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Improved spiral disk assembly for high-speed counter-current chromatography

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Abstract

The spiral disk for type-J high-speed counter-current chromatography (HSCCC) has been improved by placing short segments of PTFE (tetrafluoroethylene) tubing into the channel at regular intervals. The best results were obtained with a four-channel spiral column using 600 spacers, which significantly improved stationary phase retention and partition efficiency in both *n*-butanol–acetic acid–water (4:1:5) and poly(ethylene glycol) 1000-dibasic phosphate polymer phase systems. Based on these findings, a "bead-chain spiral disk" was designed and its performance tested with a type-J HSCCC centrifuge. This new column design substantially improved the separation of both dipeptide and protein samples in terms of stationary phase retention and partition efficiency.

Keywords: Counter-current chromatography; Instrumentation; Spiral disk assembly; Coil planet centrifuge; Peptides; Proteins

1. Introduction

High-speed counter-current chromatography has been used for separation and purification of natural and synthetic products [1,2]. However, the method fails to retain a satisfactory amount of stationary phase of polymer phase systems [3] used in the separation of proteins.

Recently, a new column design "spiral disk assembly" has been developed for separation of biopolymers with high-speed counter-current chromatography [4,5]. It consists of a set of inert plastic disks stacked together to form a separation column. Each disk is equipped with single or multiple spiral channels. The system improved retention of polymer phase systems by producing a radial centrifugal force gradient formed when eluted with the lower mobile phase from the inner terminal toward the periphery or the upper phase from the outer terminal toward the inner terminal. In the past, the system was studied using three sets of two-phase solvent systems, i.e. 1-butanol–acetic acid–water (4:1:5) for the separation of dipeptides, and poly(ethylene glycol) (PEG) 1000 and dibasic potassium phosphate, each 12.5% (w/w)

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in water for separation of proteins, and 4% (w/w) PEG 8000 and 5% (w/w) dextran T500 (molecular mass: 500 000) in water to determine the retention of the stationary phase. The results indicated that the spiral disk produces a satisfactory level of stationary phase retention for all the solvent systems including a viscous PEG dextran system, especially in the high pitch spiral channels. However, the partition efficiency of the protein on the PEG phosphate system was found to be unsatisfactory probably due to a laminar flow of the mobile phase along the spiral channel which would limit the interfacial area available for mass transfer between the two phases. Therefore, an effort has been made to improve the partition efficiency of the system by introducing various inserts into the channel. It was found that inserting glass beads or fine PTFE thread as a mixer into the channel produced extensive carryover of the stationary phase resulting in detrimental loss of partition efficiency. However, inserting short segments of polytetrafluoroethylene (PTFE) tubing into the channel at regular internals significantly improved the partition efficiency especially for the PEG–phosphate polymer phase system. Based on this finding, a new column "bead-chain spiral disk" has been designed.

The present paper describes the results of our studies on a spiral disk with PTFE tubing insert and the performance of the bead-chain spiral disks in terms of stationary phase retention and partition efficiency.

2. Experimental

2.1. Apparatus

The present studies employed a type-J coil planet centrifuge (P.C. Inc., Potomac, MD, USA) with a 10 cm revolution radius. A spiral disk assembly (single disk) was mounted on one side and a counterweight on the other side to balance the centrifuge system.

The design of the spiral disk assembly has been described earlier [4,5] and therefore, it is only briefly reviewed here. Fig. 1 shows a photograph of the spiral disk assemblies (bottom) and their elements. The single spiral disk assembly used in the present studies (bottom, left) consists of a pair of flanges (top) and a plastic disk (high-density polyethylene) with a single or four spiral grooves (middle, left) and a pair of PTFE sheet septa (middle, right). The spiral disk is sandwiched by a pair of PTFE septa and tightly held between a pair of flanges using a multiple set of screws in the inner and outer edges of the disk. The upper flange (top, left) is equipped with a plastic planetary gear which is engaged to the stationary sun gear mounted on the central axis of the centrifuge. The lower flange (top, left) is equipped a pair of screws which rigidly fastens the assembly to the rotary shaft.

