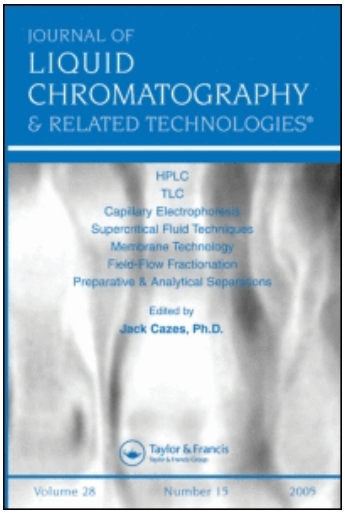

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Protein and Cell Separations using Nonsynchronous Coil Planet Centrifuge

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To cite this Article Shinomiya, Kazufusa , Kobayashi, Koji , Oshima, Hisashi , Okada, Tadashi , Yanagidaira, Kazuhiro and Ito, Yoichiro(2007) 'Protein and Cell Separations using Nonsynchronous Coil Planet Centrifuge', Journal of Liquid Chromatography & Related Technologies, 30: 18, 2681 — 2698

To link to this Article: DOI: 10.1080/10826070701560579

URL: http://dx.doi.org/10.1080/10826070701560579

Journal of Liquid Chromatography & Related Technologies®, 30: 2681–2698, 2007

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070701560579

Protein and Cell Separations using Nonsynchronous Coil Planet Centrifuge

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Abstract: The nonsynchronous coil planet centrifuge has a unique mode of planetary motion in that it allows a freely adjustable rotational rate of the coiled separation column at a given revolution speed. This paper describes a series of studies using the apparatus fabricated in our laboratory on the countercurrent chromatographic separation of proteins with aqueous-aqueous polymer phase systems, experimental and theoretical analysis of the effect of planetary motion on protein separation, and application to elutriation of cells such as blood cell components and mast cells using a single-phase physiological buffer solution.

Keywords: Nonsynchronous coil planet centrifuge, Countercurrent chromatography, Planetary motion, Protein separation, Polymer phase system, Acceleration, Elutriation, Blood cell separation, Mast cell separation

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INTRODUCTION

Countercurrent chromatography (CCC) is known as one of liquid partition chromatography, but without using a solid support that sometimes presents such problems as loss of samples by adsorption and chemical degradation of compounds.[1] Since the advent of CCC in 1970, many CCC instruments have been developed and widely used for the separation and purification of natural and synthetic products.[2–6] Among those CCC systems, the type-J multilayer coil planet centrifuge (CPC) and the cross-axis CPC have proven to be the most useful and effective 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. The cross-axis CPC, on the other hand, 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, where the type-J multilayer CPC performs excellent separation with organic-aqueous two-phase solvent systems, whereas the cross-axis CPC is especially used for aqueous two-phase solvent systems (ATPSs), which have a low interfacial tension and a small density difference between the two phases. 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[7] is considered most versatile in that it provides desirable combination between rotation (about its own axis) and revolution (around the centrifuge axis) of the coil holder.[7–11] This system offers a unique separation method for cells and macromolecules by freely adjustable coil rotation under a given centrifugal force field.

Previous studies demonstrated that this apparatus was useful for partition of cells[7,9,11] and plasmid DNA[9] using ATPSs as well as elutriation of cells according to their size and density.[7–10,12] The present paper describes a series of studies using the apparatus on the protein separation with ATPSs,[13] experimental[14] and theoretical analysis[15] of the effect of planetary motion on protein separation, and elutriation of blood cell components and mast cells with a physiological buffer solution.[16]

EXPERIMENTAL

Apparatus

The apparatus has a distinctive feature which allows a freely adjustable rotational rate of the coiled separation column (between 0 and ± 60 rpm) at

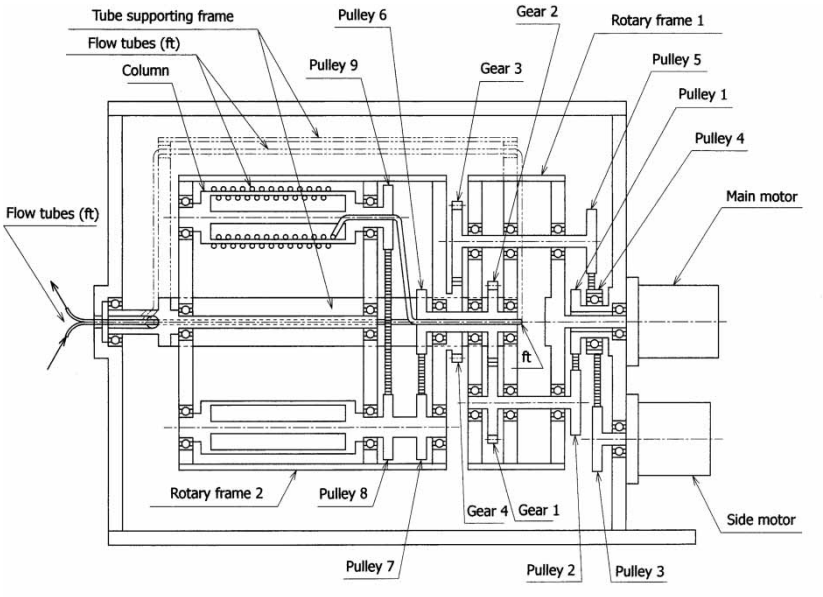

Figure 1. Schematic drawing of the cross-sectional view of the nonsynchronous CPC fabricated in our laboratory. Cited from Figure 1 of Ref. [13].

any given revolution speed while the effluent is eluted through the rotating column without the use of conventional rotary seal device.

