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IMPROVED CROSS-AXIS SYNCHRONOUS FLOW-THROUGH COIL PLANET CENTRIFUGE FOR PERFORMING COUNTER-CURRENT CHROMATOGRAPHY

I. DESIGN OF THE APPARATUS AND ANALYSIS OF ACCELERATION

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SUMMARY

A novel design of the cross-axis synchronous flow-through coil planet centrifuge is introduced. The apparatus holds a pair of large coil holders symmetrically, one on each side of the rotary frame, at a lateral position 12.5 cm from the center of the holder shaft held 10 cm from the centrifuge axis. Mathematical analysis of acceleration generated by the planetary motion of the apparatus revealed a unique centrifugal force field which promises high retention of the stationary phase in the multilayer coil to perform efficient preparative-scale counter-current chromatography.

INTRODUCTION

Counter-current chromatography (CCC) is a true liquid–liquid partition chromatography which totally eliminates the use of a solid support[1]. Being a support-free system, the method offers a number of advantages over other chromatographic methods by minimizing various problems arising from the use of solid supports such as adsorptive loss and deactivation of samples, tailing of solute peaks, contamination, etc. In practice, CCC provides its greatest advantage in preparative-scale separations where high-performance liquid chromatography (HPLC) suffers loss of partition efficiency and high expenditure of the solvents and solid support materials.

Recently, partition efficiency of preparative CCC has been remarkably improved by development of a new centrifuge system called the cross-axis synchronous flow-through coil planet centrifuge (X-axis CPC) which performs multigram separations at a high partition efficiency[2–7]. A series of previous studies has shown that in the X-axis CPC the position of the coil on the rotary frame and helical diameter of the coil play a vital role in both stationary phase retention and partition efficiency. The experimental data obtained from the most recent model of the X-axis CPC with a 20-cm revolutional radius[4,5] further indicated that the column performance can be

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improved by (1) increasing the helical diameter of the coiled column and (2) lateral shift of the coil holder along the holder shaft.

In the present studies, a new model of the X-axis CPC is designed to accommodate a pair of large column holders each at a lateral location 12.5 cm from the center of the holder shafts. In Part I, principle and design of the apparatus are described in detail, and unique hydrodynamic effects produced by the present model are discussed in the light of the acceleration analysis together with the experimental results obtained from the existing X-axis CPC instruments.

PRINCIPLE AND DESIGN OF THE APPARATUS

Fig. 1 illustrates a set of rotary-seal-free flow-through centrifuge systems developed for performing CCC. In each diagram, a cylindrical coil holder with a bundle of flow tubes revolves around the main axis of the centrifuge and simultaneously rotates about its own axis at the same angular velocity, as indicated by a pair of arrows. The bundle of flow tubes with one end tightly supported at the central axis above the centrifuge does not twist because the synchronous planetary motion of the holder steadily unwinds the twist of the tube bundle caused by revolution. Consequently, all these systems permit continuous elution through the rotating column without the use of the rotary seal device which would become a potential source of various complications such as leakage, contamination, etc.

Type I shown at the top of the figure has a vertical orientation of the holder which can be modified in two different ways: in the left column, the holder is tilted toward the

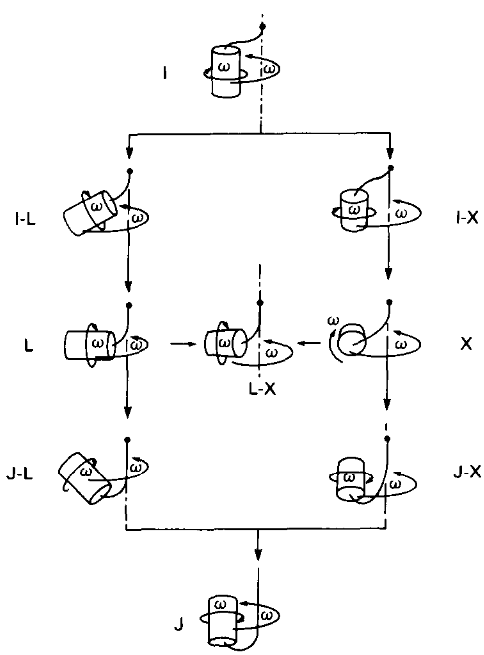

Fig. 1. A series of rotary-seal-free flow-through coil planet centrifuge systems for counter-current chromatography.

