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Cross-Axis Synchronous Flow-Through Coil Planet Centrifuge Free of Rotary Seals for Preparative Countercurrent Chromatography. Part I. Apparatus and Analysis of Acceleration

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Abstract

A novel type of the coil planet centrifuge is introduced. The apparatus holds a coil holder in such a way that the axis of the holder is positioned perpendicular to and at a fixed distance away from the centrifuge axis. In maintaining the above orientation the holder undergoes synchronous planetary motion, i.e., revolution around the central axis of the centrifuge and rotation about its own axis at the same angular velocity. Mathematical analysis of acceleration generated by this planetary motion revealed a unique distribution pattern of centrifugal force vectors which promises a high performance of the present scheme in countercurrent chromatography.

INTRODUCTION

Countercurrent chromatography (CCC) utilizes complex hydrodynamic interaction of two immiscible solvent phases in a tubular column space free of solid support matrix (*1*). Retention of the stationary phase and solute partitioning take place in an open space of the column by the aid of suitable acceleration field and column configuration. In the past a series of flow-through centrifuge systems has been developed for performing CCC (*2*). Among those, the most useful systems were found to be the coil planet centrifuges which produce synchronous planetary motion (rotation and revolution being fixed at the same rate) of the column holder.

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Figure 1 illustrates orientation and motion of a cylindrical coil holder in various types of the synchronous flow-through coil planet centrifuge free of rotary seals. In Type I the holder revolves around the central axis of the centrifuge system while it synchronously counterrotates about its own axis at the same angular velocity. This synchronous counterrotation of the holder steadily unwinds the twist of the flow tubes made by revolution, thus totally eliminating the need for the conventional rotary seal device. This twist-free flow-through mechanism works equally well in other types with tilted (Types I-L and I-X), horizontal (Types L and X), dipping (Types J-L and J-X), and inverted (Type J) orientations of the holder. Among those planetary centrifuge systems, Type I (3, 4), Type I-L (5), Type L (6, 7), Type J-L (8, 9), and Type J (10, 11) have been successfully constructed to evaluate their performance in CCC.

The present paper introduces a novel system called the cross-axis synchronous flow-through coil planet centrifuge which is assigned as Type X in Fig. 1. In this article (i.e., Part I) the design of the prototype apparatus is described and the acceleration field generated by this synchronous planetary motion is mathematically analyzed. Comparative studies with various other types of synchronous planetary motion strongly suggest that the present system will yield a high performance in CCC.

DESIGN OF THE APPARATUS

As described earlier, the planetary motion of the present scheme is illustrated in Fig. 1 as Type X at the middle portion of the right column. The cylindrical column holder revolves around the central axis of the centrifuge while it rotates about its own axis at the same angular velocity as indicated by a pair of arrows. While doing so, the holder maintains the same orientation with respect to the central axis of the centrifuge. Consequently, the horizontal axis of the holder and the vertical axis of the centrifuge are always kept perpendicular to each other and with a fixed distance. This forms a "cross" pattern, as indicated by the name. This planetary motion of the holder allows the flow tubes to rotate around the central axis of the centrifuge without twisting as seen in all other synchronous planetary motions illustrated in Fig. 1.

The design of our prototype centrifuge is shown in Fig. 2. The motor (a) drives the central shaft (b) and the rotary frame around the central axis of the centrifuge via a pair of toothed pulleys coupled with a toothed belt. The rotary frame consists of a pair of side-plates (c) rigidly bridged with links (d) to support a column holder (e) and a counterweight holder

