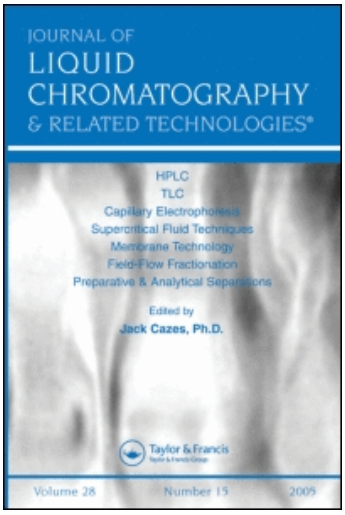

Journal of Liquid Chromatography & Related Technologies

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

Elutriation of Blood Cell Components and Mast Cells by Nonsynchronous Coil Planet Centrifuge

Kazufusa Shinomiya[a]; Tadashi Okada[b]; Yoichiro Ito[c]
[a] College of Pharmacy, Nihon University, Funabashi-shi, Chiba, Japan [b] Department of Physiology, Aichi Medical University, Aichig, Japan [c] Laboratory of Biophysical Chemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland, USA

To cite this Article Shinomiya, Kazufusa , Okada, Tadashi and Ito, Yoichiro(2005) 'Elutriation of Blood Cell Components and Mast Cells by Nonsynchronous Coil Planet Centrifuge', Journal of Liquid Chromatography & Related Technologies, 28: 6, 835 — 846
To link to this Article: DOI: 10.1081/JLC-200051469
URL: http://dx.doi.org/10.1081/JLC-200051469

Journal of Liquid Chromatography & Related Technologies®, 28: 835–846, 2005

ISSN 1082-6076 print/1520-572X online
DOI: 10.1081/JLC-200051469

Elutriation of Blood Cell Components and Mast Cells by Nonsynchronous Coil Planet Centrifuge

Kazufusa Shinomiya
College of Pharmacy, Nihon University, Funabashi-shi, Chiba, Japan

Tadashi Okada
Department of Physiology, Aichi Medical University, Aichig, Japan

Yoichiro Ito
Laboratory of Biophysical Chemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland, USA

Abstract: The nonsynchronous coil planet centrifuge was applied to the separation of blood cell components and mast cells using a physiological buffer solution. The optimal separation of sheep blood samples was performed at the counterclockwise revolution and the clockwise rotation of coiled separation column by the head to tail elution mode. Under the high speed of revolution of 600–1000 rpm, sheep and human blood samples were completely separated and eluted into two peaks, where all cell components, other than erythrocytes such as platelets and leukocytes, were eluted at 0 rpm and erythrocytes at 10 rpm of coil rotation. The normal elution pattern was achieved at 800 rpm of revolution for sheep blood and at 700 rpm for human blood samples. The separation of mast cells, large cells with a high density, was also performed using an eluent composed of RPMI 1640 medium +10% FCS. Mast cells were satisfactorily separated from other cell components under a high speed revolution combined with a low speed of coil rotation (2–5 rpm). The overall

Address correspondence to Kazufusa Shinomiya, College of Pharmacy, Nihon University, 7-7-1, Narashinodai, Funabashi-shi, Chiba 274-8555, Japan. E-mail: kshinomiya@pha.nihon-u.ac.jp

results indicate that the nonsynchronous CPC can be effectively used for separation of blood cell components and mast cells according to their sedimentation rates.

Keywords: Nonsynchronous coil planet centrifuge, Elutriation, Blood cell separation, Erythrocytes, Mast cell separation, Centrifugation

INTRODUCTION

The coil planet centrifuge (CPC) was first introduced to perform particle separation, purification, and analysis of chemicals and, also, for testing the osmotic fragility of erythrocytes.[1] Among a variety of CPC instruments developed in the past, the nonsynchronous CPC is considered most versatile in that it provides a desirable combination between rotation (about its own axis) and revolution (around the centrifuge axis) of the coil holder.[2–8] In the past, Ito et al. studied the separation between sheep and human erythrocytes with an isotonic buffered saline solution,[2] while the detail experimental conditions for obtaining the optimum separation remains to be further investigated. Sutherland et al. also studied the separation of red blood cells (mixtures of dog and sheep, and dog and human) using an isotonic polymer phase system composed of polyethylene glycol 6000-dextran.[3] In addition, the nonsynchronous CPC has been used for the separation of plasmid DNA with the polymer two-phase system[5] and for *Salmonella typhimurium* cells, according to their differences in their surface lipopolysaccharides.[6]