central axis of the centrifuge to form types L and J and their hybrids, whereas in the right colum the holder is rotated sideways to form types X and J and their hybrids. In the past, most of those centrifuge systems (except for types I–X and J–X) were constructed for performing CCC. Among those, types J and X were found to be most useful because of their unique capability to form unilateral distribution of the two solvent phases in the coiled column which is in turn utilized for performing high-speed CCC[8]. Type J is ideal for semipreparative to analytical-scale separations, while type X is most suitable for preparative-scale separations.

The original X-axis CPC was derived from type X as described elsewhere[2]. In the present apparatus, type X is further modified by shifting the position of the holder along its axis to form a hybrid between types L and X or type L–X as indicated at the center of Fig. 1. As described later, this new orientation of the holder can produce remarkable improvement in the retention of the stationary phase in the coiled column under the proper mode of elution and planetary motion.

The design of the present apparatus is illustrated in Fig. 2, where A shows a side view of the apparatus and B, a cross-sectional view through the central axis of the coil holders in a horizontal plane. The motor (1) (Fig. 2A, bottom) drives the central shaft (2) and the rotary frame around the central axis of the centrifuge. (In the actual design, the motor drives the rotor via a pair of toothed pulleys coupled with a toothed belt.) The rotary frame consists of four side-plates, a pair of inner plates (3) and a pair of outer plates (4), all rigidly bridged together with multiple aluminum links (not shown in the figure). A pair of horizontal plates, the upper (5) and the lower (6) plates (Fig. 2A), connects the pair of inner side-plates to the central shaft. The inner and the outer side-plates horizontally hold a coil holder shaft (7) symmetrically on each side of the rotary frame at a distance of 10 cm from the central axis of the centrifuge. A pair of identical coil holders (8) is mounted around the holder shafts symmetrically, one on each side of the rotary frame between the inner and the outer side-plates at a location about 12.5 cm from the center of the holder shaft as shown in Fig. 2B.

The planetary motion of each coil holder is established by the use of a set of miter gears and toothed pulleys as follows: A stationary miter gear (45°) (9) is rigidly mounted on the bottom plate of the centrifuge coaxially around the central shaft (2). This stationary sun gear is coupled to a pair of identical planetary gears (10) each attached to the proximal end of a countershaft (11) which radially extends toward the periphery of the rotary frame through the lower portion of the inner side-plate. This gear arrangement produces synchronous rotation of each countershaft on the rotating rotary frame. This motion is further conveyed to each coil holder by coupling a toothed pulley (12) mounted at the peripheral end of the countershaft to the identical pulley (13) on the respective coil holder shaft with a toothed belt (14). Consequently, each coil holder undergoes the desired planetary motion, *i.e.*, revolution around the central axis of the centrifuge and rotation about its own axis at the same angular velocity.

In the actual design of our prototype, each coil holder is made from three portions, *i.e.*, a main holder shaft supporting the coil holder and the toothed pulley, an auxiliary shaft placed on the opposite side, and a central pipe connecting these two shafts where the positions of the main holder shaft and the auxiliary shaft are mutually exchangeable on each side of the rotary frame. Each shaft is removable from the rotary frame by loosening the screws on each bearing block. Three pairs of spool-shaped coil holders were fabricated each with hub diameter of 10, 15, or 20 cm and equipped with