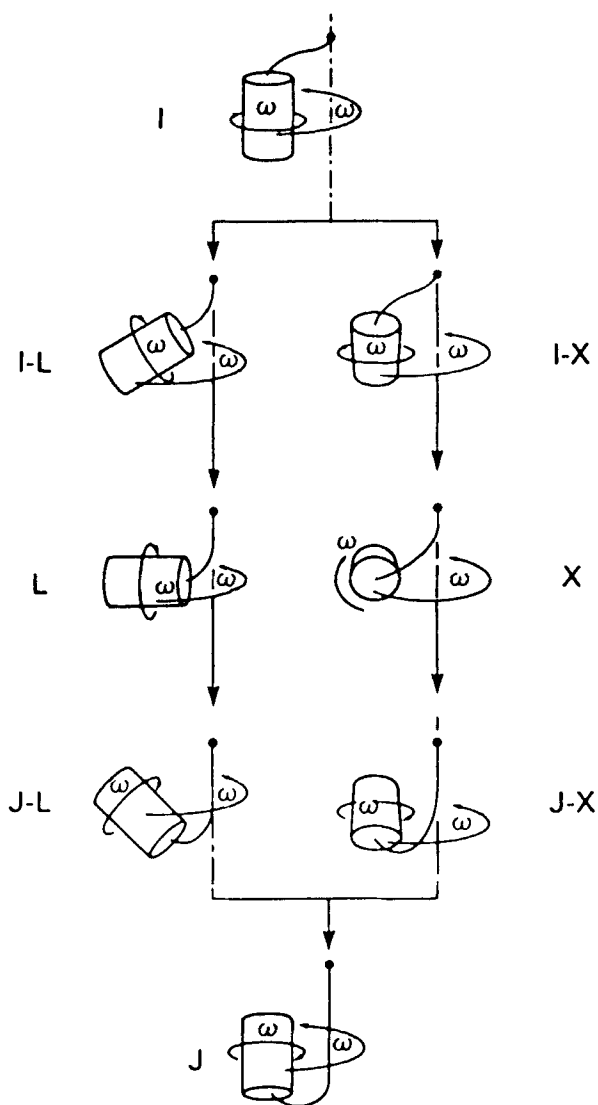


FIG. 1. Diagrammatic illustration of planetary motion in various types of synchronous flow-through coil planet centrifuge free of rotary seals.

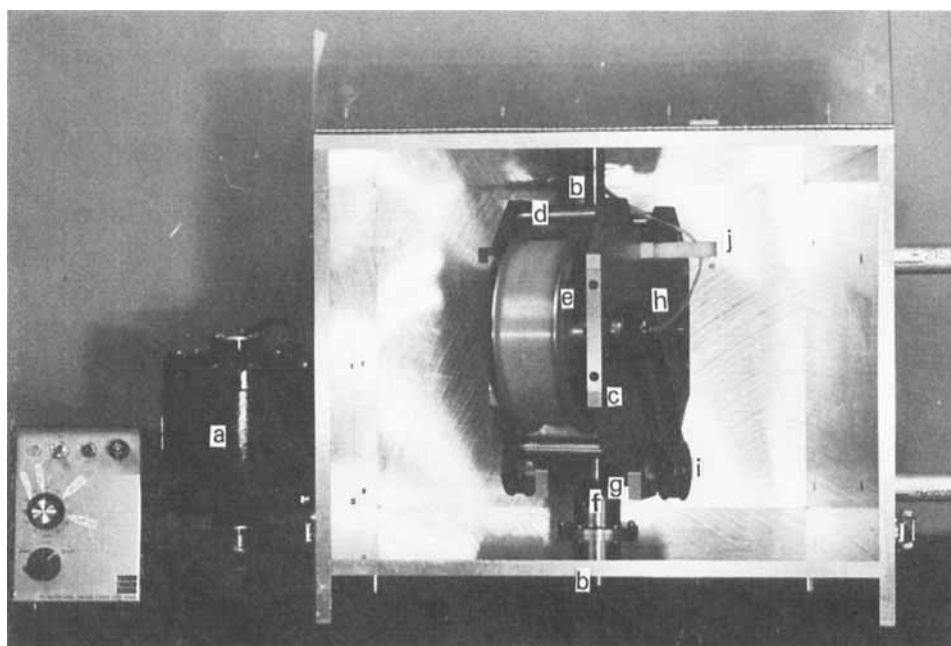


FIG. 2. The first prototype of the cross-axis synchronous flow-through coil planet centrifuge.

horizontally in the symmetrical positions at a distance of 10 cm from the central axis of the centrifuge. At the lower portion of the side-plates a pair of countershafts is radially mounted in the symmetrical positions through ball bearings. The stationary miter gear (45°) (f) rigidly mounted on the bottom plate of the centrifuge coaxially around the central shaft is coupled to the identical miter gear (g) on the proximal end of each countershaft. This gear coupling results in synchronous rotation of each countershaft on the revolving rotary frame. This motion is further conveyed to the column holder and the counterweight holder by coupling a pair of identical toothed pulleys, one (h) mounted on each holder shaft and the other (i) on the distal end of the respective countershaft. Consequently, both the column holder and the counterweight holder undergo the desired synchronous planetary motion, i.e., rotation about its own axis and revolution around the central axis of the centrifuge at the same angular velocity.

In order to facilitate column preparation and balancing in the centrifuge system, both the column holder and counterweight holder

were designed so that they are easily removed from the rotary frame by loosening a pair of screws in each bearing block. The separation column was prepared by winding a long piece of PTFE (polytetrafluoroethylene) tubing typically 2.6 mm i.d. (Zeus Industrial Products, Raritan, New Jersey) directly onto the holder hub making either single layer or multiple coiled layers. Also other types of columns with potential usage include a toroidal coil made by winding a coiled tube again around the holder hub and multiple parallel coils which are connected in series and mounted around the periphery along the axis of the holder.

