Journal of Chromatography, 538 (1991) 59–66
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 861

Cross-axis synchronous flow-through coil planet centrifuge (Type XLL)

I. Design of the apparatus and studies on retention of stationary phase

YOICHIRO ITO*, EIICHI KITAZUME and MOLINA BHATNAGAR
Laboratory of Biophysical Chemistry, National Heart, Lung, and Blood Institute, Building 10, Room 7N-322, National Institutes of Health, Bethesda, MD 20892 (U.S.A.)
and
FRANKLIN D. TRIMBLE
Biomedical Engineering and Instrumentation Branch, Division of Research Services, National Institutes of Health, Bethesda, MD 20892 (U.S.A.)

ABSTRACT

The fourth prototype holds a pair of column holders in the lateral position at 15 cm from the center of the rotary shaft horizontally mounted on the rotary frame at 7.6 cm from the central axis of the apparatus. Using short coils of 2.6 mm I.D. PTFE (polytetrafluoroethylene) tubing with 7.6 cm and 24 cm helical diameters, retention of the stationary phase was measured in ten pairs of two-phase solvent systems under various experimental conditions. Satisfactory retention was obtained by choosing proper combinations of three factors, *i.e.*, the direction of planetary motion, head–tail elution mode, and inward–outward elution mode. The polar butanol solvent systems showed excellent retention from 65 to 80% in the 7.6 cm helical diameter left-handed coil.

INTRODUCTION

The cross-axis synchronous flow-through coil planet centrifuge (X-axis CPC) has a unique feature among coil planet centrifuges in that the column holder axis is perpendicular to the central axis of the centrifuge [1]. In the past, three different models of the X-axis CPC were fabricated and their capability for performing counter-current chromatography (CCC) was examined using various two-phase solvent systems [1–8]. Hydrodynamic studies on retention of the stationary phase in the coiled column indicated that the system provides more reliable retention of the stationary phase for viscous polar solvent systems compared with the high-speed CCC centrifuge based on the type J synchronous planetary motion [1,2]. Further studies have shown that the phase retaining capacity of the X-axis CPC is enhanced by

0021-9673/91/$03.50

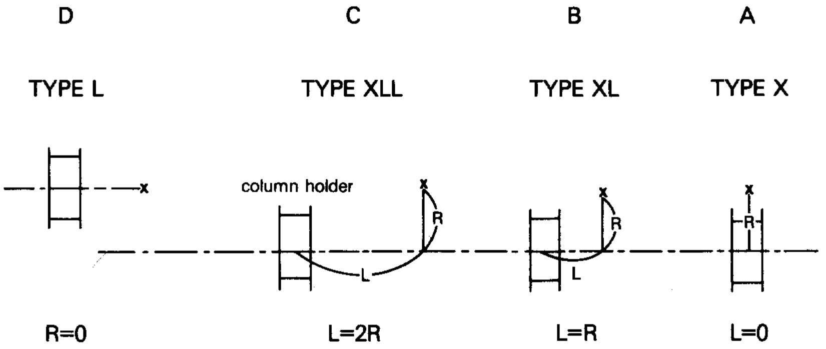

Fig. 1. Orientation of the column holder on the axis of rotation in four types of coil planet centrifuge. × = Axis of revolution; - - - - = axis of rotation; R = rotation radius; L = revolution radius.

laterally shifting the position of the column holder along the holder shaft, probably due to the asymmetry of the laterally acting force field between the upper and the lower halves of the rotating coil [3]. The degree of the lateral shift of the column holder may be conveniently expressed by L/R where L is the distance from the center of the holder shaft to the coil holder and R, the distance from the centrifuge axis to the holder shaft (revolution radius) (Fig. 1). The latest model [8] with $L/R = 1$ (Fig. 1B) has produced substantially higher stationary phase retention for the polar solvent systems compared with the original model [1,2] with the central column position ($L = 0$, Fig. 1A). These results suggest that shifting the position of the column holder even further along the rotary shaft would permit the retention of highly viscous polar solvent systems including aqueous–aqueous polymer phase systems used for the partition of macromolecules and cell particles [9].