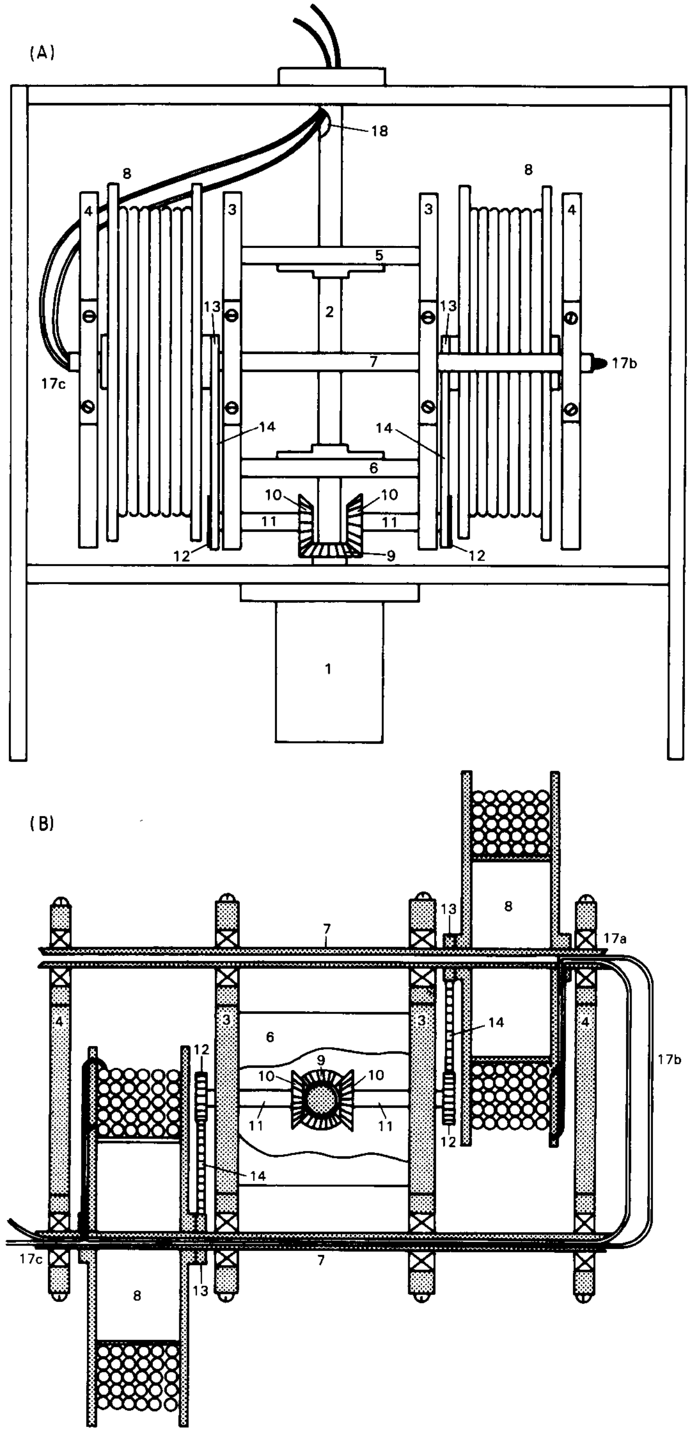

Fig. 2. The design of the present cross-axis synchronous flow-through coil planet centrifuge. (A) Side view of the apparatus. (B) Cross sectional view through the holder axis in the horizontal plane: 1 = motor; 2 = central shaft; 3 = inner side-plate; 4 = outer side-plate; 5 = upper plate; 6 = lower plate; 7 = coil holder shaft; 8 = coil holder; 9 = stationary miter gear; 10 = planetary miter gear; 11 = countershaft; 12 and 13 = toothed pulleys; 14 = toothed belt; 17a–c = flow tubes; 18 = side hole of the central shaft.

a pair of flanges of 24 cm in diameter, spaced 5 cm apart. The coiled column was prepared by winding a piece of PTFE (polytetrafluoroethylene) tubing (Zeus Industrial Products, Raritan, NJ, U.S.A.) directly around the holder hub, making either a single layer or multiple layers of coil as described in detail in Part II[9]. Here should be noted the advantages of the present design of the apparatus over that of the original X-axis CPC with the central coil position[2]. The lateral shift of the coil holder permits accommodation of a larger diameter coil holder which can extend in space beyond the center line of the rotary frame. This lateral coil position further provides a favorable hydrodynamic condition for most of the two-phase solvent systems in retention of the stationary phase, as discussed later in detail.