The flow tubes from the separation column were first passed through the center hole of the holder shaft and then led through the side-hole of the central shaft to exit the centrifuge at the top plate where they were tightly held by a clamp. The tube support (j) on the side-plate of the rotary frame in Fig. 1 was found to be unnecessary (it often impairs the flow tubes by friction). The flow tubes should be thoroughly lubricated with grease and protected with a piece of Tygon tubing at each hole to prevent contact with metal parts. If this caution is followed, they can maintain integrity for many months of use.

The apparatus can be operated at a desired revolutionary speed ranging from 50 to 1000 rpm with a speed control unit (Bodine Electric Company, Chicago, Illinois). It was found that the prototype centrifuge produces a considerable noise at the site of the metal gears, thus limiting the use of high revolutionary speeds in the present prototype. This problem may be largely eliminated by the use of plastic gears upon reducing the load with a thrust bearing which supports the weight of the rotary frame at the bottom of the centrifuge.

The present prototype apparatus was designed to study the potential capability of the new centrifuge system. Because the apparatus has a simple and mechanically stable design, the sample loading capacity can be easily scaled up by increasing both the revolutionary radius and the column dimensions. The counterweight may be replaced by another column of the same size so that the two columns can be used either individually or connected in series to double the column capacity.

ANALYSIS OF ACCELERATION

Acceleration produced by the present mode of synchronous planetary motion has been mathematically analyzed in two steps. The first analysis is performed on an arbitrary point located in the central plane of the holder across the holder axis. In this particular case, the arbitrary point, the radius of revolution, and the central axis of the centrifuge are all

confined in the same plane. In the second analysis, the arbitrary point can be chosen at any location on the holder, thus yielding generalized formulas for computing acceleration.

1. Acceleration Acting on the Central Plane of the Holder

We consider a circular body with radius r and undergoing a planetary motion in such a way that it revolves around the central axis of the centrifuge (AB) and simultaneously rotates about its own axis (Q) at the same angular velocity (ω) as illustrated in Fig. 3. In so doing, the circular body always maintains its axis perpendicular to and at the same distance ($\overline{OQ} = R$) away from the central axis. Then, we wish to study the motion of an arbitrary point (P) located at the periphery of the circular body ($\overline{QP} = r$).

Figure 4 shows an x - y - z coordinate system where the z -axis coincides with the central axis and the center of the circular body (Q) circles around Point O in the x - y plane. The arbitrary point on the circular body and the center of the circular body are initially located on the x -axis at P_0 and Q_0 , respectively. The circular body undergoes a synchronous planetary motion as described above, i.e., it revolves around the z -axis and rotates

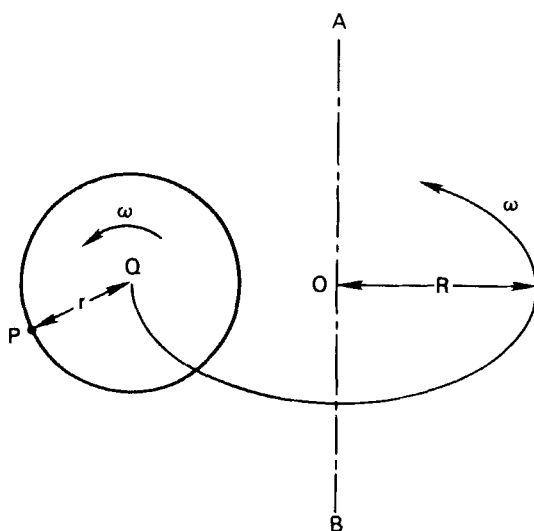


FIG. 3. Orientation and motion of the circular body for analysis of acceleration.

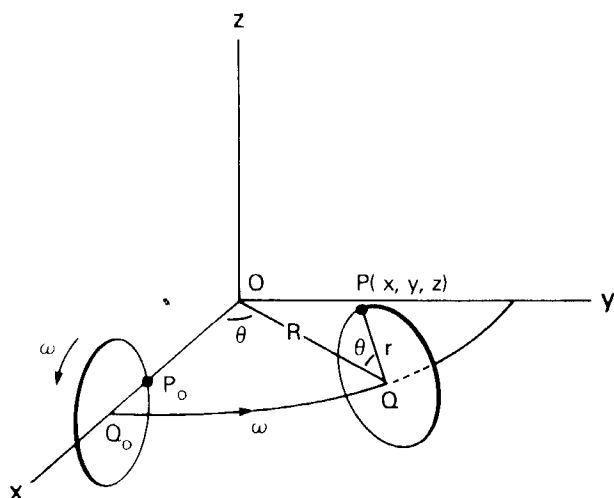


FIG. 4. The x - y - z coordinate system for analysis of acceleration acting on the circular body.

about its own axis at the same angular velocity, ω . Consequently, after a lapse of time t , the arbitrary point moves to Point P and the center of the circular body to Point Q . Then the location of Point $P(x, y, z)$ is expressed in the following equations:

$$x = R \cos \theta - r \cos^2 \theta \quad (1)$$

$$y = R \sin \theta - r \sin \theta \cos \theta \quad (2)$$

$$z = r \sin \theta \quad (3)$$

where $R = \overline{OQ}$, $r = \overline{QP}$, and $\theta = \omega t$.