The present paper deals with the fourth model of the X-axis CPC equipped with a pair of column holders at $L/R = 2$ (Fig. 1C). In this paper we describe the design of the apparatus and hydrodynamic studies on retention of the stationary phase using short coils mounted on the column holders with two different hub diameters.

APPARATUS

The basic design of the apparatus has been reported earlier [7]. The present apparatus has $L/R = 2$ to provide a strong lateral force field to retain viscous polar solvent systems. Fig. 2 schematically illustrates the horizontal cross-section of the apparatus equipped with a pair of multilayer coils.

The motor (not shown in the diagram) drives the central shaft (1) and the rotary frame around the central axis of the centrifuge. The rotary frame consists of two pairs of side plates (2) rigidly bridged by a pair of horizontal plates (3) and holds a pair of rotary shafts (4) horizontally at a distance of 7.6 cm from the central axis of the centrifuge. A pair of column holders (5) are mounted one on each side of the rotary frame between the outer and inner side plates (2) at a distance 15 cm from the center of the rotary shaft. The desired synchronous planetary motion of the holders is produced as follows: The toothed pulley (6) mounted on each holder shaft is coupled

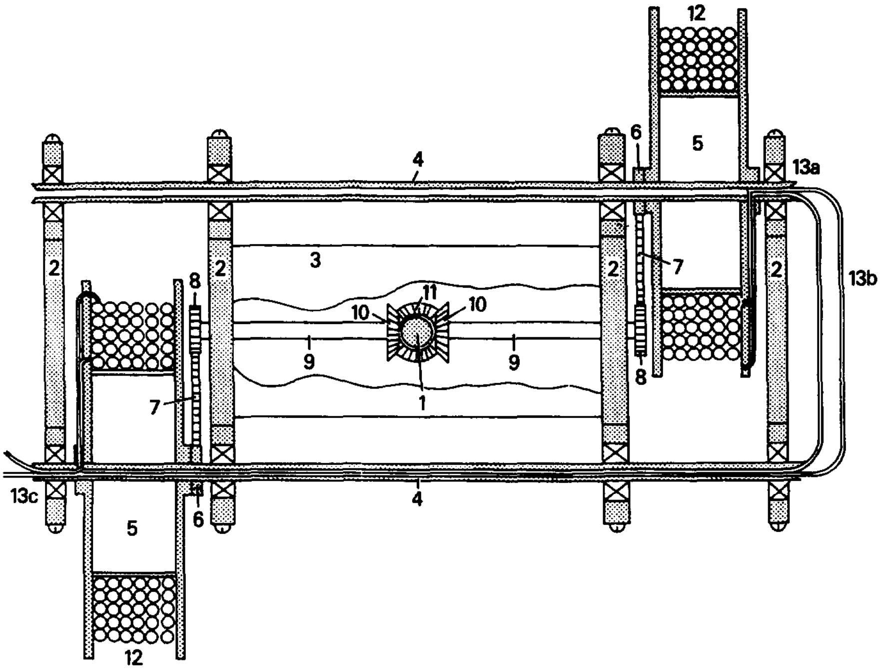

Fig. 2. Cross-sectional view of the type XLL coil planet centrifuge. 1 = Central shaft; 2 = side plates; 3 = bottom plate; 4 = rotary shafts; 5 = column holders; 6 and 8 = toothed pulleys; 7 = toothed belt; 9 = countershafts; 10 = planetary miter gears; 11 = stationary miter gear; 12 = multilayer coil separation columns; 13a–c = flow tubes.

with a toothed belt (7) to an identical pulley (8) on the countershaft (9) equipped with a miter gear (45°) (10) which is in turn engaged to the identical stationary sun gear (11) mounted on the bottom plate of the centrifuge. A pair of coiled columns (12) mounted on the holders are serially connected with flow tubes (13a–c) as illustrated in the diagram. The above mechanical arrangement prevents twisting of the flow tubes and continuous elution can be performed through the rotating column without the use of rotary seals. Three pairs of interchangeable coil holders were fabricated with hub diameters of 7.6, 15 and 24 cm, respectively. They can be easily removed from the rotary frame by loosening the screws on each bearing block.