The pair of coiled columns on the rotary frame is connected in series to double the column capacity. The layout of the flow tubes (0.85 mm I.D. PTFE) is illustrated in Fig. 2A and B. A pair of flow tubes (17a) from the second column (Fig. 2B, right) first passes through the center hole of the holder shaft and, by making a loop (17b), enters the opening of the other holder shaft to reach the first column holder (Fig. 2B, left), where one flow tube (interconnecting tube) joins one end of the first column while the other bypasses the column. The flow tube from the other end of the first column and the latter flow tube from the second column exit the holder shaft together (17c) and then enter the side hole (18) (Fig. 2B) of the central shaft to reach the stationary tube-guide projecting down from the top plate of the centrifuge. These flow tubes are lubricated with grease and protected with a sheath of tygon tubing to prevent direct contact with metal parts. Under ordinary conditions, the flow tubes can maintain their integrity for many months of operation. As described earlier, these tubes are free from twisting and, therefore, serve for the continuous elution through the rotating coils without complications such as leakage and contamination.

Revolutional speed of the apparatus can be regulated up to 1000 rpm with a speed control unit (Bodine Electric Co., Chicago, IL, U.S.A.). In our laboratory the solvents are pumped with a Milton Roy metering pump while the effluent is continuously monitored with an LKB Uvicord S and fractionated with an LKB fraction collector.

ANALYSIS OF ACCELERATION GENERATED BY L–X PLANETARY MOTION

Analysis of the acceleration field produced by the original X-axis CPC at the central and lateral positions on the holder shaft has been reported earlier[2]. In order to discuss the hydrodynamics involved in the present system, it is desirable to briefly review the analysis of acceleration in the lateral position which can be directly applied to the L–X planetary motion illustrated in Fig. 1.

Fig. 3A shows planetary motion of an X-axis CPC where a cylindrical coil holder with radius r revolves around the central axis of the centrifuge system and simultaneously rotates about its own axis at the same angular velocity, ω, in the indicated directions. While doing so, the cylinder maintains the axial orientation perpendicular to and at a distance R from the central axis of the centrifuge. An arbitrary point, P, is located at the periphery of the cylinder at a distance l from point M on the central plane, as illustrated. Then, we observe the motion of point P as the cylinder undergoes the planetary motion described above.

In the reference x–y–z coordinate system for analysis, illustrated in Fig. 3B, the

