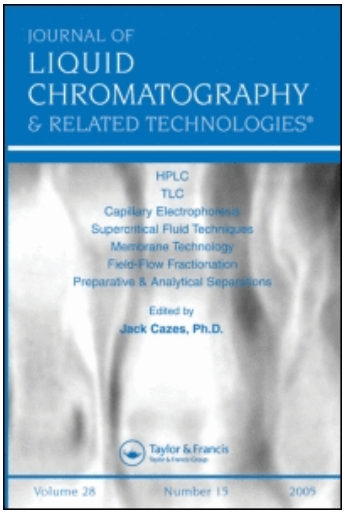

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Triangular Helical Column for Centrifugal Countercurrent Chromatography

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Triangular Helical Column for Centrifugal Countercurrent Chromatography

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Abstract: Effective column space and stationary phase retention have been improved by changing the configuration of the helical column originally used for toroidal coil countercurrent chromatography. The use of an equilateral triangular core for the helix column doubles effective column space and retains the stationary phase over 40% of the total column capacity without increasing the column pressure. The present results suggest that the stationary phase retention and the peak resolution will be further improved using new column designs fabricated by a new technology called ''laser sintering for rapid prototyping.''

Keywords: Dipeptides, Effective column space, Laser sintering for rapid prototyping, Retention of the stationary phase, Toroidal coil countercurrent chromatography, Triangular helical column

INTRODUCTION

Hydrostatic countercurrent chromatography (CCC) reported in the past used a coiled column mounted around the periphery of the centrifuge bowl to carry out analytical separations. The system can produce highly

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efficient analytical separation as reported earlier.[1–4] In this separation, the centrifugal force retains the stationary phase in each turn of the coil, while the mobile phase entirely occupies the other half. Since the mobile phase steadily percolates through the stationary phase by partially displacing it, the volume of the stationary phase retained in the separation column becomes substantially less than 50% or typically about 30% of the total column space.[4] This low retention of the stationary phase limits the partition efficiency of the system.

The present paper introduces a new configuration of the toroidal coil using an equilateral triangular core which improves both retention of the stationary phase and peak resolution. The performance of this triangular helical tube is demonstrated on the CCC separation of dipeptide samples with a two-phase solvent system composed of 1-butanol-acetic acid-water at a volume ratio of 4:1:5 using a rotary-seal-free continuous flow centrifuge system.

EXPERIMENTAL

Apparatus

The present study uses a rotary-seal-free centrifuge fabricated by Pharma-Tech Research Corporation, Baltimore, Maryland, USA. It holds an aluminum rotary plate about 34 cm in diameter to hold a toroidal coil separation column. The column is made by winding a 0.46 mm ID FEP (fluorinated ethylene propylene) (Zeus Industrial Products, Orangeburg, SC, USA) onto a 2 m length of equilateral triangular PTFE (polytetrafluoroethylene) tubing (6 mm each side) making about 2000 turns with a 6.7 ml capacity. The coiled column is arranged at the periphery of the rotary plate by making two and a half spiral turns in such a way that one side of the triangular column is facing to the plate. Each terminal of the coiled column is connected to 0.47 mm ID PTFE flow tube using a set of tubing connectors (Upchurch Scientific, Palm Springs, CA, USA). These flow tubes are put together and passed through the center of the central shaft downward, the hollow horizontal shaft of a miter gear, then led upward into the vertical hollow tube support, and finally exit the centrifuge from the center of the upper plate where they are tightly supported with a pair of clamps.

Reagents

1-Butanol was purchased from Fisher Scientific, Fair Lawn, NJ, USA and acetic acid from Mallinckrodt Chemicals, Phillipsburg, NJ, USA. Dipeptide samples including tryptophyl-tyrosine (trp-tyr) and valyl-tyrosine (val-tyr) were obtained from Sigma Chemicals, St. Louis, MO, USA.

Preparation of Two-Phase Solvent System and Sample Solution

A two-phase solvent system composed of 1-butanol-acetic acid-water (4:1:5, v/v) was used to separate dipeptide samples, trp-tyr and val-tyr. The solvent mixture was thoroughly equilibrated in a separatory funnel by vigorous shaking and degassing several times, and the two phases separated shortly before use. The sample solution was prepared by dissolving 25 mg of trp-tyr and 100 mg of val-tyr in 20 ml of the upper phase of the above two-phase solvent system, and 50 μl was charged in each run.