Figure 1 illustrates the schematic drawing of the nonsynchronous CPC fabricated at the Machining Technology Center of Nihon University, Chiba, Japan. Figure 2 also shows the revolution mechanism of the apparatus.

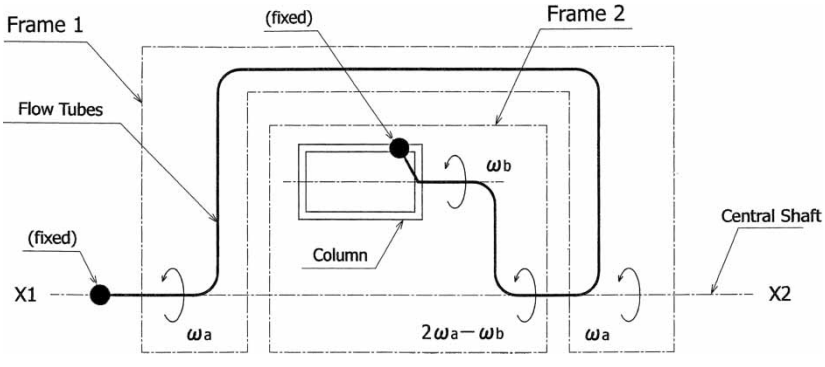

Figure 2. Schematic illustration of revolution mechanism of the nonsynchronous CPC. Cited from Figure 1 of Ref. [15].

Table 1. Angular velocities of motors and rotary frames on nonsynchronous coil planet centrifuge

Main motor	Side motor	Rotary frame 1	Rotary frame 2	Column		
				Rotation	Revolution	Total
$+\omega_a$	0	$+\omega_a$	$+2\omega_a$	0	$+2\omega_a$	$+2\omega_a$
0	$+\omega_b$	0	$-\omega_b$	$+\omega_b$	$-\omega_b$	0
$+\omega_a$	$+\omega_b$	$+\omega_a$	$+2\omega_a - \omega_b$	$+\omega_b$	$+2\omega_a - \omega_b$	$+2\omega_a$

The main motor drives the rotary frame 1 at an angular velocity, ω_a, around the central axis of the centrifuge. Rotary frame 1 carries a pair of countershafts which convey the motion to rotary frame 2: the lower countershaft rotates frame 2 via pulleys 3, 4, 5, and gears 3 and 4, whereas the upper countershaft rotates pulley 6 on a center piece via pulley 1 (stationary) and 2, and gears 1 and 2. When the side motor is at rest, these two countershafts synchronously rotate at ω_a on rotary frame 1, and therefore, both rotary frame 2 and pulley 6 (on the center piece) rotate at the same doubled speed at $2\omega_a$. In this case, the column holder simply revolves with rotary frame 2 without rotating about its own axis.

When the side motor rotates at ω_b, this motion is conveyed through the upper countershaft changing the rotation speed of rotary frame 2 at $2\omega_a - \omega_b$. The difference in rotation rate between rotary frame 2 and pulley 6 on the center piece is then conveyed to the coil holder shaft through pulleys 7, 8, and 9, causing the column holder to rotate at ω_b about its own axis. Thus, the rotation-revolution ratio of the column holder is expressed as $\omega_b/(2\omega_a - \omega_b)$. The relationships in angular velocity between the motors and rotary frames are summarized in Table 1.

Protein Separation[13]

The problem that the type-J multilayer CPC generally fails to retain the ATPS is largely alleviated in the present nonsynchronous CPC system by applying a low coil rotation rate, which gives the two phases enough time to settle under a strong centrifugal force field. Protein separation using ATPSs was performed to evaluate the capability of the apparatus.

Figure 3A illustrates a set of CCC chromatograms obtained using a multilayer coil (0.8 mm I.D.) with an ATPS composed of 12.5% (w/w) polyethylene glycol (PEG) 1000–12.5% (w/w) dibasic potassium phosphate. Using the lower phase as the mobile phase, the best separation was attained by the 39 mL capacity coil at 10 rpm rotation in the head to tail elution mode where the peak resolution between cytochrome *c* and myoglobin peaks was 1.6 and that between myoglobin and lysozyme peaks, 1.9 with the stationary phase retention at 35.0%. With the upper phase mobile (Figure 3B), the best