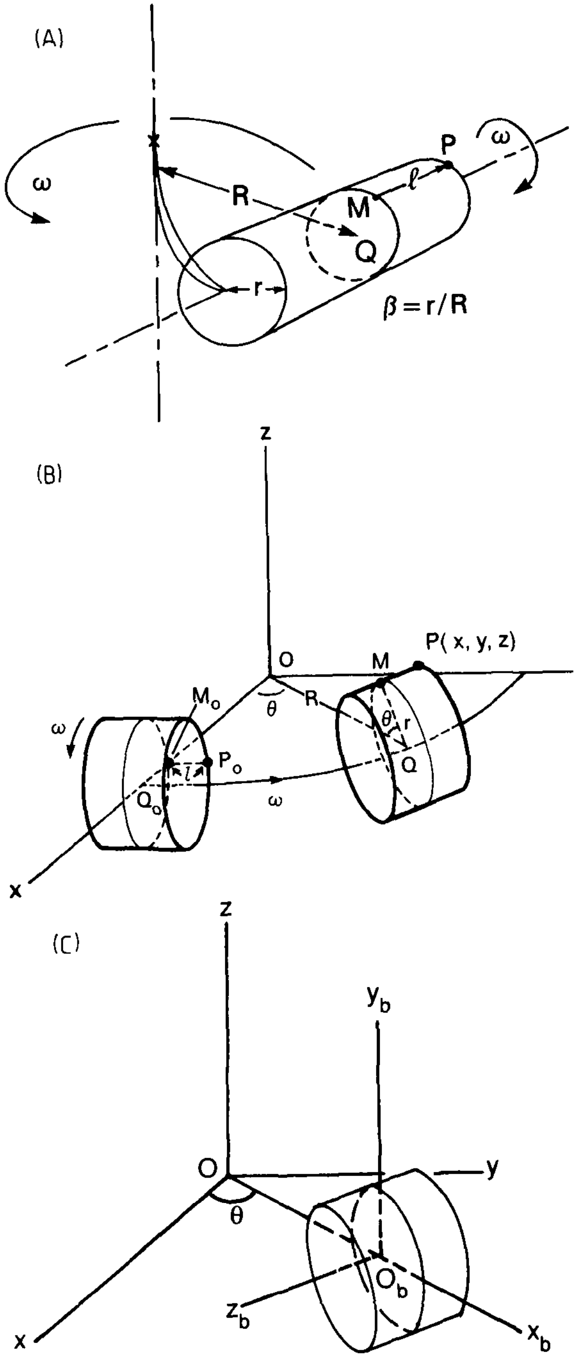

Fig. 3. Analysis of acceleration. (A) Mode of planetary motion. (B) Reference x–y–z coordinate system for analysis. (C) Relationship between the reference x–y–z coordinate system and x_b–y_b–z_b body coordinate system.

cylinder revolves around the z-axis at angular velocity ω and simultaneously rotates about its own axis at the same angular velocity in the indicated directions. The arbitrary point to be analyzed initially locates at point P_0 $(R-r,l,0)$ and, after a lapse of time t, moves to point P (x, y, z) which can be expressed in the following equations:

$$x = R\cos\theta - r\cos^2\theta - l\sin\theta \tag{1}$$

$$y = R\sin\theta - r\sin\theta\cos\theta + l\cos\theta \tag{2}$$

$$z = r\sin\theta \tag{3}$$

The acceleration acting on the arbitrary point is then obtained from the second derivatives of these equations as follows:

$$\mathrm{d}^2x/\mathrm{d}t^2 = -R\omega^2(\cos\theta - 2\beta\cos 2\theta) + l\omega^2\sin\theta \tag{4}$$

$$\mathrm{d}^2y/\mathrm{d}t^2 = -R\omega^2(\sin\theta - 2\beta\sin 2\theta) - l\omega^2\cos\theta \tag{5}$$

$$\mathrm{d}^2z/\mathrm{d}t^2 = -R\omega^2\beta\sin\theta \tag{6}$$

where $\beta = r/R$.

In order to visualize the effects of acceleration on the objects rotating with the cylinder, it is more appropriate to express the acceleration vectors with respect to the body frame or the x_b–y_b–z_b coordinate system illustrated in Fig. 3C. Transformation of the vectors from the original reference coordinate system to the body coordinate system may be performed according to the following equations:

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

$$\alpha_{y_b} = \mathrm{d}^2z/\mathrm{d}t^2 = -R\omega^2\beta\sin\theta \tag{8}$$

$$\alpha_{z_b} = (\mathrm{d}^2x/\mathrm{d}t^2)\sin\theta - (\mathrm{d}^2y/\mathrm{d}t^2)\cos\theta = -R\omega^2 2\beta\sin\theta + l\omega^2 \tag{9}$$

where α_{x_b}, α_{y_b}, and α_{z_b} indicate the acceleration vectors acting along the corresponding coordinate axes. Eqns. 7–9, thus obtained, may serve as general formulae of acceleration generated by three types of synchronous planetary motion, illustrated in Fig. 1, by designating two parameters, R and l, where $l/R = \pm 1$ for type L–X (the present scheme), $l = 0$ for type X, and $R = 0$ for type L.

From these equations, the centrifugal force vectors (same magnitude with the acceleration but acting in the opposite direction) at various points on the cylinder are computed for three types of planetary motion, *i.e.*, type L ($R = 0$), type L–X ($l/R = -1$) and type X ($l = 0$), and diagrammatically illustrated in Fig. 4A–C. In order to express 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 x_b-axis, whereas the third force vector, $-\alpha_{z_b}$, which acts perpendicularly to the x_b–y_b plane, is drawn as a vertical column along the y_b-axis. The ascending column indicates the force acting upward ($z_b > 0$) and the descending

