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NEW HORIZONTAL FLOW-THROUGH COIL PLANET CENTRIFUGE FOR COUNTER-CURRENT CHROMATOGRAPHY

I. PRINCIPLE OF DESIGN AND ANALYSIS OF ACCELERATION

YOICHIRO ITO
Laboratory of Technical Development, The National Heart, Lung and Blood Institute, Bethesda, Md. 20205 (U.S.A.)
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SUMMARY

The new design of the horizontal flow-through coil planet centrifuge permits continuous elution through a pair of coiled separation columns without the use of rotating seals. Mathematical analysis of the planetary motion discloses a characteristic pattern of the acceleration field acting on each column, one capable of efficient mixing of the two phases in a narrow-bore column and the other providing a stable retention of the stationary phase in a large-bore column. Consequently, the present scheme enables both micro-scale and preparative-scale separations with a high partition efficiency.

INTRODUCTION

In the past, various types of coil planet centrifuges have been developed to evaluate their capabilities for performing counter-current chromatography[1–11]. Of all of these schemes the flow-through coil planet centrifuge[2–4,6] enables the most efficient analytical-scale separations while the horizontal flow-through coil planet centrifuge[10,11] is the most suitable for preparative-scale separations. The new horizontal flow-through coil planet centrifuge introduced here holds a pair of coiled separation columns, one identical to that in the flow-through coil planet centrifuge and the other identical to that in the horizontal flow-through coil planet centrifuge. Consequently, the present scheme combines the capabilities of the above two centrifuge schemes to perform both micro-scale and large-scale separations with a high partition efficiency. In addition, the apparatus has a unique design which eliminates the use of rotating seals. The principle and capability of the apparatus have been briefly reported earlier[12].

The present paper describes the principle of design and analysis of the acceleration field produced by each column holder. In the light of the results obtained by the above analysis, hydrodynamic behavior of the two immiscible solvent phases in the coiled separation columns is elucidated.

DESIGN PRINCIPLE

Fig. 1 illustrates the principle of the present flow-through coil planet centrifuge. Each diagram labelled A–D shows the orientation of a cylindrical column holder undergoing a synchronous planetary motion. A bundle of flow tubes connected to the cylinder is tightly supported by a stationary member marked "×" on the central axis of the centrifuge. In A the holder maintains a parallel orientation of its axis with respect to the axis of the centrifuge. The synchronous counter-rotation of the holder prevents twisting the flow tubes as in the flow-through coil planet centrifuge reported earlier[3]. The orientation of the holder, however, can be altered without twisting the flow tubes by lifting the axis of the holder by 45° (B), 90° (C) and even 180° (D).

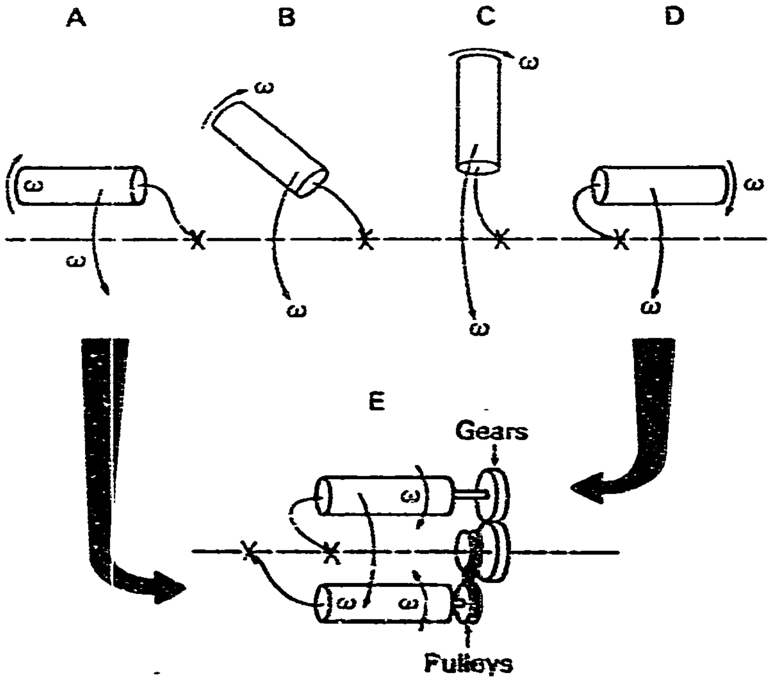

Fig. 1. Principles of the rotating-seal-free design of the horizontal flow-through coil planet centrifuge. A–D show various types of planetary motion of the column holder which prevent twisting of the flow tubes. Systems A and D can be combined in one apparatus (E) without interfering with each pathway of the flow tubes.

