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Origin and Evolution of the Coil Planet Centrifuge: A Personal Reflection of My 40 Years of CCC Research and Development

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Origin and Evolution of the Coil Planet Centrifuge: A Personal Reflection of My 40 Years of CCC Research and Development

Yoichiro Ito
Center for Biochemistry and Biophysics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA

Abstract: The view by its founder of countercurrent chromatography history through the evolution of the coil planet centrifuge hydrodynamic chromatograph. Originally built for the separation of lymphocytes, the coil planet centrifuge took an unexpected course to become an efficient instrument that is currently widely used for separation and purification of natural and synthetic products.

Keywords: Countercurrent chromatography, coil planet centrifuge, liquid stationary phase, history

INTRODUCTION

In the 1950s a countercurrent distribution method (CCD) (1) was used for purification of various natural products such as penicillin and insulin and working with two nonmiscible liquid phases. The apparatus was operated by repeating three steps, i.e., mixing, settling, and transfer of an upper phase to the next unit. One separation cycle by the instrument with 1000 partition units could take many days. Mainly because of its time-consuming operation and the fragile and bulky apparatus, the method has been replaced by column chromatography.

Late in the 1960s, development of a new separation technique called countercurrent chromatography (CCC) started by retaining an important

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advantage of CCD over column chromatography: it was using two liquid phases and so it was free from complications arising by the use of a solid support. Practical development of this support-free chromatographic technique depended on overcoming several serious problems:

1. Retention of the liquid stationary phase in the column.
2. Division of the column space into numerous partition units.
3. Mixing each phase to reduce mass transfer resistance.
4. Prevention of laminar flow spreading of the solute bands.

All these problems have been solved today by using a coiled separation column. The development of efficient CCC instruments has required over 20 years of steady effort by a number of scientists and engineers. This review article covers the history of development of CCC beginning with the construction of the original coil planet centrifuge in Japan.

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ORIGIN OF THE COIL PLANET CENTRIFUGE

A Young Student in the 1960s and the First Prototype

About half a century ago when I was a medical student in Japan, I was interested in lymphocytes, whose function at that time was unknown. They were merely classified as large, medium, and small. After graduating from the Osaka City University Medical School and completing 1 year of rotating internship at Yokosuka U.S. Naval Hospital in Japan, I became a resident in the Pathology Department at Cuyahoga Metropolitan General Hospital, Cleveland, Ohio, USA, since it provided each resident an opportunity to participate in a research activity after the first year of training. In the second year, I attempted the separation of blood lymphocytes using a density gradient centrifugation method as shown in Figure 1A. After filling the centrifuge tube with an albumin density gradient, blood cells were layered over the gradient column and subjected to centrifugation.

Although the method worked well and lymphocytes were mostly separated from other cell components (2), I wished to separate lymphocytes into their subgroups. I noticed that the cell sedimentation distance in the centrifuge tube was no more than several centimeters and at this point I thought how I might increase it many times as shown in Figure 1B.

A long piece of tubing was compactly wound into a coil, which was then rotated around its own axis. This motion creates an Archimedean screw force to move particles along the coil at various rates according to their size and density. In order to examine the practicality of this idea, it was necessary to construct a particular type of centrifuge that produces a planetary motion in the coiled column. Such centrifuges were not available. Professor Lloyd Arnold in Loyola University in Chicago and his engineer friend, Mr. Don Vern, kindly helped me build a coil planet centrifuge at their own expense.

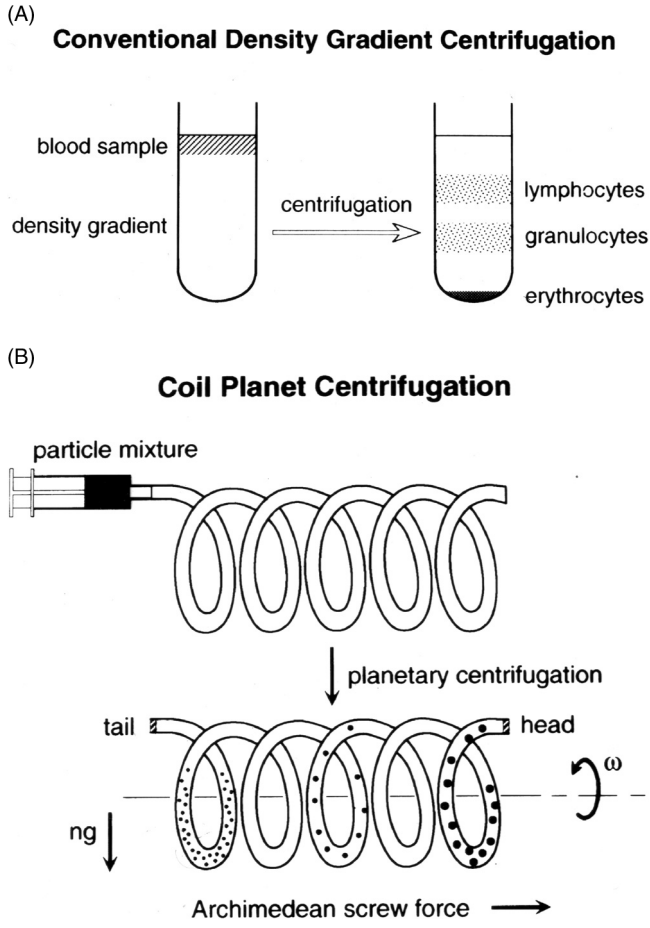

Figure 1. (A) Blood cell separation by density gradient centrifugation; (B) Particle separation by rotating coiled tube.

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Unfortunately, my Fulbright scholarship fund was about to expire, and I had to return to Japan without completing the project.

In Japan I worked under Professor Eiichi Kimura at the Department of Physiology in Osaka City University Medical School. Professor Kimura kindly introduced me to the Kubota Centrifuge Corporation in Tokyo where construction of the coil planet centrifuge would take place. Mr. Yonezo Kubota, the president and founder of the company, was interested in my idea, and he successfully fabricated a prototype with his own hands, despite his advanced years.

The design of his prototype is shown in Figure 2A, where the rotary plate of the centrifuge supports two sets of worm gears, one at the center and the

other at the periphery to rotate the coiled separation column. Each worm gear reduces the coil rotation rate by 1/25 so that the coil rotates about its own axis once during 625 revolutions. Using this instrument we were able to investigate the capability of the system. The results of our preliminary studies were published in Ikakikaigaduzashi in 1966 (3).

