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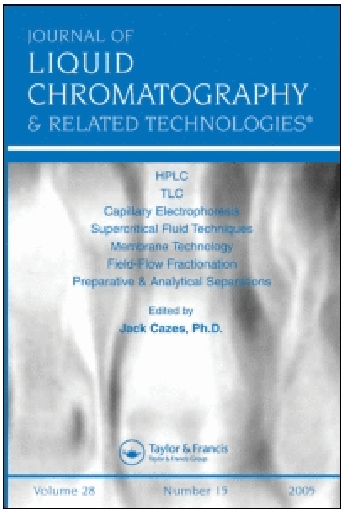

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Analysis of Acceleration Produced by Planetary Motion in a Nonsynchronous Coil Planet Centrifuge

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Abstract: The nonsynchronous coil planet centrifuge has a unique mode of planetary motion such that it provides freely adjustable coil rotation under a given centrifugal force field, and it produces an acceleration which fluctuates in a plane perpendicular to the axis of the holder. The two modes of planetary motion may be expressed as $P_{forward}$ (coil rotation and revolution in the same direction) (revolution/rotation = clockwise CW/CW or counterclockwise CCW/CCW) and $P_{backward}$ (coil rotation and revolution in the opposite directions) (revolution/rotation = CW/CCW or CCW/CW). The present study describes the analysis of acceleration produced by planetary motion in this apparatus. Our analysis is based on the equation for the absolute value of acceleration (A) in a given point on the column holder. A is calculated from each value obtained at the maximum and the minimum. The result indicated that the maximum value of A in $P_{forward}$ is larger than that in $P_{backward}$ under the same rotational rate, while the minimum value of A in $P_{forward}$ is smaller than that in $P_{backward}$. A higher rotational rate increases the difference of the value between $P_{forward}$ and $P_{backward}$. The overall results may support that the difference of maximum A and minimum A (ΔA) between $P_{forward}$ and $P_{backward}$ affects the retention of the stationary phase. Differences were observed experimentally in a

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previous study on protein separation with an aqueous two-phase solvent system using the nonsynchronous CCC apparatus.

Keywords: Countercurrent chromatography, Nonsynchronous coil planet centrifuge, Planetary motion, Acceleration

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INTRODUCTION

In the past, various types of rotary-seal-free flow-through centrifuges have been developed for performing countercurrent chromatography (CCC).[1–5] The most distinctive feature of this CCC method is a system based on liquid-liquid partition between two immiscible liquids, without a solid support that sometimes presents such problems as loss of samples by adsorption and chemical degradation of compounds. Among those instruments, both type-J multilayer coil planet centrifuge (type-J multilayer CPC) and cross-axis coil planet centrifuge (X-axis CPC) have proven to be the most useful models. The type-J multilayer CPC produces a synchronous planetary motion of the separation column, which revolves around the central axis of the centrifuge and simultaneously rotates about its own axis at the same angular velocity in the same direction. On the other hand, the X-axis CPC produces a synchronous planetary motion of the column in such a way that it revolves around the vertical axis of the centrifuge, while rotating about its horizontal axis at the same angular velocity. The difference in the planetary motion between these two instruments provides distinctive use of the two-phase solvent systems: The type-J multilayer CPC performs excellent separation with organic-aqueous two-phase solvent systems, whereas the X-axis CPC is used for highly polar two-phase solvent systems, such as aqueous two phase solvent systems (ATPS). However, this synchronous planetary motion limits the versatility of the methods, especially in the separation of cells where slower rotational speeds are required for the sedimentation of cell particles.

