Journal of Chromatography, 538 (1991) 3–25
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 863

Review

Recent advances in counter-current chromatography

YOICHIRO ITO
Laboratory of Biophysical Chemistry, National Heart, Lung, and Blood Institute, Building 10, Room 7N-322, National Institutes of Health, Bethesda, MD 20892 (U.S.A.)

ABSTRACT

During the past several years, counter-current chromatography (CCC) technology has been advanced to cover a broad spectrum of applications, from large-scale preparative to analytical-scale separations. These advances include liquid–liquid dual CCC, foam CCC and partition of macromolecules with aqueous–aqueous polymer phase systems. For these developments the synchronous coil planet centrifuge scheme has been used, which relies on a relatively simple mechanical design. Future developments in CCC may be focused on the improvement of the more intricate non-synchronous coil planet centrifuge scheme which has a greater potential for the separation of biopolymers and cell particles.

CONTENTS

1. DEVELOPMENTAL BACKGROUND OF COUNTER-CURRENT CHROMATOGRAPHY

Since the first introduction of counter-current chromatography (CCC) in 1970 [1], the method has been steadily improved to shorten elution times so that efficient chromatographic separations can be completed within a few hours.

Early developments in hydrostatic CCC systems such as helix CCC [1–3], droplet CCC [2–4] and locular CCC [2,3] have been superseded by more efficient

0021-9673/91/$03.50

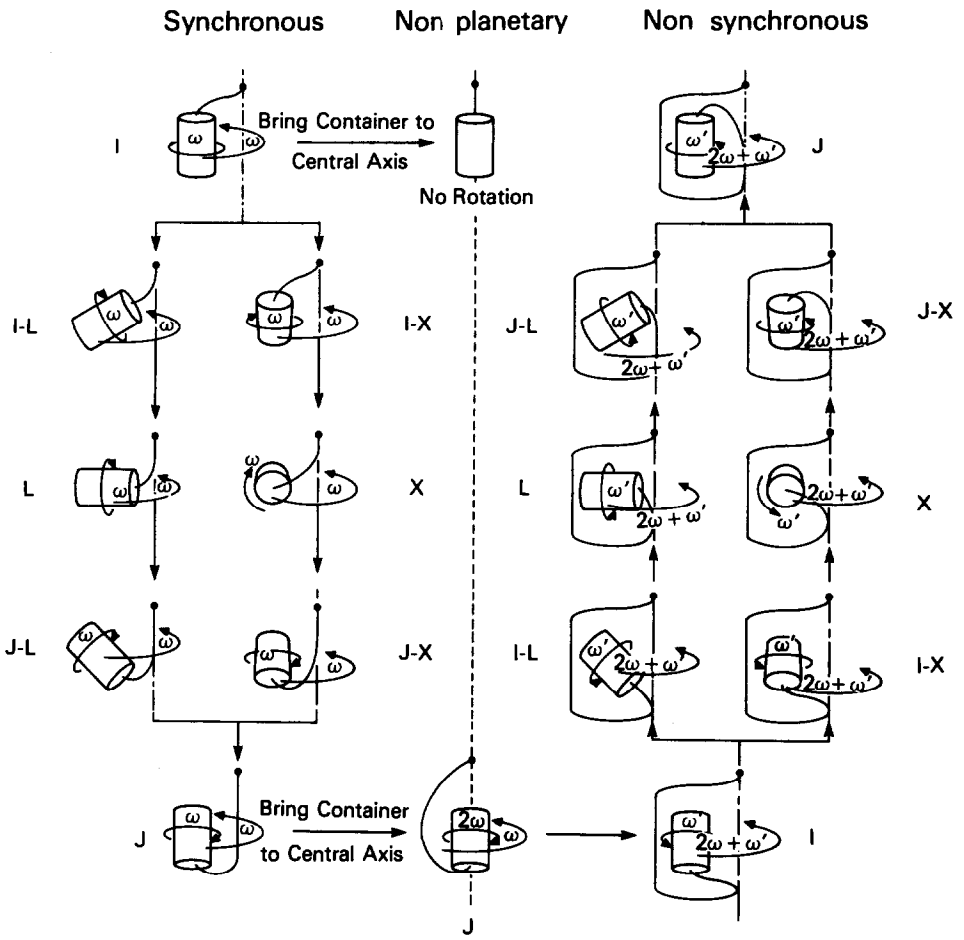

Fig. 1. Rotary-seal-free flow-through centrifuge systems for performing CCC. Each diagram indicates orientation and motion of a cylindrical column holder with a bundle of flow tubes, the other end being tightly supported on the central axis of the centrifuge. These centrifuge systems are divided into three categories according to their mode of planetary motion as indicated at the top of the diagram. In the type I synchronous planetary motion, the holder revolves around the central axis of the centrifuge and simultaneously counter-rotates about its own axis at the same angular velocity. The counter-rotation of the holder steadily unwinds the twist of the tube bundle, thus eliminating the need for the rotary seal. This principle works equally well with other synchronous systems in tilted (I-L and I-X), horizontal (L and X), dipping (J-L and J-X) and finally inverted (J) orientation of the holder. Here, the I–L–J series is obtained by tilting the holder radially toward the central axis of the centrifuge whereas the I–X–J is formed by rotating the holder tangentially relative to the revolutional orbit. This type J is a transitional form to the non-planetary system J. When the holder of type I synchronous planetary motion is moved to the central axis as indicated by an arrow, the holder becomes stationary due to cancellation of the rotation of the holder by revolution. However, when the same treatment is applied to the type J synchronous planetary motion, the holder gains the angular velocity by ω, because the revolution of the holder is added to the rotation. Thus, the holder rotates at 2ω around the central axis of the centrifuge. This non-planetary system provides a base for the non-synchronous systems. The holder of the non-planetary system is again moved away from the central axis to undergo synchronous planetary motions of types I, L, X, J and their hybrids. Although these planetary motions are synchronous with respect to the base, the net revolutional speed of the holder is the sum of the revolution rates of the top and the base. Since the revolution of the base is independent of the top planetary motion, the rotation/revolution ratio of the holder becomes freely adjustable.