The acceleration acting on the arbitrary point is then given by the second derivatives of these equations and

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

$$d^2y/dt^2 = -R\omega^2 \sin \theta + 2r\omega^2 \sin 2\theta = -R\omega^2(\sin \theta - 2\beta \sin 2\theta) \quad (5)$$

$$d^2z/dt^2 = -r\omega^2 \sin \theta = -R\omega^2\beta \sin \theta \quad (6)$$

where $\beta = r/R$.

In order to visualize the effects of acceleration on the objects rotating with the circular body, it is more convenient to express the acceleration vectors with respect to the body frame or the X_b - Y_b - Z_b coordinate system illustrated in Fig. 5. Transformation of the vectors from the original x - y - z coordinate system to the X_b - Y_b - Z_b coordinate system may be performed according to the following equations:

$$\alpha_{x_b} = (d^2x/dt^2) \cos \theta + (d^2y/dt^2) \sin \theta = -R\omega^2(1 - 2\beta \cos \theta) \quad (7)$$

$$\alpha_{y_b} = d^2z/dt^2 = -R\omega^2\beta \sin \theta \quad (8)$$

$$\alpha_{z_b} = (d^2x/dt^2) \sin \theta - (d^2y/dt^2) \cos \theta = -R\omega^2 2\beta \sin \theta \quad (9)$$

where α_{x_b} , α_{y_b} , and α_{z_b} indicate the acceleration vectors acting along the corresponding coordinate axes.

From Eqs. (7)–(9), the centrifugal force vectors (same magnitude with the acceleration but acting in the opposite direction) at various points on the circular body are computed and diagrammatically illustrated in Fig. 6. In order to illustrate the three-dimensional pattern of the centrifugal force vectors on a two-dimensional diagram, two force vectors, $-\alpha_{x_b}$ and $-\alpha_{y_b}$, are combined into a single arrow forming various angles from the

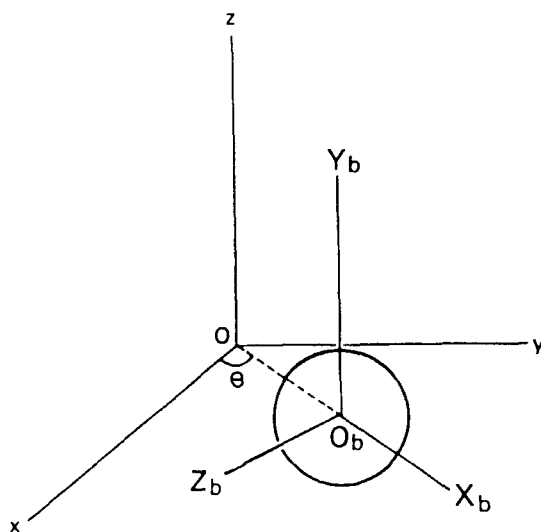


FIG. 5. Relationship between the reference x - y - z coordinate system and the X_b - Y_b - Z_b body coordinate system.