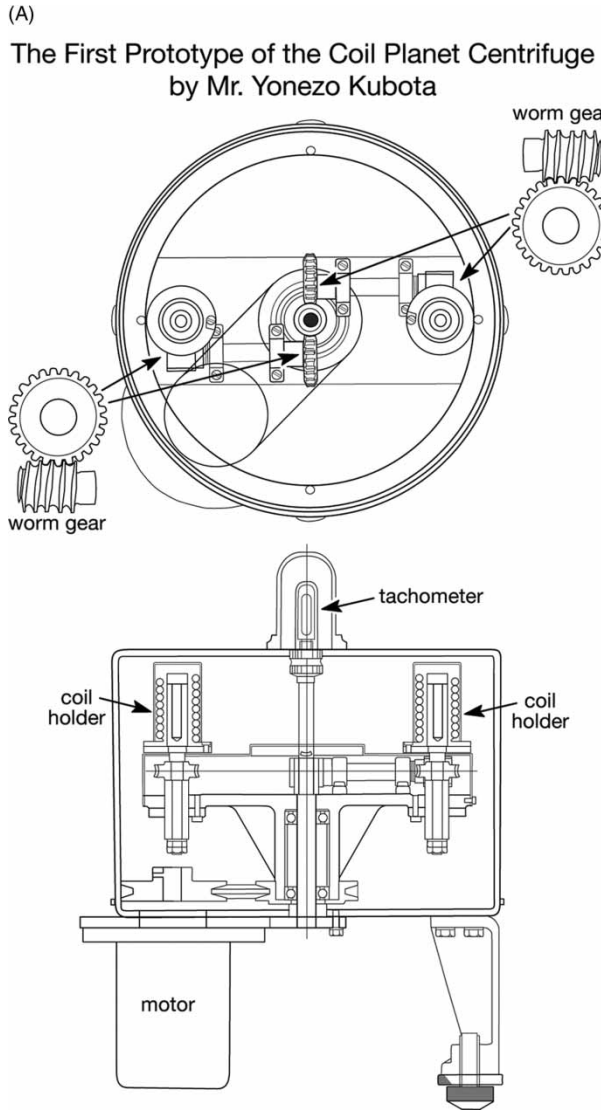

Figure 2. (A) Design of the first prototype of the coil planet centrifuge by Yonezo Kubota (1964); (B) Improved design of the coil planet centrifuge by Sanki Engineering, Ltd. (1966).

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(B)

Figure 2. Continued.

Our studies showed that further improvement was required in terms of adjustable rotation/revolution ratio, a higher centrifugal force field, and a longer column length. Realizing that Mr. Kubota had retired from his position, I looked for a company that might be able to improve our prototype. Thanks to the kind help of Mr. Reizo Matsumoto, a worker at the Suzuki Patent Office, I found Sanki Engineering (Kyoto, Japan), which was manufacturing a variety of gears while constructing prototypes of various new scientific instruments. Mr. Kanich Nunogaki, the president of the company, and Mr. Yoshiaki Nunogaki, his younger brother and vice president, were interested in building a new model of the coil planet centrifuge with various improved features.

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The Second Prototype and First Applications

Shortly after our meeting a second prototype of the "coil planet centrifuge" was successfully fabricated by Sanki Engineering, and this is shown in Figure 2B. The central shaft and the rotary frame rotate at slightly different rates so that the gear coupling between the central shaft and the coil holder on the rotary frame produces a slow rotation of the holder in a strong centrifugal force field. A pair of long column holders accommodates six coils each. I examined all features of this unique apparatus.

I found three basic applications as shown in Figure 3A where 6 coils are each shown as a straight tube. In the first application, the coil is filled with

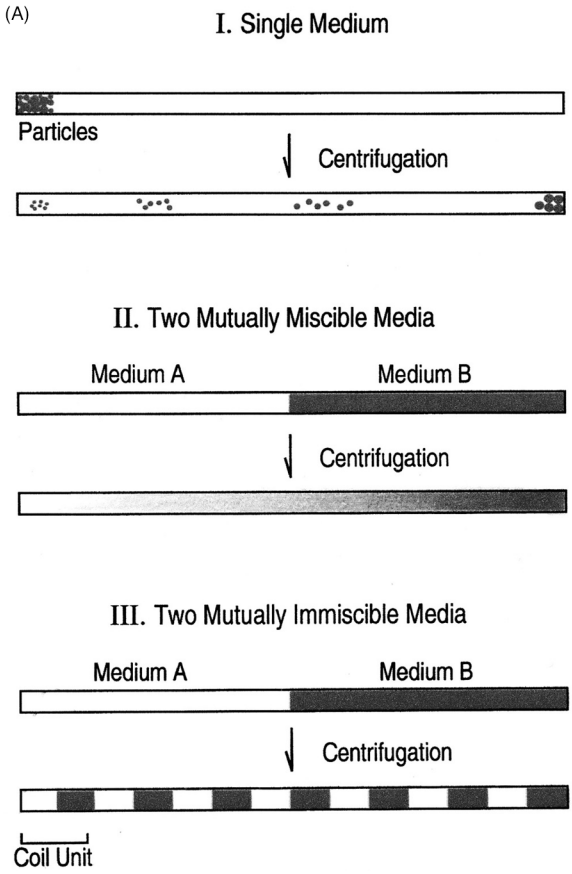

Figure 3. (A) Three basic applications of the coil planet centrifuge; (B) Polystyrene particle separation by the coil planet centrifuge; (C) Osmotic fragility test of human blood samples; (D) Countercurrent chromatographic separation of dyes.

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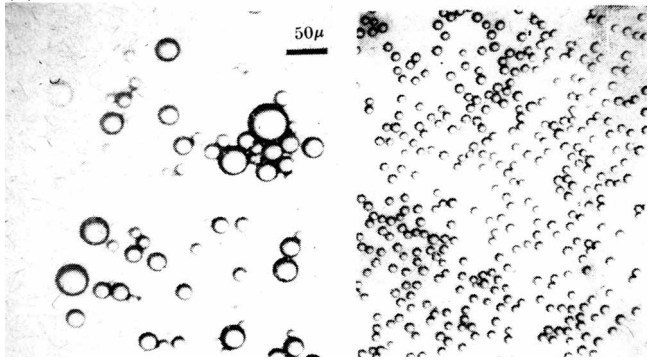

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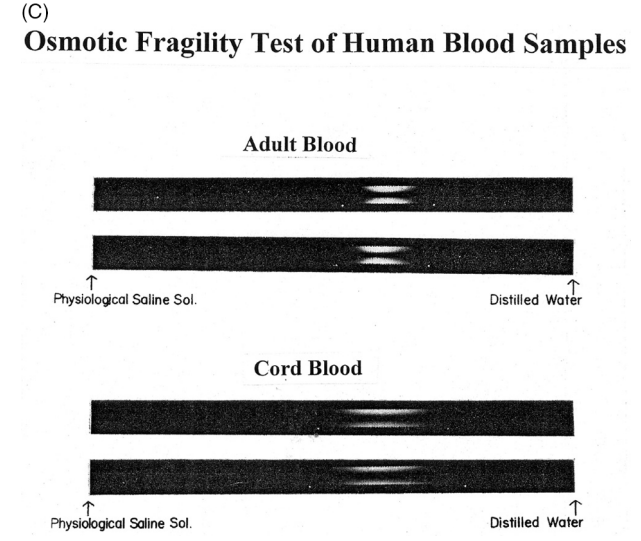

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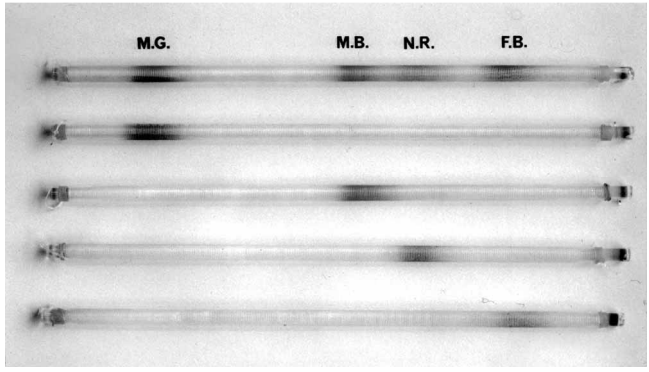

Figure 3. Continued.

water and sample particles are charged at one end. Then, coil planet centrifugation, as expected, separated the particles according to their size and density. Figure 3B shows the separation of polystyrene particles. These separated particles are about 10 microns in diameter and this is about the size of lymphocytes. The motion of the particles in the rotating coiled tube was analyzed by Dr. Marvin A. Weinstein, an excellent physicist and former professor in physics in Nairobi University. His mathematical analysis was published in *Nature*, together with the results of our preliminary studies (4).

