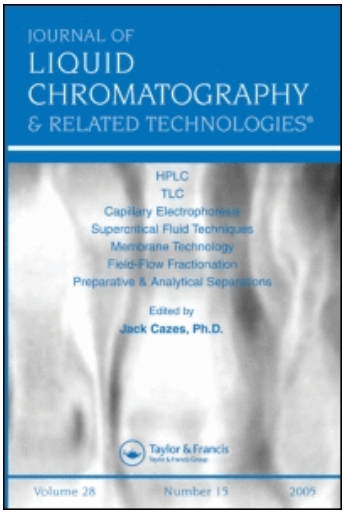

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Spiral Disk Assembly for HSCCC: Column Design and Basic Studies on Chromatographic Resolution and Stationary Phase Retention

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ABSTRACT

A set of four separation disks equipped with spiral channel(s) was designed for our type-J high speed countercurrent chromatography (J-HSCCC) centrifuge to improve retention of the stationary phase of polar solvent systems. Four different spiral disks were tested: two had a single spiral channel with different depths and the other two had four spiral channels connected in series to provide a greater spiral pitch.

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Performance of each disk was tested in terms of chromatographic resolution and/or stationary phase retention using three different two-phase solvent systems, including 1-butanol/acetic acid/water (4 : 1 : 5, v/v/v) for dipeptide separation; 12.5% (w/w) polyethylene glycol (PEG) 1000–12.5% (w/w) dibasic potassium phosphate for protein separation; and 4% (w/w) PEG8000–5% (w/w) dextran T500 in 10 mM dibasic sodium phosphate for determination of stationary phase retention. The results show that the spiral column retains a satisfactory amount of stationary phase for all solvent systems, even at a relatively high mobile phase flow rate where the spiral pitch plays a significant role. Separation of dipeptides with the butanol solvent system was possible using 10 mL/min flow rate of the mobile phase and 4-spiral-disks with their greater spiral pitch. In protein separations with the PEG–phosphate system, the single-spiral disks yielded the best separation using the upper phase in tail-to-head elution mode at 1 mL/min, while the 4-spiral disks show higher retention of the stationary phase. The retention of the PEG–dextran system is improved in the 4-spiral disks, which exceed 60% at a flow rate of 0.5 mL/min. Various parameters, which affect the performance of the spiral disk separation column are discussed.

Key Words: Spiral disk assembly; Countercurrent chromatography; Aqueous two phase system; Peptide separation; Protein separation.

INTRODUCTION

Generally speaking, high-speed countercurrent chromatography (HSCCC) can be done using two types of the planetary centrifuge: one is called the type-J multilayer coil planet centrifuge (J-CPC) and the other the cross-axis coil planet centrifuge (X-CPC).[1] The J-CPC types provide efficient mixing of the two solvent phases in the coiled column to produce excellent peak resolution, but it fails to retain a satisfactory amount of stationary phase for two-phase solvent systems low interfacial tension, such as the aqueous two phase systems (ATPS). The X-CPC, on the other hand, provides satisfactory levels of stationary phase retention for most of the solvent systems, while the peak resolution is substantially lower than that of the J-CPC. In addition, the X-CPC requires a complex mechanical design, which makes the commercial model more expensive.

The present paper introduces a novel column design for the J-CPC to improve the retention of the stationary phase for polar solvent systems while retaining its high partitioning performance. Below, we describe the principle and design of the separation column together with its performance on chromatographic resolution and retention of the stationary phase obtained using a set of polar, low-interfacial-tension ATPSs.

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EXPERIMENTAL

Principle and Design of the Separation Column

Traditionally, the separation column of the HSCCC centrifuge is fabricated from Teflon tubing, which is wound around the spool-shaped column holder to form multiple coiled layers (multilayer coil).[1,2] The planetary motion of the coiled column produces an Archimedean screw force, which acts on two solvent phases in the column causing their efficient mixing of the two phases while one is permanently retained in the column. In the conventional type J high-speed CCC, the retention of the stationary phase almost entirely depends upon this force. However, retention of the stationary phase can be improved if the column is made into a spiral configuration. This is due to the fact that the planetary motion induces a radially acting centrifugal force gradient with the heavier phase tending to move outward and the lighter phase inward along the spiral path. Previously, the performance of the spiral column has been tested using a spirally wound Teflon tube to improve the stationary phase retention.[3,4] However, the effect of the radial gradient depends largely upon the pitch of the spiral, and, in such a column design; the spiral pitch is limited by the outer diameter of the Teflon tubing. In addition, the connection of each spiral to form a long separation column is difficult. The solid column design described below solves all these problems and, if mounted on the conventional J-CPC, it provides universal application of two-phase solvent systems including the polymer phase systems.