The nonsynchronous CPC, introduced first in 1979,[6] is a particular type of planet centrifuge which provides a desirable combination between rotation (about its own axis) and revolution (around the centrifuge axis) of the coil holder.[6–10] Previous studies demonstrated that this apparatus was useful for partition of cells[6,8,10] and plasmid DNA[8] using ATPSs as well as elutriation of cells according to their size and density.[6–9,11]

Our previous studies also revealed that this apparatus can be used for partition of proteins with ATPSs[12] and the head to tail elution mode produced somewhat better stationary phase retention (Sf approaching 0.5) and higher peak resolution regardless of the choice of the mobile phase, where the best result was obtained by the clockwise (CW) coil rotation for the lower mobile phase or by the counterclockwise (CCW) coil rotation for the upper mobile phase, both under CCW revolution of the rotor.[13]

The present study describes the analysis of acceleration produced by planetary motion in the nonsynchronous CPC.

EXPERIMENTAL

Apparatus

The nonsynchronous CPC employed in the present studies was constructed at the Machining Technology Center of Nihon University, Chiba, Japan. The design of the apparatus was previously described in detail[8,9,12] and a brief description is given here. The apparatus has a distinctive feature which allows a freely adjustable rotational rate of the coiled separation column (between 0 and $\pm$ 80 rpm) at any given revolution speed. The effluent is eluted through the rotating column without the use of conventional rotary seal device.

Figure 1 is a simplified drawing to comprehensively illustrate the rotational rate of various parts of the system. The rotary frame is divided into 2 parts, frame 1 directly driven by the main motor, and frame 2 supporting the column holder. When the side motor is at rest, the main motor drives frame 1 at an angular velocity ω_a, and frame 2 and the column holder at a doubled speed of $2\omega_a$. When the side motor rotates at ω_b, the rotation rate of the frame 2 is modified in such a way that it rotates at $2\omega_a - \omega_b$. Thus, the rotation-revolution ratio of the column is expressed by $\omega_b/(2\omega_a - \omega_b)$.

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Analysis of Acceleration

Considering a discoid body with radius r that undergoes a nonsynchronous planetary motion in such a way that it revolves around the central axis at

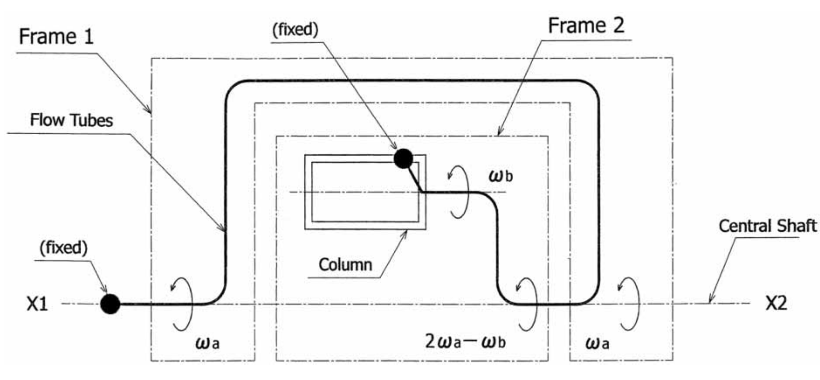

Figure 1. Schematic illustration of revolution mechanism of the nonsynchronous CPC.

ω_a, with a revolution radius, R, and simultaneously rotates about its own axis at ω_b. Then, studying the motion of an arbitrary point on the periphery of the discoid body, the following treatment can be proposed.

Figure 2 shows an $x-y$ coordinate system where the center of the discoid body initially locates at point Q_0 $(R, 0)$ and the arbitrary point at P_0 $(R + r, 0)$. The center of the discoid body circles around point 0 in $x-y$ plane and, at time t, moves angle $\omega_a t$ to reach point Q $(R \cos \omega_a t, R \sin \omega_a t)$. Therefore, the location of the arbitrary point on the discoid body is expressed by $P(x, y)$ according to the following equations:

$$\begin{aligned} x &= R\cos\omega_a t + r\cos(\omega_a t + \omega_b t), \\ y &= R\sin\omega_a t + r\sin(\omega_a t + \omega_b t). \end{aligned} \tag{1}$$