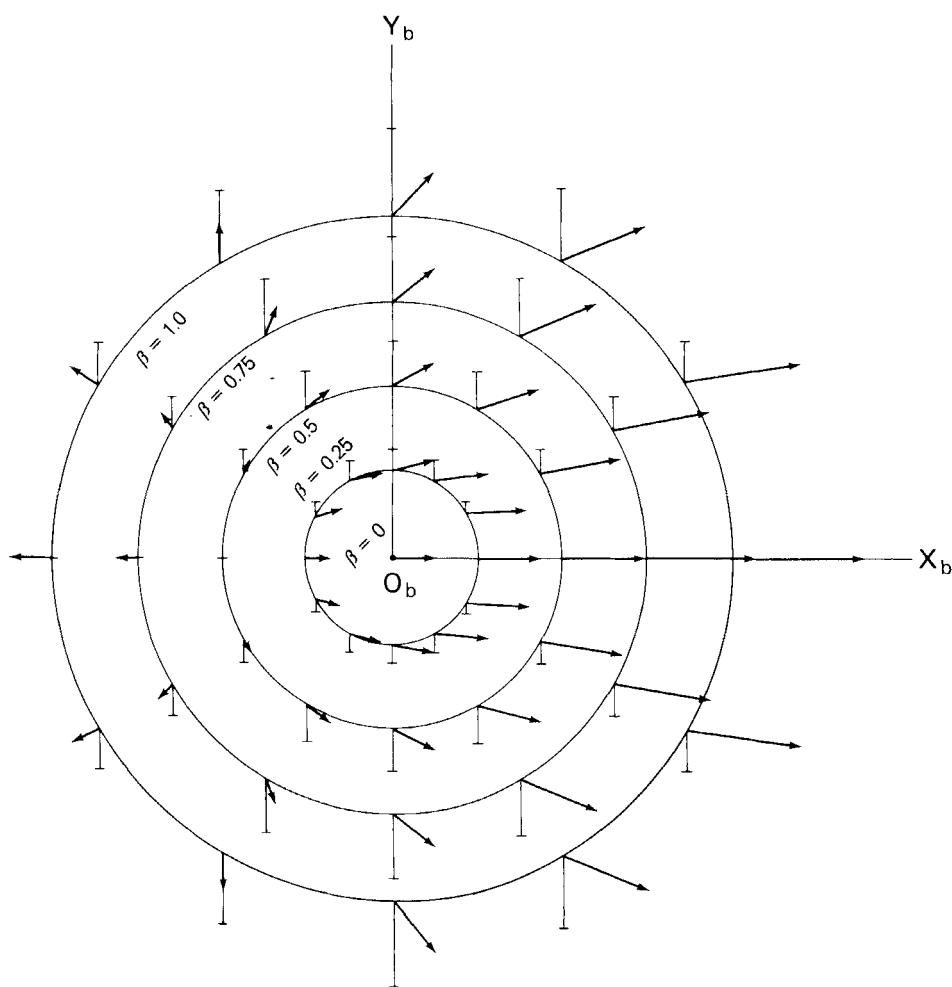


FIG. 6. Force distribution diagram for the cross-axis synchronous flow-through coil planet centrifuge (Type X).

X_b -axis, whereas the third force vector, $-a_{Z_b}$, which acts perpendicular to the X_b - Y_b plane, is drawn as vertical columns along the Y_b -axis. The ascending column indicates the force acting upward ($Z_b > 0$) and the descending column, the force acting downward ($Z_b < 0$). Several concentric circles around Point O_b (the center of the circular body) indicate the location on the circular body corresponding to various β values labeled in the diagram.

While the force distribution diagram thus produced (Fig. 6) indicates the distribution of centrifugal force vectors acting on the circular body at any given moment, an arbitrary point on the circular body is rotating clockwise around the center of the body coordinate system (O_b) at a uniform angular velocity equal to revolution. Consequently, during one revolutional cycle, the arbitrary point is subjected to a series of centrifugal force vectors indicated by the force distribution diagram.

As illustrated in Fig. 6 the force distribution diagram for the cross-axis synchronous coil planet centrifuge reveals complex three-dimensional fluctuation of the centrifugal force field during each revolutional cycle where both magnitude and direction of force vectors change with β or the location of the point on the circular body. The X_b - Y_b component (expressed as arrows) shows a mirror image between the upper and the lower halves of the circular body but it displays a great asymmetry along the X_b -axis. At small β or the locations close to the center of the circular body, all force vectors are directed toward the right, indicating that the force rotates around each point as the circular body revolves. When β becomes greater than 0.5, however, the force vectors are always directed outwardly from the circle indicating that the rotation of the force around each point changes into the oscillatory motion. On the other hand, the Z_b -component (expressed as columns) displays perfect symmetry around the center of the circular body while it gradually increases both the magnitude and amplitude of oscillation as β becomes greater.

The above findings from the force distribution diagram strongly suggest the great potential capability of the present scheme in performing CCC as discussed later in detail.

2. Acceleration Acting on the Arbitrary Point on the Holder

Figure 7 shows a x - y - z coordinate system where a cylindrical body (column holder) revolves around the z -axis and synchronously rotates about its own axis positioned in the x - y plane. The cylindrical body contains the circular body drawn as a thin circle at the middle portion together with Points P_0 and P which were subjected to the preceding analysis. An arbitrary point for the present analysis is located at distance l away from Point P along the axis of the cylindrical body. As shown in Fig. 7, the arbitrary point initially locates at $P'_0 (R - r, l, 0)$ and after a lapse of t moves to Point $P' (x', y', z')$ which is expressed in the following equations:

$$x' = R \cos \theta - r \cos^2 \theta - l \sin \theta \quad (10)$$

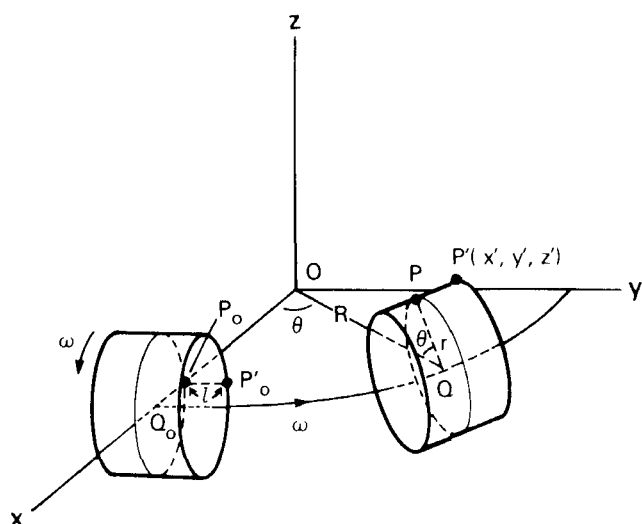


FIG. 7. The x - y - z coordinate system for analysis of acceleration acting on the cylindrical body.

$$y' = R \sin \theta - r \sin \theta \cos \theta + l \cos \theta \quad (11)$$

$$z' = r \sin \theta \quad (12)$$

The acceleration acting on Point P' is similarly obtained from the second derivatives of the above equations and

$$d^2x'/dt^2 = -R\omega^2(\cos \theta - 2\beta \cos 2\theta) + l\omega^2 \sin \theta \quad (13)$$

$$d^2y'/dt^2 = -R\omega^2(\sin \theta - 2\beta \sin 2\theta) - l\omega^2 \cos \theta \quad (14)$$

$$d^2z'/dt^2 = -R\omega^2\beta \sin \theta \quad (15)$$

where $\beta = r/R$.

According to the procedure used in the preceding analysis, these acceleration vectors expressed in reference to the original x - y - z coordinate system are further transformed into the X_b - Y_b - Z_b body coordinate system (see Fig. 5) by the following equations:

$$\alpha'_{x_b} = (d^2x'/dt^2) \cos \theta + (d^2y'/dt^2) \sin \theta = -R\omega^2(1 - 2\beta \cos \theta) \quad (16)$$

$$\alpha'_{Y_b} = d^2z'/dt^2 = -R\omega^2\beta \sin \theta \quad (17)$$

$$\alpha'_{Z_b} = (d^2x'/dt^2) \sin \theta - (d^2y'/dt^2) \cos \theta = -R\omega^2\beta \sin \theta + l\omega^2 \quad (18)$$

Comparison between the two sets of equations, Eqs. (7)–(9) and Eqs. (16)–(18), clearly shows that deviation of the point from the central plane of the holder has no effect on the acceleration components acting in the X_b - Y_b plane. The above effect is only found on the acceleration vectors acting along the Z_b -axis where the magnitude of all vectors is uniformly affected by $l\omega^2$ regardless of the values for R and r . Consequently, deviation of the point along the holder axis causes asymmetric distribution of the laterally acting force field in such a way that the degree of asymmetry increases as r becomes smaller.

Equation (18) may be written as

$$\alpha'_{Z_b} = -\omega^2(2r \sin \theta - l) \quad (19)$$

indicating that when $l \geq 2r$, $\alpha'_{Z_b} \geq 0$, that is, the direction of the acceleration (or the centrifugal force) vector acting along the Z_b -axis becomes unilateral.

COMPARATIVE STUDIES ON FORCE DISTRIBUTION DIAGRAMS

In the past, various types of synchronous flow-through coil planet centrifuges (which include Type I, Type I-L, Type L, Type J-L, and Type J shown in Fig. 1, left and middle columns) have been successfully constructed to evaluate their performance in CCC in terms of retention of the stationary phase and partition efficiency. These studies have demonstrated a close correlation between the hydrodynamic behavior of the two solvent phases and the applied centrifugal force field and that the use of suitable coil orientation can yield highly efficient separations with sample size ranging from analytical to preparative scales.

Figure 8 illustrates force distribution diagrams produced from the above series of the synchronous planetary motion (8). In order to facilitate comparison, all diagrams are drawn according to the same format where magnitude of the force vectors (arrows and columns) are all expressed in the same unit. As in the force distribution diagram shown in Fig. 6, arrows indicate the force components acting in the X_b - Y_b plane of the body coordinate system and columns, the force components acting along the Z_b -axis. Observation of these force distribution diagrams from the top to the bottom reveals successive changes in the force distribution

