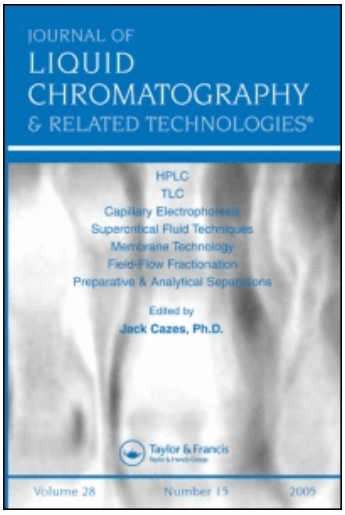

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Development of a New Preparative Spiral-Coil Low-Speed Rotary Countercurrent Chromatographic (Spiral-Coil LSRCCC) Method

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ABSTRACT

A new prototype, large-scale countercurrent chromatograph was constructed. It was equipped with a three-layer spiral column made from a continuous piece of convoluted Teflon tubing (8.5 mm I.D. and 9.7 m in length), which was accommodated in a spirally carved foam plastic holder. Two different types of two-phase solvent systems

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were used to evaluate stationary phase retention and mixing effect between stationary and mobile phases, i.e., chloroform/water (1 : 1, v/v) for separation of caffeine and theophylline; and 1-butanol/acetic acid/water (4 : 1 : 5, v/v/v) for separation of dipeptides, valyl-tyrosine (val-tyr), and tryptophyl-tyrosine (trp-tyr). A set of different elution modes (inside–outside and head–tail), and rotational speed (0–400 rpm) was assessed for stationary phase retention and separation efficiency. The elution mode of inside to outside of the spiral for the lower mobile phase or the opposite direction for the upper mobile phase, is rated the best for stationary phase retention due to observed spiral effect for liquid movement. Different operational parameters, such as rpm and flow rate of the mobile phase were evaluated for separation efficiency and speed. The obtained data revealed that the spiral effect and Archimedean screw force were two important contributing factors for yielding the enhanced speed and higher partition efficiency, compared to conventional low-speed rotary countercurrent chromatography.

Key Words: Low-speed rotary CCC; Spiral coil assembly; CCC; Caffeine; Theophylline; Dipeptides; Scale-up.

INTRODUCTION

Countercurrent chromatography (CCC) has been routinely used for separation and purification of various natural and synthetic products.[1,2] Low-speed rotary countercurrent chromatography (LSRCCC) has proven to be a suitable system for large-scale fractionation.[3–5] However, a few shortcomings are noted, such as its low retention of stationary phase and long operation time. When a polar two-phase solvent system was used, a long settling time of the two phases, laminar flow effect and radial phase distribution in the coiled column are the main causes affecting the peak resolution.

Most recently, an improved spiral disk assembly was used to replace the conventional multilayer coil for high-speed CCC operation, offering a fresh approach that enables high flow-rate elution even with a highly hydrophilic solvent system. Elimination of laminar flow effects and utilization of a spiral flow pattern provide high retention of the stationary phase even at a high flow rate of the mobile phase, resulting in efficient and speedy separation.[6,7] Based on this spiral disk geometry, we designed a large-scale rotary CCC apparatus to assess and evaluate operation parameters, such as centrifuge speeds, flow rates of the mobile phase, and elution modes.

EXPERIMENTAL

Apparatus

The spiral tube assembly for our prototype of the LSRCCC was fabricated by Pharma-Tech Research Corporation (Baltimore, MD) based on the design of an NIH US patent application.[8] A continuous piece of convoluted Teflon tubing (8.5 mm average ID) (Zeus Industrial Products, Raritan, NJ) was mounted on a foam plastic holder reinforced with an aluminum plate on each side (see Fig. 1), forming three spiral layers with a total capacity of about 550 mL. The pitch of the spiral was ca. 3.7 cm. The spiral starts at 6.7 cm and ends at 22.0 cm from the center of rotation, forming nearly