In the latter case (D), the system becomes identical to that in the horizontal flow-through coil planet centrifuge[10,11] where the holder synchronously rotates about its own axis in the same direction as the revolution around the central axis of the centrifuge. Because of the different geometry of the flow tubes together with the symmetrical orientation of the holders, systems A and D are conveniently paired in one apparatus as shown in E where a pair of identical gears and pulleys provide a respective planetary motion to each column holder. Consequently, separations can be carried out simultaneously in both columns without the use of rotating seals. While these paired column holders undergo similar planetary motions, *i.e.*, one rotating in the same direction (gear-side) and the other rotating in the opposite direction (pulley-side) with respect to the centrifugal revolution, the resulting centrifugal force field on each holder is quite different as described later in detail. The gear-side holder gives stable retention of the stationary phase in a large-bore preparative column whereas the pulley-side holder provides efficient mixing of the phases in a narrow-bore tube and enables micro-scale separations with extremely high partition efficiency. Obviously, this unique feature of this present apparatus has a great advantage over the previous models in that both large-scale and small-scale separations are possible in one apparatus.

ANALYSIS OF ACCELERATION FIELD

As in other counter-current chromatographic schemes, the results of the present scheme are highly dependent upon the behavior of the two-phase solvent system in the coiled column. This behavior is determined by the column geometry and the applied centrifugal force field. In order to achieve an efficient separation, an optimum operational condition must be applied to satisfy two basic requirements for counter-current chromatography, *i.e.*, retention of the stationary phase and efficient mixing of the two phases. In other words, the applied centrifugal force field must be strong enough to retain a satisfactory amount of the stationary phase in the column while it also induces vigorous agitation of the two phases to minimize mass transfer resistance. Although these two requirements seem somewhat mutually conflicting, in practice it is relatively easy to optimize the operational conditions if one acquires a sufficient knowledge of the acting patterns of the centrifugal-force field on the coiled column. The acceleration field produced by the present scheme has been analyzed earlier for both the pulley-side[6] and the gear-side[11] columns. In the following, these analyses are reviewed in further detail to elucidate the contrasting nature of the paired columns in the present apparatus.

Acceleration field acting on the pulley-side column. Fig. 2A shows a schematic diagram of the pulley-side column holder undergoing planetary motion. The holder revolves around the central axis of the centrifuge (center of revolution) at angular velocity ω and synchronously counter-rotates about its own axis (center of rotation) located parallel to and at a distance R from the center of revolution. Acceleration acting on the holder at an arbitrary point P distanced r from the center of rotation can be analyzed by the aid of a coordinate system shown in Fig. 2B. The coordinate system is chosen so that the center of revolution is located at the center of the coordinate system and the center of rotation is on the x-axis. An arbitrary point initially located at P_0 forms an angle θ_0 with the x-axis. Then after time t, where $\omega t = \theta$, location of the point P (x, y) is expressed by

$$x = R\cos\theta + r\cos\theta_0 \quad (1)$$

$$y = R\sin\theta + r\sin\theta_0 \quad (2)$$

From these equations, the orbit of the arbitrary point is easily obtained by eliminating the variable, θ, and:

$$(x - r\cos\theta_0)^2 + (y - r\sin\theta_0)^2 = R^2 \quad (3)$$

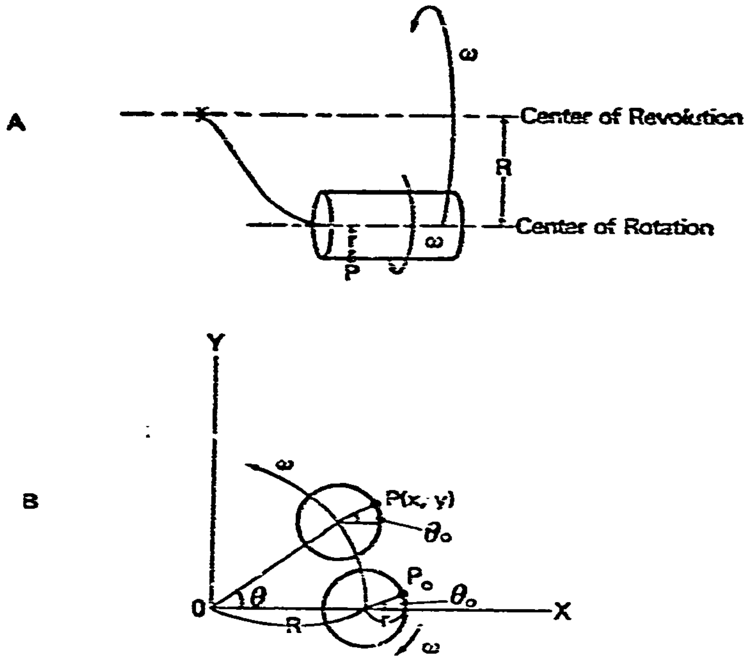

Fig. 2. Analysis of acceleration field acting on the pulley-side column holder. A, Orientation and motion of the pulley-side column holder; B, coordinate system for analysis of acceleration field at the arbitrary point on the pulley-side holder.

which indicates a circle with radius R centered at point ($r \cos \theta_0$, $r \sin \theta_0$). The fact that the radius of the circle, R, is independent of θ_0 indicates that any point located on the holder travels a circular orbit with the same radius R.

The net magnitude, α, and acting angle, γ (with respect to the x-axis), of the acceleration at the arbitrary point are also obtained from eqns.1 and 2 as

$$\alpha = [(\mathrm{d}^2x/\mathrm{d}t^2)^2 + (\mathrm{d}^2y/\mathrm{d}t^2)^2]^{1/2} = R\omega^2 \quad (4)$$

$$\gamma - \pi = \tan^{-1}[(\mathrm{d}^2y/\mathrm{d}t^2)/(\mathrm{d}^2x/\mathrm{d}t^2)] = \omega t = \theta \quad (5)$$

These results clearly indicate that the arbitrary point is subjected to a constant magnitude of the acceleration, $R\omega^2$, which rotates around the point at a uniform rate of ω. Furthermore, it is extremely important to note that the magnitude of the acceleration and its acting angle are both independent of r and θ_0 which are the whole determinants for the location of the point on the holder. This clearly indicates that at any given moment every location on the holder is subjected to the identical acceleration field acting in a plane perpendicular to the axis of the holder. This unique feature of the present scheme permits freedom to mount multiple columns around the holder at any location to produce the same effect, provided that the axis of the coiled columns is parallel to the axis of the holder.

Acceleration field acting on the gear-side column. Fig. 3A shows the orientation and planetary motion of the gear-side column holder. The holder revolves around

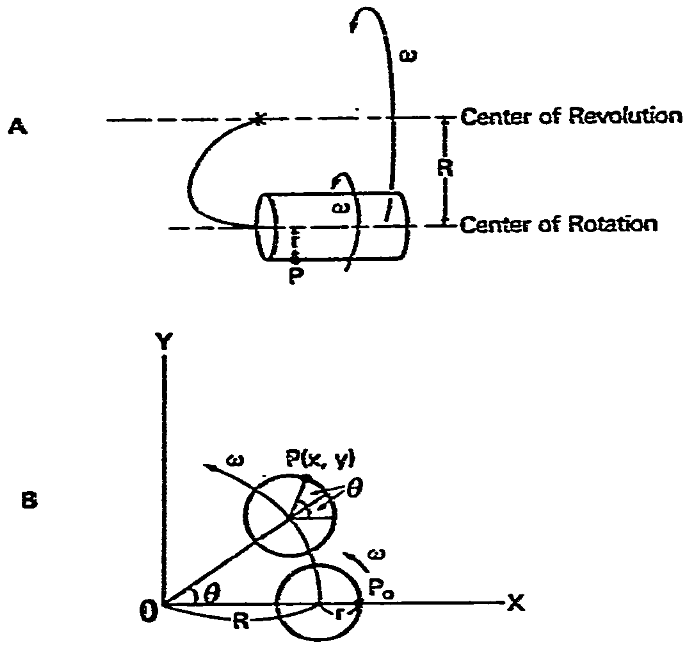

Fig. 3. Analysis of acceleration field acting on the gear-side column holder. A, Orientation and motion of the gear-side column holder; B, coordinate system for analysis of acceleration field at the arbitrary point on the gear-side column holder.