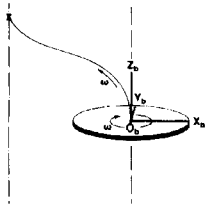
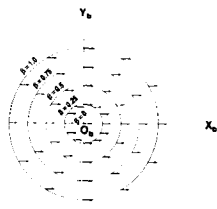
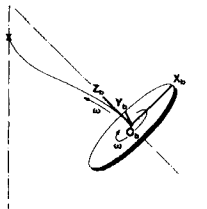
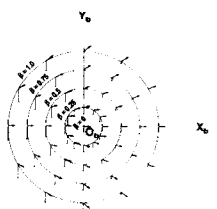
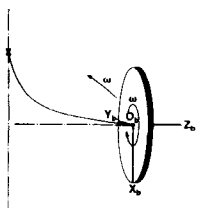
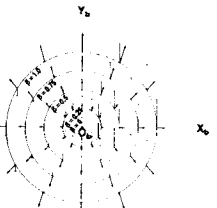
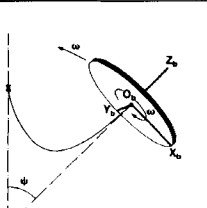
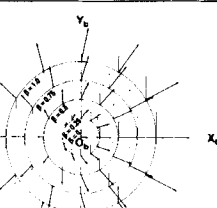
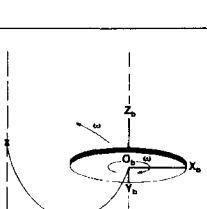
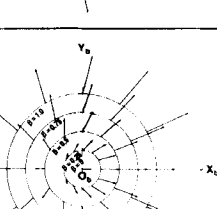
New Nomenclature of Synchronous Planetary Motion (Old Terms)	Angle ψ	Planetary Motion and Reference Coordinate Frame	Force Distribution Diagram
Type I (Scheme I)	180°		
Type I-L (Scheme II)	135°		
Type L (Scheme III)	90°		
Type J-L	45°		
Type J (Scheme IV)	0°		

FIG. 8. Force distribution diagrams for various types of synchronous flow-through coil planet centrifuges studied in the past (8).

pattern. Among those, two extreme forms of Type I and Type J display two-dimensional force distributions while showing striking contrast in their patterns.

The Type I synchronous planetary motion produces a homogeneous distribution of centrifugal force vectors all with a unit magnitude ($R\omega^2$). Every force vector uniformly rotates around each point as the circular body revolves. In order to utilize this centrifugal force field efficiently, coils are mounted parallel to the holder axis (Z_h -axis) so that the Archimedean screw force can exert a strong hydrodynamic effect on two immiscible solvent phases present in the coil. Under this coil orientation, two solvent phases quickly establish a hydrodynamic equilibrium in the rotating coil where each phase competitively occupies about half the space from one end of the coil called the head while any excess of either phase quietly remains at the other end called the tail.

Thus, elution of either phase from the head toward the tail of the coil retains the other phase stationary in the coil where retention of the stationary phase is usually less than 50% of the total column capacity. Application of the reversed elution mode, i.e., introduction of the mobile phase from the tail toward the head usually results in total loss of the stationary phase from the coil. With a proper elution mode and a moderate flow rate, the system enables highly efficient analytical-scale separations with a narrow-bore coil.

In the Type J synchronous planetary motion, force vectors display a highly complex heterogeneous distribution, varying in both magnitude and direction according to the β values and also during each revolutionary cycle. At the vicinity of the holder axis ($\beta < 0.25$), all force vectors are directed toward the right, indicating that each vector rotates around the acting point as in the Type I synchronous planetary motion. As β becomes greater than 0.25, all vectors are directed outwardly from each circle, indicating that the force vectors display oscillatory motion where amplitude of oscillation becomes smaller as β increases. This unique distribution of the centrifugal force vectors produces entirely different hydrodynamic effects according to the orientation of the coiled column on the holder.

When the coiled column is mounted onto the periphery of the holder either by arranging it parallel to the holder axis (eccentric parallel coil) or winding it again around the holder (toroidal coil), the outwardly acting force field ($\beta > 0.25$) traps the heavier phase in the outer portion and the lighter phase in the inner portion of each helical turn to establish the so-called hydrostatic equilibrium. Consequently, introduction of either phase through either end of the coil results in retention of the other phase in each helical turn while oscillating force field produces efficient mixing

of the two solvent phases to promote partition process. Here again the stationary phase volume retained in the coil is usually less than 50% of the total column capacity. These hydrostatic schemes provide universal application of conventional two-phase solvent systems and yield efficient separations under a moderate flow rate of the mobile phase.