Fig. 2A shows the design of the original spiral disk with a single channel. The spiral groove (1.5 mm wide and 3.7 mm deep) starts at the inner terminal (I) and ends at the outer terminal (O) with a total capacity of about 24 ml. The heavier mobile phase introduced at inlet I reaches outlet O after 13 spiral turns. It then flows through a hole to reach the other side of the disk and follows the radial groove (dotted line) to reach the point behind inlet I where it exits the disk through the hole made on the lower flange. In this disk design, the spiral pitch (radial distance between the spiral) is only 4 mm which is increased to 16 mm in the four-spiral channel design illustrated in Fig. 2B. This high pitch spiral disk has a set of four-spiral channels, each labeled I_1–O_1, I_2–O_2, I_3–O_3, and I_4–O_4, which are all connected in series through a set of radial channels on the opposite side of the disk (dotted line). The heavier mobile phase introduced at I_1 reaches O_1 after three and a quarter turns. Then, it flows through a hole at O_1 to reach the opposite side and follows the radial channel and through another hole to come up at I_2 which leads the second spiral channel to the terminal O_2. Repeating this process four times, the mobile phase finally reaches O_4, where it exits the disk through a hole. The present disk design enhances the pitch four times without wasting the disk space (the first spiral channel is highlighted to visualize the enhanced pitch).

Fig. 3A shows a single-channel spiral disk with inserts of short segments of PTFE tubing (3 mm o.d., 2 mm i.d., 2–3 mm long) at ca. 1 cm intervals, dividing the channel into ca. 320 compartments called "locules." Fig. 3B similarly shows a four-channel spiral disk with similar inserts at ca. 5 mm intervals with ca. 600 locules.

Fig. 4 shows the bead-chain spiral disks, a single-channel (A) and four-channel (B) disks each with about 1460 wells (2.8 mm diameter and 2 mm deep) connected with a narrow duct (0.5 mm long,

