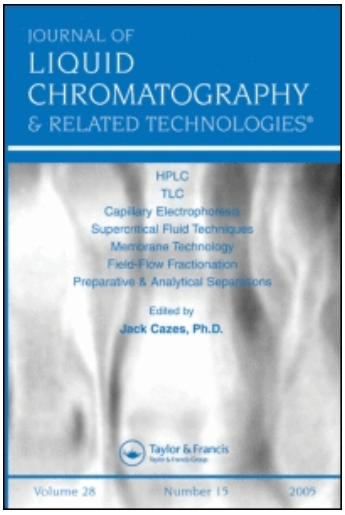

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CENTRIFUGAL COUNTERCURRENT PARTITION CHROMATOGRAPHY WITH A CHAIN WINDING COLUMN

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ABSTRACT

A new kind of centrifugal countercurrent partition chromatographic column has been developed. The shape of the column looks like the chain of a bicycle. Using the column, retention ratio of the stationary phase was measured with three kinds of solvent systems under various experimental conditions. The chloroform solvent systems showed retention from 52 to 66% when the upper phase was used as the stationary phase. The performance of the column has been successfully demonstrated in the separation of dinitrophenyl (DNP) amino acids, Weimeisu and an extract of branch-leaves of *Taxus cuspidate*.

INTRODUCTION

A simplified centrifugal countercurrent partition chromatography (CCPC) without using planet motion was developed in 1980's.[1] Its column can be easily wound because it consists of a helical coil winding of a PTFE tubing.

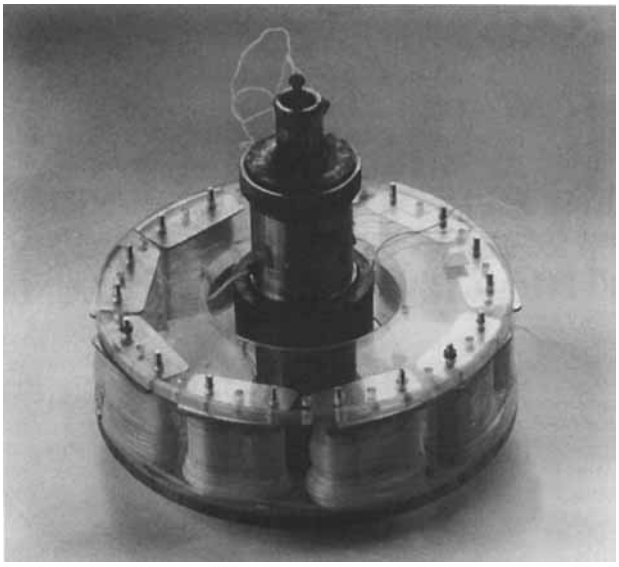

Figure 1. Photograph of the rotor contains the chain winding column.

Owing to the rotation of the rotor around its axis, the rotor can be easily employed in a normal laboratory centrifuge. The CCPC was used as an analytical scale with injection range of 50-200μg solute. But its retention ratio of stationary phase was only up to 50%.

In order to increase this ratio, several attempts were tried from the view point of the instrumentation in author's laboratory. A chain winding column was designed for this purpose and the helical coil column was replaced by it in CCPC. In this paper, the construction of the chain winding column was described. Its capability of retaining stationary phase was studied. Separations of DNP-amino acids, Weimeisu, and extract of branch-leaves of *Taxus cuspidate* were demonstrated.