A second application is the determination of erythrocyte osmotic fragility. If the coil is filled with two mutually miscible liquids, half-and-half, as shown in this slide, the centrifugation produces a linear concentration gradient between the two liquids that soon reaches a stable state. A gradient between isotonic saline and water is made externally and introduced into the coil, and a small amount of blood is introduced at the saline side. The planetary motion causes the red cells to move down the gradient so that they are exposed to gradually decreasing osmotic pressure. At a critical saline concentration they release hemoglobin, which stays at the point of hemolysis. Consequently, after all cells are hemolyzed, one can obtain a hemolysis curve that is measured by a densitometer. My first and last Ph.D. student, Dr. Reiko Harada, completed this study. The results of her experiments were published in the *Japanese Journal of Physiology* (5). A commercial model of this coil planet centrifuge was called "CPC" and it was used in many hospitals in Japan. Figure 3C shows the results of an osmotic fragility test for normal human adult and cord blood. The latter shows a broadened hemolytic band due to its high osmotic fragility.

The third and most important application is shown at the bottom. Two mutually immiscible liquids are similarly introduced half-and-half into the coil. Centrifugation distributes these two liquids along the coil to form alternating segments as shown in this slide. This suggests that solutes introduced at the original interface would be distributed along the coil according to their partition coefficients. With Dr. Ichiro Aoki, an associated professor in our department, this idea was examined using a long, fine coiled tube under a strong centrifugal force field. Figure 3D shows the separation of dyes by this countercurrent chromatography method. The coil was first filled with a two-phase solvent system, the lighter phase on one side and the heavier phase on the other, followed by injection of the sample solution at the interface. The four dyes separated as shown in the top coil. The rest of the coils show separation of each component (6).

These results demonstrated an ability of the coil planet centrifuge to perform CCC in an end-closed coiled tube. The next step was to introduce a flow-through mechanism to the coil so that the system can be used for continuous elution, monitoring, and fractionation as is done in liquid chromatography.

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TWO BASIC CCC SYSTEMS

In 1967 Mr. K. Nunogaki and I attended the International Conference on Medical and Biological Engineering in Sweden where we met Dr. Robert L. Bowman, the chief of the Laboratory of Technical Development in the Heart and Lung Institute of NIH. On our way home we visited his laboratory and after my short seminar, Dr. Bowman invited me to be a visiting scientist for continued development of CCC. In Dr. Bowman's laboratory, I considered two basic approaches with biphasic liquid systems: One is called the hydrostatic equilibrium system and the other, the hydrodynamic equilibrium system as shown in Figure 4 (7).

The hydrostatic system uses a stationary coiled tube. The coil is first filled with the liquid stationary phase and the mobile phase is introduced at one end of the coil (Figure 4 left). The mobile phase then percolates through the stationary phase segments on one side of the coil. Consequently, solutes introduced locally at the inlet of the coil are subjected to a continuous partition process between the two liquid phases and separated according to their partition coefficients in a manner analogous to liquid chromatography, but in the absence of solid support.

In the hydrodynamic system the partition efficiency is improved by rotation of the coil (Figure 4, right). This motion creates an Archimedean screw force to move all objects of different density competitively toward one end of the coil. This end is called the head and the other end, the tail. Under slow rotation of the coil, the mobile phase introduced at the head of the coil is mixed with the stationary phase to establish hydrodynamic

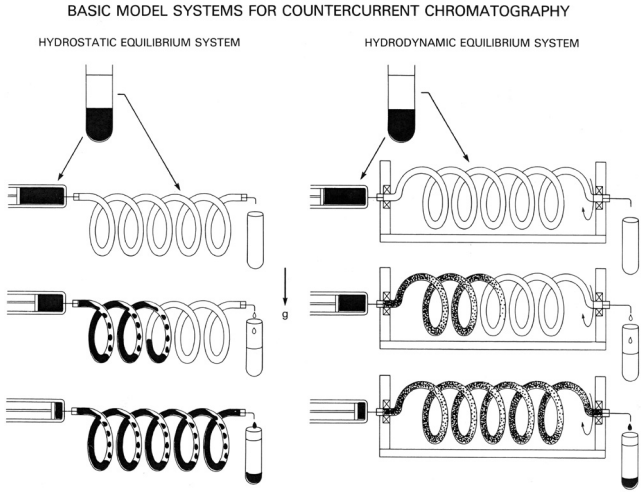

Figure 4. Two basic CCC systems. Hydrostatic equilibrium system (left) and hydrodynamic equilibrium system (right).

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equilibrium where about half a volume of the liquid stationary phase is permanently retained in the coil while constantly undergoing mixing with the mobile phase. Consequently, the hydrodynamic system yields more efficient separation by the additional mixing induced by the rotation of the coil.

DEVELOPMENT OF HYDROSTATIC SYSTEMS

In the early 1970s, we developed the basic hydrostatic system into several efficient CCC schemes for both analytical and preparative separations as shown in Figure 5 (7). In the analytical scheme first developed, both internal diameter and helical diameter of the coil were reduced while the column was subjected to a strong centrifugal force field to establish retention of the stationary phase (8). Figure 6A shows our prototype of this helix (toroidal coil) countercurrent chromatograph. A long coiled tube was mounted around the periphery of the centrifuge bowl. The mobile phase was eluted through the coil from a rotating syringe while the effluent from the outlet of the coil was fractionated through a rotating seal made between the syringe plunger and the syringe drive. This system gave a highly efficient separation of dinitrophenyl(DNP)-amino acid samples as shown in Figure 6B. The result was published in Science with the title of "Countercurrent Chromatography: Liquid–Liquid Partition Chromatography Without Solid Support" (8). There, the name "countercurrent chromatography" was proposed for the first time. It was firmly established with the CCC acronym by the numerous coming publications.

Subsequently, a preparative system called "droplet CCC" was developed in collaboration with Dr. Takenori Tanimura from Tokyo University. In this preparative scheme (Figure 5, upper left), the inefficient portion of the coil

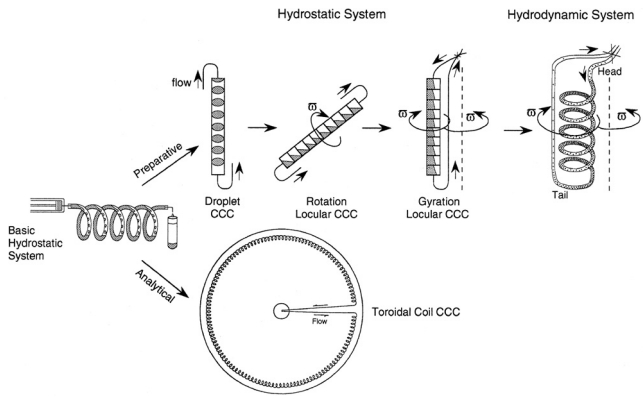

Figure 5. Development of hydrostatic CCC systems.