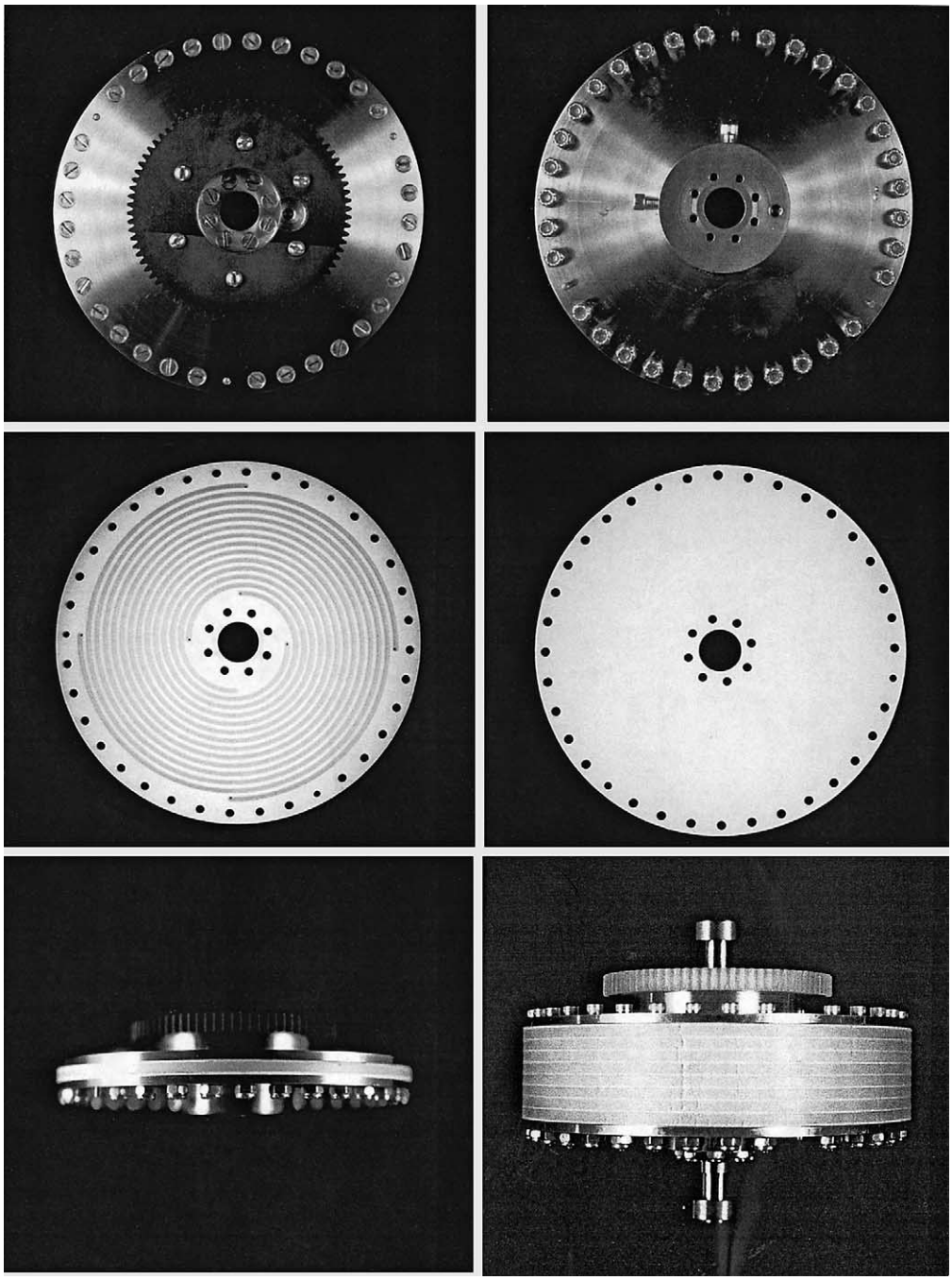

Fig. 1. Photographs of spiral disk assemblies and their components. Upper flange equipped with a plastic gear (upper, left); lower flange (upper, right); spiral disk (middle, left); PTFE sheet septum (middle, right); single spiral disk assembly (bottom, left); and multiple spiral disk assembly (eight units) (bottom, right).

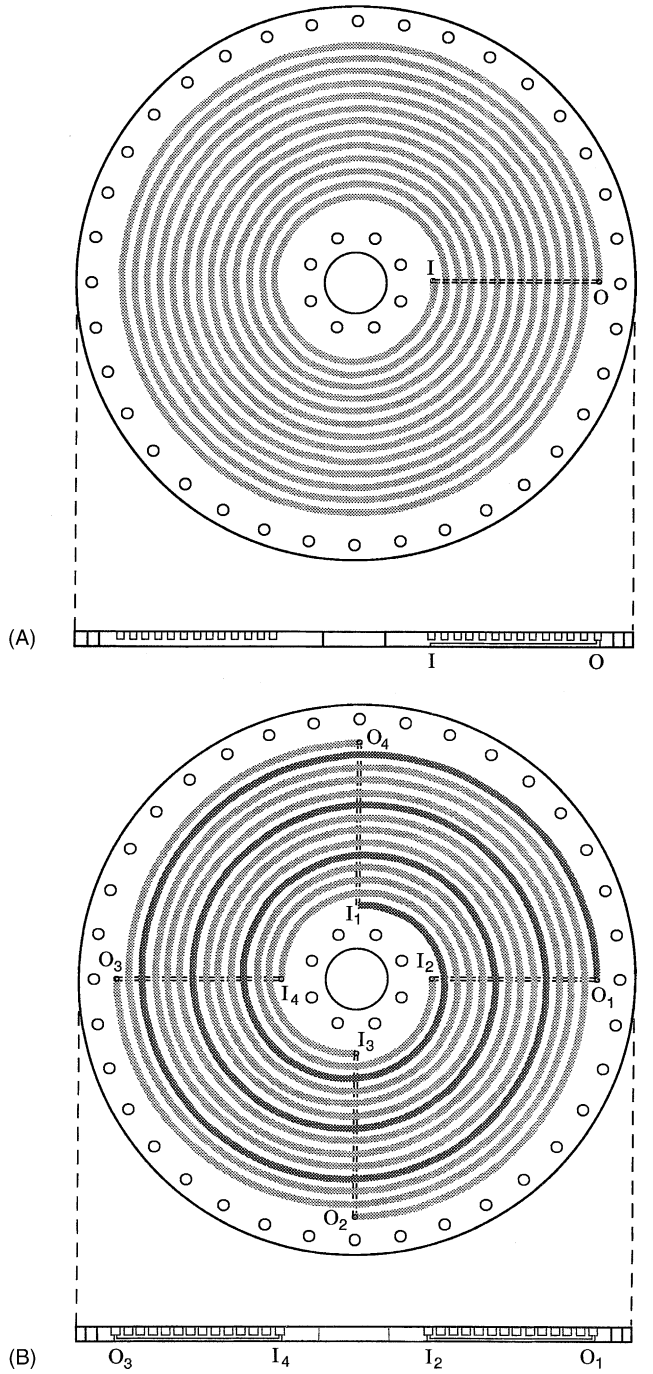

Fig. 2. Drawing of the spiral disks. (A) Single-channel spiral; (B) four-channel spiral disk.