hydrodynamic CCC systems which utilize synchronous planetary motion of the coiled column to produce efficient mixing of the two solvent phases promoting the partition process. Fig. 1 illustrates a series of flow-through centrifuge systems developed for performing CCC [5]. All these systems permit continuous elution through a rotating column without the use of a rotary seal device (see figure caption for details). These rotary-seal-free flow-through centrifuge systems provide various advantages such as leak-free elution under high back pressure, use of multiple flow channels without cross-contamination, elimination of the risks associated with heating or clogging of channels at the site of the rotary seal, and negligible dead space responsible for band broadening. Early designs based on these centrifuge systems have demonstrated that synchronous schemes I [6,7], I-L [8] and L [9,10] produce efficient analytical-scale separations, while synchronous scheme J [11,12] yielded excellent preparative-scale separations. However, in all these schemes the volume of the stationary phase retained in the separation column was usually limited to less than 50% of the total column capacity and the application of a high flow-rate of the mobile phase sharply decreased the retention volume of the stationary phase, resulting in serious loss in peak resolution.

In the early 1980s a great advance in the CCC technology was made by the discovery of a new hydrodynamic phenomenon in a rotating coiled tube [13]. When a coil containing two immiscible solvent phases is rotated around its horizontally placed axis at a critical angular velocity (*ca.* 100 rpm), the two solvent phases are unilaterally distributed along the length of the coil in such a way that one phase entirely occupies the head side and the other phase the tail side of the coil. Here, the head–tail orientation of the coil is referred to an Archimedean screw force which drives all objects in a rotating coil competitively toward the head of the coil. This particular hydrodynamic behavior of the two solvent phases can be used for performing CCC to retain a large volume of the stationary phase in the coiled column as described later in this article. A similar hydrodynamic phenomenon has been observed in the coil coaxially mounted on the holder in the scheme J synchronous coil planet centrifuge [14]. The application of the centrifugal force field has enhanced the hydrodynamic motion of the two solvent phases resulting in an excellent chromatographic separation in a short period of time [15,16]. More recently, it was found that this high-speed CCC system can also be obtained from other synchronous planetary motions such as schemes J-L [17,18] and X [19,20].

2. RECENT DEVELOPMENTS IN COUNTER-CURRENT CHROMATOGRAPHY

2.1. Large-scale preparative counter-current chromatography

The feasibility of large preparative CCC has been examined using a slowly rotating coiled tube [21]. A simple rotary coil assembly was used to study hydrodynamic distribution of the two immiscible solvent phases in a rotating glass coil (Fig. 2).

In each experiment, the coil was filled with about equal volumes of the two phases. In order to facilitate observation of the interface between the two phases, a small amount of a dye was added to color one of the phases. Then, the coil was sealed at both ends and rotated at a desired speed. After a steady state hydrodynamic equilibrium was established, rotation was stopped to allow the two phases to settle in

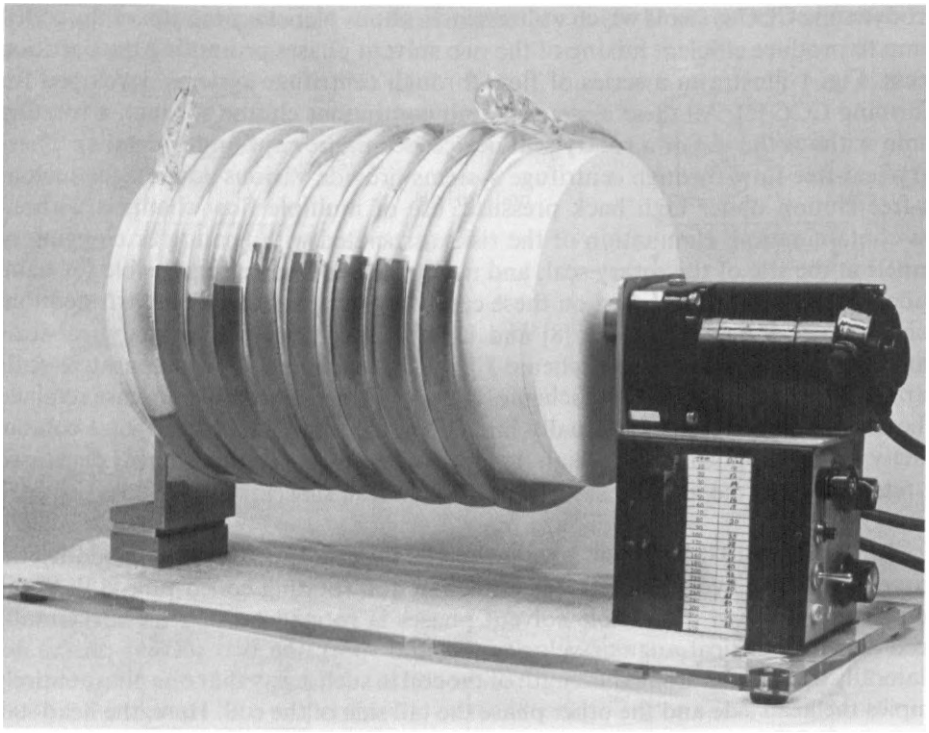

Fig. 2. Rotating coil assembly.

each turn of the coil. The volume of each phase occupying the head side of the coil was then measured. The experiment was repeated after changing the rotational speed of the coil.