Figure 1A shows the design of the spiral disk assembly, which consists of multiple disk units sandwiched between a pair of stainless-steel flanges. The top flange is equipped with a plastic gear, which engages an identical stationary gear mounted on the central shaft of the CPC machine. Each component is shown in Fig. 1B. Each disk made of high-density polyethylene has a single or several spiral grooves, each of which forms a spiral channel when sealed with a Teflon septum. As shown in the diagram, each spiral disk is separated with a Teflon septum equipped with a transfer hole (see Fig. 1B), which provides the liquid flow from one unit to the next. In the present study, the following four different types of spiral columns were made (see Table 1): single spiral columns and four-spiral columns, each made with two different dimensions of grooves as indicated in Table 1. The design of the individual spiral disks and Teflon septum are shown in Fig. 2A for the single pitch spiral design, and Fig. 2B for the four spiral design.

Figure 2A schematically illustrates the design of the single spiral column where the heavier phase is introduced from the inner terminal (I) while it exits at the outer terminal (O), and vice versa for the lighter mobile phase. A narrow radial groove is made on the other side of the disk to lead the mobile phase to

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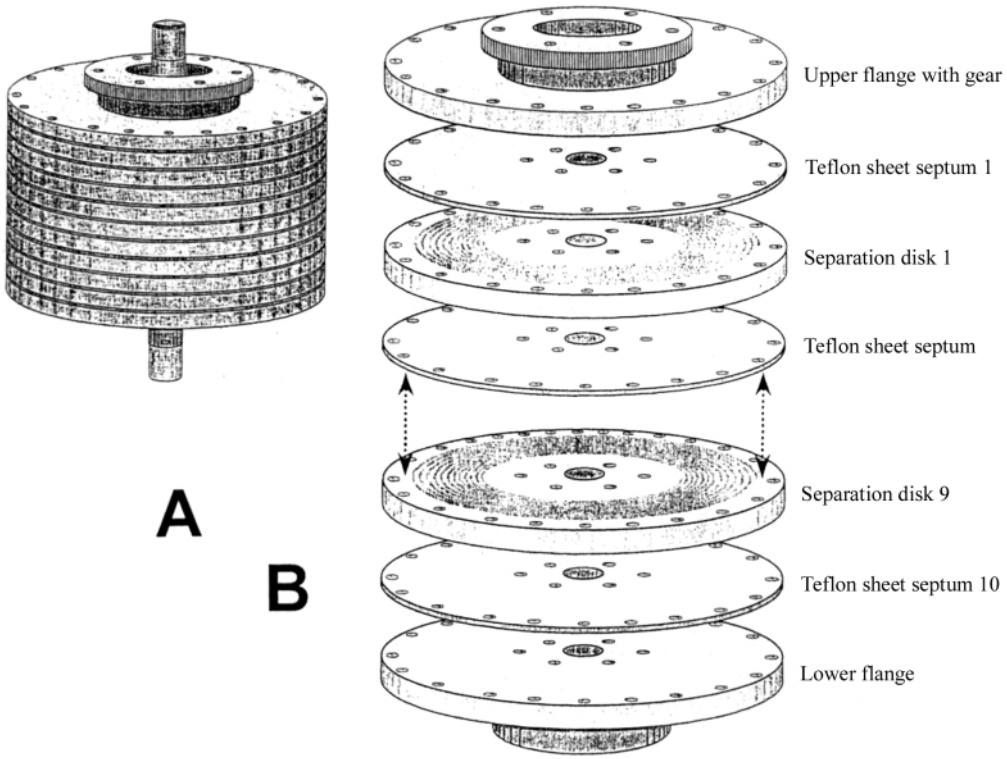

Figure 1. Spiral disk assembly. (A) Sketch of the assembly consisting of nine disk units; (B) Exploded view of the assembly.

Table 1. Dimensions of spiral grooves of four different separation disks.

Column	Number of spirals	Width[a] (mm)	Depth[a] (mm)	Pitch (mm)	Capacity (mL)
I	1	2.6	2.0	4	23
II	1	1.5	3.7	4	25
III	4	2.6	2.0	16	23
IV	4	1.5	3.7	16	25

Note: In the spiral column assembly the width becomes the sedimentation distance and the depth, the width of the channel.
[a]Values indicate the dimensions of the spiral grooves.

the exit of the column, or the inlet of the neighboring disk, through a hole in the Teflon septum. The channel design of the four spiral disk is shown in Fig. 2B (the first channel from I_1 to O_1 is highlighted to visualize its enhanced pitch). The four channels are each labeled I (inlet) and O (outlet) with subscript to indicate the channel for the heavier mobile phase (for the lighter mobile phase I and O should be reversed as well as the number at each terminal). The heavier mobile phase is introduced through the inner terminal (I_1) and flows through the spiral path toward the outer terminal (O_1), where it moves to the other side of the disk through a hole and then through the radial connection channel (dotted line), reaching the inlet of the next spiral channel through the hole I_2. By repeating this process, the mobile phase flows through the four spiral channels and finally reaches the outer terminal of the channel 4 (O_4) where it goes to the other side of the disk and exits the disk through the radial channel.

Figure 3 shows the composite photographs of individual parts and a whole spiral disk assembly consisting of eight single-spiral disks. The spiral disk assembly was mounted on a multilayer coil planet centrifuge (P.C. Inc. Potomac, MD) with a 10 cm revolution radius. A counterweight of a similar shape was mounted on the other side of the rotary frame at a similar level to obtain dynamic balance of the centrifuge system. The rotational velocity of the apparatus was regulated using a speed control (Bodine Electric Company, Chicago, IL).