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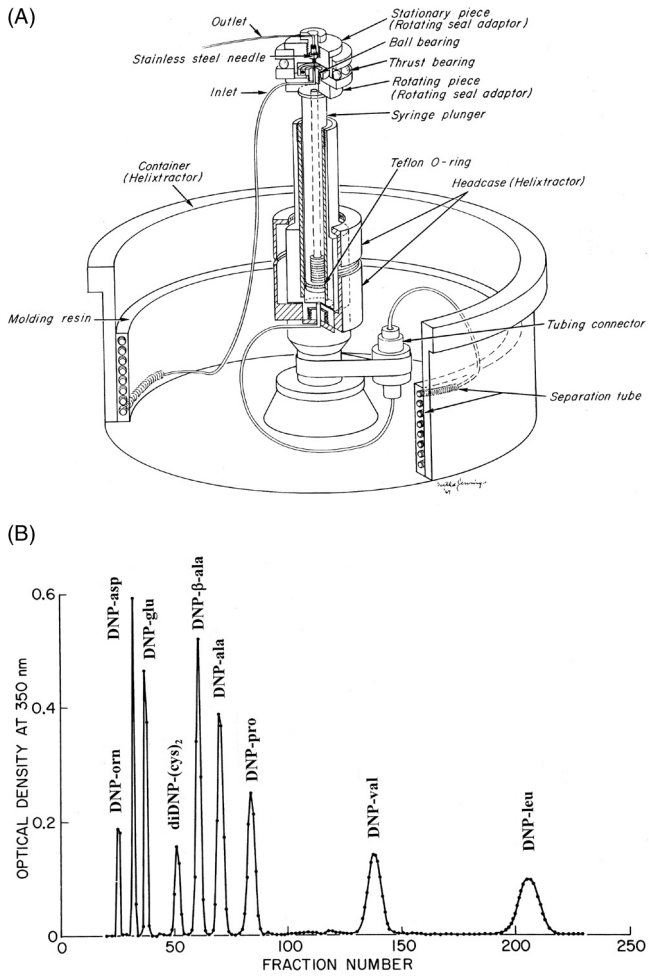

Figure 6. (A) Helix CCC, design of the apparatus; (B) DNP-amino acid separation by helix CCC.

entirely occupied by the mobile phase was reduced to a narrow transfer tube while the efficient portion of the coil was replaced by a straight tubular column. The mobile phase introduced into the vertical column forms multiple droplets in the stationary phase to divide the column space into numerous partition units.

Figure 7A shows our prototype of the droplet CCC instrument consisting of 300 glass tubes, each 2.6 mm ID and 60 cm in length. The system separated DNP amino acids with a high efficiency of about 900 theoretical plates in 70 h (Figure 7B) (9). In this system, the necessity of proper droplet formation limits the choice of possible solvent systems.

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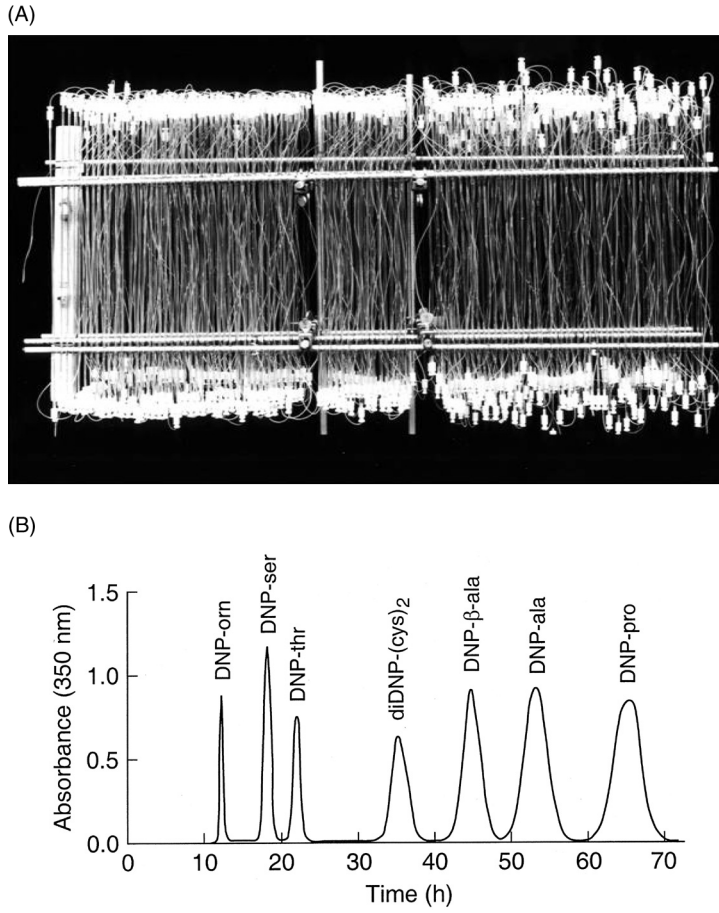

Figure 7. (A) The original prototype of the droplet CCC instrument (1970); (B) DNP-amino acid separation by droplet CCC.

This problem was solved by a locular column made by inserting centrally perforated disks into the column at regular intervals (10). In rotation locular CCC (Figure 5, upper, 2nd from the left), the retention of the stationary phase and the interfacial area were optimized by the inclination of the column while the mixing of the two phases was accomplished by the rotation of the column. This scheme permits universal application of conventional two-phase solvent systems.

This rotation locular CCC was further improved by gyration locular CCC, where the locular column was held vertical and gyrated to introduce circular motion of two phases and their interface within each compartment (Figure 5, upper, 3rd from left) (10). This gyrating motion produced more efficient mixing, but more importantly it eliminated the need for the rotary seal.

However, gyrating motion often damaged the locular column, and this suggested the alternative use of a coiled tube as the separation column (Figure 5, upper right). Surprisingly enough, a simple coiled tube mounted around the holder yielded much higher efficiency than the locular column. The combination of the coiled column and the gyrating motion apparently created an Archimedean Screw effect that improved both mixing of the two phases and retention of the stationary phase in the column. Consequently, this finding became important for the development of the hydrodynamic system. The results were published in Science as "Countercurrent Chromatography With Flow-Through Coil Planet Centrifuge" (11). Our effort was next directed toward further development of this flow-through coil planet centrifuge.

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DEVELOPMENT OF HYDRODYNAMIC SYSTEMS

The development of the hydrodynamic CCC systems was initiated by introduction of a series of flow-through centrifuge schemes free of rotary seals (Figure 8) (7). All these centrifuge schemes are divided into three classes—synchronous, nonplanetary, and nonsynchronous–according to their modes of planetary motion.

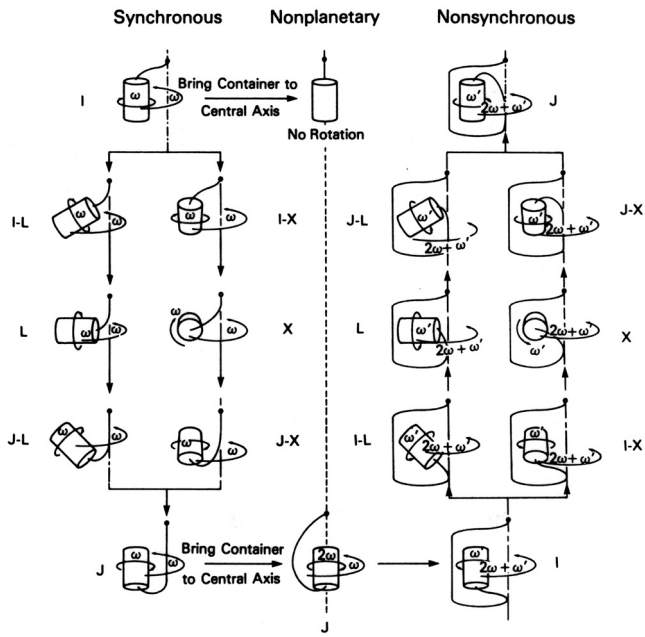

Figure 8. A series of flow-through centrifuge systems free of rotary seals.