EXPERIMENTAL

Apparatus

A newly designed rotor was employed in the same centrifuge as described before.[1] The aims of this design are: (1) it can retain more stationary phase, and

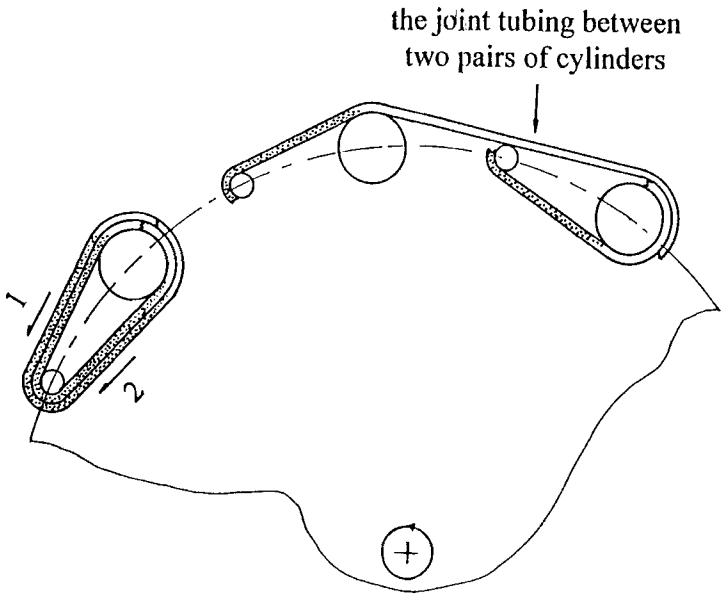

Figure 2. Diagram of the former and the tubing wound on it.

(2) fine separation performance can be retained. The rotor's rotating face-seal unit is the same as described before.[1] The rotor's separation part, chain winding column, is a fine tubing wound onto the former in series (Figure 1). The former is composed of nine pairs of cylinders. Every pair of cylinders include a larger cylinder and a smaller cylinder in diameter (Figure 2). The tubing wound on them just looks like the chain of a bicycle. About 50 turns of tubing were wound on each pair of cylinders. The diameter of the larger cylinder is 18mm, and the smaller cylinder is 6mm. The distance between them is 35mm. The height of the cylinders is 60mm. The axis of the cylinder is parallel to the axis of the rotor, the distance between them is 100mm.

When the lower phase is used as the stationary phase, the upper phase should be eluted the direction 1. Based on the principles of mechanics, the lower phase will be retained in the dotted part of the tubing (Figure 2). The dotted part is more than 70% of total column. So the stationary phase retention ratio can be higher than 50%. When the upper phase is used as the stationary phase, the lower phase should be eluted in the direction 2. The upper phase will be retained in the dotted part and higher retention ratio can be obtained.

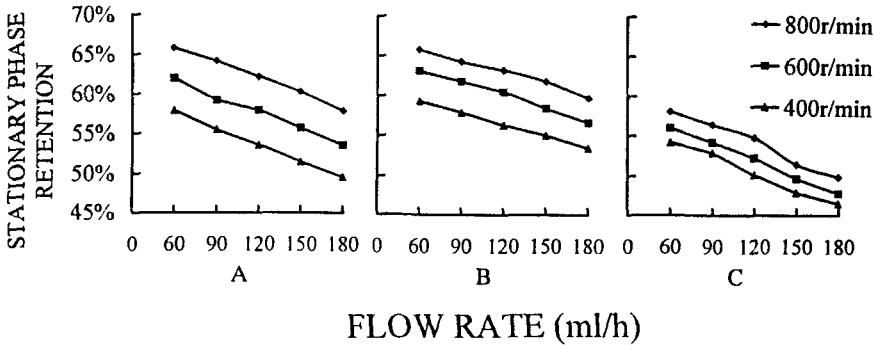

Figure 3. Retention of Stationary phase versus the flow rates for different rotating speed (rpm) conditions. Chain winding column; stationary phase, lower phase. (A) Chloroform.acetic acid/0.1N HCl (2:2:1); (B) 1-Butanol/acetic acid/ water (4:1:5); (C) n-Hexane/EtOAc/EtOH/water (6:3:2:5).