Our previous studies have demonstrated that protein separation can be successfully performed using the nonsynchronous CPC with aqueous-aqueous polymer phase systems.[8] Further studies also revealed that the partition efficiency in protein separation is remarkably affected by the rotational speed and direction of revolution of coiled separation columns using the nonsynchronous CPC.[9] The present paper describes the separation of blood cell components and mast cells based on the difference in their sedimentation rates, in an isotonic phosphate buffer using our rotary-seal-free nonsynchronous CPC.

EXPERIMENTAL

Apparatus

The design of the nonsynchronous CPC used in the present study was previously described in detail.[2–8] The apparatus has a distinctive feature, which allows a freely adjustable rotational speed of the coiled separation column (between 0 and ± 10 rpm) at any given revolution speed, while the effluent is eluted through the rotating column without the use of conventional rotary-seal device.

Preparation of Coiled Column

Both eccentric coil and multilayer coil assemblies were used in the present studies. The eccentric coil assembly was prepared by winding 0.8 mm I.D. Teflon tubing onto a set of 20 cm × 6 mm O.D. aluminum pipes making a series of tight left-handed coils. Eleven coil units were arranged symmetrically around the holder hub of 6 cm O.D. in such a way that the axis of each coil unit was parallel to the holder axis. The total column capacity was 20 mL. The multilayer coil assembly was prepared by winding 0.9 mm I.D. Teflon tubing (18 m long, 260 turns) onto the holder with a diameter of 20 mm. The total capacity was 12.5 mL. By installing an additional pump equipped with a flow tube, which was directly connected to the terminus of the multilayer coil assembly via a three-way joint mounted on the holder, the separated cells were eluted out from the outlet of the flow tube without trapping.

Reagents

The reagents used in the present studies were purchased as follows: sodium chloride (NaCl), disodium hydrogenphosphate (Na_2HPO_4), sodium dihydrogenphosphate monohydrate ($NaH_2PO_4 \cdot H_2O$), D(+)-glucose, sodium citrate, and citric acid (Wako Pure Chemicals, (Osaka, Japan), RPMI 1640 (Biofluids, Rockville, MD, USA), penicillin/streptomycin, nonessential amino acids, L-glutamine and sodium pyruvate (Flow Laboratories, McLean, VA, USA), FCS (HyClone Laboratories, Logan, Utah, USA), BSA, 2-mercaptoethanol, toluidine blue O (Sigma, St. Louis, MO, USA). All reagents were of reagent grade. Sheep blood samples were obtained from Japan Biological Material Center Co. (Tokyo, Japan).

Blood Cell Component Separation

Isotonic buffered saline solution (pH 7.4) was prepared by dissolving 90 g of NaCl, 13.65 g of Na_2HPO_4, and 2.15 g of $NaH_2PO_4 \cdot H_2O$ 2.15 g in 1 liter of distilled water and diluting 85 mL of this stock solution with distilled water to bring the final volume to 1 liter.[2]

Alsever's solution was prepared by dissolving D(+)-glucose 2.05 g, NaCl 0.42 g, and sodium citrate 0.8 g in distilled water to make the final volume of 100 mL after the pH was adjusted to 6.1 with citric acid.

Blood samples used in the present studies were prepared by adding an equal volume of Alsever's solution to prevent the sample from coagulation.

The effluent from the column outlet was collected into test tubes at 0.4 mL/tube using a fraction collector (Model SF-200, Advantec Co., Tokyo, Japan), and the collected blood fraction was diluted with 2.5 mL of

distilled water and the absorbance was measured at 230, 260, and 570 nm with a spectrophotometer (Model UV-1600, Shimadzu Seisakusho, Kyoto, Japan).

Mast Cell Separation

Conditioned RPMI was prepared by dissolving 4 mM L-glutamine, 50 μμM 2-mercaptoethanol, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 100 U/mL penicillin, and 100 μg/mL streptomycin into RPMI 1640. An eluent for separation used in the present studies was the conditioned RPMI, supplemented with 0.32% sodium citrate and 10% heat-inactivated FCS.