the central axis of the apparatus (center of revolution) at angular velocity ω while it synchronously rotates around its own axis (center of rotation) in the same direction. As in the pulley-side holder, the center of revolution always maintains a parallel orientation to the center of revolution at a fixed distance of R. The arbitrary point P chosen for analysis is located on the holder at distance r from the center of rotation. Then, the motion of the point and the resulting acceleration field can be studied with the aid of a coordinate system as illustrated in Fig. 3B.

To simplify the analysis, the coordinate system is selected so that the center of revolution is located at the central point 0 whereas both the center of rotation and the arbitrary point are initially located on the x-axis as illustrated. After the lapse of time, t, the center of rotation circles around the point 0 by $\theta = \omega t$ and the location of the arbitrary point, P (x,y), is given by

$$x = R\cos\theta + r\cos 2\theta \quad (6)$$

$$y = R\sin\theta + r\sin 2\theta \quad (7)$$

The orbit of the arbitrary point is computed from these equations by eliminating the variable, θ, and the results are illustrated in Fig. 4. These are quite different from the results obtained on the pulley-side holder. The orbit of the point on the gear-side holder displays a great variety in shape according to the locations of the point on the holder. These are conveniently expressed as the ratio between the radii of rotation and

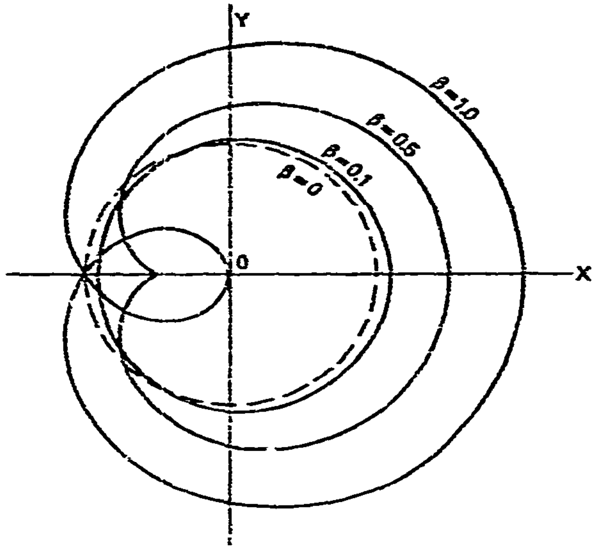

Fig. 4. Orbits of the arbitrary point for various β values on the gear-side holder.

revolution, or $\beta = r/R$. Thus the shape of the orbit changes with β values. When $\beta \leqslant 0.25$, the orbit is a single circular loop. As the β value increases, it becomes heart-shaped ($\beta = 0.5$) and then forms a double loop ($\beta = 1.0$) which gradually approaches a double circle with greater β values. These results suggest that the acceleration field not only changes its pattern according to the location of the point of the holder but also fluctuates periodically during each revolutional cycle of the holder.

The acceleration acting on the arbitrary point is further calculated from the second derivatives of eqns. 6 and 7 as

$$d^2x/dt^2 = -R\omega^2(\cos\theta + 4\beta\cos 2\theta) \quad (8)$$

$$d^2y/dt^2 = -R\omega^2(\sin\theta + 4\beta\sin 2\theta) \quad (9)$$

which gives the absolute net magnitude

$$a = [(d^2x/dt^2)^2 + (d^2y/dt^2)^2]^{1/2} = R\omega^2(1 + 16\beta^2 + 8\beta\cos\theta)^{1/2} \quad (10)$$

acting at the angle relative to the x-axis:

$$\gamma_x = \pi + \tan^{-1}[(\sin\theta + 4\beta\sin 2\theta)/(\cos\theta + 4\beta\cos 2\theta)] \quad (11)$$

provided $R \neq 0$ and $\beta = r/R$. In order to visualize the effect of the acceleration on the behavior of the two phases in the column, it is more convenient to express the acting angle of the acceleration vector with respect to the rotating holder. The angle formed between the acceleration vector and the radius of rotation, that is, the line drawn from the arbitrary point P to the center of the holder is given by

$$\gamma = \gamma_x - 2\theta - \pi = \tan^{-1}[(-\sin\theta)/(4\beta + \cos\theta)] \quad (12)$$

The change of magnitude α and the angle γ during one revolutional cycle of the holder are respectively illustrated in Figs. 5 and 6 where several curves are drawn according to the β values. In Fig. 5, α for $\beta > 0$ shows patterns undulating in such a way that it becomes greatest at $\theta = 0°$ (location of the point most distant from the axis of revolution) and smallest at $\theta = 180°$ (location of the point nearest to the axis of revolution). At $\theta = 180°$, α decreases as the β value increases from 0 to 0.25 where α becomes minimized.

Fig. 6 illustrates undulating patterns of the angle γ around the radius of rotation or $\gamma = 0$ line for various β values. The γ values on the ordinate are chosen so that the bottom line or $\gamma = -180°$ continually joins the top line or $\gamma = 180°$ to form a complete circle of 360°. Thus, curves crossing over these lines indicate angles

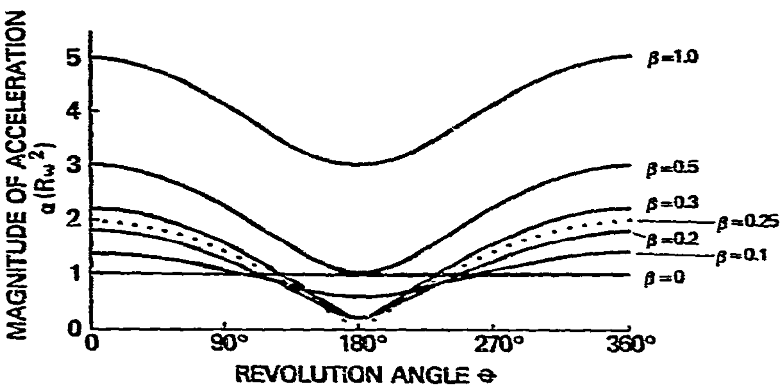

Fig. 5. Magnitude of acceleration acting at the arbitrary point on the gear-side holder for various β values during one revolutional cycle.

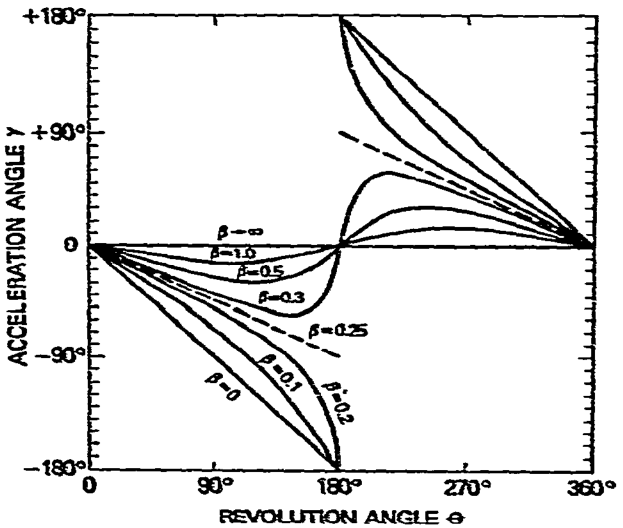

Fig. 6. Angle fluctuation of acceleration vector at the arbitrary point on the gear-side holder for various β values during one revolutional cycle.