The results of the experiments are summarized in Fig. 3 where a set of phase distribution diagrams are arranged according to the applied two-phase solvent system (top) and the coil dimensions, including internal diameters and core diameters (left), as indicated in the figure. In each diagram, the percentage of the heavier phase occupying the head side of the coil was plotted against the rotation speed of the coil. Experiments were performed with various two-phase solvent systems in 1 cm I.D. (upper panel) and 2 cm I.D. (lower panel) coils with helical diameters ranging from 2.5 to 20 cm. The solid line was obtained from the plain glass coil and the dotted line from the silicone-coated glass coil.

The results clearly show that all solvent systems display a unilateral phase distribution (100% or 0%) at a critical rpm where the head side of the coil is entirely occupied by one phase. This critical range is rather insensitive to the helical diameter of the coil in all the solvent systems. This critical rpm can be used in various ways, as illustrated in Fig. 4 where several coiled tubes are schematically drawn uncoiled to indicate the overall volume distribution of the two solvent phases along the length of the coil.

The top diagram (A) shows a bilateral phase distribution established in an

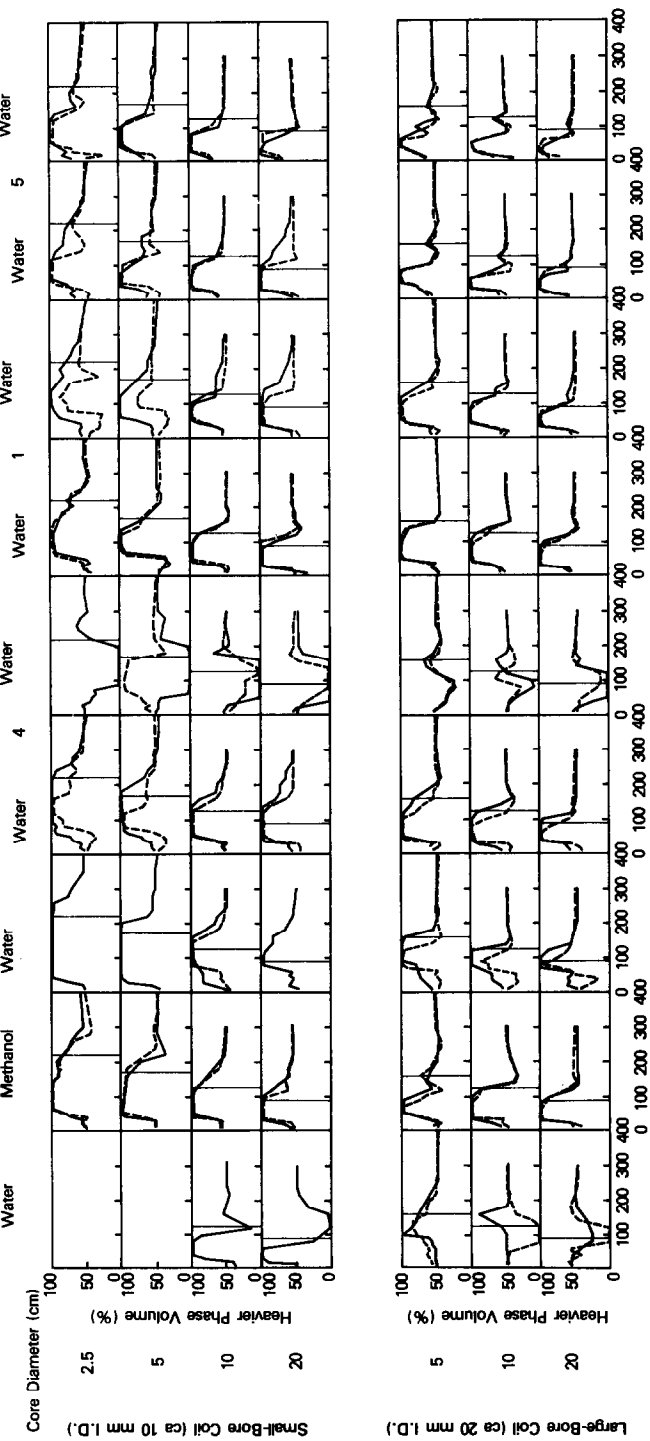

Fig. 3. Hydrodynamic phase distribution of the two immiscible solvent phases in slowly rotating coils. The coil dimensions are expressed in terms of the I.D. and core diameter as indicated on the left margin.

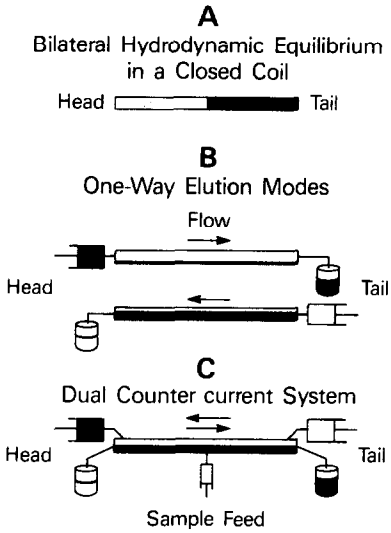

Fig. 4. Mechanism of high-speed CCC.

end-closed rotating coil with the white phase in the head side and the black phase on the tail side. This hydrodynamic distribution was previously called "unilateral" since one of the phases occupies the head side of the coil [22,23]. The white phase occupying the head side is the heavier phase in the present system, but it usually becomes the lighter phase in the centrifugal CCC schemes such as high-speed CCC. In the one-way elution mode (B), the coil is first entirely filled with the white phase followed by elution with the black phase from the head toward the tail. Alternatively, the coil is initially filled with the black phase followed by elution with the white phase from the reverse direction. In either case, the system can retain a large volume of stationary phase in the coil while efficient mixing is produced by rotation of the coil.