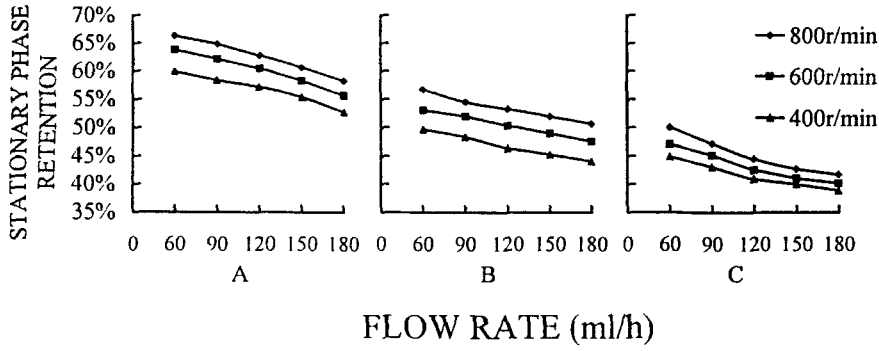

Figure 4. Retention of Stationary phase versus the flow rates for different rotating speed (rpm) conditions. Chain winding column; stationary phase, upper phase. (A) Chloroform/acetic acid/0.1N HCl (2:2:1); (B) 1-Butanol/acetic acid/water (4:1:5); (C) n-Hexane/EtOAC/EtOH/water (6:3:2:5).

Equipment

The CCPC operating system is the same as described before,[1] a piston pump(Model YSB-2, Shanghai Instruments, Academia Sinica, China) was used to pump the phases into the rotor.

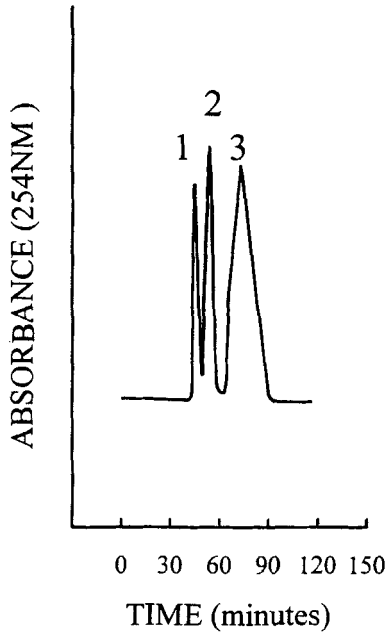

Figure 5. Chromatogram of DNP-amino acids obtained by chain winding column. Solvent system, chloroform/acetic acid/0.1N HCl (2:2:1); mobile phase, upper phase; flow rate, 180 mL/hr; rotor speed, 500 rpm; sample dose (volume), 5 mg (1 mL); column pressure, 7 kg/cm^2; peaks, 1=DNP-ornithine, 2=DNP-glutamic acid, 3=DNP-alanine.

The eluate from the outlet of the column was continuously monitored with a 8823-UV monitor (Beijing Institute of New Technology Application, China) at a suitable wavelength and the absorbency was recorded with a 3057-Portable Recorder (Sichuan Instrument Group Factory, China).

The chain winding column was prepared from a single piece of PTFE tubing of 1.6mm I.D. (Tianjin plastic works, China) by winding it tightly onto the former. The length of the tubing is about 60m, the total column volume is about 125ml.

Materials

All organic solvents used were of analytical-reagent grade. Each two-phase solvent systems was equilibrated at room temperature.