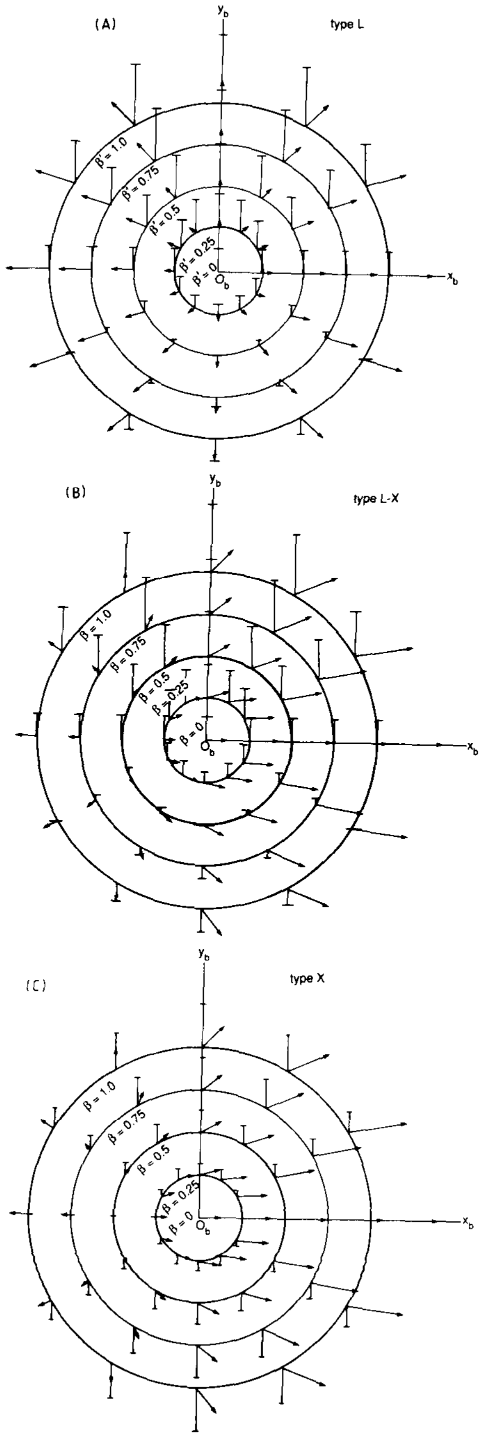

Fig. 4. Force distribution diagrams obtained from three mutually related synchronous planetary motions, type L (A), type L–X (B), and type X (C).

column, the force acting downward ($z_b < 0$). Several concentric circles around point O_b (the axis of the cylinder) indicate locations on the cylinder corresponding to parameter β or β' ($\beta' = r/l$ for type L planetary motion) indicated in the diagram. The distribution of the centrifugal force vectors in each diagram is fixed to the x_b–y_b–z_b body coordinate system and every point on the cylinder rotates around point O_b (z_b-axis) in either clockwise or counterclockwise direction as determined by the planetary motion of the cylinder.

As shown in these diagrams, the arbitrary point on the cylinder is subjected to a highly complex three-dimensional fluctuation of the centrifugal force field which varies in both magnitude and direction during each revolutional cycle. Force distribution pattern also changes with the location of the point on the cylinder where force vectors tend to increase their magnitude in the remote location from the axis of the cylinder. Each planetary motion generates a characteristic distribution of the centrifugal force vectors: type L planetary motion (Fig. 4A) forms a symmetrical distribution of outwardly radiating arrows around all circles and an asymmetric distribution of columns along the y_b-axis on the diagram. On the other hand, type X planetary motion (Fig. 4C) forms a contrasting pattern of the force distribution which consists of an asymmetric distribution of arrows along the x_b-axis and a symmetric distribution of column around point O_b. Interestingly enough, the hybrid type L–X (Fig. 4B) inherits asymmetric features from the parents forming asymmetric distributions of both arrows and columns each identical to that of the respective parent.

The effects of the above centrifugal force field to the hydrodynamic distribution and motion of two immiscible solvent phases in the coiled column is extremely complex and hardly predictable on the theoretical basis. However, it may be worthwhile to consider some possible hydrodynamic effects produced by the present L–X planetary motion in the light of the experimental results obtained from the various other types of planetary motions previously applied to CCC.