Reagents

Acetic acid, mono and dibasic potassium phosphates, dibasic sodium phosphate were obtained from Mallinckrodt Specialty Chemicals, Paris, KY, and dextran-T-500, polyethylene glycols 1000 and 8000, tryptophyl tryptophan (trp-trp), tryptophyl tyrosine (trp-tyr), leucyl-tyrosine (leu-tyr), valyl-tyrosine (val-tyr), tyrosyl glycine (tyr-gly), lysozyme, and myoglobin from

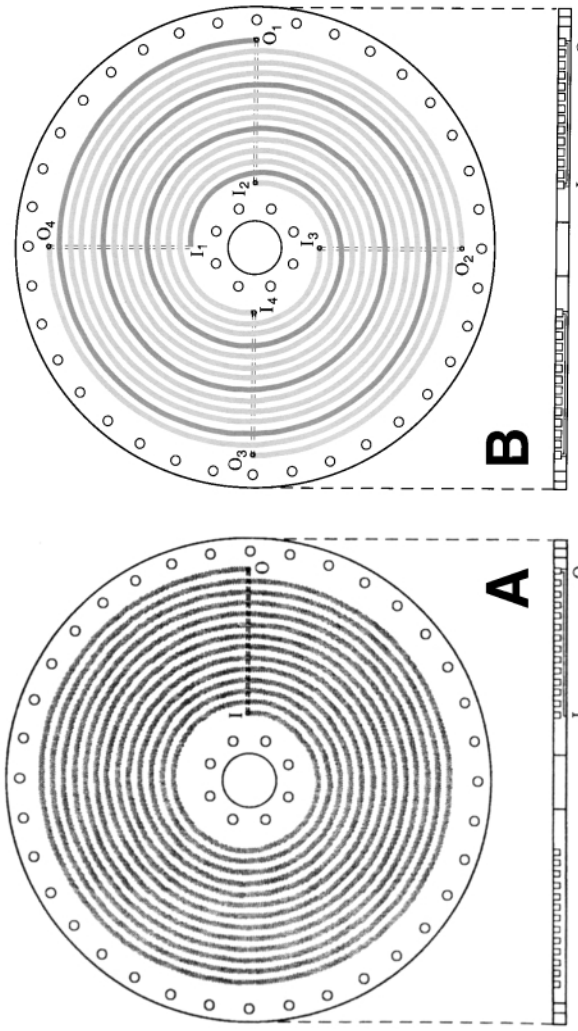

Figure 2. The spiral disks. (A) Single-spiral disk (I: inner terminal; O: outer terminal); (B) 4-Spiral disk (The first channel from I_1 to O_1 is highlighted to visualize its enhanced pitch).

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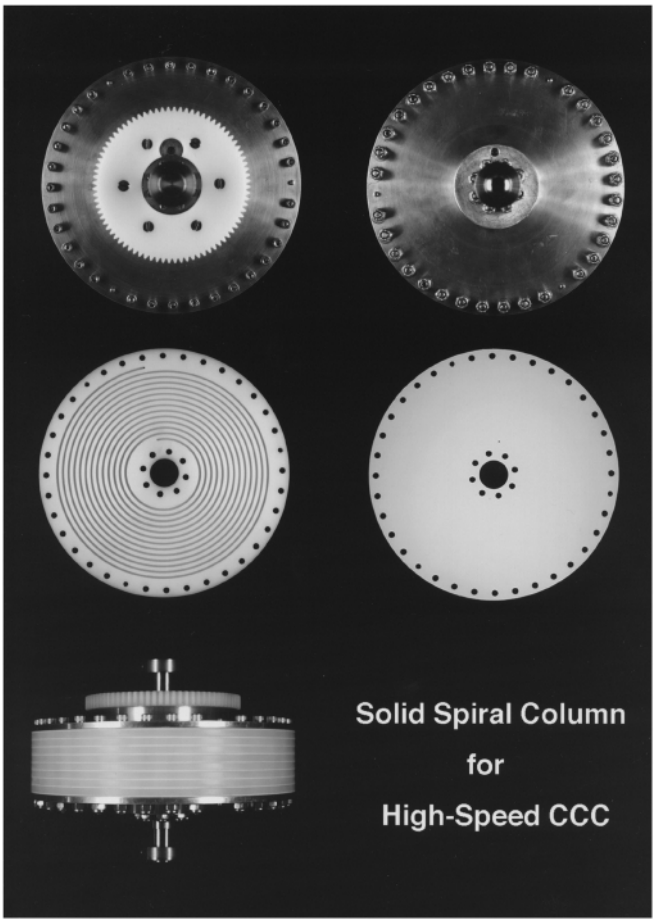

Figure 3. Photograph of individual parts and spiral disk assembly.

Sigma Chemical Co., St. Louis, MO. 1-Butanol was purchased from Fisher Scientific Co., Fair Lawn, NJ.