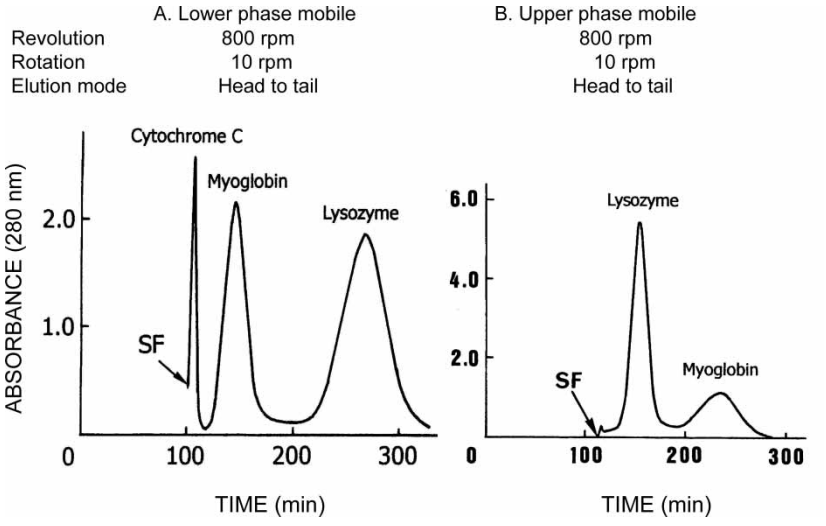

Figure 3. CCC chromatograms of proteins obtained by the nonsynchronous CPC with coaxial multilayer coils. Experimental conditions: apparatus: nonsynchronous CPC equipped with coaxial multilayer coils, 0.8 mm I.D. × 1.59 mm O.D. and 39 mL capacity; sample: cytochrome *c* (2 mg), myoglobin (8 mg) and lysozyme (10 mg); solvent system: 12.5% (w/w) PEG 1000–12.5% (w/w) dibasic potassium phosphate: (A) lower phase, (B) upper phase; flow rate: 0.2 mL/min; revolution 800 rpm; rotation 10 rpm. SF = solvent front.

separation was obtained at 10 rpm in the head to tail elution mode. Elution in the tail to head direction produced extremely low retention of the stationary phase in both lower and upper phase mobile.

When using a different ATPS of 4.4% (w/w) PEG 8000–7.0% (w/w) dextran T500 in 5 mM potassium phosphate buffer containing 2 M sodium chloride, the separation of lysozyme and myoglobin was achieved with the upper phase mobile in the head to tail elution mode as illustrated in Figure 4. The resolution between lysozyme and myoglobin peaks was 1.5 with the stationary phase retention at 19.7%.

Further experiments on partition efficiency of protein separation were performed with the eccentric coil assembly (0.8 mm I.D.) at the total capacity of 20 mL. Good separation was also obtained by eluting the lower phase in the head to tail elution mode.

Effects of Planetary Motion

Experimental Analysis[14]

The acceleration produced by the nonsynchronous planetary motion fluctuates in a plane perpendicular to the axis of the holder. In the present system using

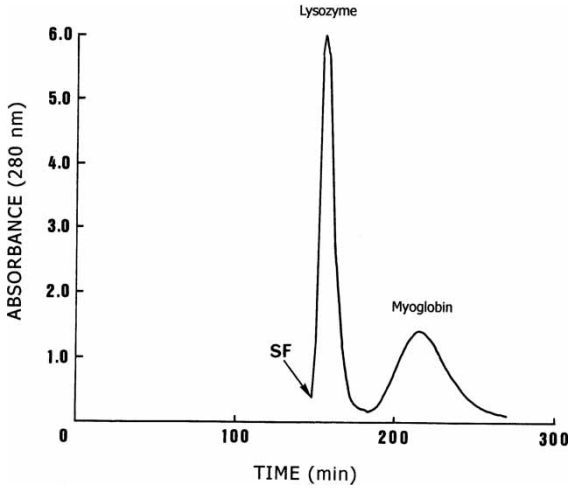

Figure 4. CCC separation of lysozyme and myoglobin by the nonsynchronous CPC with an aqueous-aqueous polymer phase system. Experimental conditions: apparatus: nonsynchronous CPC equipped with coaxial multilayer coils, 0.8 mm I.D. × 1.59 mm O.D. and 39 mL capacity; sample: myoglobin (8 mg) and lysozyme (10 mg); solvent system: 4.4% (w/w) PEG8000–7.0% (w/w) dextran T500 in 5 mM potassium phosphate buffer (pH 7.0) containing 2 M sodium chloride; mobile phase: upper phase; flow rate: 0.2 mL/min; revolution: 800 rpm; rotation: 10 rpm. SF = solvent front. Cited from Figure 6 of Ref. [13].

the combination of high speed revolution (800 rpm) and low speed coil rotation (0–60 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, the 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. This hydrodynamic condition produces the stationary phase retention of 50% maximum of the total column capacity by pumping either phase from the head end of the coil, whereas the introduction of the mobile phase from the tail end would lead to continuous carryover of the stationary phase resulting in detrimental loss of peak resolution. It has been found that the mode of planetary motion (direction of the coil rotation and the revolution) produces substantially different results in protein separation. For convenience, these 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 direction) (revolution/rotation = CW/CCW or CCW/CW).

Table 2 summarizes the analytical data computed from the chromatograms obtained using an ATPS composed of 12.5% (w/w) PEG 1000 and 12.5% (w/w) dibasic potassium phosphate by the head to tail elution

Table 2. Analytical values computed from CCC chromatograms of proteins obtained by the lower phase mobile in the head to tail elution mode using the nonsynchronous CPC

Revolution (rpm)	Rotation (rpm)	Retention time (min)			Resolution factor (Rs)		Theoretical plates (N)	Stationary phase retention (%)
		Cyt C	Myo	Lys	Cyt C/Myo	Myo/Lys		
800 (CW)	10 (CW)	62	76	120	0.6	1.1	83	29.3
800 (CW)	20 (CW)	66	82	120	1.3	1.3	225	19.0
800 (CW)	40 (CW)	70	86	117	1.3	1.2	350	12.4
800 (CW)	60 (CW)	74	88	109	1.0	1.2	418	0
800 (CCW)	10 (CW)	60	75	124	0.7	1.1	97	34.8
800 (CCW)	20 (CW)	70	88	134	1.2	1.4	213	31.0
800 (CCW)	40 (CW)	66	84	130	1.2	1.5	210	33.3
800 (CCW)	60 (CW)	72	86	124	1.1	1.3	263	28.6