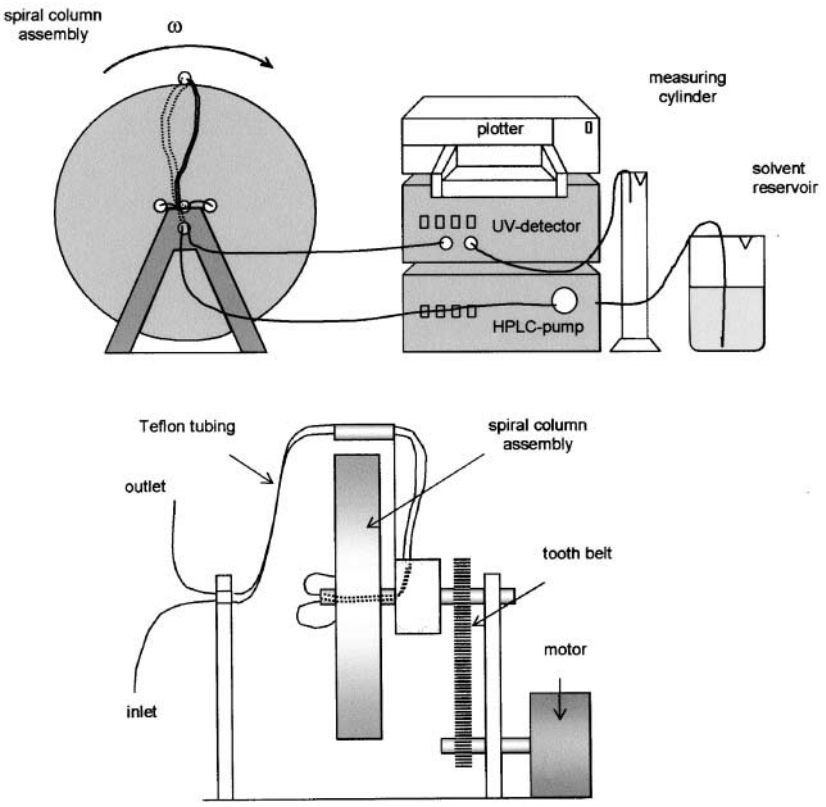

Figure 1. Schematic build-up of spiral-coil LSRCCC-upper drawing: complete system, front view; lower drawing: side view of spiral column assembly.

four spiral turns (Fig. 2). A pair of flow tubes (standard-wall 0.85 mm ID Teflon tubing, Zeus Industrial Products) from each terminal of the spiral column was led through the central axis of the apparatus, supported by a hollow plastic guide pipe, and then rigidly held at the stationary exit spot as shown in Fig. 1. This system avoids the flow tubes from twisting,[9] thus eliminating the need for a conventional rotary seal device, which often causes various complications such as leakage and cross contamination. The rotary speed of the column was regulated with a speed control. The solvents were delivered by an HPLC pump (Waters, model 515, Milford, MA).

Reagents

Butanol and chloroform were obtained from Fisher Scientific Company (Fair Lawn, NJ) and acetic acid from Mallinckrodt Baker Inc. (Paris, KY). Theophylline and caffeine were purchased from Sigma Chemical Co. (St. Louis, MO). Valyl-tyrosine (val-tyr) and tryptophyl-tyrosine (trp-tyr) were received from Bachem California Inc. (Torrance, CA).

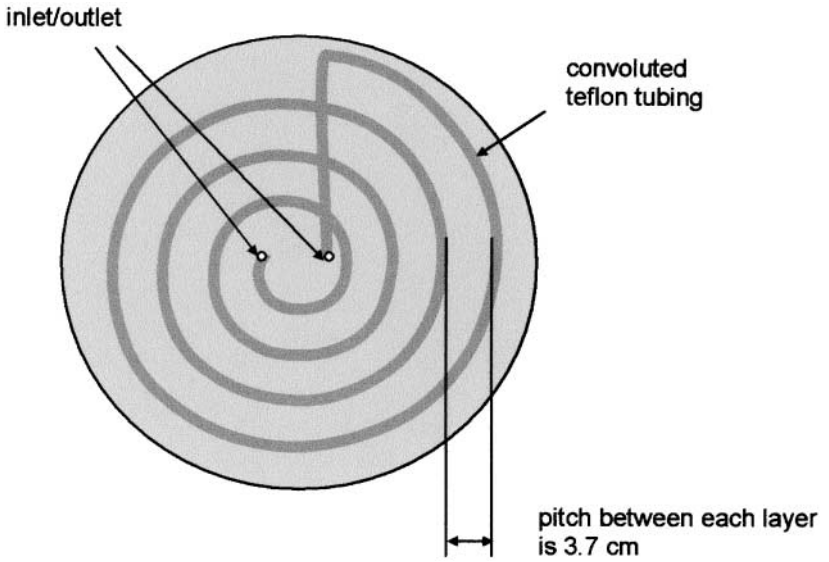

Figure 2. Schematic build-up of spiral column assembly; assembly consists of three spirals series connected.