Preparation of Two-Phase Solvent Systems and Sample Solution

The following two-phase solvent systems were prepared: (1) 1-butanol–acetic acid–water (4 : 1 : 5, v/v) for separation of dipeptides (trp-tyr, val-tyr, etc.); (2) 12.5% (w/w) polyethylene glycol 1000, 12.5% (w/w) dibasic potassium

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phosphate and 75% (w/w) distilled water for separation of proteins (lysozyme and myoglobin); (3) 4% (w/w) polyethylene glycol 8000, 5% (w/w) dextran T500 and 91% (w/w) distilled water for studies on retention of stationary phases.

Each solvent mixture was thoroughly equilibrated in a separatory funnel and the two phases separated shortly before use. The sample solutions were prepared by dissolving the sample mixture in the lower or upper phase used for the separation.

Experimental Procedures for Basic Studies with Single Disk

Using four different spiral columns listed in Table 1, experiments were carried out as follows: the column was first entirely filled with the stationary phase followed by sample injection with a syringe. Then the apparatus was rotated at 800 rpm while the mobile phase was pumped into the column at a given flow rate. The effluent was continuously monitored with a UV detector (Uvicord S, LKB/Pharmacia, Stockholm, Sweden). After the peaks were eluted, the apparatus was stopped and the column contents were emptied into a graduated cylinder to measure the volume of the stationary phase retained in the column.

The runs were performed in four different elution modes as follows:

- L-I-T: Lower phase pumped from the inner terminal in the tail to head direction.
- L-I-H: Lower phase pumped from the inner terminal in the head to tail direction.
- U-O-T: Upper phase pumped from the outer terminal in the tail to head direction.
- U-O-H: Upper phase pumped from the outer terminal in the head to tail direction.

The other four elution modes (L-O-T, L-O-H, U-I-T, and U-I-H) using the radial force gradient the opposite way, i.e., pumping the lower phase inward or the upper phase outward, resulted in much lower or no retention of the stationary phase and these modes were, therefore, eliminated from subsequent studies.

Experimental Procedure for Preliminary Separation with Spiral Disk Assembly

Preliminary separations of dipeptides and proteins were similarly performed using a spiral disk assembly consisting of 8 units of column I as follows: the column was first filled with the stationary lower phase followed by

the sample charge. Then, the upper phase was eluted through the column in the head to tail elution mode while the apparatus was run at 800 rpm. The effluent was continuously monitored at 280 nm (Uvicord S, LKB Instruments, Bromma, Sweden) and collected into test tubes using a fraction collector (Ultrorac, LKB Instruments). After the separation was completed, the volume of the stationary phase retained in the column was measured by collecting the column contents using pressured nitrogen.

RESULTS AND DISCUSSION

Prior to the present studies with the solid spiral column, we examined the performance of a single-layer coil made of rectangular Teflon tubing mounted on the type-J coil planet centrifuge, the results being compared with those obtained from a coiled column of standard Teflon tubing of a comparative size. The data clearly indicated that the rectangular tubing design could yield similar, or even slightly higher, chromatographic resolution if it is coiled with its narrow side down. In the case of similar tube dimensions, we speculated that this result was due to the increased depth of the column that provided higher retention of the stationary phase.[5]

In a recent work, Sutherland et al. showed that all phase systems from polar to non-polar behave in the same way when the Archimedean and the hydrostatic (radial gradient) forces were aligned.[6] These forces were aligned when they were flowing in the upper phase in the tail (periphery) to head (center) direction (our U-O-T way) and the lower phase in the head (center) to tail (periphery) direction (our L-I-H way). However, their study was only done with a classical J-type CCC machine with coiled regular Teflon tubing.

Basic Studies on Chromatographic Resolution and Retention of Stationary Phase

Using a single disk, each from four different spiral designs (see Table 1 for their dimensions), a series of experiments was performed to investigate their performance in terms of chromatographic resolution and retention of the stationary phase, using three different two-phase solvent systems with high polarity.

Butanol–Acetic Acid–Water (4 : 1 : 5) System

Figure 4A–D illustrate the results of basic studies on the set of four different spiral disks (column I–IV, respectively), using the butanol solvent

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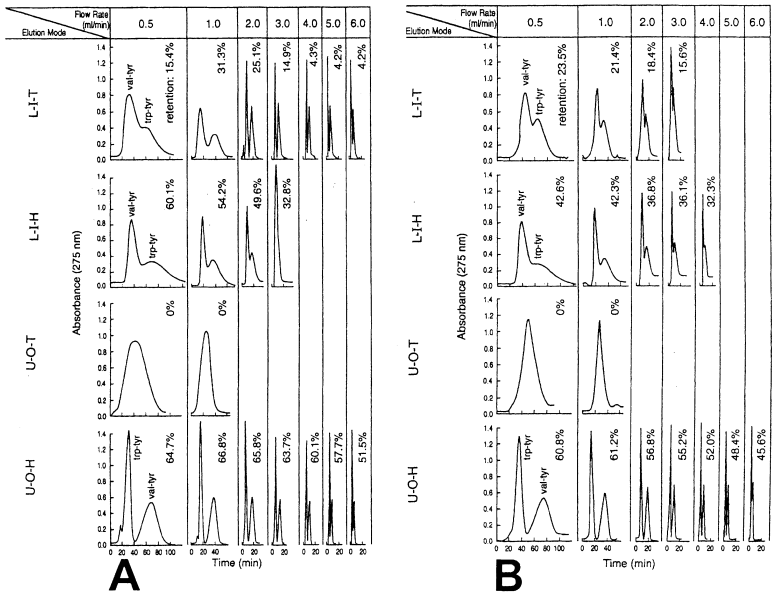