In the dual counter-current operation (C), the two solvent phases are simultaneously introduced into the coil through the respective terminals. This operation requires an additional flow tube at each end of the coil and, if desired, a sample feed port is made at the middle portion of the coil. This dual CCC system permits continuous sample feeding as well as batch sample loading. The rotary-seal-free multichannel flow-through system illustrated in Fig. 1 (non-planetary motion J) can be used to avoid various complications arising from the use of conventional multichannel rotary seal devices.

All these systems allow preparative CCC at a relatively slow rotation of the coil using the effect of the unit gravity. Consequently, this simple CCC scheme can be safely applied to a large-capacity multilayer coiled column without mechanical constraint and the system is easily automated for separations of hazardous materials such as radioactive samples.

2.2 Preparative counter-current chromatography

For laboratory-scale multigram separations, we have developed a new coil

planet centrifuge called the cross-axis synchronous flow-through coil planet centrifuge (X-axis CPC) [19,20]. It was designed according to the scheme X synchronous planetary motion previously described (Fig. 1).

Fig. 5 shows the orientation and planetary motion of the column holder of the X-axis CPC. The holder revolves around the main axis of the centrifuge while it simultaneously rotates about its own axis at the same angular velocity in the indicated direction. The bundle of flexible flow tubes supported at the central axis of the centrifuge does not twist because the synchronous rotation of the holder steadily unwinds each twist caused by revolution. Thus, the system eliminates the need for the rotary seal.

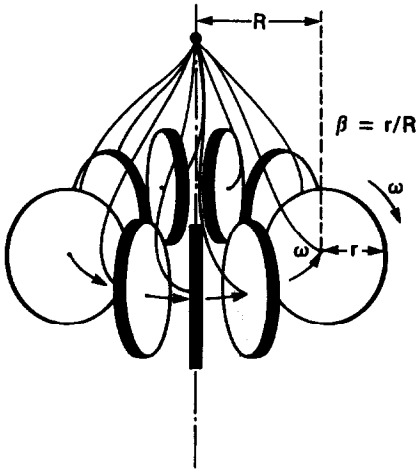

Fig. 5. Orientation and motion of the column holder in the cross-axis synchronous flow-through coil planet centrifuge.

The acceleration generated by this unique planetary motion has been mathematically analyzed [19]. The results indicate that the system produces a three-dimensional fluctuation of the centrifugal force field. The radial force field around the holder axis resembles that in the scheme J synchronous planetary motion, producing a bilateral phase distribution in the coil. The laterally-acting force field parallel to the holder axis is unique to the present system and promotes mixing of the two solvent phases across the diameter of the tube without undesirable longitudinal spreading of the sample bands.

Fig. 6 shows the original prototype of the X-axis CPC used in performing preparative separations [20]. The motor drives the rotary frame around the central axis of the centrifuge. The rotary frame consists of a pair of side plates rigidly bridged with links supporting a column holder and a counter-weight holder in symmetrical positions at a distance of 10 cm from the central axis of the centrifuge. A set of miter gears mounted at the bottom of the centrifuge drives a pair of toothed pulleys, and this motion is conveyed to each holder with a toothed belt.

The column is a multilayer coil prepared from a single piece of PTFE (polytetrafluoroethylene) tubing with a total capacity of approximately 400 ml. The

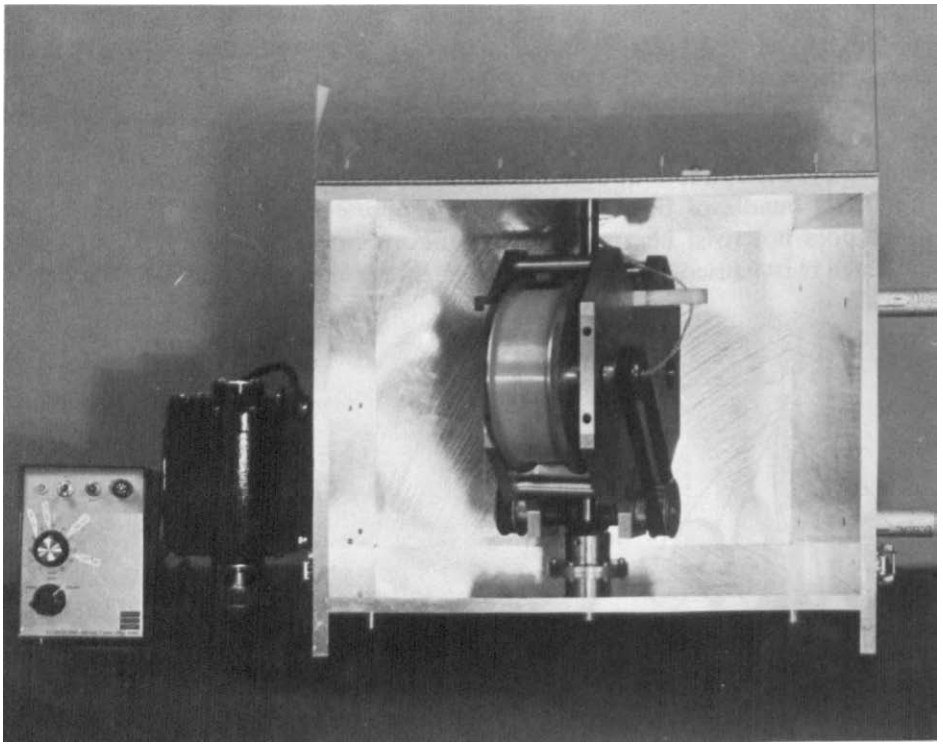

Fig. 6. Original cross-axis synchronous flow-through coil planet centrifuge.

preparative capability of this original apparatus was successfully demonstrated in separation of gram-quantities of dinitrophenyl amino acids and dipeptides [20].