Solvent Systems

The two solvent systems, chloroform/water (1 : 1, v/v) and 1-butanol/acetic acid/water (4 : 1 : 5, v/v/v), were prepared by saturating each phase in a separatory funnel at room temperature. The solvents were degassed directly before use.

Operating Procedure

Determination of Stationary Phase Retention

The spiral coil assembly was first entirely filled with the stationary phase (either upper or lower phase) using a graduated cylinder (1 L capacity) as a solvent reservoir, and the solvent in the reservoir was changed to a given volume of the mobile phase. Then, the centrifuge was rotated at a desired speed while the mobile phase was pumped through the coiled column at a flow rate of 10 mL/min, and the displaced stationary phase was collected back into the reservoir so that the volume of the solvent in the reservoir always remains the same. In this way, the retained volume of the stationary phase is calculated from the volume of the displaced stationary phase in the reservoir and, therefore, the experiment can be repeated without renewing the column contents.

A set of experiments was performed under the rotation speeds ranging from 0 to 400 rpm and different elution modes as listed below:

L–I–H: lower phase as mobile phase, elution from inside to outside and head to tail.

L–I–T: lower phase as mobile phase, elution from inside to outside and tail to head.

L–O–H: lower phase as mobile phase, elution from outside to inside, and head to tail.

L–O–T: lower phase as mobile phase, elution from outside to inside, and tail to head.

U–O–H: upper phase as mobile phase, elution from outside to inside, and head to tail.

U–O–T: upper phase as mobile phase, elution from outside to inside, and tail to head.

U–I–H: upper phase as mobile phase, elution from inside to outside, and head to tail.

U–I–T: upper phase as mobile phase, elution from inside to outside, and tail to head.

Determination of Separation Efficiency

Two sets of samples were selected for evaluating performance of each solvent system: Caffeine and theophylline (50 mg each) were separated using the chloroform solvent system and dipeptides, val-tyr (50 mg) and trp-tyr (10 mg), were separated using the 1-butanol/acetic acid/water (4 : 1 : 5, v/v/v) system.

These dipeptide samples were selected on the basis of their partition coefficient values of $K(U/L) = 0.5$ for val-tyr and 2.0 for trp-tyr, which provide similar peak distribution regardless of the choice of the mobile phase.

In each separation, the column was first completely filled with the stationary phase followed by injection of a sample solution (5 mL each phase) through the sample port. Then, the apparatus was rotated at a desired rate while the mobile phase was eluted through the column at a flow rate of 10 mL/min. The effluent from the outlet of the column was either directly monitored through a UV detector (LKB uvicord S, Stockholm, Sweden) and recorder (Rec 102, Pharmacia LKB), or collected into test tubes using a fraction collector (Ultrorac, LKB Instruments, Stockholm, Sweden). Due to a noisy baseline of the dipeptide separation, the collected fractions were manually analyzed using a UV spectrophotometer (Genesys 10 UV, Thermospectronic, Rochester, NY) at 280 nm and the absorbance was plotted against eluted volumes to draw a chromatogram for determination of peak resolution.

RESULTS AND DISCUSSION

Retention of Stationary Phase

The present studies were performed on two biphasic solvent systems having contrasting physical properties: chloroform/water (1 : 1, v/v) and 1-butanol/acetic acid/water (4 : 1 : 5, v/v/v). The chloroform/water binary system has a high interfacial tension, lower viscosity of the organic phase, and large density difference between two phases, while the 1-butanol system has low interfacial tension, high viscosity of the organic phase, and a small difference in density between two phases. As reported earlier,[10]

these two solvent systems display quite different retention curves (% retention/rotation speed) in a slowly rotating closed coil with helical diameters of 5–20 cm. When the coil is filled with about equal volumes of the two phases and rotated around its horizontal axis, the two phases soon reach a hydrodynamic equilibrium starting from the head of the coil. At rotational speed of 0–30 rpm, both solvent systems display normal hydrodynamic equilibrium in the coil where about equal volumes of each phase occupy the head end (Stage 1). As the rotational speed increased to 80–100 rpm, these two solvent systems display entirely opposite hydrodynamic behavior, i.e., in the chloroform system the upper phase completely occupies the head side, whereas in the 1-butanol system the lower phase occupies the head side (Stage 2). Further increase of the rotational speed, however, results in a sudden shift of the retention curve back toward 50% line (Stage 3), and thereafter both solvent systems maintain a stable level of the retention curve at about 50% (Stage 4).