Male SD rats (weight 250–400 g) were anesthetized with halothane and then exsanguinated. Peritoneal cells were collected by washing the abdominal cavity with a 1 : 1 mixture of 3.2% sodium citrate and conditioned PRMI containing 10% FCS.

Cells were suspended in the separation medium at a concentration of $2-3 \times 10^7$ cells/mL. In each separation, 3 mL of cell suspension were loaded into the coiled column through a three-way valve. The optimum experimental conditions were determined by applying various flow rates ranging from 0.29 to 0.6 mL/min, at a high speed revolution of 800 rpm at 4°C. Fractions were collected at 5 (Figure 4B)–10 min intervals (Figure 4A and Figure 5). Generally, elutriation was completed by 4 h and mast-cell-rich fractions were collected. The number of mast cells was counted according to the method described by Kimura et al.[10]

General Separation Procedure

Each separation was initiated by completely filling the column with an eluent, followed by the injection sample solution into the column inlet. Then, the eluent was pumped into the column using a reciprocating pump, while the column was rotated at a given combination of the rotation (0–10 rpm) and revolution (800 rpm) rates.

RESULTS AND DISCUSSION

Separation of Blood Cell Components

In the present studies, a single-phase physiological solution was used to separate blood cell components. Figure 1 illustrates the elution patterns of sheep blood samples obtained by the revolution at 800 rpm and coil rotation at 10 rpm in the head to tail elution mode with four different planetary motions as indicated in the diagram. The blood samples are partially

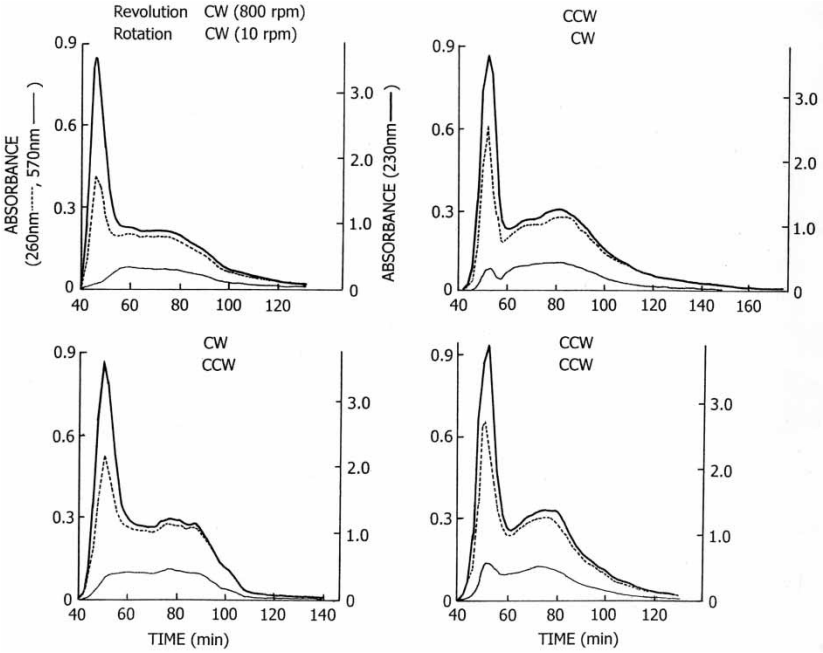

Figure 1. Effects of revolution and rotation on the separation of sheep blood cell components using the nonsynchronous CPC. Experimental conditions: apparatus: nonsynchronous CPC equipped with an eccentric coil assembly, 0.8 mm I.D. × 1.59 mm O.D. and 20 mL capacity; sample: sheep blood mixed with equal 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: 800 rpm; rotation: 10 rpm.

separated into two main peaks, while their elution patterns were only slightly different in all groups. Among those, the best separation of these two peaks was obtained by the combination of counterclockwise (CCW) revolution and clockwise (CW) rotation. This experimental condition is identical to that which produced the best protein separation, using an aqueous two-phase solvent system where the lower phase was eluted in the head to tail elution mode with the same combination of planetary motion.[9]

Figure 2 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.