In order to extend the capacity of the system, we have designed a second prototype of the X-axis CPC which has twice the revolution radius and is equipped with a long column holder measuring 25 cm in width [24–26]. A series of studies on the hydrodynamic distribution of the two solvent phases in a short coil revealed, interestingly, that the retention of the stationary phase is largely affected by the position of the coil on the holder. In the lateral coil position on the holder, the direction of the planetary motion further produced a strong effect on the stationary phase retention. Thus, with a proper combination of the planetary motion and the head–tail elution mode, the system produced extremely high retention of the stationary phase which greatly exceeded that obtained from the central coil position. The lateral coil position on the holder shaft also provides an additional advantage in that the apparatus can accommodate a pair of large diameter column holders which extend over the central axis of the apparatus. A pair of multilayer coils symmetricaly mounted on the rotary frame can be connected in series with a flow tube to double the column capacity.

In light of the above findings, a third prototype of the X-axis CPC was constructed [27,28]. Fig. 7 shows the apparatus which holds a pair of large multilayer coils in the lateral positions at 10 cm from the center of the holder shaft which in turn is

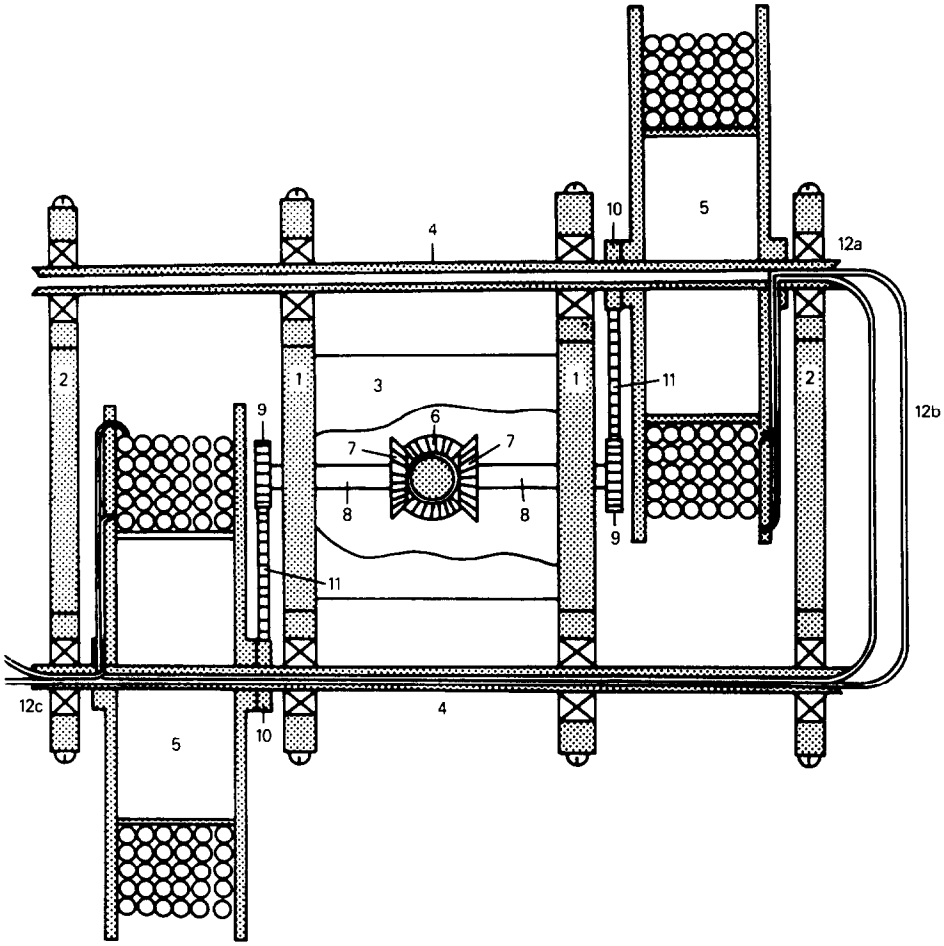

Fig. 7. Horizontal cross-sectional view of the improved cross-axis coil planet centrifuge. 1,2 = Side plates; 3 = bottom plate; 4 = column holder shafts; 5 = column holders; 6 = stationary miter gear; 7 = planetary miter gears; 8 = countershafts; 9,10 = toothed pulleys; 11 = toothed belts; 12a–c = flow tubes.

located at a distance of 10 cm from the central axis of the centrifuge. These two columns are serially connected to make up a total capacity of about 1.6 l.

The preparative capability of the apparatus was demonstrated in multi-gram separations of various biological samples [29]. Fig. 8 shows a chromatogram of crude synthetic steroids using the X-axis CPC. A 2.4-g quantity of the crude reaction mixture was efficiently separated into multiple peaks in 15 h. Five steroids corresponding to peaks 1–5 were analyzed by NMR as illustrated on the right side of the chromatogram. The desired product was found at peak 5 and over 300 mg of the crystalline material was obtained in high purity.