rotating around the arbitrary point while other curves show angles swinging around the $\gamma = 0$ line. At $\beta = 0$, or the location of the point on the axis of rotation, γ forms a straight line across the $\pm 180°$ lines indicating that the acceleration field rotates uniformly around the point as seen on the pulley-side holder but in the opposite direction. When $0 < \beta < 0.25$, the acceleration field still rotates around the point but the rate of rotation is not uniform in such a way that the field changes its direction rather slowly around $\theta = 0°$ and more quickly around $\theta = 180°$. When β becomes greater than 0.25, the rotational motion of the field changes into a swinging motion where the angle moves back and forth around the $\gamma = 0$ line with its amplitude decreasing with greater β values. Here again the acceleration changes its direction rather slowly around $\theta = 0°$ and more quickly around $\theta = 180°$ especially with β values close to 0.25. When β values becomes infinite, the γ line approaches a straight line of $\gamma = 0$ which indicates a stable acceleration field as observed in the conventional centrifuge.

The above analysis clearly discloses a versatile feature of the gear-side holder in that the magnitude and the acting pattern of the acceleration field is greatly altered by the location of the point on the holder. When a coiled column is mounted coaxially close to the axis of the holder, the column is subjected to a circulating acceleration field somewhat similar to that on the pulley-side holder. However, the same column can be mounted eccentrically on the holder to obtain a desired pattern of acceleration field, either circulating or swinging, by choosing a proper distance from the axis of the holder.

EFFECTS OF THE ACCELERATION FIELD ON THE HYDRODYNAMIC BEHAVIOR OF THE TWO PHASES IN THE COILED COLUMNS

The effect of the acceleration field on the two-phase solvent system in a rotating coiled tube is highly complex and any elaborate hydrodynamic analysis has not yet been attempted. However, observations made with a simple model system provide some useful information about the hydrodynamic motion of the two phases in a slowly rotating coiled tube in a gravitational acceleration field[13,14].

A uniformly circulating acceleration field around the coiled tube exerts an Archimedian screwing force on the droplets of one phase suspended in the other phase to establish a hydrodynamic equilibrium state of the two phases in the coil. Under this equilibrium state, the two phases occupy about equal space in each coil unit starting from one end of the coil called "the head" and any excess amount of either phase occupies the other end of the coil called "the tail". Once this hydrodynamic equilibrium state is established, the overall distribution of the two phases in the coiled tube always remains the same, while the two phases are constantly mixed with each other in the rotating coil. When the mobile phase, either upper or lower phase, is introduced at the head end of the coil to disrupt the hydrodynamic equilibrium state, the two phases instantly react to recover the equilibrium state. As a result, the stationary phase moves toward the head portion of the coil while the introduced excess amount of the mobile phase moves toward the tail, producing a counter-current flow of the two phases. Consequently, continued flow of the mobile phase displaces only the same phase leaving the stationary phase in each coil unit while the two phases are constantly mixed by rotation of the coil. Thus the system provides two essential features for solute partitioning, *i.e.*, retention of the stationary phase and mixing of the two phases. Solutes introduced locally at the head end of the coil are subjected to an efficient partition process and separated according to their partition coefficients.

In the past this uniformly circulating acceleration field has been applied to counter-current chromatography, using various coiled columns and two-phase solvent systems[1,3,4,6,13,14]. The results obtained by the flow-through coil planet centrifuge[3,4,6] reveal that the scheme enables both retention and mixing of the phases to achieve high efficiency separation in relatively narrow-bore columns. However, if low interfacial tension, viscous phase systems are eluted through a large-bore column, the mixing of the phases becomes so violent that the two phases tend to be emulsified resulting in carry-over of the stationary phase. This vigorous mixing also causes undesirable sample band broadening along the coiled tube to reduce the peak resolution. Therefore, the choice of the pulley-side holder which provides a uniformly circulating acceleration field is usually limited to micro-scale separations. Nevertheless, when the proper operational conditions are selected, the efficiency of separation attainable with the pulley-side column is extremely high, often exceeding that obtained with refined high-pressure liquid chromatography. Using 2,4-dinitrophenyl amino acids as samples, efficiencies of 10,000 theoretical plates have been reported[2].