Abbreviations: CW = Clockwise direction; CCW = Counterclockwise direction; Cyt C = Cytochrome C; Myo = Myoglobin; Lys = Lysozyme. The theoretical plates were computed from the myoglobin peak of each chromatogram.

mode of lower phase. $P_{forward}$ planetary motion (revolution/rotation = CW/CW) produces less retention of the stationary phase at higher rotation speeds, while $P_{backward}$ (revolution/rotation = CCW/CW) always gives high retention of stationary phase at around 30%, regardless of the rotation speed. The best separation in these experiments was obtained at the coil rotation speed of 40 rpm in $P_{backward}$ (CCW/CW) planetary motion and at the revolution speed of 800 rpm. These results suggest that the eccentric coil assembly used in the present studies does not display the bilateral hydrodynamic distribution as observed in the type-J multilayer CPC with organic-aqueous solvent system. It is most likely that the difference in the stationary phase retention between the above two planetary motions may be caused by the effects of the Coriolis force, as observed in the toroidal coil CCC system,[17] and in the centrifugal partition chromatography.[18]

Table 3 summarizes the analytical data computed from the chromatograms obtained by the head to tail elution of upper phase in $P_{forward}$ planetary motion (CW/CW) in the upper half, and $P_{backward}$ planetary motion (CCW/CW) in the lower half. Both groups show similar stationary phase retention around 20%, regardless of the rate of coil rotation.

Table 4 similarly summarizes the analytical data computed from the chromatograms obtained by eluting with the upper phase from the head toward the tail in $P_{backward}$ planetary motion (CW/CCW) in the upper half, and in $P_{forward}$ (CCW/CCW) in the lower half. Good stationary phase retention of over 30% was obtained in $P_{forward}$ planetary motion (CCW/CCW), and only slightly less

Table 3. Analytical values computed from CCC chromatograms of proteins obtained by the upper phase mobile in the head to tail elution mode with the same flow direction of the lower phase mobile

Revolution (rpm)	Rotation (rpm)	Retention time (min)		Resolution factor (Rs)	Theoretical plates (N)	Stationary phase retention (%)
		Lys	Myo	Lys/Myo		
800 (CW)	10 (CW)	90	113	0.5	198	17.5
800 (CW)	20 (CW)	88	108	0.5	247	24.4
800 (CW)	40 (CW)	88	112	0.8	364	20.0
800 (CW)	60 (CW)	86	119	1.3	440	24.0
800 (CCW)	10 (CW)	88	111	0.7	254	17.5
800 (CCW)	20 (CW)	88	110	0.7	366	17.5
800 (CCW)	40 (CW)	88	112	0.8	336	19.2
800 (CCW)	60 (CW)	88	114	1.0	603	17.5

Abbreviations: CW = Clockwise direction; CCW = Counterclockwise direction; Myo = Myoglobin; Lys = Lysozyme. The theoretical plates were computed from the lysozyme peak of each chromatogram.

retention of 20–30% in $P_{backward}$ planetary motion (CW/CCW). Because of higher retention of the stationary phase, the peak resolution in these groups is better than those summarized in Table 3. One clear cut finding is that partition efficiency expressed in terms of theoretical plate number (N) is strongly correlated with the coil rotation rates, i.e., the higher the coil

Table 4. Analytical values computed from CCC chromatograms of proteins obtained by the upper phase mobile in the head to tail elution mode with the reversed flow direction of the lower phase mobile

Revolution (rpm)	Rotation (rpm)	Retention time (min)		Resolution factor (Rs)	Theoretical plates (N)	Stationary phase retention (%)
		Lys	Myo	Lys/Myo		
800 (CW)	10 (CCW)	100	141	0.9	269	31.7
800 (CW)	20 (CCW)	88	115	0.9	347	30.0
800 (CW)	40 (CCW)	86	118	1.0	386	21.5
800 (CW)	60 (CCW)	89	112	0.8	430	25.0
800 (CCW)	10 (CCW)	97	136	0.9	197	33.7
800 (CCW)	20 (CCW)	87	116	0.8	327	35.0
800 (CCW)	40 (CCW)	85	122	1.1	340	34.0
800 (CCW)	60 (CCW)	87	118	1.0	376	34.0

Abbreviations: CW = Clockwise direction; CCW = Counterclockwise direction; Myo = Myoglobin; Lys = Lysozyme. The theoretical plates were computed from the lysozyme peak of each chromatogram.

rotation rate, the greater N value is obtained. This is apparently due to more efficient mixing of viscous polymer phases, which tend to form a laminar flow under gentle coil rotation.

The overall results indicate that the partition efficiency on protein separation with ATPSs using the nonsynchronous CPC is remarkably affected by the mode of planetary motion of the coiled separation column. The protein separation was optimized at $P_{backward}$ (CCW/CW) of the coil rotation for the lower phase mobile and at $P_{forward}$ (CCW/CCW) for the upper phase mobile, both in the head to tail elution mode.