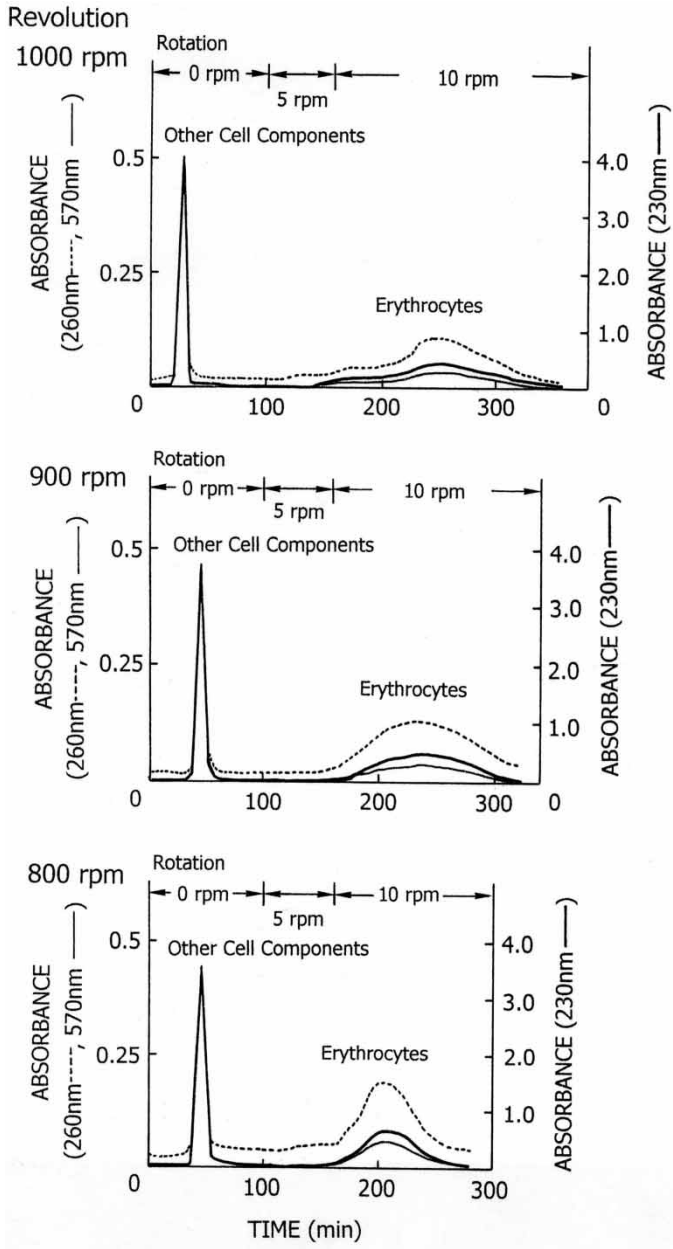

Figure 2. Effect of rotational speed on the separation of sheep blood cell components using the nonsynchronous CPC at various revolution speeds. Experimental conditions: revolution: counterclockwise direction; rotation: clockwise direction. Other conditions are same as those described in the Figure 1 caption.

The erythrocytes were retained in the column until the coil rotation rate was 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 3 illustrates a similar set of experiments using human blood samples. Experimental conditions were same as those described in Figure 2, 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.

Separation of Mast Cells

Mast cells are characterized by its large diameter 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 4A), the sufficient separation of mast cells were attained at a flow rate of 0.6 mL/min, as illustrated in Figure 4B.

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 5A illustrates 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 5B, 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 fractions of R_3 and R_4. The recovery of mast cells in these fractions was attained at 46.9%.