SPECULATION OF HYDRODYNAMIC EFFECTS OF L–X PLANETARY MOTION

Slow rotation of a coil around the axis laid horizontally in the gravitational field generates an Archimedean screw force which drives all objects of different density toward one end of the coil, which is conventionally called the head and the other end, the tail. When such a coil contains two mutually immiscible solvent phases, each phase is competitively pushed toward the head of the coil and the end result is that the two phases establish a hydrodynamic equilibrium where each phase occupies nearly equal space in each helical turn on the head side[10]. As the rotational speed of the coil is increased, the centrifugal force field produced by the rotation is superimposed on the gravitational field resulting in an asymmetrical distribution of the force field between the upper and the lower halves of the coil (Fig. 5A). This in turn alters the hydrodynamic equilibrium state in such a way that one of the phases (head phase), generally the heavier phase in this case, dominates the head of the coil. When the rotational speed reaches the critical range, the two solvent phases are completely separated along the length of the coil, the heavier phase entirely occupying the head side and the lighter phase on the tail side of the coil[10]. This unilateral hydrodynamic distribution of the two solvent phases, when combined with a strong centrifugal force field, provides the basis for the high-speed CCC[11].

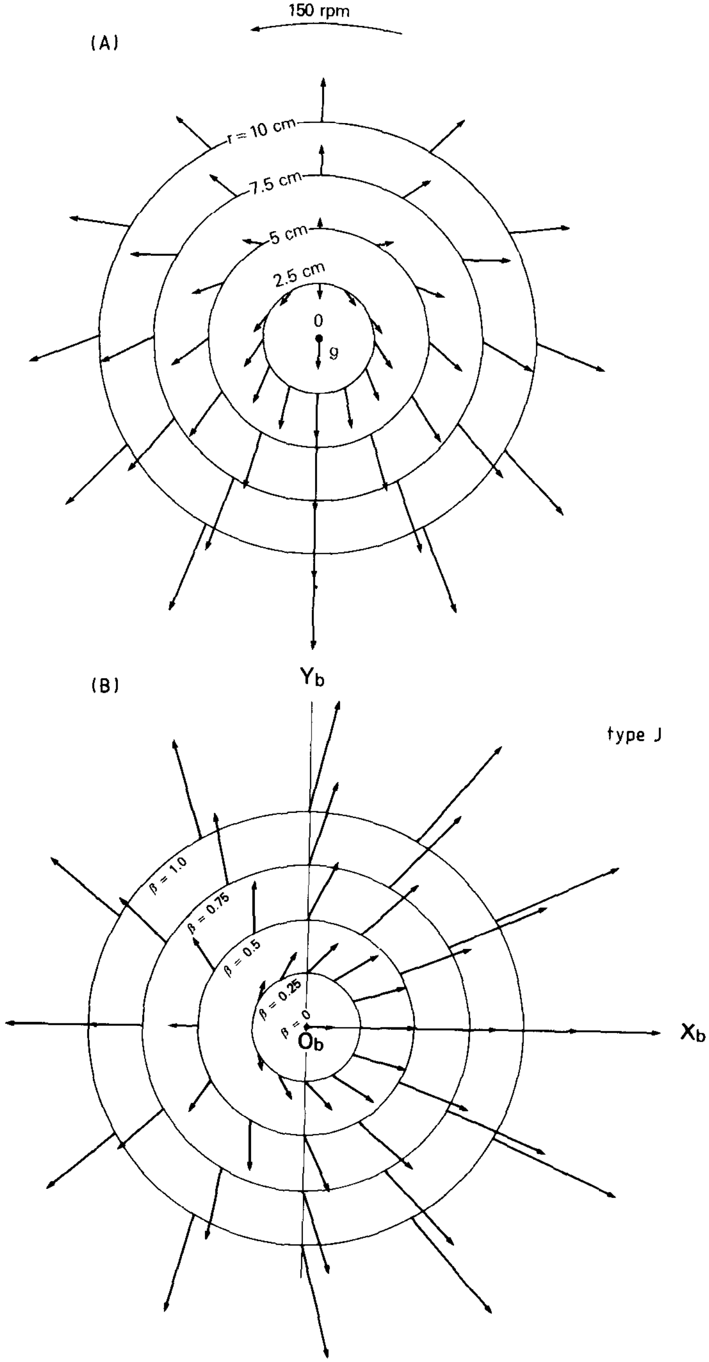

Fig. 5. Force distribution diagrams obtained from two different motions. (A) Combined force field of gravity and centrifugal force in simple rotation. (B) Centrifugal force field in type J synchronous planetary motion. Note the striking resemblance between the two.