Theoretical Analysis[15]

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 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 5 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:

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

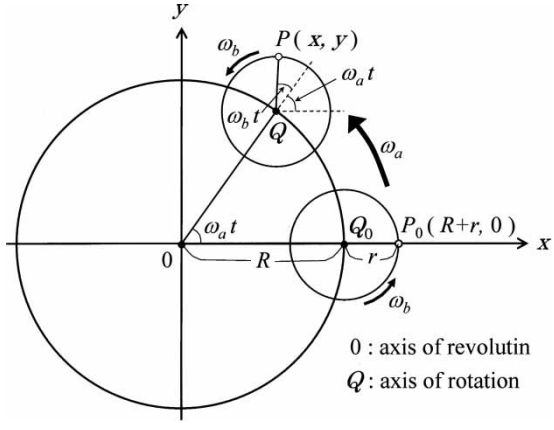

Figure 5. Nonsynchronous planetary motion of the discoid body described in the $x - y$ coordinate system. Cited from Figure 2 of Ref. [15].

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

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

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

$$A = \{(d^2x/dt^2)^2 + (d^2y/dt^2)^2\}^{1/2} \tag{3}$$

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

$$A = \{R^2\omega_a^4 + r^2(\omega_a + \omega_b)^4 + 2Rr\omega_a^2(\omega_a + \omega_b)^2 \cos \omega_b t\}^{1/2} \tag{4}$$

Figure 6 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 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:

$$\begin{aligned} A_{max} &= \{R^2\omega_a^4 + r^2(\omega_a + \omega_b)^4 + 2Rr\omega_a^2(\omega_a + \omega_b)^2\}^{1/2} \\ A_{min} &= \{R^2\omega_a^4 + r^2(\omega_a + \omega_b)^4 - 2Rr\omega_a^2(\omega_a + \omega_b)^2\}^{1/2} \end{aligned} \tag{5}$$

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

$$\Delta A = A_{max} - A_{min} \tag{6}$$

Figure 7 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 $P_{backward}$.

Our experimental studies[14] 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 at 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.

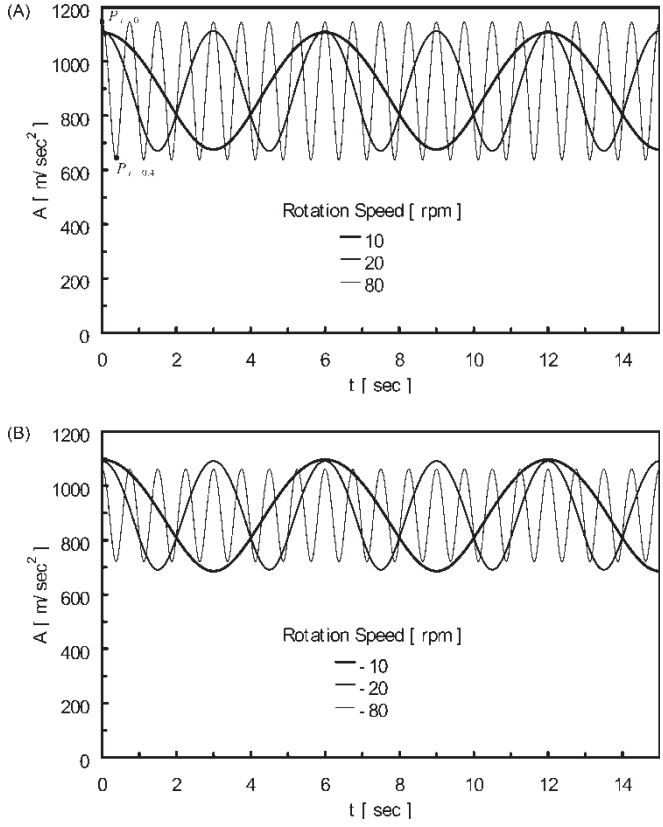

Figure 6. Change of the acceleration *A* at various rotation speeds in $P_{forward}$ (A) and $P_{backward}$ (B) modes. Cited from Figure 5 of Ref. [15].

Cell Separation[16]

Blood Cell Components

The separation of cells using the nonsynchronous CPC is generally performed by the difference in their sedimentation rates in an isotonic phosphate buffer. Figure 8 illustrates the elution patterns of sheep blood samples obtained by varying the coil rotation speed stepwise from 0 to 10 rpm in the CW mode, each under constant CCW revolutions at 1000, 900, and 800 rpm. All groups show a sharp peak at 0 rpm followed by a rather symmetrical broad peak with an intensive absorbance at 570 nm, which indicate the erythrocyte fraction. The fractions from the first sharp peak consisted of plasma proteins and cells, such as platelets and leukocytes free of erythrocytes, since it shows no absorbance at 570 nm, i.e., the absorbance maximum for hemoglobin. The erythrocytes were retained in the column until the coil rotation rate was

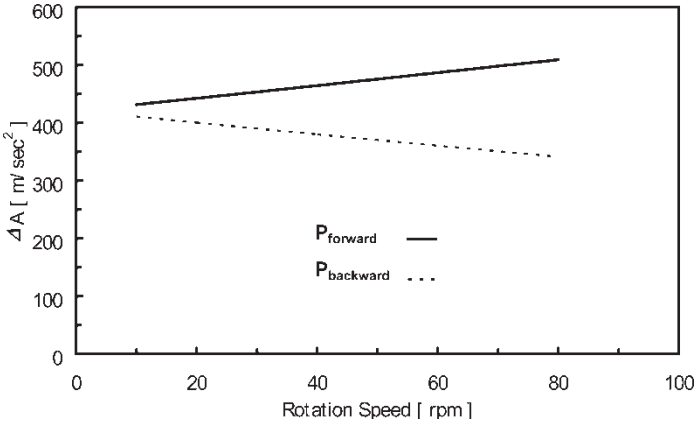

Figure 7. Change of the difference between maximum A and minimum A (ΔA) at various rotation speeds in $P_{forward}$ and $P_{backward}$ modes. Cited form Figure 6 of Ref. [15].

increased to 10 rpm. The erythrocyte peak eluted at 10 rpm coil rotation changes in shape and retention time with the revolution speeds (800, 900, and 1000 rpm). The elution pattern of erythrocytes becomes sharper and approaches the normal distribution curve as the revolution rate was decreased.