And, the acceleration caused by centrifugal force in point P given by

$$\begin{aligned} \frac{d^2x}{dt^2} &= -R\omega_a^2\cos\omega_a t - r(\omega_a + \omega_b)^2\cos(\omega_a t + \omega_b t), \\ \frac{d^2y}{dt^2} &= -R\omega_a^2\sin\omega_a t - r(\omega_a + \omega_b)^2\sin(\omega_a t + \omega_b t). \end{aligned} \tag{2}$$

The absolute value of acceleration A produced by the planetary motion is then computed from the following equation:

$$A = \sqrt{\left(\frac{d^2x}{dt^2}\right)^2 + \left(\frac{d^2y}{dt^2}\right)^2} \tag{3}$$

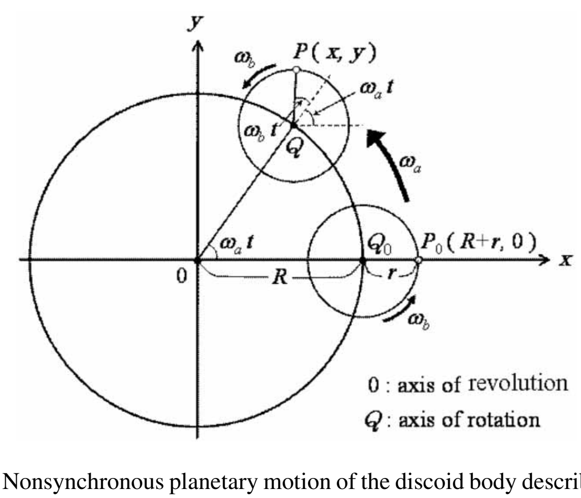

Figure 2. Nonsynchronous planetary motion of the discoid body described in the x−y coordinate system.

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Consequently, the above Eq. (2) into (3) leads to the following final equation:

$$A = \sqrt{R^2\omega_a^4 + r^2(\omega_a + \omega_b)^4 + 2Rr\omega_a^2(\omega_a + \omega_b)^2 \cos \omega_b t} \tag{4}$$

For convenience, the two modes of planetary motion (direction of rotation and revolution of the coil) may be expressed as $P_{forward}$ (coil rotation and revolution in the same direction) (revolution/rotation = CW/CW or CCW/CCW) and $P_{backward}$ (coil rotation and revolution in the opposite direction) (revolution/rotation = CW/CCW or CCW/CW).

Figure 3 illustrates a set of centrifugal force distribution diagrams produced by the nonsynchronous coil planet centrifuge. The center row labeled nonplanetary ($\omega = 0$) represents the hydrostatic equilibrium system where all centrifugal force vectors are directed radially outward from the center of revolution. In this force distribution pattern, the retention of the stationary phase becomes quite stable but always less than 50% of the total column capacity (Sf < 0.5), while the mixing effect between two solvent phases entirely depends upon the flow of the mobile phase as in centrifugal partition chromatography. From this nonplanetary system, starting the coil rotation upward in backward direction ($P_{backward}$) gradually alters the centrifuge force vectors, in both magnitude and direction, finally reaching the type-I synchronous planetary motion at the top of the diagram where the unit magnitude of centrifugal force vector uniformly rotates around every point on the rotating coil. This pattern of the centrifugal force field produces efficient mixing of the two phases while the retention of the stationary phase is again limited to less than half of the total column capacity. On the other hand, starting the coil rotation downward in forward direction ($P_{forward}$) increases the magnitude of the force vectors finally reaching the type-J synchronous planetary motion as shown at the bottom of the diagram. In this high-speed CCC system, both mixing and retention of the stationary phase becomes satisfactory for most of the organic-aqueous solvent systems whereas the system fails to retain the aqueous-aqueous polymer phase systems due to emulsification caused by violent mixing of the two phases.