In synchronous scheme I, which was also shown in the previous diagram, the vertical coil holder counterrotates about its own axis while revolving around the central axis of the centrifuge. This counterrotation of the holder unwinds the twist of flow tubes caused by revolution, thus eliminating the need for the rotary seal. This same principle can be applied equally well to other synchronous schemes with tilted (12), horizontal (13), and even inverted (14) orientations of the holder.

I would like to discuss the planetary motions of synchronous schemes "I" and the very successful "J." Although they look similar to each other, there are crucial differences between these two planetary motions: In scheme I rotation and revolution of the holder are in opposite directions whereas in scheme J they are in the same direction. Consequently, when the holder of scheme I is brought to the central axis of the centrifuge, the counterrotation of the holder cancels out the revolution effect, resulting in no movement. In contrast, when the holder of scheme J is brought to the central axis, it gains angular velocity because rotation of the holder is added to the revolution effect. Consequently, the holder rotates at doubled speed around the central axis of the apparatus as in an ordinary centrifuge system (15).

This nonplanetary scheme provides a basis for the nonsynchronous schemes. On the base of this nonplanetary system, the holder is again moved away from the central axis to undergo a synchronous planetary motion. Since the revolution rate of the base is independent of the top planetary motion, the rotation-revolution ratio of the holder becomes freely adjustable (16).

Many of these centrifuge schemes have been fabricated at the NIH machine shop to evaluate the potential capability for performing CCC. The test was conducted using a set of coiled columns mounted at the periphery of the column holder. This column geometry however, limited retention of the stationary phase to less than 50% of the total column capacity for all these CCC schemes and application of higher flow rates of the mobile phase resulted in serious loss of the stationary phase from the column.

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DEVELOPMENT OF HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY

In the early 1980s, this problem was solved by an incidental finding when the tube was coaxially wound around the holder of the scheme J coil planet centrifuge as shown in Figure 9 (17–20). To my surprise, the rotation resulted in a rapid and complete separation of two solvent phases along the end-closed coiled tube in such a way that one phase occupied the head side and the other phase the tail side of the coil.

This bilateral phase distribution led to the development of high-speed CCC as illustrated in Figure 10, where all coils are drawn as a straight tube to show the overall hydrodynamic distribution of the two liquid phases. In

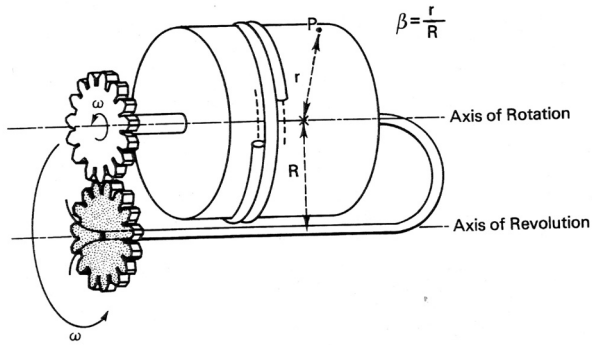

Figure 9. Coaxial coil orientation on the holder of the scheme J coil planet centrifuge for formation of the bilateral hydrodynamic distribution. r: a distance from the axis of the holder to the coil; R: revolution radius.

the top coil (Fig. 10a), two phases establish the bilateral hydrodynamic distribution where the white phase occupies the head side and the black phase occupies the tail side. This hydrodynamic equilibrium suggests that the white phase introduced from the tail end would move toward the head, and similarly the black phase introduced from the head end would move toward

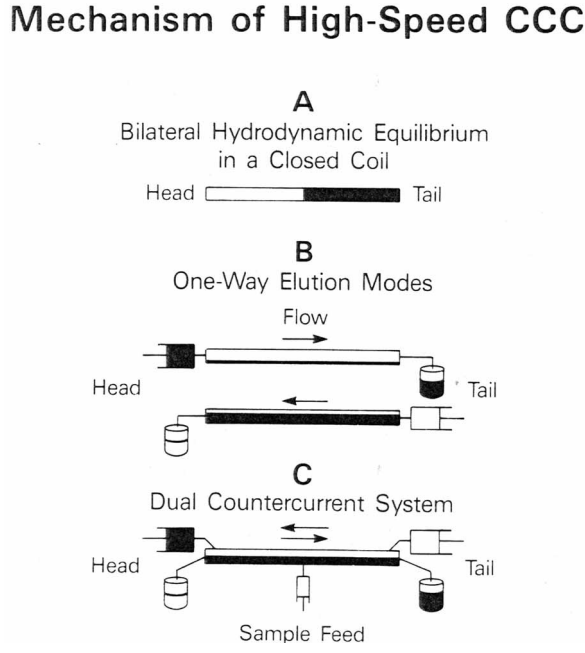

Figure 10. Mechanism of high-speed CCC.

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the tail. This hydrodynamic motion can be effectively used for performing CCC as shown in Figure 10b.

The coil is first entirely filled with the white phase and the black phase is pumped through the head end. Alternatively, the coil is entirely filled with the black phase and the white phase is pumped through the tail end. In either case the system can maintain a high retention level of the stationary phase against a high flow rate of the mobile phase. Thus, this CCC scheme is capable of yielding efficient separations in a shorter elution time (several hours instead of several days).

This system also allows simultaneous elution of the two phases through the corresponding end of the coil (Fig. 10c). This dual CCC operation requires an additional flow tube at each end of the coil to collect the effluent and if desired, the sample feed port is made at the middle portion of the coil. This dual CCC scheme has been successfully applied to foam separation of dyes (21) and natural products by Oka et al. (22).

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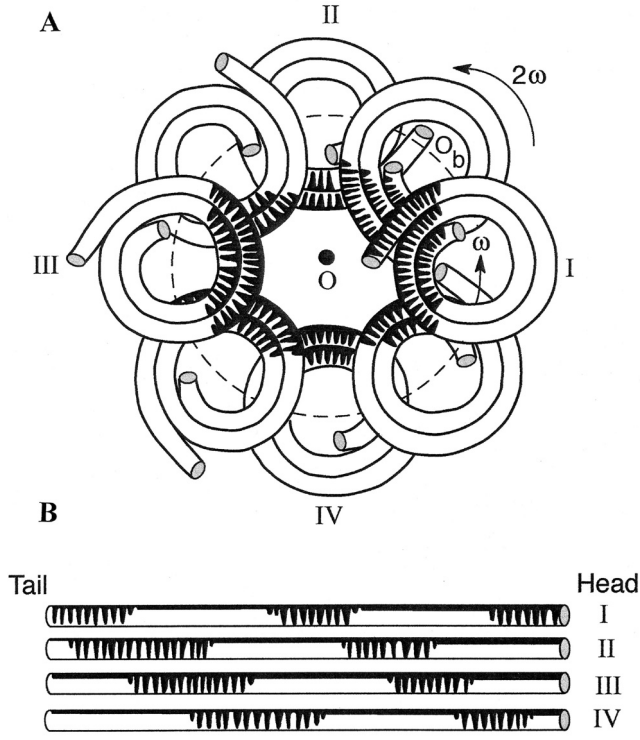

Figure 11. Schematic drawing of hydrodynamic distribution of the two solvent phases in the spiral column of the high-speed CCC centrifuge observed under stroboscopic illumination. O: Center of revolution.