Figure 9 illustrates a similar set of experiments using human blood samples. Experimental conditions were the same as those described in Figure 8, except that a lower range of revolution speeds was applied. As seen in the separation of sheep blood samples, blood cells were completely separated into two peaks: the first peak eluted at 0 rpm contained platelets and leukocytes and the second peak eluted at 10 rpm contained erythrocytes. The elution pattern of erythrocytes is only slightly different from each other at the applied range of revolution speeds from 600 to 800 rpm, while the elution time of the peak becomes considerably longer as the revolution rate is increased.

Mast Cells

Mast cells are characterized by their large diameters with a high density (over 1.085 g/mL). The optimum condition for separating rat mast cells was examined using the nonsynchronous CPC equipped with a multilayer coil assembly. An eluent composed of RPMI 1640 medium +10% FCS was used for separation at the revolution speed of 800 rpm and at the rotation speed of 5 rpm. Although, all injected cells including mast cells were eluted as a tailing peak at a flow rate of 0.4 mL/min (Figure 10A), the sufficient separation of mast cells were attained at a flow rate of 0.6 mL/min, as illustrated in Figure 10B.

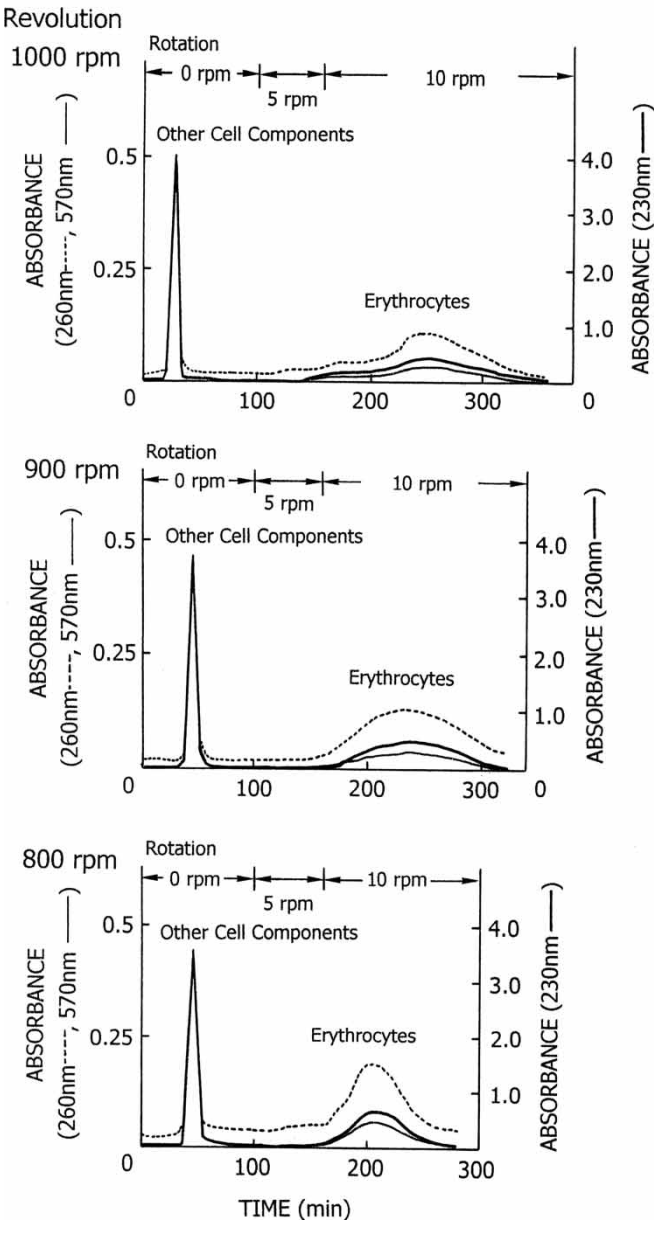

Figure 8. Effect of rotational speed on the separation of sheep blood cell components using the nonsynchronous CPC at various revolution speeds. Experimental conditions: apparatus: nonsynchronous CPC equipped with an eccentric coil assembly, 0.8 mm I.D. × 1.59 mm O.D. and 20 mL capacity; sample: sheep blood mixed with an equal volume of Alsever's solution (500 μL); eluent: isotonic phosphate buffer solution (pH 7.4); flow rate: 0.4 mL/min; fractionation: 0.8 mL/tube; revolution: CCW direction; rotation: CW direction. Cited from Figure 2 of Ref. [16].