Separation Procedure

In each separation, the coiled column was entirely filled with the stationary phase, either upper or lower phase, followed by sample injection, and the column was rotated at 1000 rpm while the mobile phase is pumped into the coiled column at a flow rate of 50 μl/min. The effluent from the outlet of the coiled column was continuously monitored with a Uvicord IIS (LKB, Stockholm, Sweden) at 280 nm and the elution curve was traced using a stripped-chart recorder (Pharmacia, Stockholm, Sweden). The separation was performed using the lower phase as the mobile phase which is introduced from either end of the coiled column. After two peaks were eluted, the rotation was stopped and the column contents were collected into a graduated cylinder to measure the amount of the stationary phase retained in the column.

Evaluation of Partition Efficiency

From the obtained chromatogram, the efficiency of the separation was expressed in terms of peak resolution (Rs) using a conventional equation,

$$Rs = 2(R_2 - R_1)/(W_1 + W_2) \quad (1)$$

where R and W indicate the retention volume and the peak width of the specified peaks, respectively.

RESULT AND DISCUSSION

Figure 1 schematically illustrates a conventional toroidal coil for CCC separation. When the coiled column is rotated, one of the phases is retained in each coiled turn while the other phase flows through it. As mentioned earlier, this toroidal coil system has a problem of low retention of the

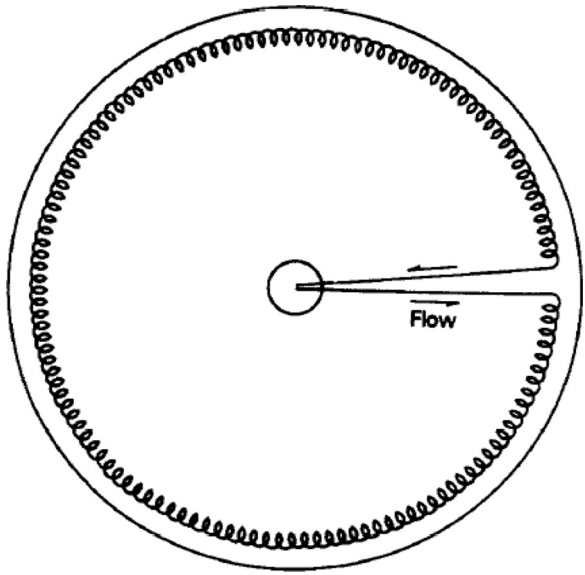

Figure 1. Diagramatic design for toroidal coil column for countercurrent chromatography.

stationary phase, since the one side of each helical turn is completely occupied by the mobile phase while the solute partition process is carried out in the other half. Consequently, the retention of the stationary phase is always limited to less than 50% of the total column space, which is sharply decreased as the flow rate of the mobile phase increased. Although increase of the effective column space for solute partitioning may be attained by using a longer separation column, it is limited by high back pressure which is increased with the number of coiled turn.[1]

The retention of the stationary phase and the effective column space, however, may be increased by changing the configuration of the coil without raising the column pressure. In the present study, the separation coil is made by winding PTFE tubing around a triangular core, which is then mounted around the periphery of the centrifuge bowl in such a way that one side of the triangular column is placed against the bottom plate of the bowl. In this way the dead space occupied by the mobile phase becomes reduced to 1/3 of the column space while the other 2/3 becomes the efficient column space, provided that the mobile phase is eluted in the right direction through the column. Consequently, the retention of the stationary phase is much improved as shown in Figure 2 that schematically illustrates one helical turn of tubing wound around round (a) and triangular (b) cores through which lower mobile phase is flowing in the suitable direction under a centrifugal force field.

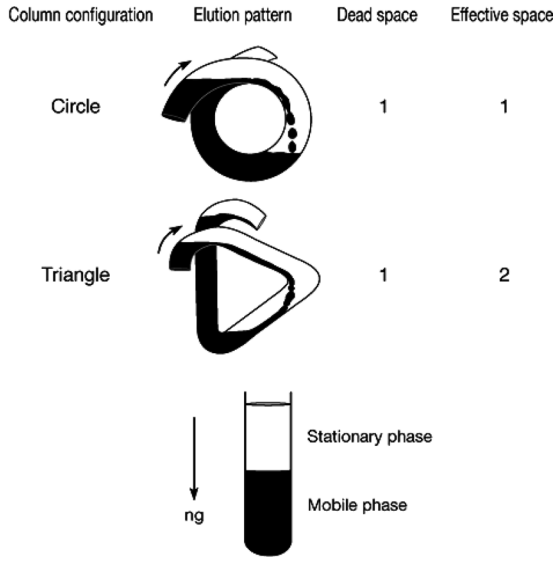

Figure 2. Comparison in relative effective column space between the conventional and the triangular helical columns.