Figure 4. Dipeptide separation with 1-butanol/acetic acid/water (4 : 1 : 5) system. (A) Column I (1 spiral, 4 mm pitch); (B) Column II (1 spiral, 4 mm pitch); (C) Column III (4 spirals, 16 mm pitch); and (D) Column IV (4 spiral, 4 mm pitch). Experimental conditions: Apparatus: type-J HSCCC centrifuge with 10 cm revolution radius; separation columns: see Table 1; solvent system: 1-butanol/acetic acid/water (4 : 1 : 5, v/v/v); sample: tryptophyl tyrosine (0.5 mg)($K_{\text{upper/lower}} = 1.7$) + valyl tyrosine (2 mg) ($K_{\text{upper/lower}} = 0.53$) in 1 mL of upper phase; elution mode: L-I-T (lower phase pumped from the inner terminal in the tail to head direction), L-I-H (lower phase pumped from the inner terminal in the head to tail direction), U-O-T (upper phase eluted from the outer terminal in the tail to head direction), U-O-H (upper phase pumped from the outer terminal in the head to tail direction); revolution speed: 800 rpm. Percentages = Sf factors.

system and a standard sample mixture of trp-tyr (0.5 mg) and val-tyr (2 mg). In each chromatogram, the UV absorbance (280 nm) was plotted against the elution time together with the percentage of retention volume of the stationary phase relative to the total column capacity. In each group, a set of chromatograms is arranged according to the four different elution modes, i.e., L-I-T, L-I-H, U-O-T, and U-O-H, where L and U indicate the lower phase mobile and upper phase mobile; I and O, pumping from internal terminal and external terminal of the spiral, and T and H, "tail to head" and "head to tail" elution mode, each, under various flow rates of the mobile phase.

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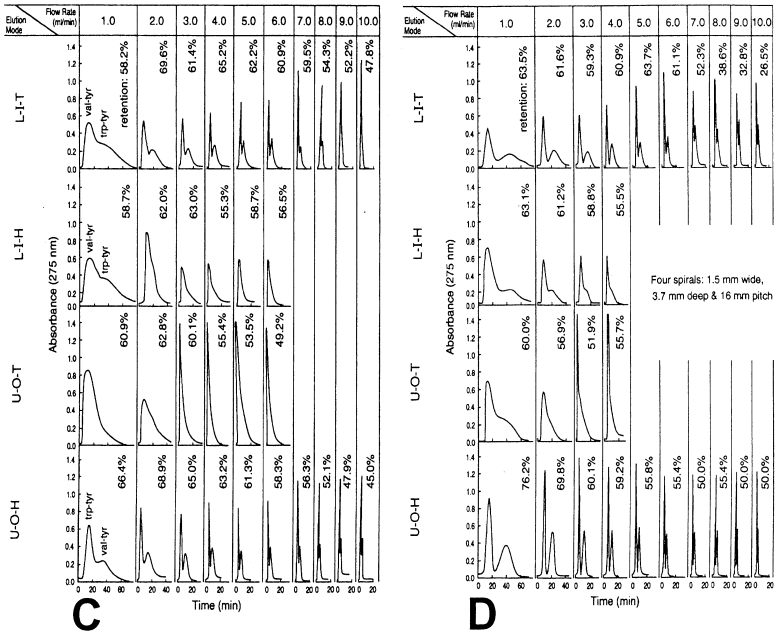

Figure 4. Continued.

In column I (Fig. 4A) pumping upper phase from the head, outer end of the spiral (U-O-H) produces the best peak resolution and the highest percentage of retention of the stationary phase. Pumping lower phase from the head, inner end of the spiral (L-I-T) also shows a good peak resolution up to the flow rate of 6 mL/min in spite of its low retention of the stationary phase. Pumping the lower phase retains a satisfactory amount of the stationary phase under the flow rate of 0.5–2 mL/min as described by Sutherland for a classical coil planet J-type centrifuge (L-I-H).[6] However, the peak resolution is much lower than those of its counterparts (L-I-T), showing that phase retention and phase mixing are not linked. Pumping the upper phase from the tail (U-O-T) showed no retention of the stationary phase, even at a low flow rate of 0.5 mL/min. These trends are also found in column II (Fig. 4B), except that the peak resolution is reduced when pumping the lower phase from the tail (L-I-T) under similar retention level of the stationary phase.