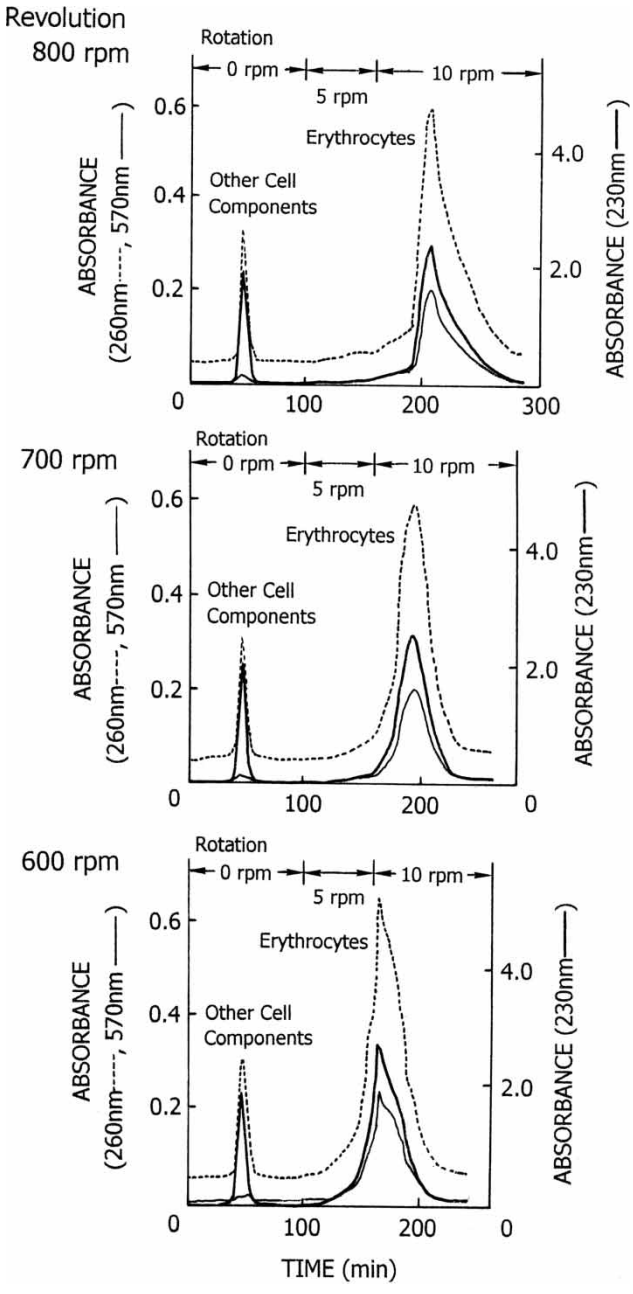

Figure 9. Effect of rotational speed on the separation of human blood cell components using the nonsynchronous CPC at various revolution speeds. Experimental conditions: sample: human blood mixed with an equal volume of Alsever's solution (500 μL). Other conditions are the same as those described in the Figure 8 caption. Cited from Figure 3 of Ref. [16].