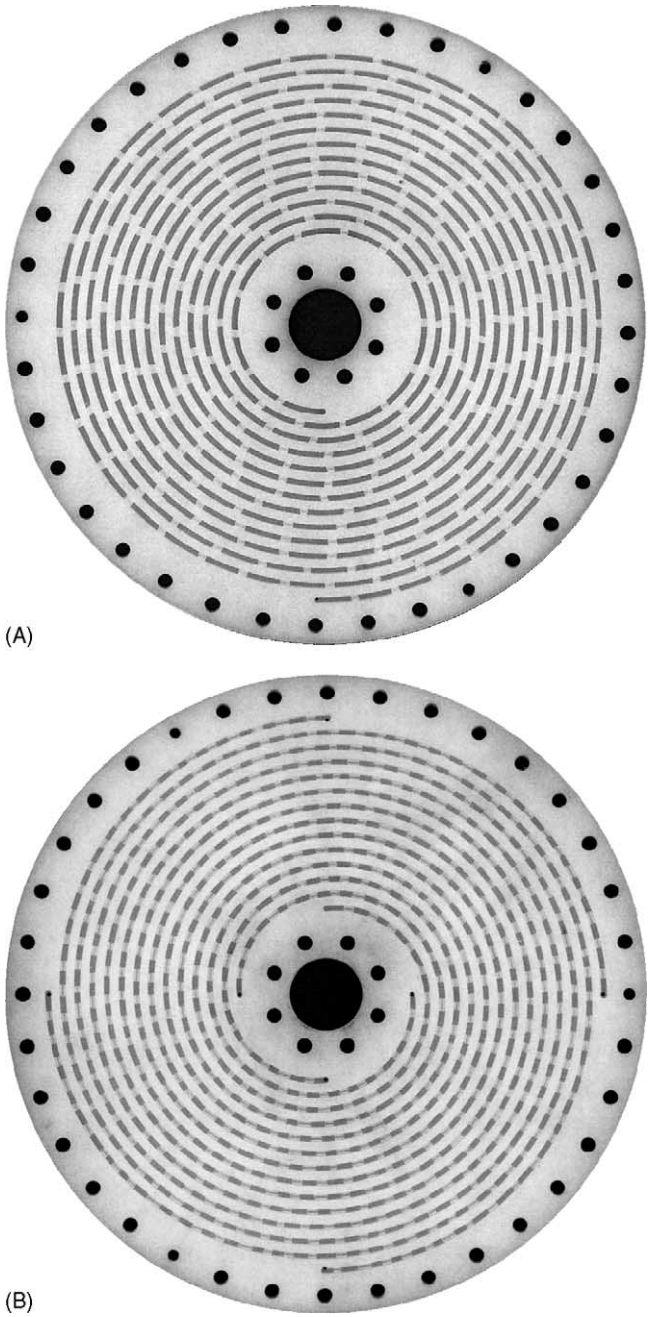

Fig. 3. Spiral disks with tubing inserts. (A) ca. 300 inserts; (B) ca. 600 inserts. Channel dimensions—1.5 mm wide and 3.7 mm deep and ca. 22 ml capacity. Inserts: PTFE tubing, 2 mm o.d. and 2–3 mm long (standard wall no.12 from Zeus Industrial Product, Orangeburg, SC, USA).