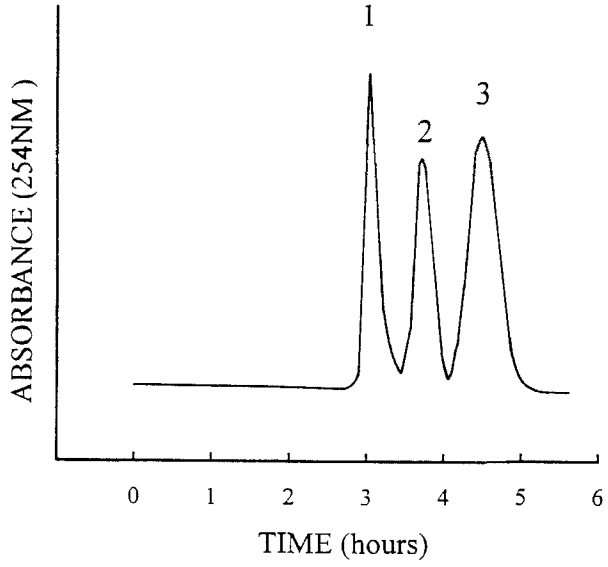

Figure 6. Chromatogram of DNP-amino acids obtained by helical coil column. Solvent system, chloroform/acetic acid/0.1N Hcl (2:2:1); mobile phase, upper phase; flow rate, 6 mL/hr; rotor speed, 500 rpm; sample dose (volume), 100 μg (20 μL); column pressure, 9 kg/cm^2; peaks, 1=DNP-ornithine, 2=DNP-glutamic acid, 3=DNP-alanine.

For studies on retention of the stationary phase, experiments were carried out with three kinds of solvent systems: chloroform/acetic acid/0.1N hydrochloric acid (2:2:1), n-hexane/ EtOAc/ EtOH/ water (6:3:2:5) and 1-butanol/acetic acid/water (4:1:5).

For demonstrating the capability of CCPC with chain winding column, experiments were carried out with DNP-amino acids (Dongfeng Chemicals, Shanghai, China), Weimeisu (a Chinese medicine developed by our Institute, extracted from soya-bean cake), and extract of branch-leaves of *Taxus cuspidate* (supplied by Professor Zong-Cheng Li and Miss Li Zhang, Qinghua University, Beijing, China).

The sample solution of DNP-amino acids was prepared by dissolving 20mg of DNP-ornithine, 20mg of DNP-glutamic acid and 10mg of DNP-alanine (total weight 50mg) in 10mL of the upper aqueous phase of chloroform solvent systems. The sample solution of Weimeisu was prepared by dissolving 1g of Weimeisu in 100mL 2 % acetic acid, centrifuged and the upper solution was chosen as sample.

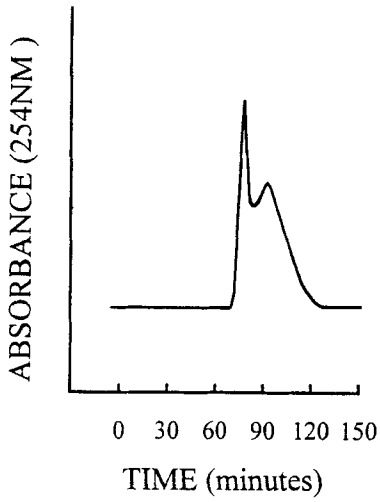

Figure 7. Chromatogram of Weimeisu obtained by chain winding column. Solvent system, 1-butanol/acetic acid/water (4:1:5); mobile phase, lower phase; flow rate, 96 mL/hr; rotor speed, 600 rpm; sample dose, 20 mg; column pressure, 5.0 kg/cm^2.

Procedure

The measurement of stationary phase retention was performed as follows: The column was first filled entirely with the stationary phase. Then the apparatus was rotated at the desired revolution speed while the mobile phase was pumped into the column at the desired flow rate.

The effluent from the outlet of the column was collected to measure the volume of the stationary phase eluted from the column as well as the total elution volume of the mobile phase.

The elution was continued until the total elution volume exceeded the column capacity. Then the centrifuge was stopped and the column contents emptied into a graduated cylinder.

The separations were performed as follows: In each experiment, the entire column was filled with the stationary phase. The sample solution was introduced into the inlet of the column, then the mobile phase was pumped into the column while the centrifuge was running at a suitable speed.