CONCLUSION

Using a physiological buffer solution, the optimal separation of blood cell components was obtained by the combination of the CCW revolution and

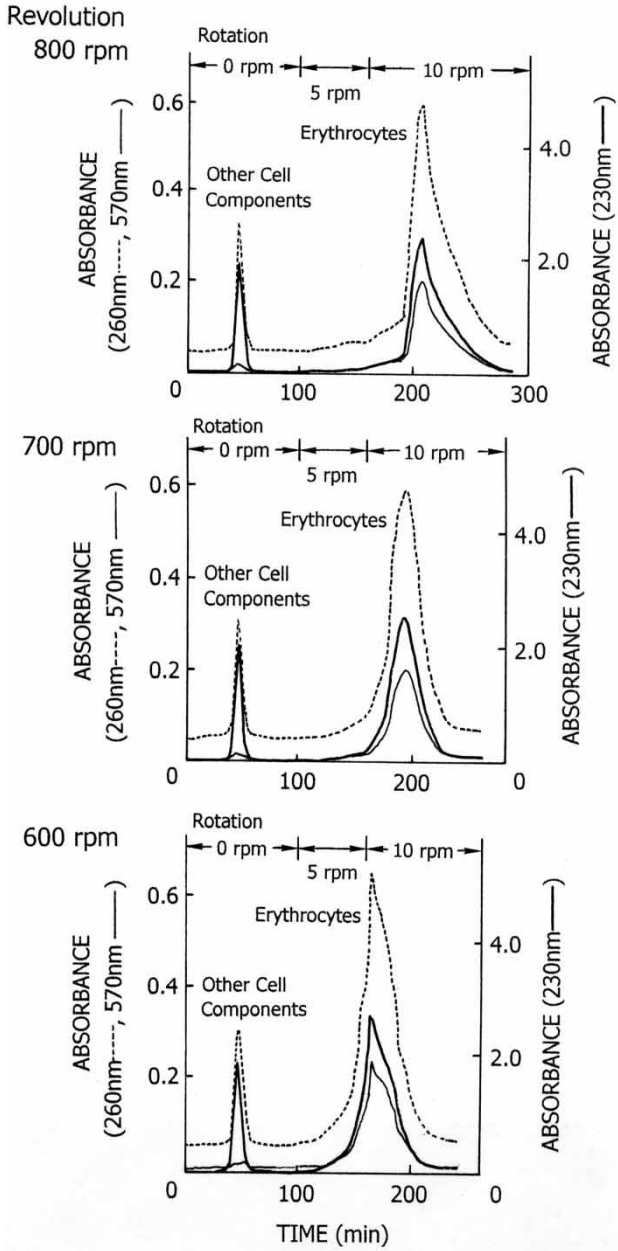

Figure 3. Effect of rotational speed on the separation of human blood cell components using the nonsynchronous CPC at various revolution speeds. Experimental conditions: sample: human blood mixed with equal volume of Alsever's solution (500 μL). Other conditions are the same as those described in the Figure 2 caption.