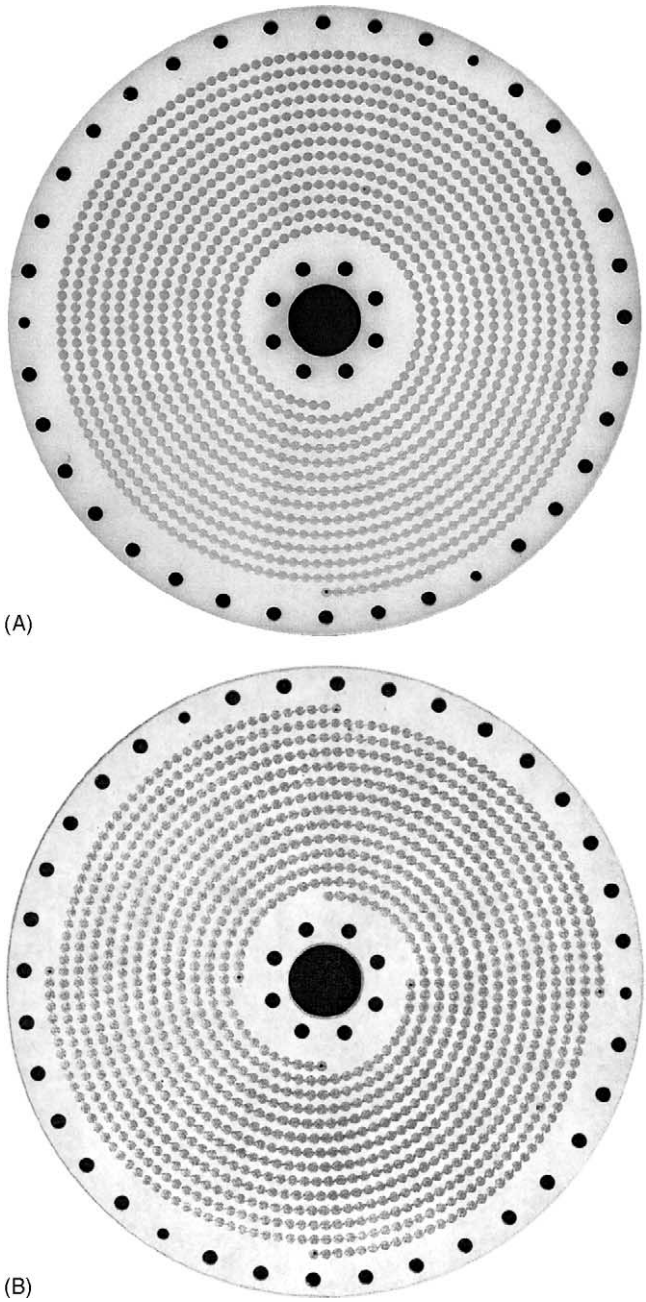

Fig. 4. Bead-chain spiral disks. (A) Single-channel disk; (B) four-channel disk. Channel dimensions—well: 2.8 mm in diameter and 2 mm in depth; connecting duct: 0.5 mm long, 2 mm deep, and 0.8 or 1.2 mm wide; capacity: 16–18 ml.

1.2 mm wide and 2 mm deep) with a total capacity of about 18 ml.

2.2. Reagents

1-Butanol was purchased from Fisher Scientific (Fair Lawn, NJ, USA), and acetic acid and dibasic potassium phosphate from Mallinckrodt Baker (Paris, KY, USA). PEG 1000 and PEG 8000, and test samples including lysozyme (chicken egg), myoglobin (horse skeletal muscle), tryptophyl tyrosine (trp-tyr), and valyl tyrosine (val-tyr) were all obtained from Sigma.

2.3. Preparation of two-phase solvent system and sample solution

The following two-phase solvent systems were prepared: 1-butanol–acetic acid–water (4:1:5, v/v/v) for separation of dipeptides (trp-tyr and val-tyr), and 12.5% (w/w) PEG 1000 and 12.5% (w/w) dibasic potassium phosphate in 75% (w/w) water for separation of proteins (lysozyme and myoglobin). Each solvent system was thoroughly equilibrated in a separatory funnel at room temperature and two phases separated shortly before use.

The sample solution of dipeptides was prepared by dissolving 10 mg of trp-tyr and 40 mg of val-tyr in 20 ml of the upper phase of 1-butanol–acetic acid–water (4:1:5), while the protein sample solution was prepared by dissolving each 20 mg of lysozyme and myoglobin in 20 ml of the upper phase of the PEG 1000-potassium phosphate system.

2.4. Experimental procedure

In each experiment the column is first entirely filled with the stationary phase (upper or lower phase) followed by injection of the sample through the sample port. Then, the apparatus is rotated at 800 rpm while the mobile phase is eluted through the column at the desired flow rate. The separation may be repeated by changing the direction of the revolution and/or elution mode (head to tail and tail to head). The effluent from the outlet of the column is continuously monitored with a UV detector at 275 nm (LKB Uvicord s, Stockholm, Sweden). The effluent was collected into a graduated cylinder to measure the volume of the stationary phase eluted from the column to compute the retention of the stationary phase.

Using spiral disks each with a different channel design, a series of experiments evaluated the performance of the disk in terms of partition efficiency and retention of the stationary phase using the two different types of two-phase solvent systems described above. The experiments were performed at various flow rates of the mobile phase using the following four different elution modes: L-I-T (lower phase pumping from the inner tail terminal); L-I-H (lower phase pumping from the inner head terminal); U-O-T (upper phase pumping from the outer tail terminal); U-O-H (upper phase pumping from the outer head terminal). In the previous studies, four other reversed elution modes including L-O-T, L-O-H, U-I-T, and U-I-H gave much less retention of the stationary phase and were not tested further.

3. Results and discussion

3.1. Effect of the spacer

The spiral disk has an advantage over the conventional multilayer coil separation column in that the channel geometry is easily modified in many ways. It also permits various inserts or spacers to be placed into the separation channel to improve its performance.

As mentioned earlier, inserting glass beads or fine PTFE tubing (both ends closed) into the channel produced intensive carryover of the stationary phase resulting in a loss of peak resolution. We assume that introducing a loose object into the channel produces vigorous vibration and emulsification of the solvents under the fluctuating centrifugal force field induced by the planetary motion of the column. However, it has been observed that placing short segments of PTFE tubing into the channel as spacers, firmly inserting them at regular intervals (Fig. 4A and B), significantly improved the partition efficiency especially for the protein separation. It is most likely that such inserts serve as a stream breaker to interrupt the laminar flow of the mobile phase, especially for the viscous polymer phase system.