Entirely different hydrodynamic phenomenon has been observed when the coil is directly wound around the holder hub (coaxial coil). Under this coil orientation, the outwardly directed force field ($\beta > 0.25$) separates the two solvent phases in such a way that the lighter phase is layered over the heavier phase along the coiled tube while the undulating force field moves one of the phases toward the head to establish a unilateral hydrodynamic equilibrium of two solvent phases. Finally, the two phases are completely separated along the length of the coil, one phase (head phase) occupying the head side and the other phase (tail phase) the tail side. This unilateral hydrodynamic equilibrium condition can be effectively utilized for performing CCC to retain a large volume of the stationary phase in the coiled column. The method allows either phase to be used as the mobile phase but each with a different elution mode: The coil is first entirely filled with the head phase followed by elution with the tail phase from the head of the coil. Alternatively, the coil is filled with the tail phase followed by elution with the head phase from the tail of the coil. In either case the system can maintain a high retention of the stationary phase against fast flow of the mobile phase, thus yielding an extremely high peak resolution of samples in a short period of time. This high-speed CCC has been successfully applied to a variety of biological samples with excellent results (12, 13).

The rest of the synchronous planetary motions including Type L and two hybrid types, I-L and J-L, produce somewhat intermediate hydrodynamic effects between the above two extremes and yield analytical to high-speed preparative separations.

The phase distribution diagram obtained from the cross-axis synchronous coil planet centrifuge (Fig. 6) bears close resemblance to those from Type J and Type J-L in terms of asymmetrical distribution of the first force component (arrows) along the X_b -axis. However, the distribution pattern of the first force component of the present scheme (Type X) differs from that of Type J in two important aspects, i.e., the relative magnitude and the critical β value where the rotary motion of the force vectors turns into an oscillatory motion as mentioned earlier: In Type X relative magnitude of the first force component is considerably reduced at a given location (β) on the holder while the critical value of β is shifted to 0.5 from 0.25 observed in other schemes (Types J and J-L). Another important feature of the present scheme (Type X) is the presence of the

second force component acting along the Z_b -axis which forms symmetrical distribution around the holder axis with a gradual increase in magnitude toward the periphery.

On the basis of these differences in the phase distribution diagram described above, the present scheme (Type X) would provide various favorable features for performing CCC. Close resemblance in radial force distributions between Types X and J strongly suggests that the present scheme would produce a unilateral hydrodynamic equilibrium of two solvent phases in a coaxially mounted coil to allow high-speed CCC as in Types J and J-L. The presence of laterally oscillating force field (columns) may produce additional phase mixing across the diameter of the tube without a risk of undesirable sample band broadening. Under this oscillating force field, two solvent layers formed within the coaxially mounted coil should laterally roll around the tube wall. This rolling motion will not only induce circular stirring in each layer but also steadily expose each solvent layer to a new wall surface, thus effectively reducing mass transfer resistance.

On the other hand, reduced radial force strength together with laterally oscillating force field would adversely affect the retention of the stationary phase in the column. This problem may be compensated by manipulating various operational factors such as revolutional speed and radius, location of the coil on the holder (β), flow rate of the mobile phase, etc.

The effect of the increased critical β value from 0.25 to 0.5 may cause some important changes in the hydrodynamic trend of solvent systems. As described in Part II, the present scheme provides the reversed hydrodynamic trend for all tested intermediate solvent systems which are characterized by the moderate hydrophobicity of the nonaqueous phases. Consequently, both intermediate and hydrophilic solvent systems display the same hydrodynamic trend, i.e., the heavier phase is distributed toward the head and the lighter phase toward the tail. This uniform behavior of the two solvent groups provides a great advantage in performing large-scale preparative separations as discussed later in Part II. Here, it is interesting to note that the hybrid scheme of Type J-L, which also forms a laterally oscillating force field at the sacrifice of the radial force strength, gives both the critical β value and hydrodynamic trend quite similar to those of the parent scheme of Type J (9).

The laterally acting force field in Type X will further render significant advantage in performing CCC with a toroidal coil. When the toroidal coil is subjected to the Type J planetary motion, the radially directed centrifugal force field fails to produce effective mixing of the solvent phases since the force vibrates along the axis of the coil, thus limiting the

mixing effect to a small portion of the coil. While this problem can be solved by mounting the coil parallel to the holder axis (eccentric parallel coil), efficient mixing is available only in the vicinity of the holder axis where the amount of the coil accommodated in space is quite limited. The use of a long coil holder to accommodate a sizable column again suffers from mechanical strain under a strong centrifugal force field which in turn necessitates reduction of the applied revolutionary speed, resulting in substantial loss of partition efficiency.

All these problems will be solved by Type X planetary motion which yields vigorous oscillation around the axis of the toroidal coil. This results in efficient phase mixing similar to that obtained from the eccentric parallel coil orientation in the Type J planet centrifuge but under a high centrifugal force field without excessive mechanical constraint on the coiled column. This method may be most suitable for analytical to semipreparative separations with a relatively small-bore toroidal coil.

In the light of the above discussion, it may be concluded that the present model of the cross-axis synchronous flow-through coil planet centrifuge deserves a series of experimental studies to explore the potential capability of performing CCC. The results of the studies on stationary phase retention and partition efficiency in coaxial coils are described in Part II.

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