The coil rotation applied in our studies are limited to less than 1/10 of the revolution as shown in the second and the fourth rows in the diagram. Nevertheless, each system ($P_{backward}$ and $P_{forward}$) bears some characteristic features of the force distribution displayed by the two different synchronous planetary motions described above. Consequently, $P_{forward}$ coil rotation can produce greater centrifugal force and higher retention of the stationary phase than $P_{backward}$ coil rotation. The β value of our nonsynchronous coil planet centrifuge is about 0.2 so that the distribution pattern and magnitude of the force vectors are close to those at = 0.25.

As reported earlier, two immiscible solvent phases in a slowly rotating coil around its horizontal axis are distributed evenly at the head of the coil

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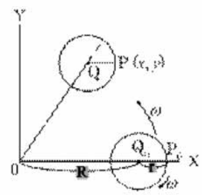
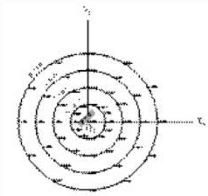
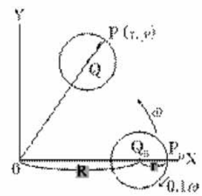
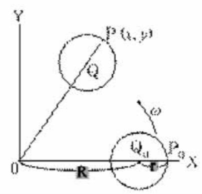
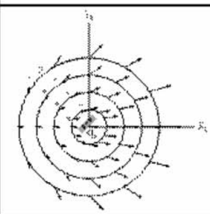
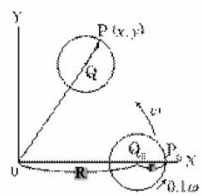
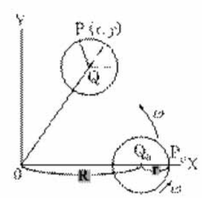
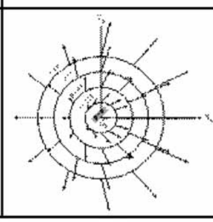
Planetary Motion	Coil Rotation	Planetary Motion and Reference Coordinate Frame	Force Distribution Diagram
Type I	$-\omega$		
Nonsynchronous ($P_{backward}$)	-0.1ω		
Nonplanetary	0		
Nonsynchronous ($P_{forward}$)	$+0.1\omega$		
Type J	$+\omega$		

Figure 3. A set of the centrifugal force distribution diagrams for five types of planetary motion.

as in the type-I synchronous planetary centrifuge.[14] In this case, either phase should be introduced from the head toward the tail to retain the other phase as the stationary phase. In the present study, the similar situation is provided by the backward coil rotation ($P_{backward}$). However, as the coil rotation is increased, the distribution of the force field becomes uneven due to the

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centrifugal force field produced by the rotation resulting in increased force field at the lower side of the coil and reduced force field at the upper side of the coil. In this situation which is somewhat similar to the forward coil rotation ($P_{forward}$), the distribution of the two phases in the coil is modified in such a way that the lower phase tends to occupy more space in the head of the coil. In this case, the elution with the lower phase from the head of the coil produces less retention of the stationary phase whereas the elution with the upper phase in the same direction yields higher retention of the stationary phase. The above assumption is well consistent with the results of proteins separation with an ATPS previously reported.[13]

Figure 4 illustrates the distribution of centrifugal force vectors produced by the planetary motion at the revolution speed of 800 rpm and at the rotation speed of 80 rpm in $P_{forward}$. In the diagram, the center of revolution coincides with the center of the *x*-*y* body coordinate system and a locus of an arbitrary point on the discoid body is described when the discoid body undergoes half