In the present studies on a spiral column eluted with the mobile phase, the above retention curves are substantially modified by combined effects of two contributing factors: Archimedean screw force displays the above four stages of hydrodynamic pattern, while the radial centrifugal force gradient of the spiral column always forces the movement of the lower phase toward the outer terminal and the upper phase toward the inner terminal. Interplay of these two factors at various rotational speeds, determines the stationary phase retention for each solvent system depending on their physical properties.

Chloroform/Water (1 : 1, v/v) System

Figure 3 shows the retention curve of the chloroform/water system (1 : 1, v/v) in the rotating spiral column, where % retention of the stationary phase is plotted against the applied rotational speed. The upper diagram is obtained by eluting the lower organic phase and the lower diagram by eluting the upper aqueous phase, as indicated in each diagram. Among eight combinations of the elution mode, the lower phase eluted from the outer terminal (L–O–H and L–O–T) and the upper phase from the inner terminal of the spiral column (U–I–H and U–I–T) show, as expected, low retention of the stationary phase regardless of the rotation speed of the column, due to the spiral centrifugal force gradient always acting against the retention of the stationary phase.

At a low rotational speed of 0–30 rpm (Stage 1) where magnitude of the centrifugal force or the spiral force gradient effect is negligible, the retention becomes much less than 50% because the high interfacial tension interferes with the movement of two phases through the spiral column, especially when the lower organic phase was used as the mobile phase (upper diagram).

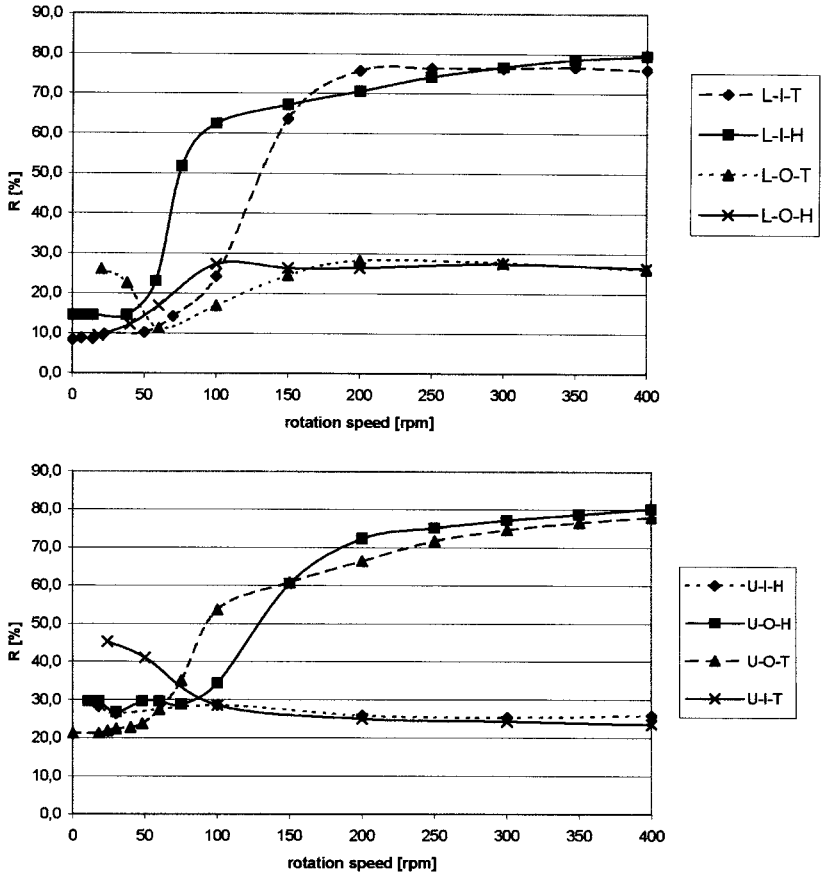

Figure 3. Retention of stationary phase at different rotation speeds: chloroform/water (1 : 1, v/v), flow rate 10 mL/min (for abbreviations cf. text).