The apparatus can be operated up to the maximum speed of 1000 rpm with a speed controller (Bodine Electric, Chicago, IL, U.S.A.).

EXPERIMENTAL

Reagents

n-Hexane, ethyl acetate, chloroform, methanol, *n*-butanol and *sec.*-butanol were all chromatographic grade and purchased from Burdick & Jackson Labs., (Muskegon, MI, U.S.A.). Acetic acid was reagent grade and obtained from Mallinckrodt (Paris, KY, U.S.A.).

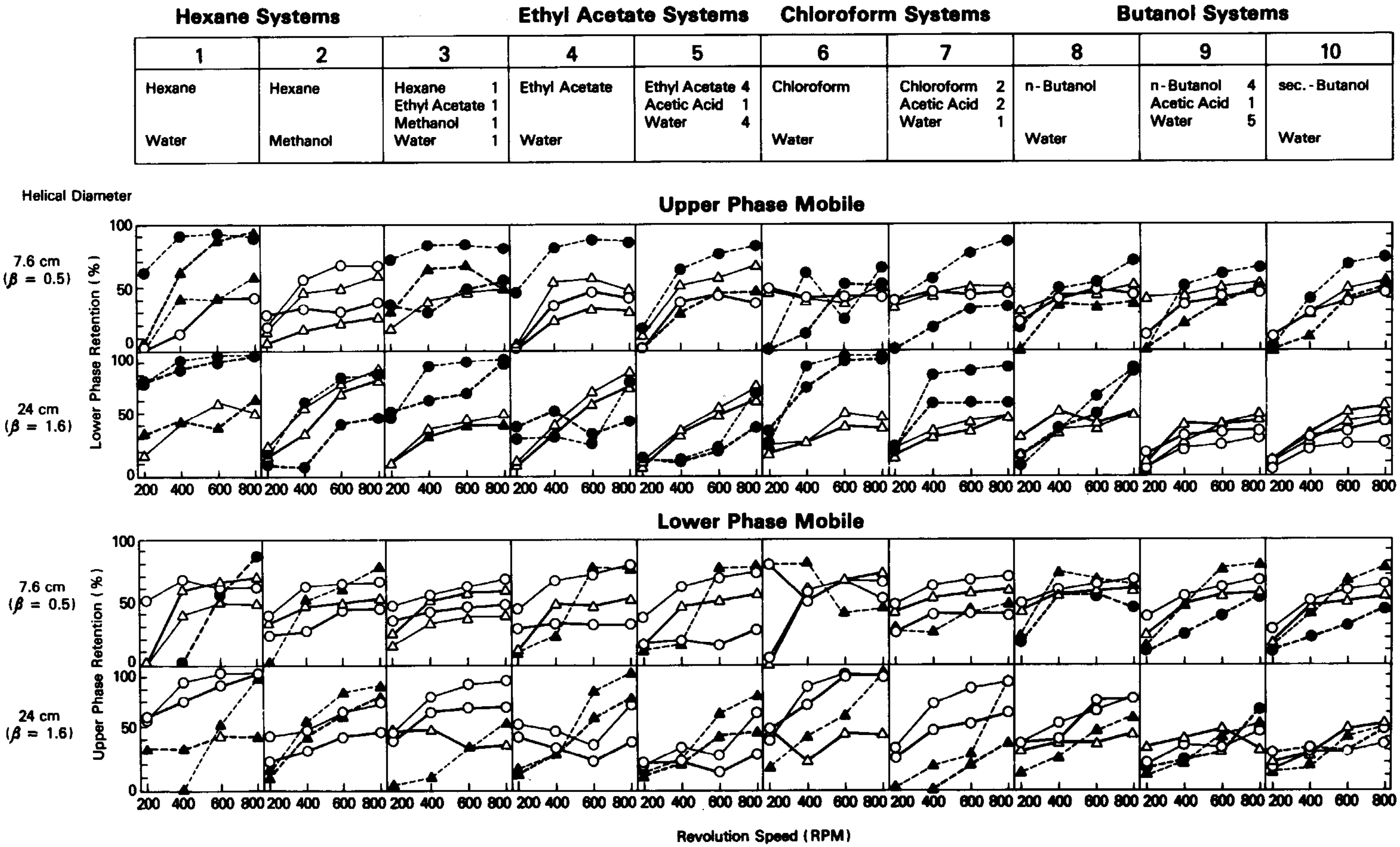

Fig. 3. A set of phase retention diagrams for ten pairs of two-phase solvent systems in two helical diameter coils. See Table I for symbols assigned for each elution mode.

Preparation of two-phase solvent systems

The following 10 pairs of solvent systems were then prepared: *n*-hexane–water, *n*-hexane–methanol, *n*-hexane–ethyl acetate–methanol–water (1:1:1:1, v/v/v/v), ethyl acetate–water, ethyl acetate–acetic acid–water (4:1:4, v/v/v), chloroform–water, chloroform–acetic acid–water (2:2:1, v/v/v), *n*-butanol–water, *n*-butanol–acetic acid–water (4:1:5, v/v/v) and *sec.*-butanol–water. Each solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases separated shortly before use.

Preparation of coiled columns

The present studies were performed with short coils of approximately 3–5 m long, 2.6 mm I.D. PTFE tubing (Zeus Industrial Products, Raritan, NJ, U.S.A.) wound directly around the holders of 7.6- and 24-cm hub diameters forming a single-layer coil with a total capacity of 20–30 ml. Both right-handed and left-handed coils were made for each holder. Each coiled column was firmly affixed on the holder with several pieces of fiber-glass reinforced adhesive tape. Each end of the column was connected to a 1 m × 0.85 mm I.D. PTFE flow tube, by inserting a series of smaller diameter PTFE tubing into one another.