Fig. 5 summarizes the effects of the spacer on partition efficiency. Short segments of PTFE tubing, 2–3 mm long and 2 mm i.d. were placed into the spiral channel of both single-channel (Fig. 3A) and

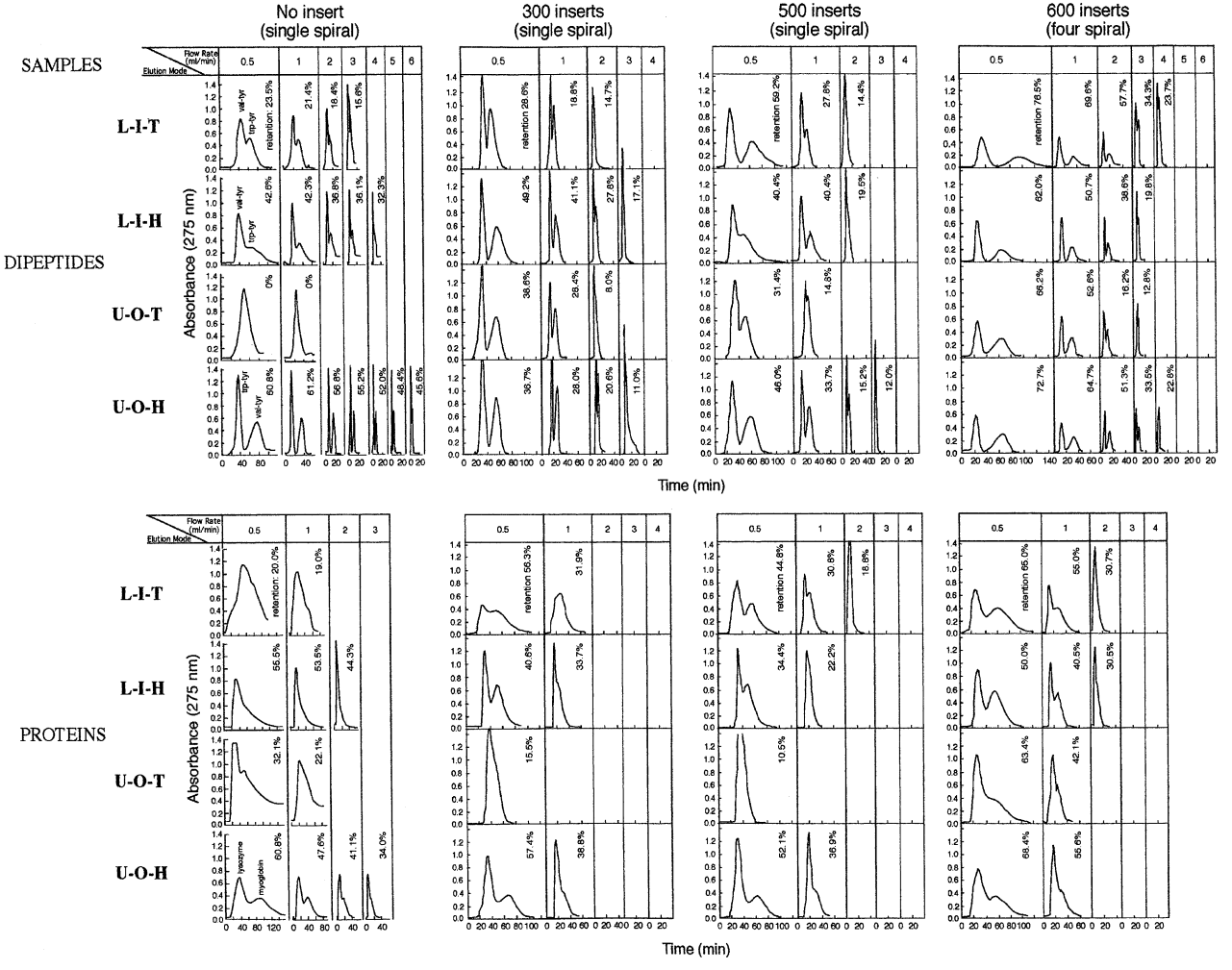

Fig. 5. Separation of dipeptides and proteins by spiral disks with PTFE tubing inserts. Experimental conditions—apparatus: type-J coil planet centrifuge with 10 cm revolution radius; spiral disks: single-channel disk (1.5 mm wide and 3.7 mm deep) with no insert, 300 inserts, 500 inserts, and four-channel spiral disk with 600 inserts as indicated on each composite diagram; sample: dipeptide (trp-tyr 0.5 mg + val-tyr 2 mg in 1 ml of upper phase) and proteins (lysozyme and myoglobin each 10 mg in 1 ml of upper phase); solvent system: 1-butanol–acetic acid–water (4:1:5, v/v/v) for dipeptide separation (upper row of composite diagrams) and 12.5% (w/w) PEG 1000 and 12.5% (w/w) potassium diphosphate in water for protein separation (lower row of composite diagrams); elution modes: L-I-T (lower phase pumped from inner tail terminal), L-I-H (lower phase pumped from inner head terminal), U-O-T (upper phase pumped from outer tail terminal), and U-O-H (upper phase pumped from outer head terminal); flow rate: 0.5–4 ml/min as indicated; rpm: 800. The retention of the stationary phase is indicated in each chromatogram.

four-channel (Fig. 3B) spiral disks. The number of spacers is shown at the top of each diagram. In each diagram, four elution modes are shown on the left, i.e. lower phase introduced from the inner tail and head terminals (L-I-T and L-I-H); and upper phase from the outer tail and head terminals (U-O-T and U-O-H). Flow rates from 0.5 to 6 ml/min were applied for the dipeptide separation, and from 0.5 to 3 ml/min for the protein separation.