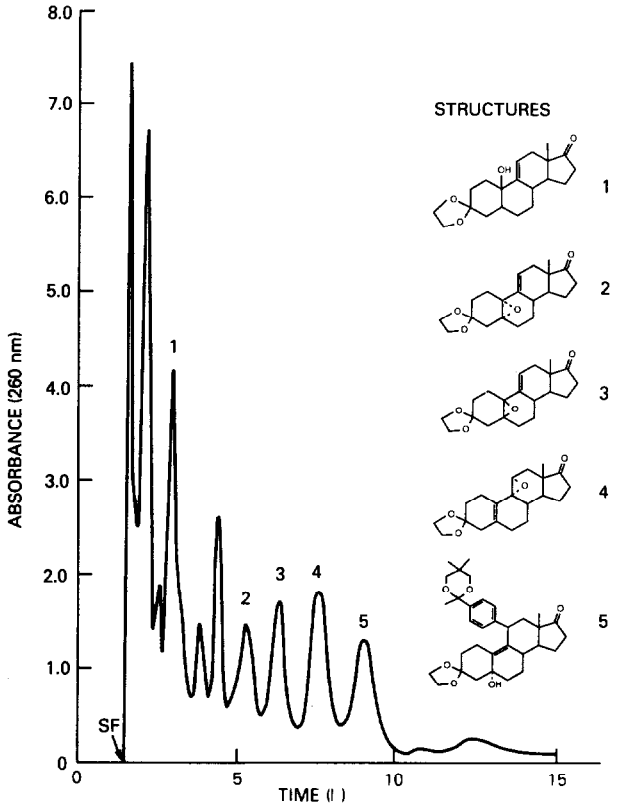

Fig. 8. Chromatogram of synthetic steroid intermediates obtained by the cross-axis coil planet centrifuge. Apparatus: cross-axis CPC, 20 cm radius; column: multilayer coils, 2.6 mm I.D., 1600 ml capacity; sample: crude steroid intermediates, 2.4 g; solvent system: hexane–ethyl acetate–methanol–water (6:5:4:2); mobile phase: lower aqueous phase; elution mode: head → tail; flow-rate: 240 ml/h; revolution: 450 rpm (P_1); retention: 71.3%.

2.3. Semi-preparative high-speed counter-current chromatography

The high-speed CCC centrifuge developed in the early 1980s has been successfully commercialized by P.C. Inc. (Potomac, MD, U.S.A.) and Pharma-Tech Research Corporation (Baltimore, MD, U.S.A.). Recently, we have made two important improvements on the performance of this apparatus.

Fig. 9 shows the principle of the first innovation [30]. The standard high-speed CCC centrifuge shown at the top holds a column holder on one side of the rotary frame and has a counterweight on the other side to balance the centrifuge system. The new design shown at the bottom eliminates the counterweight and accommodates two or more identical column holders symmetrically around the rotary frame. These holders can be interconnected with flow tubes in series so that both partition efficiency and the sample loading capacity are improved. In addition, the system totally eliminates the need for tedious adjustment of the counterweight mass and achieves perfect balancing of the centrifuge system once the column is equilibrated with the two solvent phases.

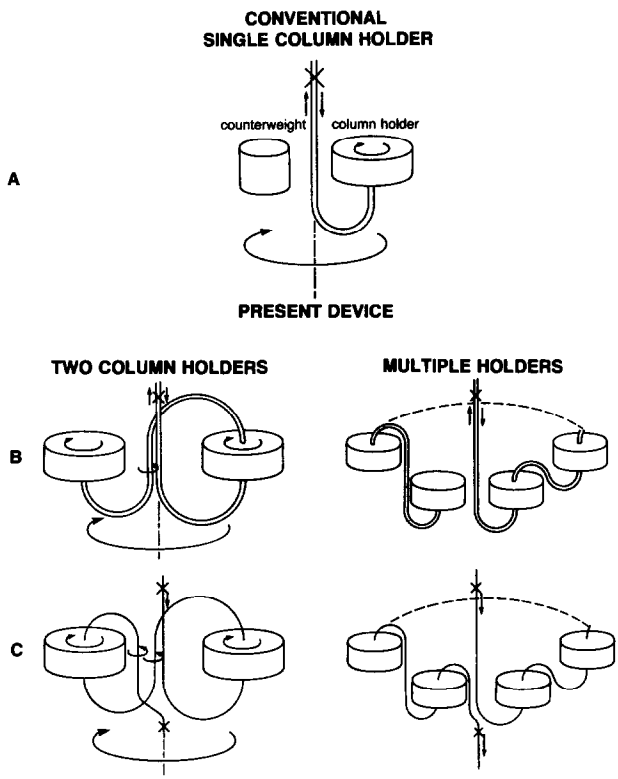

Fig. 9. Design principle of the multiholder coil planet centrifuge (type J synchronous planetary motion). (A) The conventional coil planet centrifuge equipped with a single holder and a counterweight. (B) Multiholder coil planet centrifuge systems with double passage of the flow tubes. (C) Multiholder coil planet centrifuge systems with a single passage of the flow tubes.

Fig. 10 shows our most recent high-speed CCC centrifuge equipped with three multilayer coils connected in series. This unit can be operated at 1250 rpm and yields high partition efficiencies of several thousand theoretical plates [31–33]. The capability of the apparatus has been demonstrated in separations of various samples including indole plant hormones, tetracycline derivatives, bacitracin components, flavonoids from a crude sea buckthorn extract, triterpenoic acids, and rare earth elements.

A second improvement in the apparatus involved the detection system of the effluent [34]. Since the CCC technology utilizes a two-phase solvent system, carryover of small droplets of the stationary phase from the separation column tends to disturb the uv detector. The two-solvent phases are always in a subtle equilibrium state, and any change of the ambient temperature may produce turbidity of the mobile phase due to formation of micro-droplets of the stationary phase. In addition to the above complications inherent to CCC, the pressure drop at the periphery of the flow passage may generate gas bubbles from the effluent disturbing the recording of the elution curve.