Measurement of stationary phase retention

Experiments were performed according to the standard procedure described elsewhere [8]. In each measurement, the coil 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 a flow-rate of 2 ml/min using a 50- or 20-ml capacity glass syringe driven by a syringe driver (Harvard Apparatus, Millis, MA, U.S.A.). The effluent from the outlet of the column was collected into a 25- or 50-ml graduated cylinder 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 for 12–15 min until the total elution volume exceeded the column capacity. During the elution, the temperature inside the centrifuge was controlled at 22 ± 2°C by placing an ice-bag over the top plate of the centrifuge. Then the centrifuge was stopped and the column contents emptied into a graduated cylinder by connecting the inlet of the column to a pressured nitrogen line (*ca.* 80 p.s.i.). The column was then washed with several milliliters of methanol and finally flushed with several milliliters of the stationary phase which was to be used for the next experiment.

The experiments were performed with ten different two-phase solvent systems (Fig. 3). At the maximum speed of 800 rpm, the retention of each phase was measured in each coil under eight different experimental conditions, *i.e.*, all possible combinations of the planetary motion (P_I and P_{II}), the head–tail elution mode (head to tail and tail to head), and the inward–outward elution mode determined by the handedness of the coil (right-handed and left-handed) as indicated in Table I. Choosing two best conditions which produced high retention of the stationary phase in each handedness of the coil at 800 rpm, the retention was further measured under the reduced rpm (600, 400 and 200) to obtain the phase retention diagrams described below.