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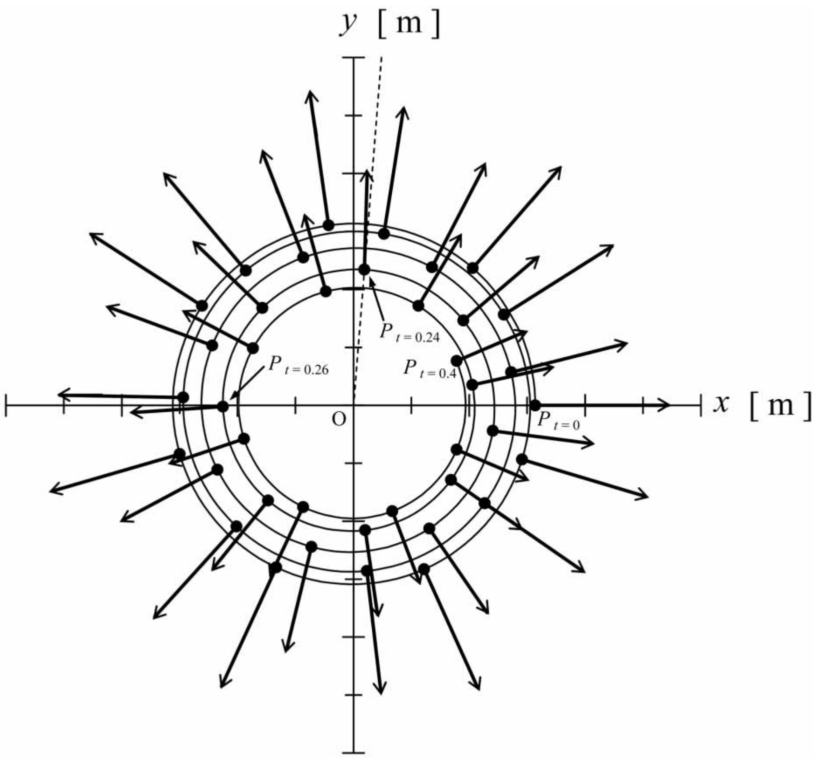

Figure 4. Distribution of the centrifugal force vectors produced by the nonsynchronous planetary motion at high speed revolution of 800 rpm and at low speed coil rotation at 80 rpm in $P_{forward}$ (revolution/rotation = CW/CW) mode during half rotation of the discoid body.

rotation. Each force vector was calculated from Eq. (2) at 10 millisecond intervals. As shown in Fig. 4, the direction of each vector slightly slips out from the line through the center of revolution axis except at $P_{t=0}$. For example, at $P_{t=0.26}$, the opposite side of $P_{t=0}$, the vector clearly slips out from the x axis and also, at $P_{t=0.24}$, the vector has already been almost parallel to the y axis. The vector at $P_{t=0}$ is larger than that $P_{t=0.4}$, where the difference corresponds to that of A value between $P_{t=0}$ and $P_{t=0.4}$ as described in Fig. 4. In general, it has been suggested that the mixing and settling of two phases are alternately carried out to perform the efficient partitioning in the rotating coil under the centrifugal force field. The change of the centrifugal force acting at a point P may affect these phase distributions in the coil with the direction of rotation and revolution. In our previous studies,[12] the satisfactory separation of proteins was successfully performed using the nonsynchronous CPC with an ATPS.

Figure 5 illustrates the change of A values in $P_{forward}$ (A) and in $P_{backward}$ (B) both at the revolution speed of 800 rpm, where the actual values of R = 127 mm and r = 30 mm were used for the calculation from Eq. (4). Each curve obtained for various rotation speeds at 10, 20, and 80 rpm depicts a cosine-like curve each with a different frequency. The maximum value of A (A_{max}) in $P_{forward}$ is larger than that in $P_{backward}$, while the minimum value of A (A_{min}) in $P_{forward}$ is smaller than that in $P_{backward}$. From the Eq.(4), A_{max} and A_{min} are given by:

$$\left.\begin{aligned} A_{max} &= \sqrt{R^2\omega_a^4 + r^2(\omega_a+\omega_b)^4 + 2Rr\omega_a^2(\omega_a+\omega_b)^2} \\ A_{min} &= \sqrt{R^2\omega_a^4 + r^2(\omega_a+\omega_b)^4 - 2Rr\omega_a^2(\omega_a+\omega_b)^2} \end{aligned}\right\} \quad (5)$$

and the magnitude of fluctuation of acceleration ΔA is expressed as follows:

$$\Delta A = A_{max} - A_{min} \quad (6)$$

Figure 6 illustrates the change of ΔA at various rotation speeds under a given revolution speed of 800 rpm. A higher rotational rate increases the difference of the value between $P_{forward}$ and $P_{backward}$.

In the present system, using the combination of high speed revolution (800 rpm) and low speed coil rotation (0–80 rpm) both in either direction, the two phases are distributed in a rotating coil at nearly equal volumes from the head end, while any excess of either phase remains at the tail end. Here, head-tail orientation of the rotating coil is defined according to the Archimedean screw effect, where all objects of different densities present in the coil are driven toward the head of the coil. The hydrodynamic condition produces the maximum stationary phase retention at 50% of the total column capacity by pumping either phase from the head end of the coil, whereas the elution of the mobile phase from the tail toward the head results in no retention of the stationary phase. However, the sufficient

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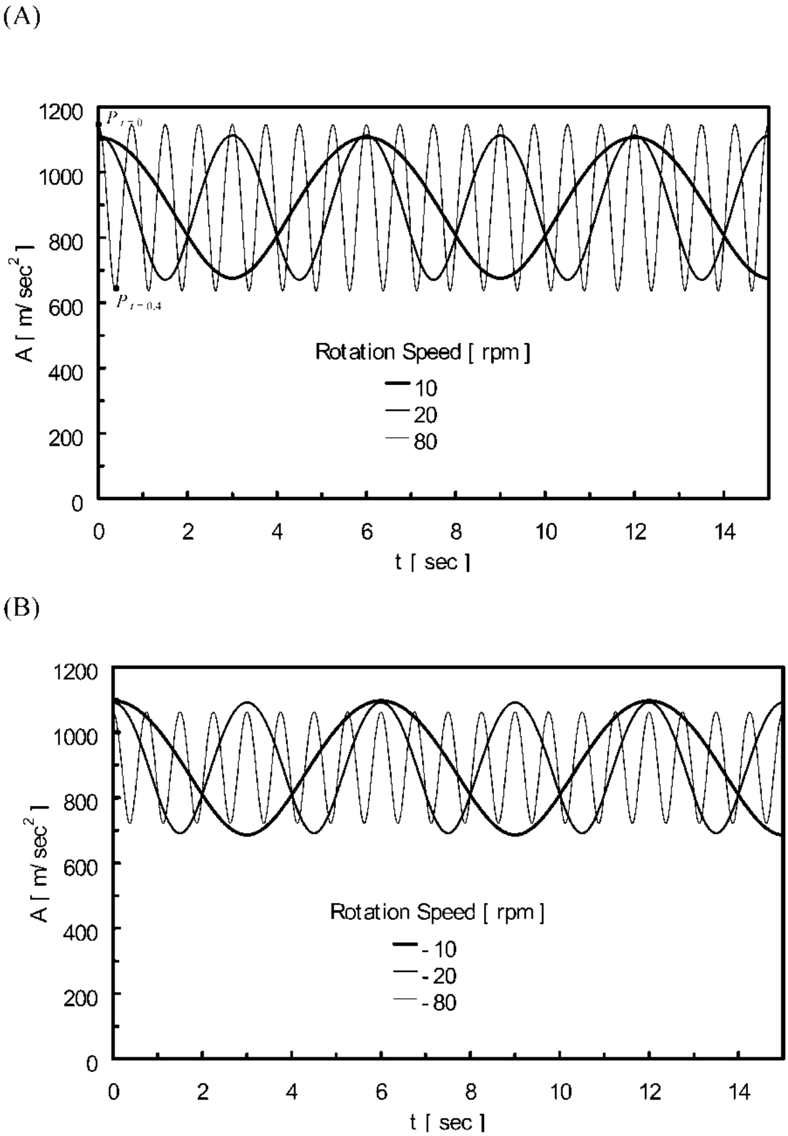