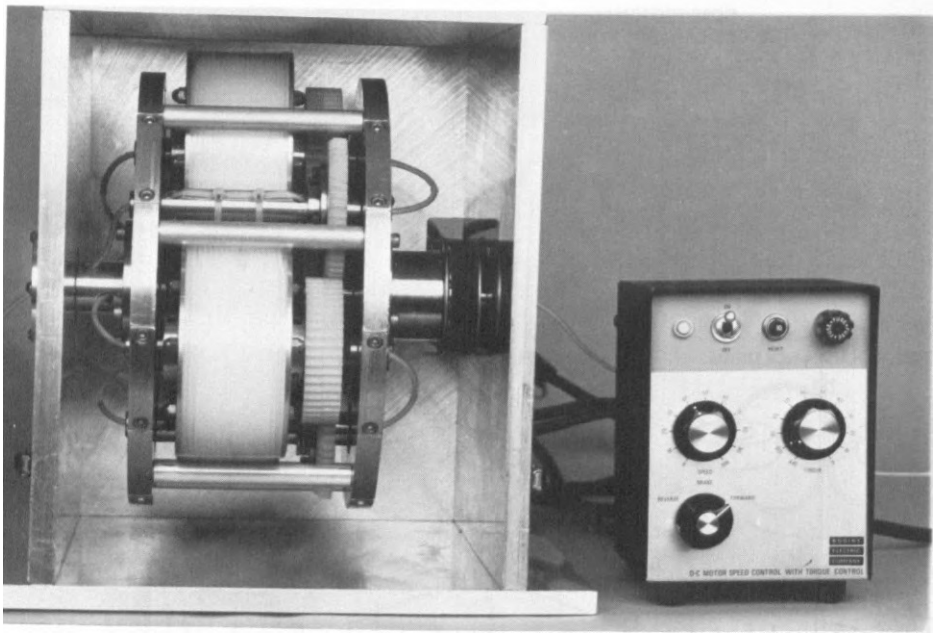

Fig. 10. High-speed CCC centrifuge equipped with three multilayer coils connected in series.

Fig. 11A shows a typical chromatogram obtained by the online UV tracing in high-speed CCC. In the chloroform solvent system, slightly altered ambient temperature in the flow cell produced turbidity of the mobile phase causing noise. This was superimposed on a higher frequency noise produced from gas bubbles generated under a reduced pressure.

These complications were totally eliminated by heating the effluent at 30°C at the inlet of the UV monitor and attaching a narrow-bore tube at the outlet of the monitor to prevent a sudden pressure drop in the flow cell. Fig. 11B shows an improved chromatogram which is comparable in quality with those obtained from high-performance liquid chromatography.

2.4. Analytical counter-current chromatography

In the past, development of the CCC instruments has been mainly directed toward preparative applications because of its long elution time. However, the recent advent of high-speed CCC has considerably shortened the separation times without sacrificing the peak resolution. Several different types of the high-speed CCC centrifuges are now available for analytical-scale separations.

Fig. 12A shows the original analytical high-speed CCC centrifuge with a 5 cm revolutional radius which can be operated up to 2000 rpm [35]. The analytical capability of the apparatus was demonstrated in separation of indole auxins at partition efficiency of 1600 theoretical plates (TP). The apparatus has been successfully interfaced to a mass spectrometer with a thermospray capillary device [36].

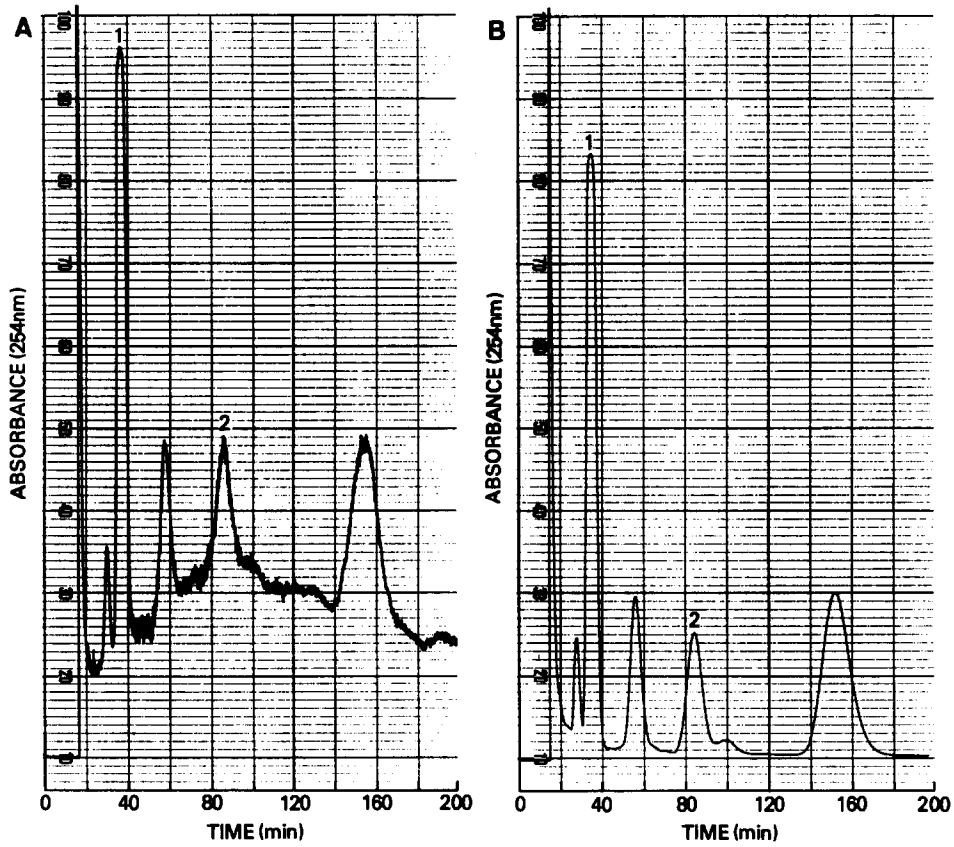

Fig. 11. High-speed CCC separation of flavonoids recorded by on-line UV monitoring with the conventional method (A) and improved method (B).

