	1.7 / 1.6	4.3
			2.0	420 - 147 / 332 - 134	156-76	1.50 / 1.29	0.90	2.1 / 2.6	5.2
			3.0	226 - 85 / 211 - 68	156-130	1.03 / 0.86	0.92	3.9 / 4.3	4.0
		800	0.5	590 - 389 / 485 - 303	303-158	2.88 / 2.86	2.23	1.2 / 1.5	2.6
			1.0	354 - 196 / 444 - 295	208-100	1.96 / 2.35	1.52	2.2 / 1.6	4.0
			2.0	314 - 199 / 442 - 271	169-127	1.42 / 1.66	1.21	2.3 / 1.7	4.1
			3.0	256 - 144 / 305 - 182	223-108	1.03 / 1.24	1.02	3.0 / 2.5	3.6
		1000	0.5	320 - 330 / 488 - 288	251-202	2.76 / 2.84	2.62	1.8 / 1.5	2.6
			1.0	354 - 356 / 676 - 286	137-124	2.41 / 2.81	1.67	1.7 / 1.2	4.1
			2.0	331 - 184 / 522 - 265	291-155	1.57 / 1.91	1.63	2.3 / 1.5	2.7
			3.0	278 - 173 / 502 - 236	278-163	1.31 / 1.55	1.33	2.7 / 1.6	2.7

TP: theoretical Plate number; Rs: peak resolution; HETP: height equivalent of one theoretical plate computed from the total column length (600 cm) divided by the average TP between the two peaks; Sf: retention of stationary phase; H: head; T: tail; ref: data obtained from original smooth cylindrical column (JCA, CCC2010). The numbers for best results in each row are marked by bold fonts.