The hydrodynamic motion of the two phases in the present scheme was observed under stroboscopic illumination. The experiment was performed in my laboratory in collaboration with Dr. Walter D. Conway from the New York State University (20, 23). The apparatus used in this study was our prototype of a high-speed CCC centrifuge equipped with a spiral column having a transparent cover for stroboscopic observation. The experiment was initiated by filling the column with the yellow-stained aqueous phase and the red-stained chloroform phase was eluted through the column while the column was rotated at 800 rpm. The stroboscopic observation revealed that the column was divided into two areas: About one-fourth of the area near the center of the centrifuge showed vigorous mixing of the two phases while in the rest of the area the two phases were separated in such a way that the heavier phase occupied the outer portion and the lighter phase the inner portion of the spiral tube.

Figure 11A schematically illustrates the hydrodynamic distribution of the two phases in the rotating spiral column observed by the experiment. The column is divided into two areas, the mixing zone occupying near the center of the centrifuge and the settling zone in the rest of the area. Figure 11B illustrates the motion of the mixing zones through the stretched spiral column from positions I to IV. It demonstrates that each mixing zone travels through the column at a rate of one round per one revolution. This indicates that at every portion of the column the solute is subjected to a partition process of mixing and settling at an very high

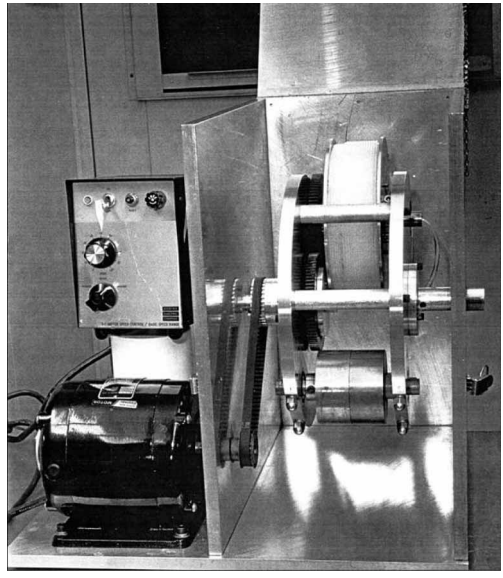

Figure 12. Original scheme J high-speed CCC centrifuge equipped with a counterweight (1981).

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frequency. For example, at 800 rpm the solute is subjected to this partition cycle at a rate of 13 times per s. Because of its high partition capability and speedy separation, the present scheme was named high-speed CCC (20).

Figure 12 shows our first prototype of the high-speed CCC centrifuge. The multilayer coil consists of 130 m long, 1.6 mm ID Teflon tubing with a total capacity of about 280 mL. A counterweight mass was placed on the opposite side of the rotary frame to balance the centrifuge system (17).

The ability of this apparatus was first demonstrated by extraction of urinary metabolites of daunorubicine by Nakazawa et al. (24) as shown in Figure 13. The upper chromatogram was obtained by HPLC analysis of a urine sample from a patient receiving daunorubicin. The lower chromatogram shows HPLC analysis of the same urine sample which had been subjected to

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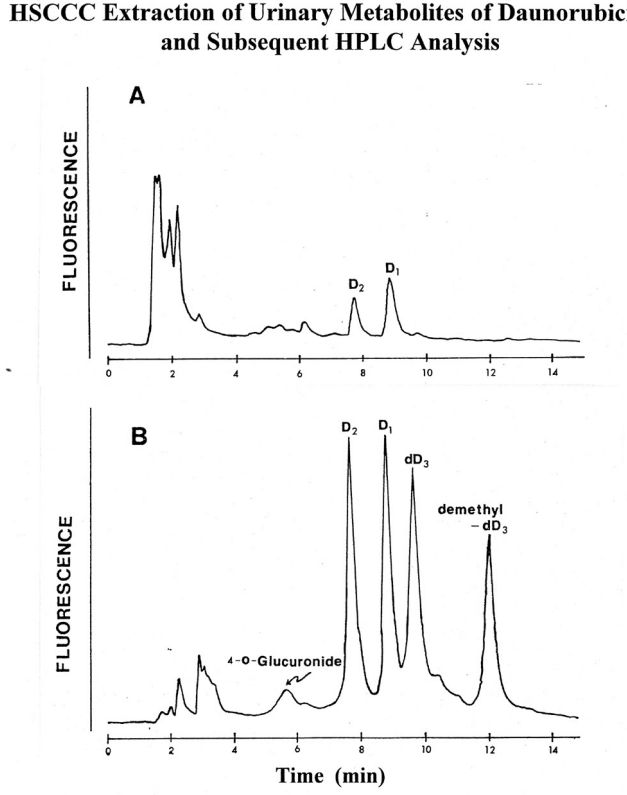

Figure 13. Extraction of urinary metabolite of daunorubicin by high-speed CCC. (A) HPLC analysis of urine before CCC extraction; (B) HPLC analysis of the same urine after CCC extraction.

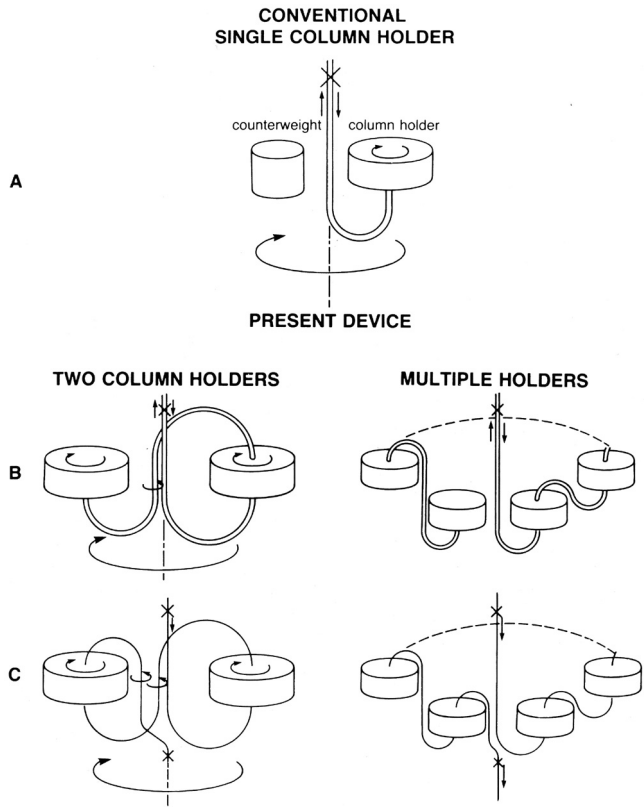

Figure 14. Principle of improved high-speed CCC without counterweight. X indicates the point where the flow tubes are affixed onto the centrifuge axis.

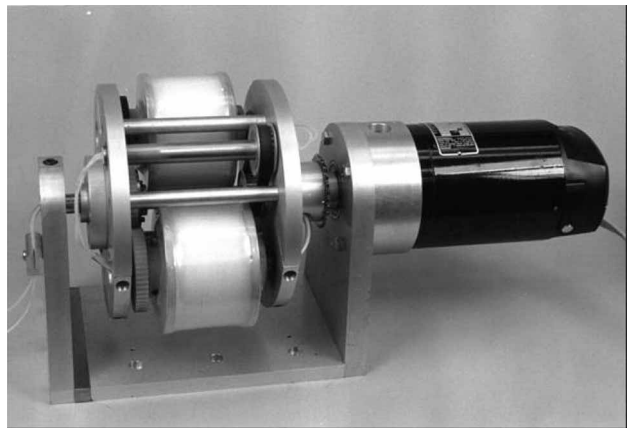

Figure 15. First prototype of the high-speed CCC centrifuge without counterweight (1988).