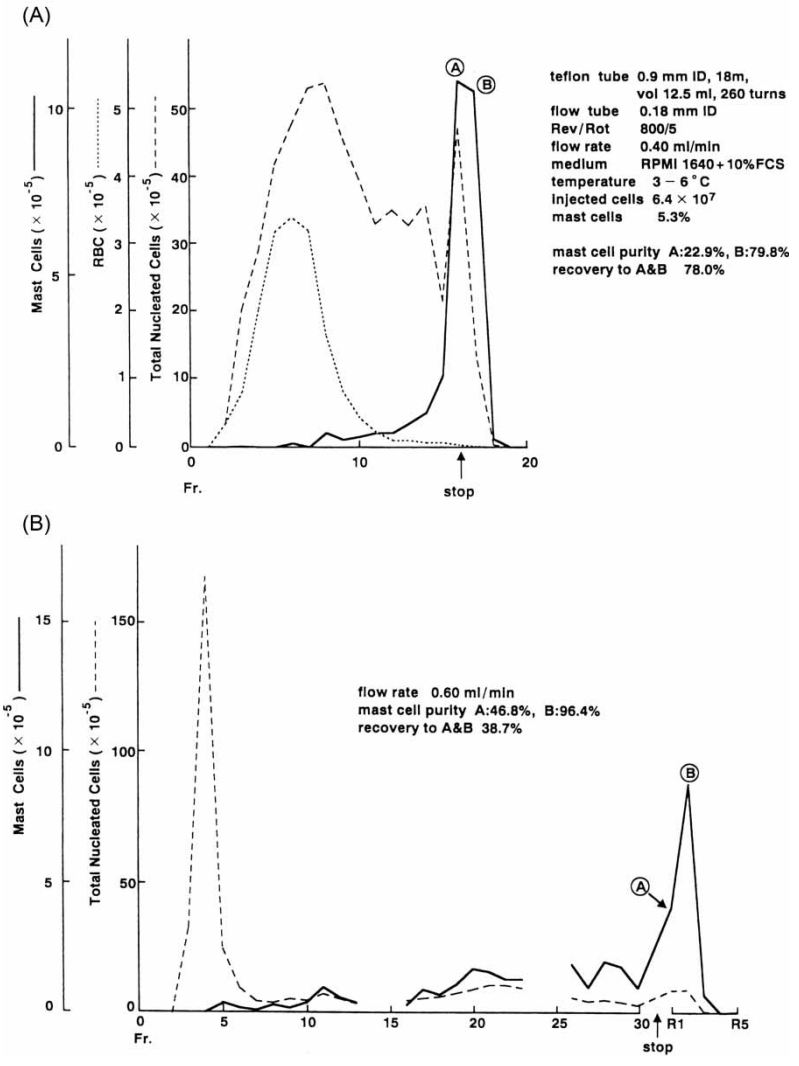

Figure 10. Elution patterns of rat mast cells using the nonsynchronous CPC. Experimental conditions: apparatus: nonsynchronous CPC equipped with multilayer coil assembly, 0.9 mm I.D. and 12.5 mL capacity; sample: rat peritoneal cell suspension (3 mL, injected cells: 6.4×10^7, mast cells: 5.3%); column temperature: 3–6°C; eluent: RPMI 1640 medium +10% FCS; flow rate: (A) 0.4 mL/min, (B) 0.6 mL/min; revolution: 800 rpm; rotation: 5 rpm. Cited from Figure 4 of Ref. [16].

Tailing of the peak may be also caused by the interaction between mast cells and the internal wall surface of the tubing. Therefore, the flow tubing and the column were pretreated with a protein rich separation medium (RPMI 1640 with 10% FCS) for 5 h before separation. Figure 11A illustrates

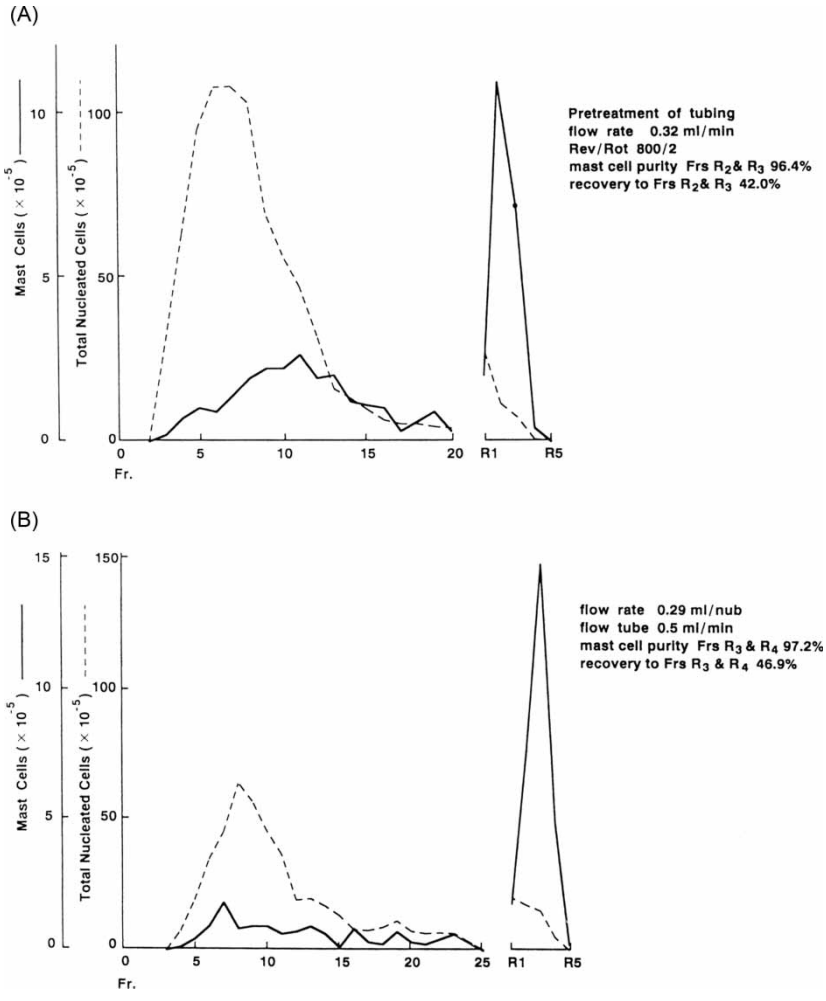

Figure 11. Elution pattern of rat mast cells using the nonsynchronous CPC. Experimental conditions: column: a multilayer Teflon coil tubing pretreated with serum protein; flow rate: (A) 0.32 mL/min, (B) 0.29 mL/min. Other conditions are same as those described in the Figure 10 caption. Cited from Figure 5 of Ref. [16].

an elution pattern of mast cells at a flow rate of 0.32 mL/min under high speed revolution of 800 rpm and low speed coil rotation of 2 rpm, where mast cells were satisfactorily separated from the other cell components in spite of the low flow rate. Furthermore, as illustrated in Figure 11B, a decreased flow rate of 0.29 mL/min also improved the separation of mast cells up to their purity of 97.2% in the fraction of R3 and R4. The recovery of mast cells in these fractions was attained at 46.9%.

CONCLUSION

A series of our studies demonstrate that the nonsynchronous CPC is useful for protein separation with ATPSs. The best result was obtained by the $P_{backward}$ (CCW/CW) of the coil rotation for the lower phase mobile and at $P_{forward}$ (CCW/CCW) for the upper phase mobile, both in the head to tail elution mode. The theoretical analysis of acceleration may explain this experimental result in such a way that increased ΔA could produce less retention of the stationary phase in the coiled column in the present system. Separations of blood cell components and mast cells were also successfully performed according to the difference in the sedimentation rates in the physiological buffer solution.

ACKNOWLEDGMENTS

This work was supported in part by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and also from College of Pharmacy, Nihon University, Chiba, Japan.

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Received February 5, 2007
Accepted April 25, 2007
Manuscript 6098