Figure 4C and D show the chromatograms obtained from the 4-spiral disks under otherwise identical experimental conditions. It clearly indicates that the retention of the stationary phase is much improved in all elution modes. Although the peak resolution of the two dipeptides was substantially

reduced by the loss of stationary phase, the flow rate could be increased up to 10 mL/min to yield a moderate degree of peak resolution, as emphasized by column IV (Fig. 4D). The Sf factor in all four elution modes was higher than 55% up to 4 mL/min. Only the L-I-T and U-O-H modes gave satisfactory chromatographic resolution (or phase mixing). This suggests that if one used the two latter modes and a multichannel spiral disk with a large pitch, the separation of peptides might be achieved in a very short elution time.

PEG1000-Dibasic Potassium Phosphate Polymer Phase System

Figure 5A–D similarly illustrates the peak resolution between two protein samples (lysozyme and myoglobin, each 10 mg injected) in the PEG/phosphate system obtained from the four different spiral disks. In the single-spiral disks (Fig. 5A and B), the best results were obtained by pumping

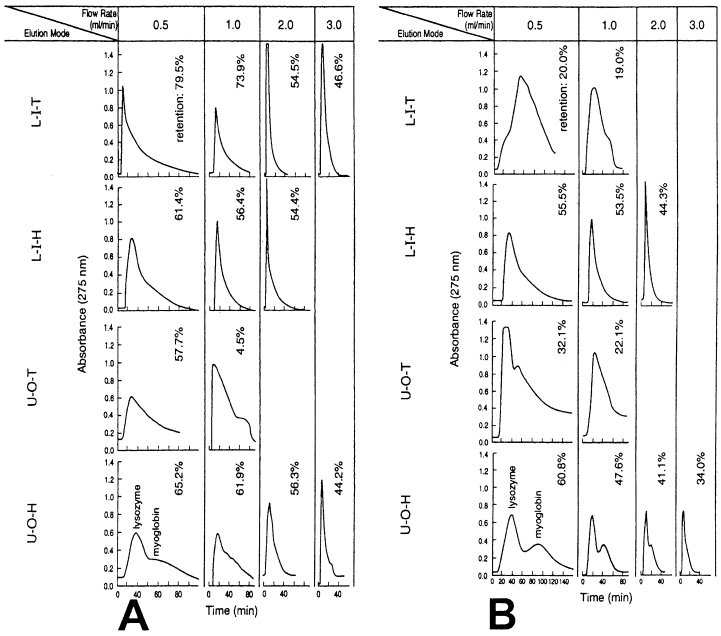

Figure 5. Protein separation with PEG1000/dibasic potassium phosphate system. See Figure 4 caption. (A) Column I; (B) Column II; (C) Column III; and (D) Column IV. Experimental conditions: see Fig. 4 caption; solvent system: 12.5% (w/w) PEG1000 and 12.5% (w/w) in 75% (w/w) 0.1 M dibasic potassium phosphate aqueous solution; sample: lysozyme and myoglobin each 10 mg in 1 mL of equal volumes of each phase; elution mode: see the Fig. 4 caption; revolution speed: 800 rpm. Percentages = Sf factors.

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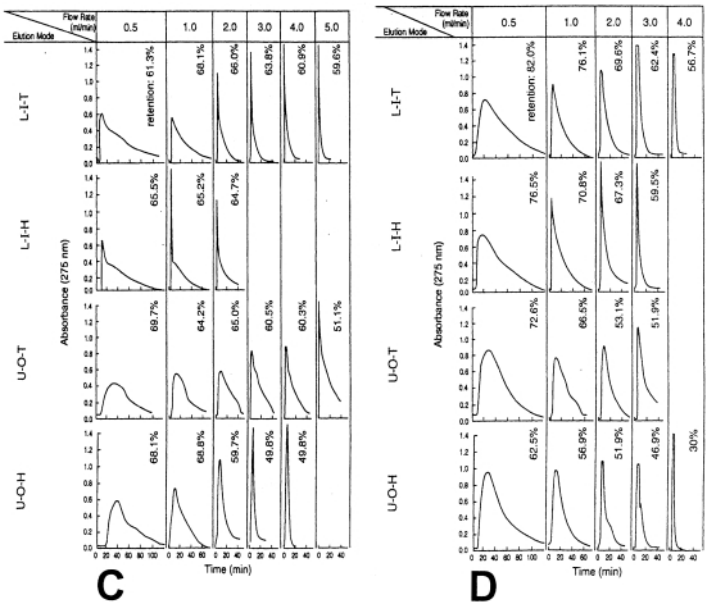

Figure 5. Continued.

the viscous upper PEG-rich mobile phase in the head-to-tail direction and from the outer terminal (U-O-H). All other modes resulted in low or no resolution. Except for L-I-T and U-O-T in column II, all groups show a satisfactory level of stationary phase retention with a flow rate of 0.5 mL/min ($Sf > 54\%$). Since there is good stationary phase retention and no solute separation, it means that the mixing of the two phases in the spiral column is poor. This poor mixing is more pronounced in the 4-spiral disks (Fig. 5C and D), which show higher stationary phase retention ($Sf > 60\%$) but without any peak resolution. Comparing columns in Fig. 5A and B shows the latter to yield somewhat higher peak resolution, probably due to the shallow channel which facilitates mass transfer between the two phases. This suggests that the separation might be further improved by reducing the width of the spiral groove.