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extraction by high-speed CCC. Six metabolites of daunorubicin were detected after CCC extraction.

The original design of the high-speed CCC centrifuge was improved later by eliminating the counterweight as illustrated in Figure 14. The top diagram shows the original design of the apparatus equipped with a counterweight (19). In the new design shown below, two or more identical columns are symmetrically arranged around the rotary frame to achieve a stable balancing of the centrifuge system. All columns are connected in series to increase the partition efficiency and the sample loading capacity. Figure 15 shows the first prototype of scheme J high-speed CCC centrifuge equipped with a pair of coil holders (25). Figure 16A shows the most advanced design of the

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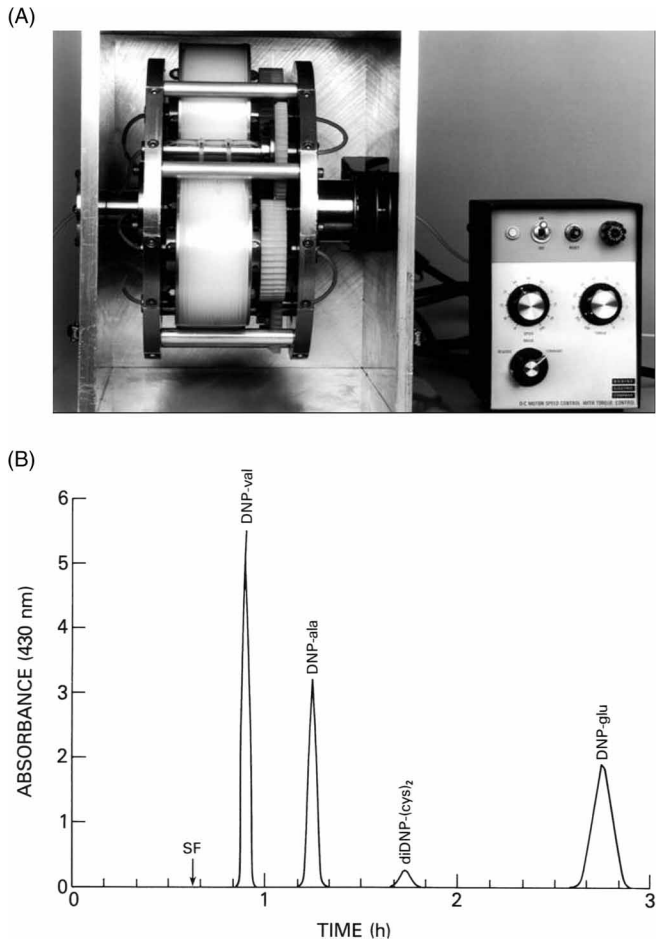

Figure 16. (A) Triplet multilayer coil planet centrifuge (1989); (B) DNP-amino acid separation by the triplet multilayer coil planet centrifuge.

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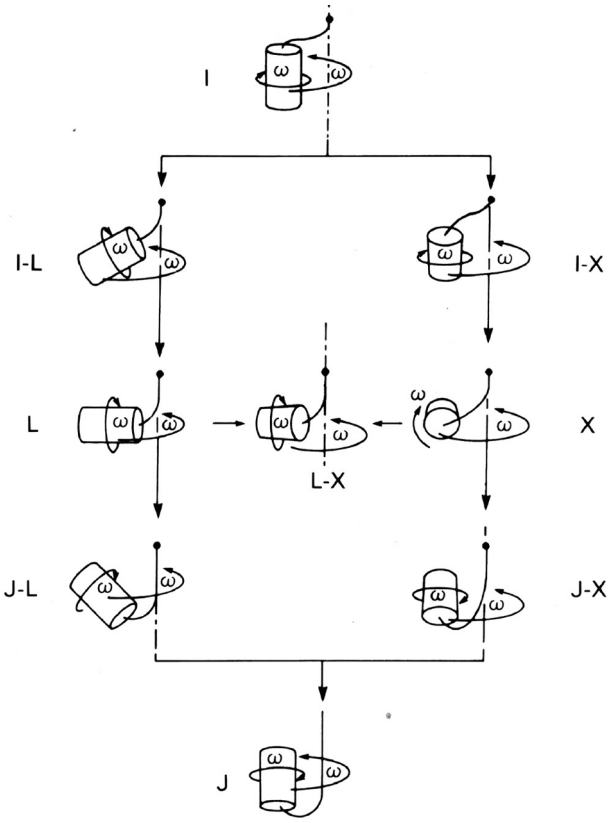

Figure 17. Planetary motion of synchronous scheme L-X coil planet centrifuge.

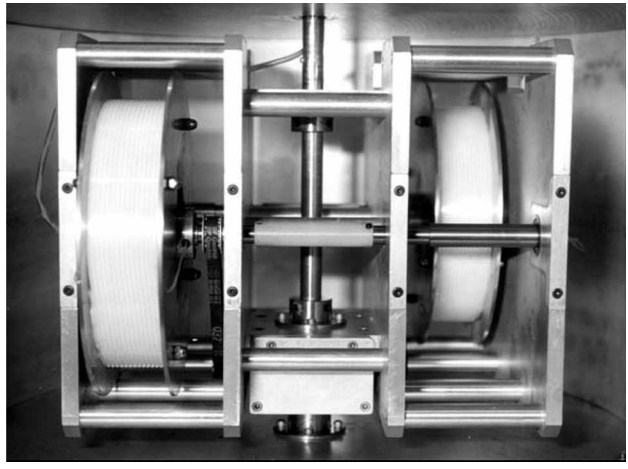

Figure 18. Photograph of a large cross-axis coil planet centrifuge (1989).

high-speed CCC centrifuge equipped with a set of three column holders (26). Each column consists of 100 m long Teflon tubing with a 90 mL capacity. Three multilayer coils are connected in series to triple the capacity to 270 mL. Figure 16B shows a chromatogram obtained from this triplet multilayer coil planet centrifuge. A 10 mg quantity of a DNP-amino acid mixture was separated at a high partition efficiency of several thousand theoretical plates in few hours.

One disadvantage of this scheme J high-speed CCC is that it often fails to retain polymer phase systems useful for the separation of biopolymers such as proteins and nucleic acids. In late eighties it was found that the scheme L-X hybrid planetary motion shown in Figure 17 was more suitable for this purpose (27). In this scheme the holder revolves around the central axis of the centrifuge and simultaneously rotates about its horizontal axis. Because the axes of the rotation and revolution cross each other, this apparatus is called the cross-axis coil planet centrifuge (28).

Figure 18 shows a large cross-axis coil planet centrifuge used for preparative separations (27). The synchronous planetary motion of the holder is produced by a set of miter gears mounted at the bottom of the centrifuge shaft. The motion of the miter gear is conveyed to the column holder by a pair of toothed pulleys coupled with a toothed belt. The system provides stable retention of a large volume of the stationary phase of polar solvent systems for performing efficient preparative separations.