The actual effect of the triangular column configuration on the retention of the stationary phase and partition efficiency is shown in Figure 3. The experiment was performed on separation of dipeptides with a two-phase solvent system composed of 1-butanol-acetic acid and water at a volume ratio of 4: 1: 5 using the lower phase as the mobile phase at a flow rate of 50 µl/min under a revolution speed of 1,000 rpm. The chromatogram on the left was obtained by eluting the lower mobile phase in the wrong direction where effecting space is limited to one side of the equilateral triangle or 1/3 of the total column capacity, yielding the peak resolution (Rs) at 2.33 with retention of the stationary phase at 21.3%. The chromatogram on the right is obtained by eluting the same mobile phase in the right direction to provide the effective space at 2/3 of the total column capacity, yielding higher peak resolution (Rs) at 2.7 with improved stationary phase retention of 42.9%. These retention figures indicate that about 1/3 of the effective column space is occupied by the mobile phase. The present results agree with the previous data[4] obtained from the conventional helical column wound on the circular core of similar size that produced the stationary phase retention of 29 – 32% or the average value of the above two retention data.

Our experimental results clearly indicate that the retention of the stationary phase in the toroidal coil will be further improved by increasing

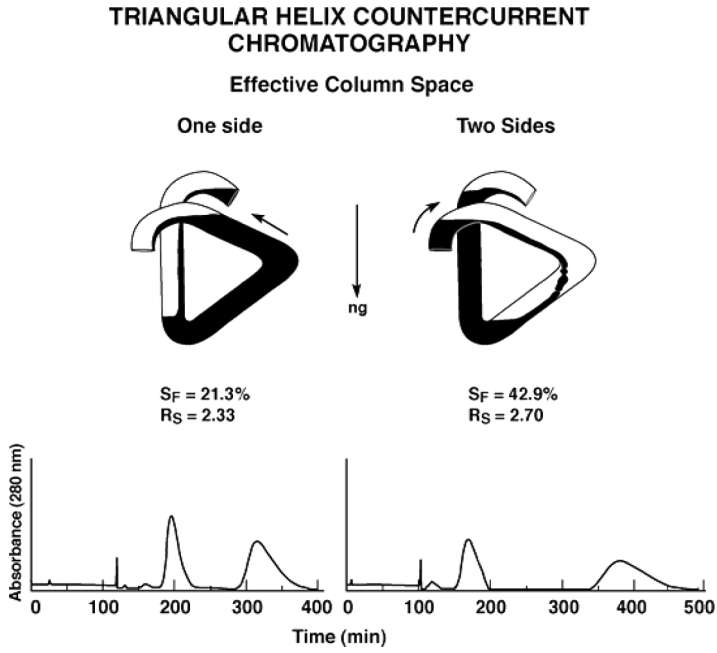

Figure 3. Comparison in stationary phase retention and peak resolution (Rs) in the triangular helical column between two elution modes.

the height of the triangular core and/or reducing the length of the side of triangle providing the dead space. Although such column configuration is not easily held on the rotary plate in the right orientation, a similar effect can be created by a recently developed process called "laser sintering for

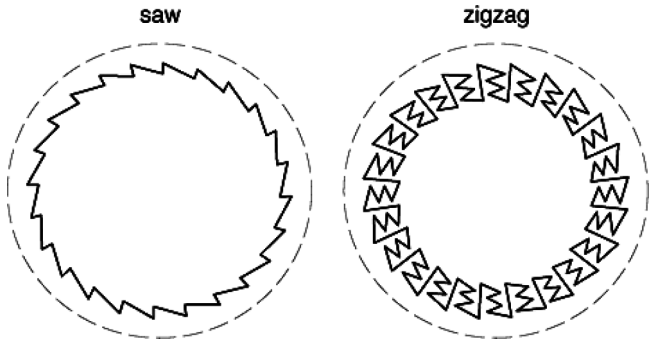

Figure 4. Schematic designs for the new column by the laser sintering for rapid prototyping technology. Left: saw pattern; right: zigzag pattern.

rapid prototyping'' which can make a variety of shapes of plastic models by laser imaging irradiation. This technology can form any desired shape and depth of grooves on a rigid plastic disk in which fine tubing can be accommodated into a multilayer fashion. The examples of these configurations are shown in Figure 4. In the saw pattern shown on the left, the groove is a series of repetitive triangular form which provide a long effective column space. In the zigzag pattern shown on the right, each bending point may serve as a good mixing space of the two phases. In both designs, the effective column space is much greater than that in the conventional or equilateral triangular helix column without increasing the column pressure. These columns can be mounted on either on planetary[5] or non-planetary centrifuge[1–4] for performing a small-scale preparative separation and analysis of food[6] and drinks.[7] It may also be interfaced to mass spectrophotometer (CCC/MS).[8]

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