Fig. 5 (upper four composite diagrams) shows the separation of a standard sample mixture of trp-tyr (0.5 mg) and val-tyr (2 mg) in 1 ml of the upper phase with a two-phase solvent system composed of 1-butanol–acetic acid–water (4:1:5). The dipeptides were well resolved in all columns by the mobile upper phase from the outer head terminal (U-O-H). On the other hand, the control column with no insert (left) yielded less efficient separation with the lower phase mobile (L-I-T and L-I-H), which is substantially improved by the spacers. In the single spiral column, best results were obtained with 300 spacers (second from the left) where the effects of head–tail elution modes on peak resolution become somewhat obscure. The peptide separation was further improved by the four-spiral column with ca. 600 spacers (right) apparently due to its high retention of the stationary phase as indicated in each chromatogram. The two dipeptides were completely resolved regardless of the choice of the mobile phase at flow rate of 0.5 and 1.0 ml/min.

Fig. 5 (lower four composite diagrams) similarly illustrates the peak resolution between two protein samples (lysozyme and myoglobin, each 10 mg) in 1 ml of the upper phase with the PEG 1000-dibasic potassium phosphate system. In the single-channel spiral disks, the separation of the protein samples was substantially improved by the spacers up to 300 and 500, where the lower mobile phase started to produce partial separation of two proteins. Here again, the disk having four-spiral channels with 600 spacers produced the best separation among all.

3.2. Bead-chain spiral disks

The above results obtained with the PTFE tubing spacers prompted us to design a new design of the spiral disk that resembles a beaded chain in appearance as shown in Fig. 4A and B. It consists of about 1460 round holes each 2.8 mm in diameter and 2 mm deep, which are connected with short channels of width 0.8 or 1.2 mm. The capacity of each disk is about 16 ml for 0.8 mm channel and 18 ml for 1.2 mm channel. The disks with both single and four-spiral channels were made as shown in Fig. 4.

Fig. 6 similarly illustrates the performance of these "bead-chain" spiral disks. The separation obtained by the original spiral disk (standard disk) is shown on the left. The column design of the bead-chain disks is shown above each diagram.

In the dipeptide separation with 1-butanol–acetic acid–water (4:1:5) shown in the top four composite diagrams, the bead-chain spiral disks show substantial improvement over the original disk when the lower phase was used as the mobile phase. It is observed that the width of the connecting duct substantially effects the separation. The wider duct produced better separation in both single- and four-spiral disks. As in the spiral disk with PTFE tubing inserts, the effect of head–tail elution mode becomes reduced in the bead-chain spiral disks. Among the bead-chain disks, either wider channel or greater pitch distance substantially improved the separation for both mobile phases, and the best separation was attained with the four-channel bead-chain disk with the wider channels (1.2 mm). In the protein separation (lower four composite diagrams), the bead-chain spiral disk substantially improves the resolution especially with wider channel and higher pitch. Here again, the bead-chain spiral disk with a high spiral pitch (four-spiral channels) is least affected by the head–tail elution mode as in the disks with tubing insert described earlier.

It was found that in the bead-chain spiral disk the width of the connecting channel played an important role in both retention and peak resolution. The wider channel produced better stationary phase retention and peak resolution. The optimum width of the channel may exist for a given dimension of the well. This should be investigated further.

It should be noted here that each chromatogram presented in the present paper was obtained from a single spiral disk. In the practical separation, several spiral disks are stacked to form a multiple disk assembly as shown in Fig. 1 (bottom, right) where two