Figure 5. Change of the acceleration *A* at various rotation speeds in P_{foward} (A) and $P_{backward}$ (B) modes.

separation of proteins was experimentally achieved using an ATPS while the stationary phase retention was low, around 30% (Sf = 0.3) as described in our previous studies.[12] This result indicates that the volume of the stationary phase retained in the column is weak but still enough for the separation even the ratio is less than 50%.

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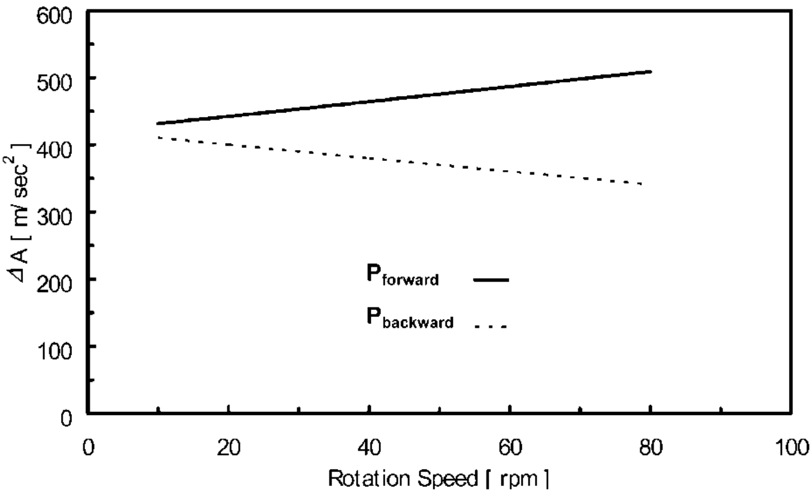

Figure 6. Change of the difference between maximum A and minimum A (ΔA) at various rotation speeds in $P_{forward}$ and $P_{backward}$ modes.

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Our previous studies on protein separation by the nonsynchronous CPC[13] revealed that $P_{forward}$ planetary motion (revolution/rotation = CW/CW) produced less retention of the upper stationary phase at high rotation speeds, while $P_{backward}$ (revolution/rotation = CCW/CW) always gave relatively high retention of the stationary phase of around 30% regardless of the rotation speed. The above analysis of acceleration may explain this phenomenon in such a way that increased ΔA could result in less retention of the stationary phase in the coiled column in the nonsynchronous CPC. Therefore, the $P_{backward}$ mode with the reduced ΔA may produce the possibility of new machinery design of the type-I multilayer synchronous CPC for the separation of proteins with low interfacial tension two-phase solvent systems while the cross-axis CPC can be used for the protein separation.

CONCLUSION

The absolute value of acceleration A produced by the planetary motion in the nonsynchronous CPC was analyzed mathematically for two different operation modes of $P_{forward}$ and $P_{backward}$. The A maximum in $P_{forward}$ is larger than that in $P_{backward}$ and the A minimum in $P_{forward}$ is smaller than that in $P_{backward}$ under otherwise identical operation conditions. A higher rotational rate increases the difference of A values (ΔA) between $P_{forward}$ and $P_{backward}$. This may affect the retention of the stationary phase of ATPSs in protein separation. Compared to the J-type coil planet centrifuge, the weak point of the non-synchronous CPC is its low ability to retain the liquid stationary phase with a maximum Sf always lower than 0.5 (50%), its strong point is that it is able to retain the very polar ATPS aqueous-aqueous liquid systems with a Sf ratio that are very useful in protein and biological separations.

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