Similar unilateral phase distribution is observed in the coil subjected to various types of planetary motion such as type J, type X and their hybrids, all of which generate an asymmetrical centrifugal force field between the proximal and distal position of the rotating coil. However, in these centrifuge systems, the mode of unilateral phase distribution varies according to the physical properties of the solvent system or, in a more convenient term, the settling time of the two solvent phases in the gravitational field[12]. In hydrophobic binary solvent systems with short settling times of 3–10 s, the lighter phase is always the head phase, whereas in hydrophilic butanol solvent systems with long settling times of 30–60 s, the heavier phase becomes the head phase. In the rest of the solvent systems with an intermediate range of settling times of 10–30 s, the head phase is determined by the mode of the planetary motion and further modified by the location of the coil on the holder expressed by β.

The type J planetary motion (Fig. 5B), which generates a strong two-dimensional centrifugal force field, usually distributes the lighter phase of the intermediate solvent systems toward the head of the coil[13]. This tendency is further enhanced in the remote location (large β value) on the holder where the magnitude of the radiating force vectors are increased in all directions. In the type X planetary motion (Fig. 4C), which generates a three-dimensional centrifugal force field, the magnitude of the main centrifugal force vectors is substantially reduced due to the formation of the lateral components. Subsequently, the intermediate solvent systems generally distribute the heavier phase toward the head[3]. However, it has been recently discovered that the hydrodynamic phase distribution in the type X planetary motion is significantly altered by shifting the position of the coil along the axis of the holder[5].

As shown in Fig. 4C, the type X planetary motion generates a lateral force field (columns) symmetrically on the upper and the lower segments of the coil. This symmetrical distribution of the lateral force vectors is altered by lateral shift of the coil position along the holder axis, while the main force field (arrows) remains unchanged. When this shift is applied toward the left, the lateral force component acting toward the left is added in all portions of the coil, resulting in an enhanced force field in the upper portion and a decreased force field in the lower portion of the coil as illustrated in Fig. 4B. According to the previous observation[5], hydrodynamic effects of this asymmetrical lateral force field may be divided into the following two categories: one on the head–tail phase distribution and the other on the retention of the stationary phase with respect to the direction of the planetary motion of the coil holder.

The lateral shift of the coil along the holder shaft adds a lateral force vector at the proximal and distal portions of the coil which may create an effect similar to that of an increased β value. In this situation, the intermediate solvent systems generate an inverse hydrodynamic trend to distribute the lighter phase toward the head as in the hydrophobic solvent systems. This effect becomes greater as the deviation ($\pm l$) of the coil position from the center of the holder shaft increases. Consequently, all intermediate solvent systems would eventually distribute the lighter phase toward the head of the coil as in the type J planetary motion.

The lateral shift of the coil further produces a peculiar effect on the retention of the stationary phase, particularly in the intermediate solvent group. In the type X planetary motion ($l = 0$), where the laterally acting force vectors display a symmetrical arrangement between the upper and the lower halves of the coil, retention of the stationary phase is not affected by direction of the planetary motion, whether it is counterclockwise (P_I) or clockwise (P_{II}) revolution, as far as the proper mode of the head–tail elution is applied to the coil. However, in the lateral coil position where the laterally acting force field becomes asymmetrical, the retention of the stationary phase is determined by combination of the head–tail elution mode and the planetary motion. Among four possible combinations, *i.e.*, head–tail/P_I, head–tail/P_{II}, tail–head/P_I, and tail–head/P_{II}, one particular condition yields the highest level of stationary phase retention which in most cases substantially exceeds that obtained from the type X planetary motion ($l = 0$).

The above description about the effect of lateral coil shift is mostly based on the findings previously obtained from the X-axis CPC with a large revolutional radius ($R = 20$ cm) equipped with a 25-cm wide coil holder where the ratio of the lateral shift (l) to the revolutional radius or l/R is limited to $\pm$ 0.625 (ref. 4). The present X-axis CPC model with a 10-cm revolutional radius holds the coil holder in the lateral positions to provide $l/R = -1.25 \pm 0.25$ and, therefore, will significantly enhance the above effects to improve the retention of the stationary phase in the coil.

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