The acceleration field produced by the motion of the gear-side holder has a characteristic nature in that the pattern of the field varies according to the location of the point on the holder. The overall results of the foregoing analysis suggest that both magnitude and acting pattern of the acceleration field favor the heavier phase to stay at the outer portion and the lighter phase at the inner portion of the column with respect to the holder. This tendency increases with greater β values for the location of the column on the holder. This uneven phase distribution is utilized effectively for retention of the stationary phase by mounting the coiled column eccentrically onto the holder. With this orientation of the column, the acceleration field separates the two phases in the coiled column, the heavier phase occupying the outer portion and the lighter phase, the inner portion of each coil unit. As a result, the two phases are distributed throughout the column to form alternating segments each occupying the column space about a half turn of the coil. Thus, the eluted mobile phase (either heavier or lighter phase) percolates through the segment of the stationary phase trapped in each coil unit while the undulating acceleration field induces oscillations of the phase segments synchronously with the revolution of the holder to effectively reduce the mass transfer resistance. Compared with the pulley-side column, the gear-side column gives more stable retention of the stationary phase and less violent mixing of the two phases. This tendency is more pronounced on a coiled column mounted more remotely from the axis of rotation. This renders a great versatility to the gear-side column holder in that a large-bore column can be used for a variety of two-phase solvent systems with minimum risk of emulsification and carry-over of the stationary phase.

CONCLUSION

The design of the present flow-through coil planet centrifuge allows continuous elution simultaneously through a pair of separation columns without complications arising from the use of rotating seals. Mathematical analysis of the acceleration field acting on each column revealed a characteristic pattern which provides its own specific advantage for performing counter-current chromatography. These unique features of the gear-side and pulley-side columns are summarized in Table I.

TABLE I

CHARACTERISTIC FEATURES OF THE PULLEY-SIDE AND GEAR-SIDE COLUMNS

	Pulley-side column	*Gear-side column*
Mode of planetary motion	Synchronous rotation in the opposite direction	Synchronous rotation in the same direction
Magnitude of acceleration	Homogeneous at all locations during each revolutional cycle	Heterogeneous at different locations and during each revolutional cycle
Pattern of acceleration field	Uniform circulation at all locations	Non-uniform circulations to swinging motion depending on β values
Retention of stationary phase	Retention dependent upon interfacial tension and viscosity of the two phases	Very stable. Stability increases with β values
Mixing of solvent phases	Very efficient	Less efficient especially at great β values
Partition efficiency	Extremely high with narrow-bore columns when the solvent is not emulsified	Excellent for large-bore columns Good for small-bore columns
Choice for application	Micro-scale separation with small-bore columns	Universal but particularly suitable for large-scale separation with large-bore columns

When a proper choice is made for the column holder, the present apparatus gives great versatility in performing counter-current chromatography for both large-scale and small-scale separations.

REFERENCES

1 Y. Ito, I. Aoki and E. Kimura, *Anal. Chem.*, 41 (1969) 1579–1584.
2 Y. Ito and R. L. Bowman, *Anal. Chem.*, 43 (1971) 69A–75A.
3 Y. Ito and R. L. Bowman, *Science*, 173 (1971) 420–422.
4 R. E. Hurst and Y. Ito, *Clin. Chem.*, 18 (1972) 814–820.
5 Y. Ito, R. L. Bowman and F. W. Noble, *Anal. Biochem.*, 49 (1972) 1–8.
6 Y. Ito and R. L. Bowman, *J. Chromatogr. Sci.*, 11 (1973) 284–291.
7 Y. Ito and R. L. Bowman, *Science*, 182 (1973) 391–393.
8 Y. Ito, R. E. Hurst, R. L. Bowman and E. K. Achter, *Separ. Purif. Methods*, 3 (1974) 133–165.
9 Y. Ito and R. L. Bowman, *Anal. Biochem.*, 65 (1975) 310–320.
10 Y. Ito and R. L. Bowman, *Anal. Biochem.*, 82 (1977) 63–68.
11 Y. Ito and R. L. Bowman, *J. Chromatogr.*, 147 (1978) 221–231.
12 Y. Ito, *Anal. Biochem.*, in press.
13 Y. Ito and R. L. Bowman, *Anal. Biochem.*, 78 (1977) 506–512.
14 Y. Ito and R. L. Bowman, *J. Chromatogr.*, 136 (1977) 189–198.