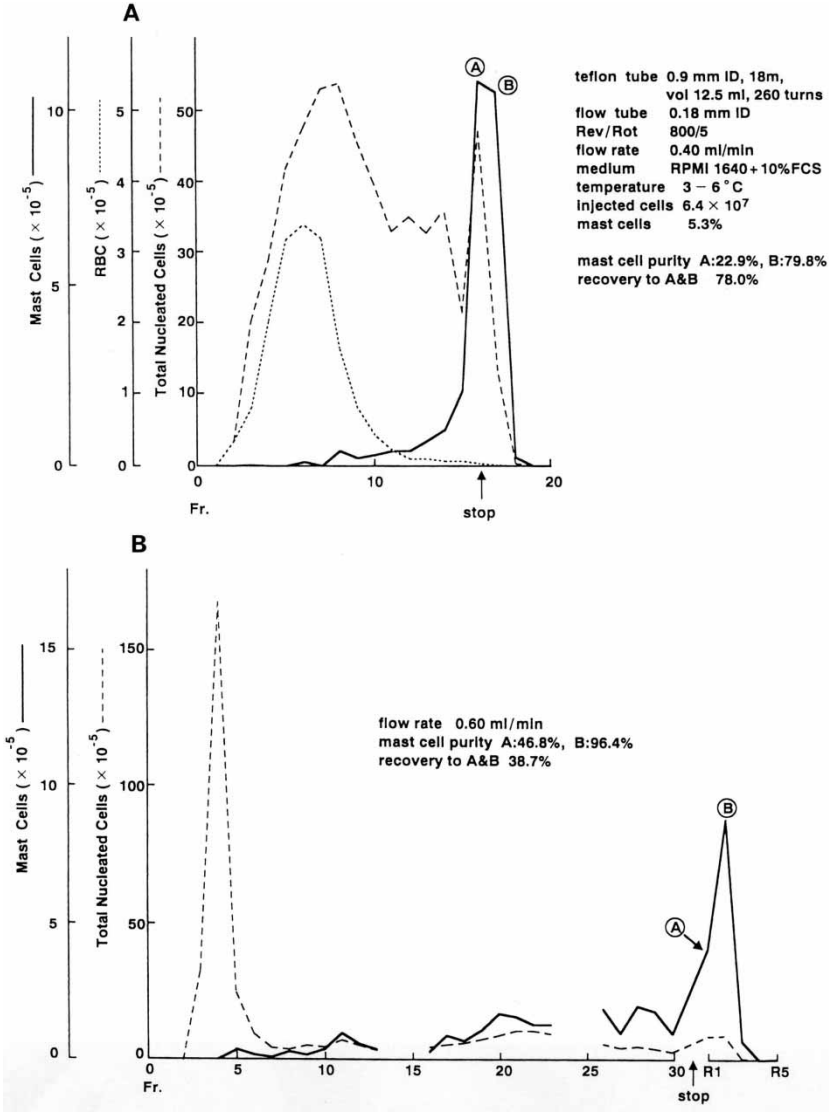

Figure 4. Elution patterns of rat mast cells using the nonsynchronous CPC. Experimental conditions: apparatus: nonsynchronous CPC equipped with multilayer coil assembly, 0.9 mm I.D. and 12.5 mL capacity; sample: rat peritoneal cell suspension (3 mL, injected cells: 6.4×10^7, mast cells: 5.3%); column 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.

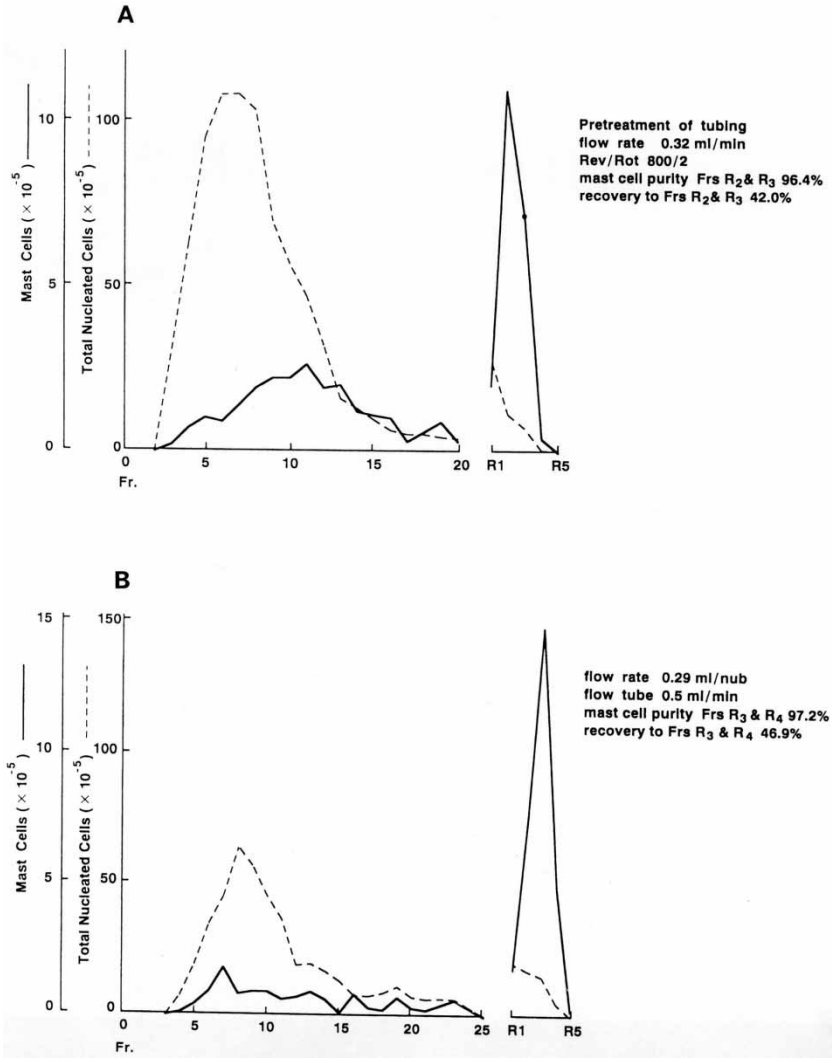

Figure 5. Elution patterns of rat mast cells using the nonsynchronous CPC. Experimental conditions: column: a multilayer Teflon coil tubing pretreated with serum protein; flow rate: (A) 0.32 mL/min, (B) 0.29 mL/min. Other conditions are the same as those described in the Figure 4 caption.