When the rotational speed was increased to 80 rpm (Stage 2), the retention curve rose above 50% line in two elution modes by introducing either the lower organic phase from the inner head terminal (L–I–H in the upper diagram), or the upper aqueous phase from outer tail terminal (U–O–T, in the lower diagram). In these two elution modes, the spiral force gradient effect is superimposed on the Archimedean screw effect resulting in the enhancement of the retention. As the rotation speed exceeds 120 rpm (Stage 3), where the Archimedean screw effect is reversed to move the lower phase toward the head terminal of the spiral column, the retention curve of two other groups (L–I–T and U–O–H) started to rise crossing over the retention

curves of their counter-part (L–I–H and U–O–T). Finally, when the rotational speed exceeds 200 rpm, all these four curves reach over 75% due to enhanced effects of the radial force gradient of the spiral column.

1-Butanol/Acetic Acid/Water (4 : 1 : 5, v/v/v) System

Figure 4 similarly shows the retention curve of the 1-butanol solvent system where effective four elution modes are plotted in the same diagram as indicated on the right. Because its low interfacial tension minimizes the wall effect, the system can maintain 30–50% retention at very low rotational speeds where the two phases undergo smooth exchange forming a linear flow along the wall. At the rotational speed of 30–40 rpm (Stage 1), however, the stationary phase retention sharply drops to near 0% in all elution modes. This reduced retention may be caused by emulsification of the two phases when the increased rotational speed changes the linear flow to the droplet flow at the vertical section of the spiral column, while the centrifugal force acting on the horizontal portions of the spiral loop is not strong enough to settle the emulsion. As the rotational speed is increased to 50–80 rpm (Stage 2) where the Archimedean screw force moves the lower phase toward the head (note that this effect is opposite to that in the chloroform system), the retention of the two elution modes of introducing either the lower phase from the inner tail terminal (L–I–T) or the upper phase from the outer head terminal (U–O–H) sharply increases 50% crossing line. Further increase of rotation speed to

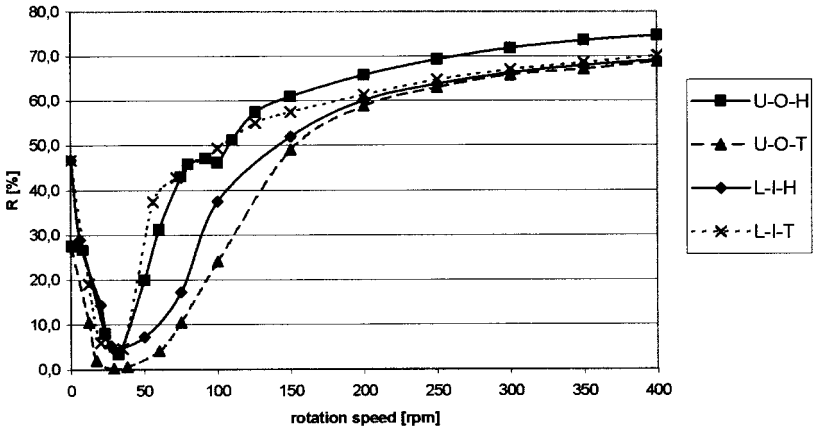

Figure 4. Retention of stationary phase at different rotation speeds: butanol/acetic acid/water (4 : 1 : 5, v/v/v), flow rate 10 mL/min (for abbreviations cf. text).

over 100 rpm (Stage 3), the effect of the Archimedean screw force on the phase distribution becomes reversed in such a way that the upper phase moves toward the head of the spiral. This results in a sharp rise of the retention curves of the other two elution modes of introducing either the lower phase from the inner head terminal (L–I–H) or the upper phase from outer tail terminal (U–O–T), while slightly inhibiting the retention of their counter-parts forming a notch on the curve. As the rotational speed passes 160 rpm (Stage 4), the radial centrifugal force gradient effect intensifies to override the influence from the Archimedean screw effect, yielding the stationary phase retention at over 50% in all four elution modes.