SUMMARY

Figure 19 summarizes the evolution of the coil planet centrifuge during the past 40 years. In the mid 1960s, the coil planet centrifuge first demonstrated the potential capability of CCC in an end-closed coiled tube. A few years later, the seal-free flow-through mechanism was introduced to the coil planet centrifuge so that the system became capable of continuous elution,

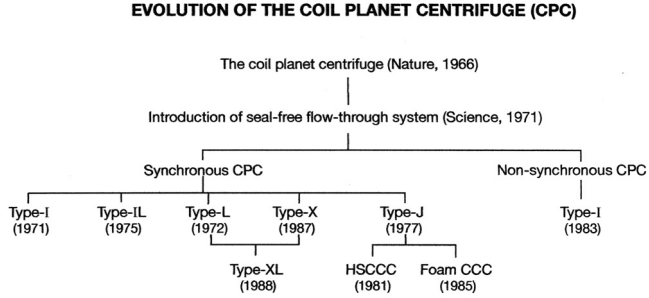

Figure 19. The evolution path of the original coil planet centrifuge during the past 40 years.

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monitoring, and fractionation as in liquid chromatography. During the 1970s a series of seal-free flow-through coil planet centrifuge schemes was developed including schemes I, L, J, and their hybrids.

In the early 1980s, the most important breakthrough in the CCC technology came from the combination of the most successful scheme J synchronous planetary motion and coaxial coil orientation on the holder that drastically shortened the separation times without sacrificing the peak resolution. In 1987 our hydrodynamic studies on the scheme X synchronous planetary motion led to the development of the cross-axis coil planet centrifuge, which provides stable retention of polymer phase systems for the preparative separation of biopolymers.

The coil planet centrifuge originally developed for separation of lymphocytes has evolved through an unexpected course to become an efficient chromatographic system called high-speed CCC that is now widely used for separation and purification of a variety of natural and synthetic products. In the early 21st century, all commercially available CCC coil planet centrifuges are based on the scheme J synchronous planetary motion.

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REFERENCES

1. Craig, L.C. (1962) Chapter 1: Countercurrent Distribution in *Comprehensive Biochemistry*; Elsevier Publishing Co.: Amsterdam, Vol. 4, 1–38.
2. Ito, Y. (1964) Improved gradient method for separation of lymphocytes and neutrophils from human peripheral blood. *Osaka City Med. J.*, 10 (1): 33–40.
3. Ito, Y., Harada, R., Weinstein, M.A., and Aoki, I. (1966) The coil planet centrifuge, principle and application. *Ikakikaigakuzashi*, 36 (7): 1–13 (in Japanese).
4. Ito, Y., Weinstein, M.A., Aoki, I., Harada, R., Kimura, E., and Nunogaki, K. (1966) The coil planet centrifuge. *Nature*, 212: 985–987.
5. Harada, R., Ito, Y., and Kimura, E. (1969) A new method of osmotic fragility test of erythrocytes with coil planet centrifuge. *Jap. J. Physiol.*, 19: 306–314 (in Japanese).
6. Ito, Y., Aoki, I., Kimura, E., Nunogaki, K., and Nunogaki, Y. (1969) New micro liquid-liquid partition techniques with the coil planet centrifuge. *Anal. Chem.*, 41: 1579–1584.
7. Ito, Y. (1981) Countercurrent chromatography. *J. Biochem. Biophys. Methods*, 5 (2): 105–129.
8. Ito, Y. and Bowman, R.L. (1970) Countercurrent chromatography: Liquid-liquid partition chromatography without solid support. *Science*, 167: 281–283.
9. Tanimura, T., Pisano, J.J., Ito, Y., and Bowman, R.L. (1970) Droplet countercurrent chromatography. *Science*, 169: 54–56.
10. Ito, Y. and Bowman, R.L. (1970) Countercurrent chromatography: Liquid-liquid partition chromatography without solid support. *J. Chromat. Sci.*, 8: 315–323.

11. Ito, Y. and Bowman, R.L. (1971) Countercurrent chromatography with flow-through coil planet centrifuge. *Science*, 173: 420–422.
12. Ito, Y. and Bowman, R.L. (1975) Angle rotor countercurrent chromatography. *Anal. Biochem.*, 65: 310–320.
13. Ito, Y., Bowman, R.L., and Noble, F.W. (1972) The elution centrifuge applied to countercurrent chromatography. *Anal. Biochem.*, 49: 1–8.
14. Ito, Y. and Bowman, R.L. (1977) Horizontal flow-through coil planet centrifuge without rotating seals. *Anal. Biochem.*, 82: 63–68.
15. Ito, Y., Suaudeau, J., and Bowman, R.L. (1975) New flow-through centrifuge without rotating seals applied to plasmapheresis. *Science*, 189: 999–1000.
16. Ito, Y., Bramblett, G.T., Bhatnagar, R., Huberman, M., Leive, L., Cullinane, L.M., and Groves, W. (1983) Improved non-synchronous flow-through coil planet centrifuge without rotating seals. Principle and application. *Sep. Sci. Technol.*, 18 (1): 33–48.
17. Ito, Y. (1981) New continuous extraction method with a coil planet centrifuge. *J. Chromatogr.*, 207: 161–169.
18. Ito, Y. (1981) Efficient preparative countercurrent chromatography with a coil planet centrifuge. *J. Chromatogr.*, 214: 122–125.
19. Ito, Y., Sandlin, J.L., and Bowers, W.G. (1982) High-speed preparative countercurrent chromatography (CCC) with a coil planet centrifuge. *J. Chromatogr.*, 244: 247–258.
20. Ito, Y. (1986) High-speed countercurrent chromatography. *CRC Crit. Rev. Anal. Chem.*, 17 (1): 65–143.
21. Ito, Y. (1985) Foam countercurrent chromatography based on dual countercurrent system. *J. Liq. Chromatogr.*, 8: 2131–2152.
22. Oka, H., Harada, K., Suzuki, M., Nakazawa, H., and Ito, Y. (1989) Foam countercurrent chromatography of bacitracin: Part I. Batch separation with nitrogen and water free of additives. *J. Chromatogr.*, 482: 197–205.
23. Conway, W.D. (1990) *Countercurrent Chromatography: Apparatus, Theory, and Applications*; VCH Publishers: Weinheim, Germany.
24. Nakazawa, H., Andrews, P.A., Bachur, N.R., and Ito, Y. (1981) Isolation of daunorubicin derivatives by countercurrent chromatography with the horizontal flow-through coil planet centrifuge. *J. Chromatogr.*, 205: 482–485.
25. Ito, Y. and Chou, F.-E. (1988) New high-speed countercurrent chromatograph equipped with a pair of separation columns connected in series. *J. Chromatogr.*, 454: 382–386.
26. Ito, Y., Oka, H., and Slemp, J.L. (1989) Improved countercurrent chromatograph with three multilayer coils connected in series I. Design of the apparatus and performance of semipreparative columns in DNP amino acid separation. *J. Chromatogr.*, 475: 219–227.
27. Ito, Y., Oka, H., and Slemp, J.L. (1989) Improved cross-axis synchronous flow-through coil planet centrifuge for performing countercurrent chromatography. I. Design of the apparatus and analysis of acceleration. *J. Chromatogr.*, 463: 305–316.
28. Ito, Y. (1987) Cross-axis synchronous flow-through coil planet centrifuge free of rotary seals for preparative countercurrent chromatography. Part I. Apparatus and analysis of acceleration. *Sep. Sci. Technol.*, 22 (8–9): 1971–1988.

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