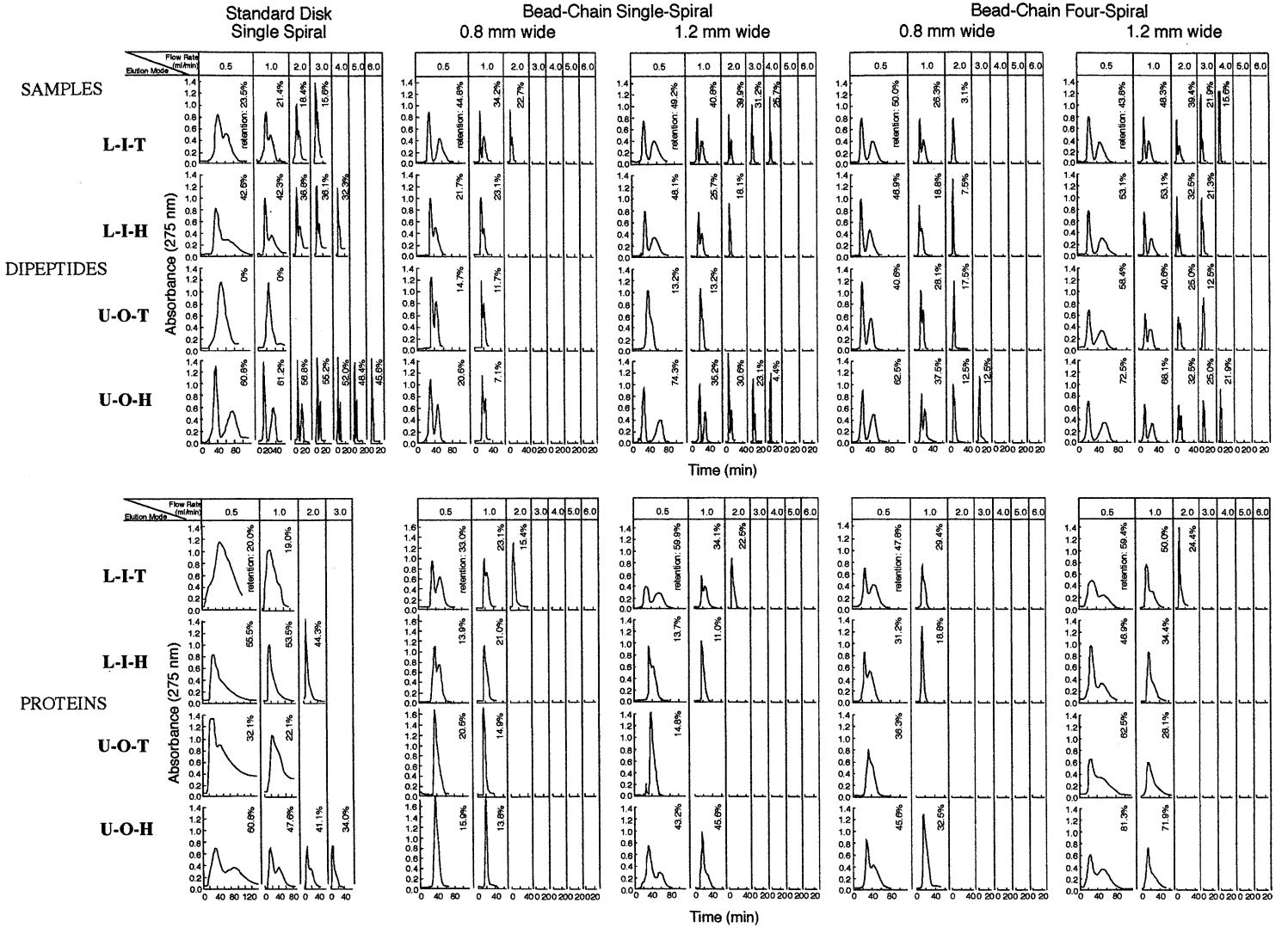

Fig. 6. Separations of dipeptides and proteins with bead-chain spiral disks. Experimental conditions—spiral disks: standard disk (left); 1.5 mm wide, 3.7 mm deep, and 24 ml capacity; bead-chain spiral disk with single- and four-channel as indicated at the top of each composite diagram. The rest of the conditions are identical to those in the Fig. 5 caption.

to three of such assemblies can be connected in series and mounted on the rotary frame of a type-J HSCCC centrifuge. This will increase the partition efficiency as well as the sample loading capacity of the present system many times.

4. Conclusions

The rectangular spiral channel embedded in a solid plastic disk applied to the type-J HSCCC centrifuge enhances the retention of stationary phase for viscous, low interfacial tension two-phase solvent systems. The performance of the spiral disk is substantially improved by embedding a set of spacers or modifying the geometry of the channel into multiple pits interconnected with narrow ducts. The main advantages of these spiral disks are

(1) Applicability of polymer phase systems for separation of biopolymers (proteins, DNA and RNA, and polysaccharide).
(2) Reliable retention of the stationary phase for polar or low interfacial tension solvent systems which are useful for the separation of bioactive compounds.
(3) Improved stationary phase retention against emulsification.
(4) Possible application of the large column for industrial-scale separations by mounting the column assembly on the slowly rotating horizontal shaft.

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