Separation

The results obtained from the separation of caffeine and theophylline using a solvent system of chloroform/water, were compared to those of the conventional LSRCCC system equipped with a multilayer coil separation column made of similar convoluted Teflon tubing.[4] In Fig. 5, the elution modes of L–I–H and L–I–T generated comparable separation efficiency with better resolution for caffeine in much shorter elution time. For the spiral rotary device, separation efficiency for both elution modes of L–I–H and L–I–T are comparable at three different rotation speeds of 100, 200, and 300 rpm (Fig. 5, Table 1), except for less resolution in short elution time at 100 rpm, which is caused by low retention of less than 30% (Fig. 3, upper diagram). The peak broadening of theophylline at high rotational speed may be caused by enhanced longitudinal mixing of the two phases.

For the hydrophilic solvent system of 1-butanol/acetic acid/water (4 : 1 : 5), the conventional LSRCCC system can only operate at a rotational speed below 50 rpm. In contrast, spiral rotary CCC can be operated at higher rotational speed, with an optimum at 60 rpm for L–I–T mode and 75 rpm for U–O–H mode with a flow rate of 10 mL/min. In Fig. 6, separation efficiency was evaluated for L–I–T for four rotational speeds (60, 75, 100, and 200 rpm). Both 60 and 75 rpm produced, similarly, best peak resolution for val-tyr and trp-tyr, while 100 and 200 rpm gave poor results despite higher retention of the stationary phase (cf. Table 2). These data showed that mixing and settling effects play an important role beside stationary phase retention. In Fig. 7, the elution mode of U–O–H was applied for four rotation speeds (7, 75, 100, and 200 rpm) and only 75 rpm turned out to give a partial peak resolution for val-tyr and trp-tyr (cf. Table 2).

From these experiments, we found intriguingly different results based on variation of choice of the mobile phase, inlet position, elution direction, and

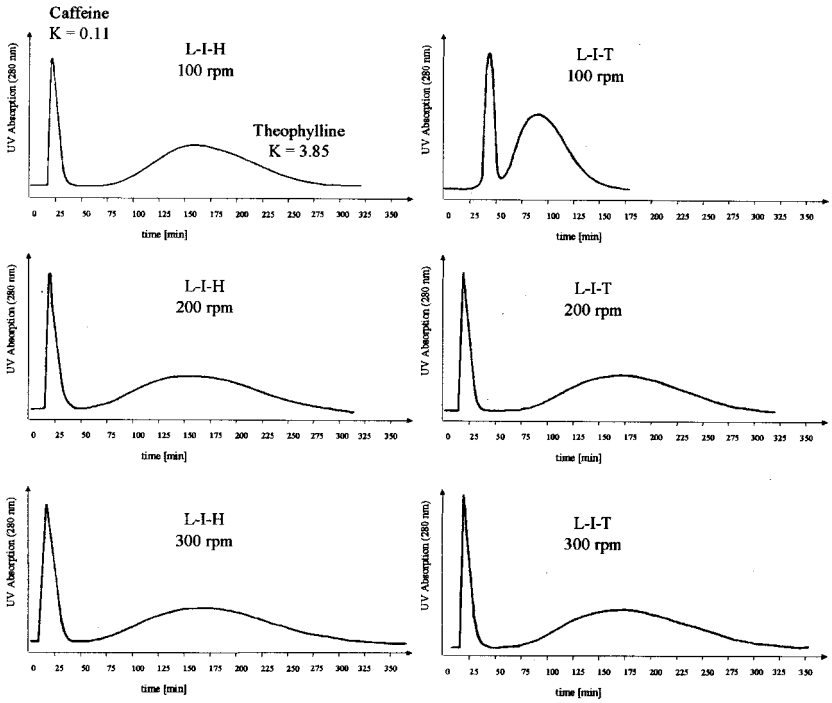

Figure 5. Spiral-coil LSRCCC-separations of caffeine and theophylline at 100, 200, and 300 rpm in L–I–H and L–I–T elution modes, 10 mL/min flow rate, chloroform/water (1 : 1, v/v), for abbreviations cf. text.

Table 1. Separation of caffeine and theophylline in chloroform/water (1 : 1, v/v).

	L–I–H		L–I–T	
rpm	*R* (%)	R_s	*R* (%)	R_s
100	62.5	1.42	24.2	0.94
200	70.5	1.13	75.8	1.33
300	76.5	1.16	76.4	1.16

Note: *R* (%), retention of stationary phase in per cent; R_s, peak resolution of caffeine and theophylline; for abbreviations (L–I–H and L–I–T) cf. text.