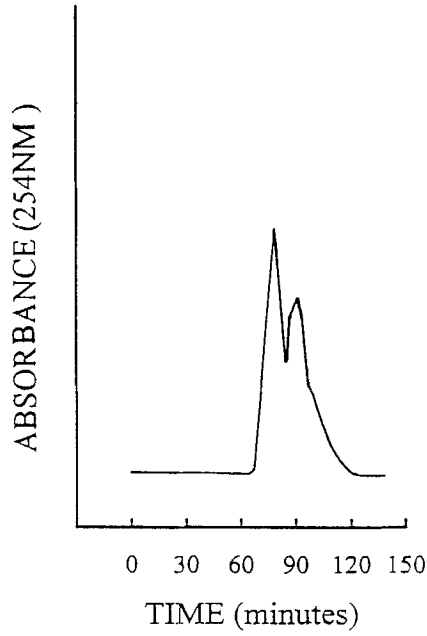

Figure 8. Chromatogram of Weimeisu obtained by helical coil column. Solvent system, 1-butanol/acetic acid/water (4:1:5); mobile phase, lower phase; flow rate, 27 mL/hr; rotor speed, 600 rpm; sample dose, 1 mg; column pressure, 5.5 kg/cm^2.

RESULTS AND DISCUSSION

Figure 3 and Figure 4 illustrate a set of phase retention diagrams for three kinds of solvent systems, the percentage of retention increases with rotating speed but decreases when the flow rate increases. In most conditions, the retention ratio exceed 50%. The highest one reaches 66%. Figure 5 shows a typical chromatogram obtained from a mixture of DNP-amino acids using the chain winding column. The separation was performed at a flow rate of 180ml/h using the upper aqueous phase as the mobile phase at a rotating speed of 500rpm. All three components were eluted within 1.5 h. Figure 6 shows the separation of same sample by helical coil column[1] (tubing of 0.6mm I.D., 19m length). The results in Figures 5 and 6 are nearly same: chain winding column separates a larger dose in shorter time. Figure 7 shows the chromatogram of Weimeisu obtained by chain winding column and Figure 8 shows the helical coil column results for the same sample obtained using the helical coil column[2] (tube of 0.6mmI.D., 15.5m length). The results indicate that the chain winding column can separate a larger sample dose, which increased from 1mg to 20mg.

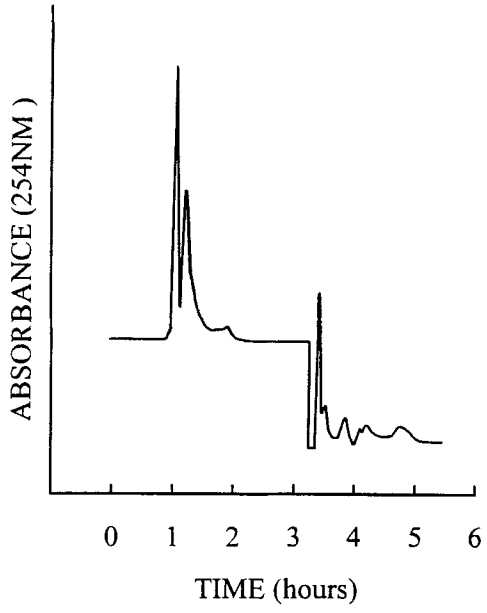

Figure 9. Chromatogram of extract of branch-leaves of *taxus cuspidate* obtained by chain winding column. Solvent system, hexane/EtOAc/EtOH/water (6:3:2:5); floww rate, 60 mL/hr; rotor speed, 500 rpm; sample dose, 0.8 mL; column pressure, 9.0 kg/cm^2; in the first 3 hours, upper phase as mobile phase; after 3 hours, lower phase as mobile phase.

Figure 9 shows the chromatogram of extract from branch-leaves of *Taxus cuspidate* obtained by chain winding column. The upper phase was used as mobile phase at first and two peaks emerged. Then the lower phase was used as mobile phase and flow direction was altered; several peaks emerged. There are two peaks in the chromatogram of separation of extract of branch-leaves of *Taxus cuspidate* reported by Jian-Qiao Gu, etc.[3]

The performances of chain winding column described above show that it's capability of retaining stationary phase is higher and exceed 50%, which up to 66%. The separation of mg-scale sample can be finished in hours.

ACKNOWLEDGMENT

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