PEG8000-Dextran 500 Polymer Phase System

A series of experiments was performed to examine the phase retention of the PEG-dextran polymer ATPS without injecting a sample. The results are

Table 2. Stationary phase retention factors, Sf, for 4% PEG8000–5% Dextran T 500 in 10 mM Na_2HPO_4 system at different flow rates.

	Column			
Flow rate (mL/min)	I 0.5–1.0	II 0.5–1.0	III 0.5–1.0–2.0	IV 0.5–1.0–2.0
L-I-T	40.0–14.8	14.3–3.3	61.1–50.0–14.3	55.6–33.3–4.0
L-I-H	37.1–2.0	10.9–6.5	53.9–47.8–17.4	52.0–33.3–9.6
U-O-T	71.8–32.3	31.0–17.4	67.8–65.7–50.0	64.4–56.0–42.7
U-O-H	61.9–28.0	15.1–10.1	59.8–46.8–45.1	58.5–54.5–44.0

shown in Table 2 where the stationary phase retention factors, Sf, are indicated at flow rates of 0.5 to 1 or 2 mL/min for each spiral column. The results indicated that among the single-spiral disks (I and II), column I with a deep channel retained over 60% of the lower dextran-rich stationary phase (U-O-H and U-O-T) at a low flow rate of 0.5 mL/min, while in other conditions, the retention was substantially less than 50% of the total column capacity. In contrast, the 4-spiral disks (columns III and IV) attained remarkably improved Sfs of over 50% for all elution modes at 0.5 mL/min flow rate. The results, again, indicate the effect of the greater spiral pitch, which favors the retention of the stationary phase.

Preliminary Separation with a Spiral Disk Assembly

The performance of the present column design was examined using a spiral disk assembly consisting of 8 units of column I with a total capacity of 165 mL. The results are shown in Fig. 6A, B. In Fig. 6A, a set of five dipeptides were well resolved and eluted in 2 hours. The upper organic phase of the 1-butanol solvent system was pumped at a flow rate of 4 mL/min with an excellent 60% Sf factor. Similar separations have been shown with the standard multilayer coils in HSCCC, but only with more stable butanol solvent systems.[1]

Figure 6B shows the separation of two stable proteins (lysozyme and myoglobin) using a PEG1000/K_2HPO_4 polymer phase system at a flow rate of 1 mL/min, where they were well resolved in slightly over 5 hours (Sf = 70%). As suggested by the basic studies, the separation may be substantially improved using the column II disk assembly, which provides a shallower channel to facilitate the mass transfer in viscous polymer phases.

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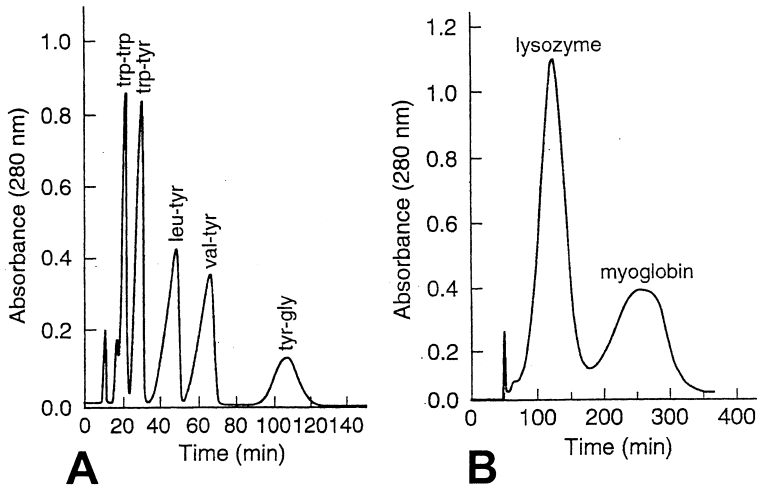

Figure 6. Preliminary separation a spiral disk assembly. A. Separation of five dipeptide samples. Experimental conditions: separation column: 8 units of column I with a total capacity of 165 mL; Sample: a mixture of five dipeptide samples, total 35 mg; including trp-trp ($K_{\text{lower/upper}} = 0.22$), typ-tyr ($K = 0.59$), leu-tyr ($K = 1.18$), val-tyr ($K = 1.89$), and tyr-gly ($K = 3.33$); solvent system: 1-butanol/acetic acid/water (4 : 1 : 5); Elution mode: U-O-H or upper phase pumped from head to tail, inward; flow-rate: 4 mL/min; Sf = 60%. B. Separation of protein samples. Experimental conditions, separation column: 8 units of column I with a total capacity of 165 mL; sample: a mixture of lysozyme ($K_{\text{lower/upper}} = 0.59$) and myoglobin ($K = 1.96$) each 50 mg in 5 mL of upper phase; solvent system: 12.5% (w/w) PEG1000, 12.5% (w/w), 75% (w/w) 0.1 M K_2HPO_4; elution mode: U-O-H or upper phase pumped from head to tail, inward; flow-rate: 1 mL/min; Sf = 70%.