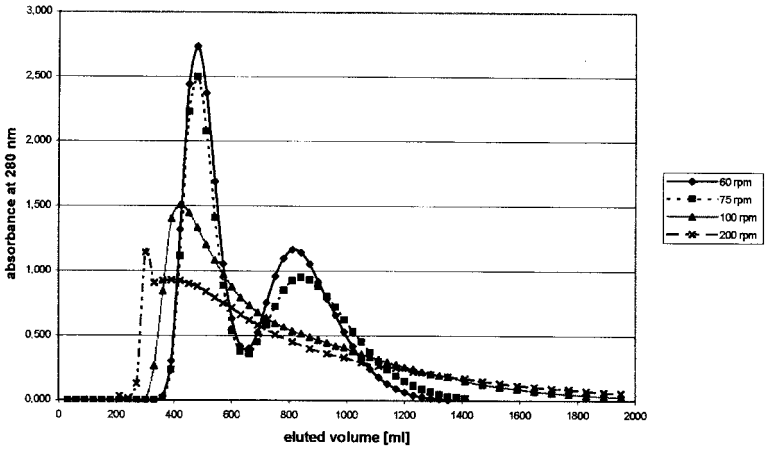

Figure 6. Separation of val-tyr and trp-tyr with the solvent system butanol/acetic acid/water (4 : 1 : 5, v/v/v), flow rate 10 mL/min (L–I–T), for abbreviations cf. text.

rotational speed. Those are the results of interplay of two contributing parameters: Archimedean screw force and radial centrifugal gradient of the spiral column.

CONCLUSIONS

This new spiral coil assembly provides enhanced stationary phase retention and solute partitioning for successful separations of both hydrophobic and

Table 2. Separation of val-tyr and trp-tyr in butanol/acetic acid/water (4 : 1 : 5, v/v/v).

	L–I–T		U–O–H	
rpm	*R* (%)	R_s	*R* (%)	R_s
60	36.5	0.95	–	–
75	42.0	0.85	43.1	0.88
100	48.0	*	45.3	*
200	62.2	*	63.4	*

Note: *R* (%), retention of stationary phase in per cent; R_s, peak resolution of val-tyr and trp-tyr; *, not available; R_s not available; for abbreviation (L–I–T and U–O–H) cf. text.

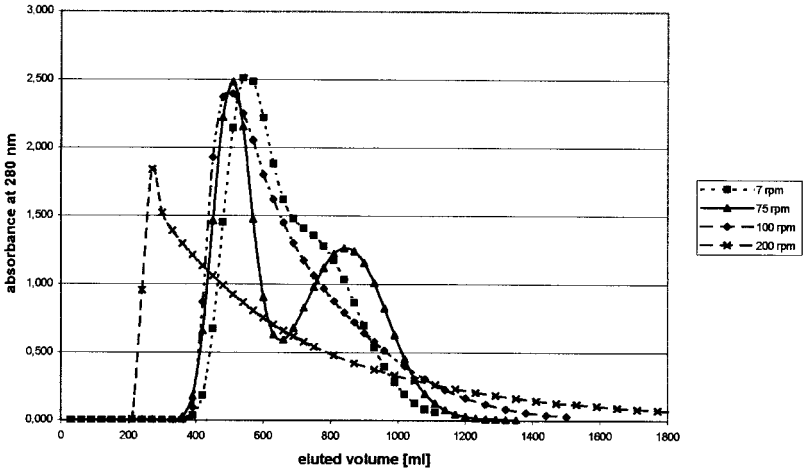

Figure 7. Separation of val-tyr and trp-tyr with the solvent system butanol/acetic acid/water (4 : 1 : 5, v/v/v), flow rate 10 mL/min (U–O–H), for abbreviations cf. text.

hydrophilic samples using a slow rotary CCC device. Compared to multilayer coil slow rotary CCC, the present device offers higher flow rate for the mobile phase due to the combined contribution of spiral effect and Archimedean screw force. In addition, adequate stationary phase retention observed at higher rotational speed, facilitates separation efficiency for other solvent systems with a broad spectrum of hydrophobicity. The goal of retaining larger sample load and speedy separation has been demonstrated from the experiments, which will encourage us greatly for the design and fabrication of larger scale CCC (5 L or more) for industrial applications of 100 g to 1 kg sample loads.

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