Phase retention diagram

From each measurement, retention of the stationary phase was expressed as a

TABLE I

EIGHT DIFFERENT ELUTION MODES IN X-AXIS CPC

Planetary motion		Head–Tail elution mode	Inward–Outward elution mode (handedness of coil[a])	Combined elution mode[b]	Symbolic signs in PDD[c]
P_I		Head → Tail	Inward (R)	P_I-H-I	○——○
		Head → Tail	Outward (L)	P_I-H-O	○——○
		Tail → Head	Inward (L)	P_I-T-I	●----●
		Tail → Head	Outward (R)	P_I-T-O	●----●
P_{II}		Head → Tail	Inward (L)	P_{II}-H-I	△——△
		Head → Tail	Outward (R)	P_{II}-H-O	△——△
		Tail → Head	Inward (R)	P_{II}-T-I	▲----▲
		Tail → Head	Outward (L)	P_{II}-T-O	▲----▲

[a] R: Right-handed. L: Left-handed.
[b] H: Head → Tail. T: Tail → Head. I: Inward. O: Outward.
[c] PDD: Phase Distribution Diagram (Phase Retention Diagram).

percentage relative to the total column capacity according to the expression $100(V_C, + V_F - V_S)/V_C$, where V_C is the total capacity of the coil; V_F free space in the flow tubes; and V_S the volume of the stationary phase eluted from the coil. From these retention data the hydrodynamic distribution of the two solvent phases in the coil was summarized in a phase retention diagram constructed by plotting percentage retention of the stationary phase as a function of revolutional speed for each mobile phase. A group of retention curves produced by different elution modes but otherwise identical experimental conditions can be illustrated in the same diagram. In order to distinguish each elution mode in the phase retention diagram, a set of symbolic designs was used to draw phase retention curves as illustrated in Table I.

RESULTS AND DISCUSSION

Phase retention diagrams

Fig. 3 illustrates a set of phase retention diagrams for ten different two-phase solvent systems with a broad range in hydrophobicity. Each column was obtained from the solvent system indicated at the top and arranged from left to right according to the degree of hydrophobicity of the major organic component. The upper panel shows the retention of the lower stationary phase and the lower panel, the retention of the stationary upper phase. In each panel, the first row was obtained from the coil mounted on the 7.6 cm diameter holder (β = 0.5) and the second row from the coil mounted on the 24 cm diameter holder (β = 1.6) as indicated on the left margin ($\beta = r/R$ where r is the distance from the holder axis to the coil and R, the distance from the holder axis to the central axis of the centrifuge).

In each diagram, four retention curves are drawn against the applied revolution speeds from 200 to 800 rpm. Among those four curves, two were obtained from the right-handed coil and the other two from the left-handed coil, both groups being selected among four possible combinations of the elution modes (see Table I for symbolic designs assigned for each elution mode). In general 50% retention is considered satisfactory, but at higher retention, better peak resolution is expected.

The results show that the majority of the retention curves rise with increased rpm approaching a plateau at the highest speed of 800 rpm. All solvent systems showed a satisfactory phase retention of 50% or greater at 800 rpm, regardless of the choice of the mobile phase or β values. The most important finding is that the hydrophilic and low interfacial tension solvent systems such as *n*-butanol–acetic acid–water (4:1:5) and *sec.*-butanol–water show excellent phase retention ranging from 65 to 80% in the 7.6 cm helical diameter coil ($\beta = 0.5$). These retention figures substantially exceed those obtained from the existing X-axis CPC including types X [1–6], XXL [3–6] and XL [7,8].