Consideration on Various Parameters Governing Performance of Spiral Column

Depth and Width of the Spiral Channel

Differing from the ordinary coiled column prepared from Teflon tubing, the channel of the spiral column has a rectangular cross section. Since the centrifugal force acts along the radius of the disk, the width of the groove on the disk becomes the depth of the channel, and the depth of the groove becomes the width of the channel. Here, it is reasonable to consider that a greater depth of the channel favors the retention of the stationary phase, while it provides a less efficient solute mass transfer. In the present studies, we have

used two different dimensions of the spiral channel: columns I and III are 2.6 mm in depth (or width of the groove) and 2.0 mm in width (or depth of the groove) while columns II and IV are 1.5 mm in depth and 3.7 mm in width. The experimental data clearly indicated that with few exceptions, the deeper column (columns I and III) produced a better retention of the stationary phase. This trend is most clearly observed in the viscous PEG–dextran system in the single-channel spiral disks (columns I and II).

The effects of channel geometry on chromatographic resolution in terms of peak resolution gives a more complex picture: in the dipeptide separation with the butanol solvent system, the deeper channel of the single spiral disk (column I) produced a substantially better peak resolution, especially in the lower phase mobile (L-I-T), whereas the shallower channel in the 4-spiral disk (column IV) produced somewhat better separation than its counterpart. We do not understand the reason why higher peak resolution is obtained in the deeper channel of column I in L-I-T despite the low retention of the stationary phase. In the protein separation with the PEG–phosphate system, the shallower spiral channel of the single-spiral disk gave the best separation in the upper PEG-rich mobile phase, whereas both 4-spiral disks (columns III and IV) showed less efficient separations.

The overall results suggest that the shallower channel gives better separation of proteins using the PEG–phosphate polymer phase system and that the deeper channel favors the retention of viscous PEG–dextran solvent systems.

Spiral Pitch

Before discussing the effect of the spiral pitch, the effect of the β value of the spiral channel should be considered. The present column was designed for the polar solvent systems such as butanol/acetic acid/water (4 : 1 : 5) and ATPSs. These solvent systems have a small difference in density and a high difference in viscosity between the two phases. They seem to be less affected by the Archimedean screw effect. Therefore, the spiral channel was made between 2.5 cm and 7.5 cm from the axis of the holder, i.e., the β values range from 0.25 to 0.75, respectively. However, it is well known that many moderately polar two-phase solvent systems tend to reverse their hydrodynamic trend at a low β value (<0.5) in an ordinary multilayer coil, hence, affecting the retention of the stationary phase.[7] This may indicate, that if these solvent systems are used, the portions of the spiral channel within 5 cm radius of the disk would become an inefficient dead space entirely occupied by the mobile phase, unless the spiral channel configuration alters the above hydrodynamic trend. In the present channel design, the effect of the spiral pitch becomes relatively greater in the inner spiral loop since the channel loop

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becomes longer in proportion to its radius, while the spiral pitch remains the same. Consequently, this relatively large spiral pitch near the center of the disk may cancel out the adverse hydrodynamic effect by the Archimedean screw force to improve the retention of the stationary phase for these moderately hydrophobic two-phase solvent systems. The validity of this hypothesis will be examined in the future.

The effects of the spiral pitch on the present studies are seen by comparing the chromatograms between columns I and III for the deep channel and between columns II and IV for the shallow channel. It is clearly observed, that in dipeptide separation with 1-butanol solvent system the greater pitch results in lower peak resolution at a given flow rate, while it provides higher retention of the stationary phase that allows a higher flow rate of the mobile phase to reduce the separation time. Similar effects are also seen in the protein separation with the PEG–phosphate solvent system. In the PEG–dextran system, a greater pitch provides a substantial increase in retention of the stationary phase, some exceeding over 50% retention at a flow rate of 1 mL/min.

Flow Rate of the Mobile Phase

The flow-rate of the mobile phase is an important parameter, which determines the elution time and volume of stationary phase retained. In the dipeptide separation, the optimum flow-rate was used producing the best peak resolution in all spiral disks. In the 4-spiral disks (columns III and IV) which retain a large volume of the stationary phase, the application of a high flow rate up to 10 mL/min is possible to reduce the separation time without substantial loss of peak resolution. In separation of proteins with PEG–phosphate the low flow rate produced the best peak resolution with higher retention of the stationary phase and longer duration for the separation.

CONCLUSION

The overall results of our experiments indicate that the spiral disk assembly provides satisfactory retention of the stationary phase for polar two-phase solvent systems, which are often a problem for the conventional multilayer coil separation column. Both the stationary phase retention factors and, especially, the mixing of the two liquid phases behave differently in a classical J-type coiled planet centrifuge[7] and in the spiral disk assembly. This assembly can be made by molding the disks, which will facilitate the commercialization of the instrument.

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