the CW rotation at the head to tail elution mode. Both sheep and human blood samples were each separated into two peaks, where all cell components other than erythrocytes were eluted at 0 rpm, and while erythrocytes were eluted at 10 rpm of coil rotation. A normal elution pattern was attained for sheep

erythrocytes at 800 rpm of revolution and for human erythrocytes at 700 rpm. The mast cells were also separated using an eluent composed of RPMI 1640 medium +10% FCS at a low speed of coil rotation, under a high speed revolution of the coiled separation column. The overall results indicate that the nonsynchronous CPC is useful for the separation of cell components according to the difference in their sedimentation rates. It may be feasible to fractionate leukocyte components by optimizing revolution and rotation rates of the coil under a reduced flow rate.

ACKNOWLEDGMENTS

The authors would like to thank Misters Tomoaki Tanaka and Hayato Watanabe for their technical assistance, and Mr. Kazuhiro Yanagidaira (Machining Technology Center, College of Science and Technology, Nihon University, Chiba, Japan) for the maintenance of the nonsynchronous CPC apparatus. The authors are also indebted to Dr. Kuniko Kusama and Mr. Atsuhiro Suda (College of Pharmacy, Nihon University, Chiba, Japan) for their valuable suggestions. This work was supported in part by a grant from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

REFERENCES

1. Ito, Y.; Weinstein, M.; Aoki, I.; Harada, R.; Kimura, E.; Nunogaki, K. The coil planet centrifuge. Nature **1966**, *212*, 985 (No. 5066).
2. Ito, Y.; Carmeci, P.; Sutherland, I.A. Nonsynchronous flow-through coil planet centrifuge applied to cell separation with physiological solution. Anal. Biochem. **1979**, *94*, 249.
3. Sutherland, I.A.; Ito, Y. Cell separation using two-phase polymer systems in a nonsynchronous flow-through coil planet centrifuge. Anal. Biochem. **1980**, *108*, 367.
4. Ito, Y.; Carmeci, P.; Bhatnagar, R.; Leighton, S.B.; Seldon, R. The nonsynchronous flow-through coil planet centrifuge without rotating seals applied to cell separation. Sep. Sci. Technol. **1980**, *15*, 1589.
5. Ito, Y.; Bramblett, G.T.; Bhatnagar, R.; Huberman, M.; Leive, L.L.; Cullinane, L.M.; Groves, W. Improved nonsynchronous flow-through coil planet centrifuge without rotating seals: principle and application. Sep. Sci. Technol. **1983**, *18*, 33.
6. Leive, L.; Cullinane, L.M.; Ito, Y.; Bramblett, G.T. Countercurrent chromatographic separation of bacteria with known differences in surface lipopolysaccharide. J. Liq. Chromatogr. **1984**, *7*, 403.
7. Okada, T.; Metcalfe, D.D.; Ito, Y. Purification of mast cells with an improved nonsynchronous flow-through coil planet centrifuge. Int. Arch. Allergy Immunol. **1996**, *109*, 376.
8. Shinomiya, K.; Kabasawa, Y.; Yanagidaira, K.; Sasaki, H.; Muto, M.; Okada, T.; Ito, Y.J. Chromatogr. A B *1005*, 103.

9. Shinomiya, K.; Ito, Y. Effect of the planetary motion of a coiled column on protein separation by the nonsynchronous coil planet centrifuge. J. Liq. Chrom. & Rel. Technol. **2004**, *27* (20), 1.
10. Kimura, I.; Moritani, Y.; Tanizaki, Y. Basophils in bronchial asthma with reference to regain-type allergy. Clin. Allergy **1973**, *3*, 195.

Received October 14, 2004
Accepted November 10, 2004
Manuscript 6522