On some occasions, the effluent from the coil persistently showed cloudy appearance apparently due to steady carryover of the stationary phase. This phenomenon was more often observed in the solvent system with low interfacial tension, especially when the mobile phase was pumped in a tail-to-head elution mode. This continuous carryover of the stationary phase resulted in an uneven phase distribution in the coil, *i.e.*, gradual depletion of the stationary phase starting at the beginning of the coil. However, such carryover of the stationary phase does not significantly affect the actual separation, if the solute peak is eluted before a considerable loss of the stationary phase takes place. In the present experiments, the retention volume of the stationary phase was measured after the elution volume exceeded the total column capacity so that solutes with $K(\mathrm{Cm/Cs}) \geqslant 1$, if introduced at the beginning of the experiment, would be eluted before the end of the experiment. Here, $K(\mathrm{Cm/Cs})$ is the partition coefficient given by solute concentration in the mobile phase divided by that in the stationary phase.

The majority of the retention diagrams also show that the left-handed coil (thin lines) gives the highest retention levels especially in the 7.6 cm helical diameter coils. The comparison between the upper and lower panels reveals that two particular combinations of the elution mode dominate in each group, *i.e.*, P_{I}–T–I and P_{II}–H–I in the upper panel and P_{II}–T–O and P_{I}–H–O in the lower panel (see Table I for symbols), indicating that the inward–outward elution mode plays a more significant role than the head–tail elution mode in the retention of the stationary phase. It is important to note that all the optimum conditions are solely provided by use of left-handed coils (see Table I).

In practical use the multilayer coil column as shown in Fig. 2 (No. 12) usually consists of right-handed and left-handed coils alternating in each layer. In this configuration, efficient separations are obtained only if each coiled layer retains a satisfactory volume of the stationary phase at a given elution mode. However, the present experimental results clearly indicate that left-handed coils generally yield much higher retention levels than right-handed coils. In order to make the best use of this hydrodynamic trend the configuration of the conventional multilayer coil should be modified in such a way that all layers are also exclusively left-handed. This can be either done by connecting each layer with a narrow transfer tube or using a continuous piece of tubing which is directly returned to the starting position after completing each coiled layer. Although this modification may cause minor deformation of the multilayer coil by accommodating the connection flow tube between each layer, it promises a good retention of the stationary phase of viscous polar solvent systems such as butanol systems which tend to produce carryover problems in other types of the CCC apparatus. Because of the excellent phase retention obtained from the most viscous *sec.*-

butanol–water solvent system tested in the present study, the present system may also retain aqueous–aqueous polymer phase systems [9].

As described above, the results of the present studies provide useful information on a new configuration of the multilayer coil column used in XLL coil planet centrifuge. In the following article (Part II) [10], the phase retention data obtained in the present experiments are statistically analyzed to study the unique hydrodynamic mechanism involved in the present X-axis CPC system.

ACKNOWLEDGEMENT

The authors of this paper wish to thank Dr. Henry M. Fales for his assistance in reviewing this paper.

REFERENCES

1 Y. Ito, *Sep. Sci. Technol.*, 22 (1987) 1971.
2 Y. Ito, *Sep. Sci. Technol.*, 22 (1987) 1989.
3 Y. Ito and T.-Y. Zhang, *J. Chromatogr.*, 449 (1988) 135.
4 Y. Ito and T.-Y. Zhang, *J. Chromatogr.*, 449 (1988) 153.
5 T.-Y. Zhang, Y.-W. Lee, Q.-C. Fang, C. E. Cook, R. Xiao and Y. Ito, *J. Chromatogr.*, 454 (1988) 185.
6 Y. Ito and T.-Y. Zhang, *J. Chromatogr.*, 455 (1988) 151.
7 Y. Ito, H. Oka and J. L. Slemp, *J. Chromatogr.*, 463 (1989) 305.
8 M. Bhatnagar, H. Oka and Y. Ito, *J. Chromatogr.*, 463 (1989) 317.
9 P. Å. Albertsson, *Partition of Cell Particles and Macromolecules*, Wiley-Interscience, New York, 1986.
10 Y. Ito, *J. Chromatogr.*, 538 (1991) 67.