Because the narrow thermospray capillary required to limit flow into the mass spectrometer creates a high back pressure, direct interfacing is difficult. Therefore, the effluent from the outlet of the CCC column was split into two streams with the smaller flow proceeding into the mass spectrometer. Fig. 12B shows a total ion chromatogram of an alkaloid mixture obtained by high-speed CCC–mass spectrometry using a thermospray device in the mass spectrometer. This chromatogram revealed an isomer of vincine which was not resolved in high-performance liquid chromatographic analysis [36].

Fig. 13A shows a compact model of the analytical high-speed CCC centrifuge with a 2.5 cm revolutional radius [37]. This unit can be operated at a maximum speed of 4000 rpm while the capacity of the multilayer coil (0.85 mm I.D.) was limited to about 10 ml. The apparatus can yield efficient microgram-quantity separations as demonstrated in a rapid separation of flavonoids in a crude sea buckthorn extract (Fig. 13B).

Most recently, we have reevaluated the analytical performance of the toroidal coil centrifuge (helix CCC) by mounting a long coiled column made of narrow-bore (0.3–0.4 mm I.D.), thick-wall PTFE tubing. In order to speed the separation, mobile

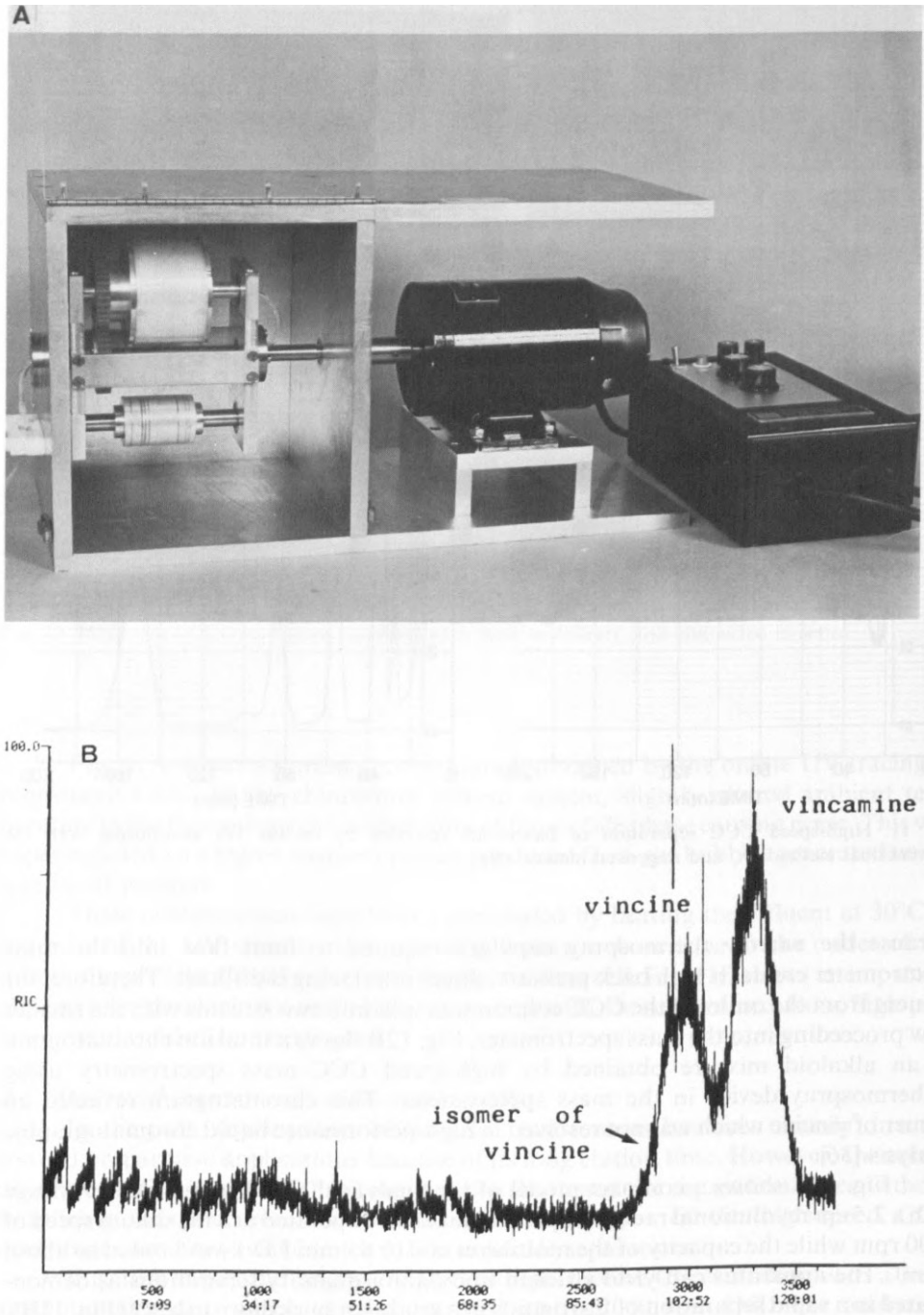

Fig. 12. (A) Original analytical high-speed CCC centrifuge. (B) Total ion chromatogram of alkaloids obtained by high-speed CCC–mass spectrometry with a thermospray capillary device; sample: vincamine–vincine mixture; horizontal scales: top, scan No.; bottom, time in min:s.

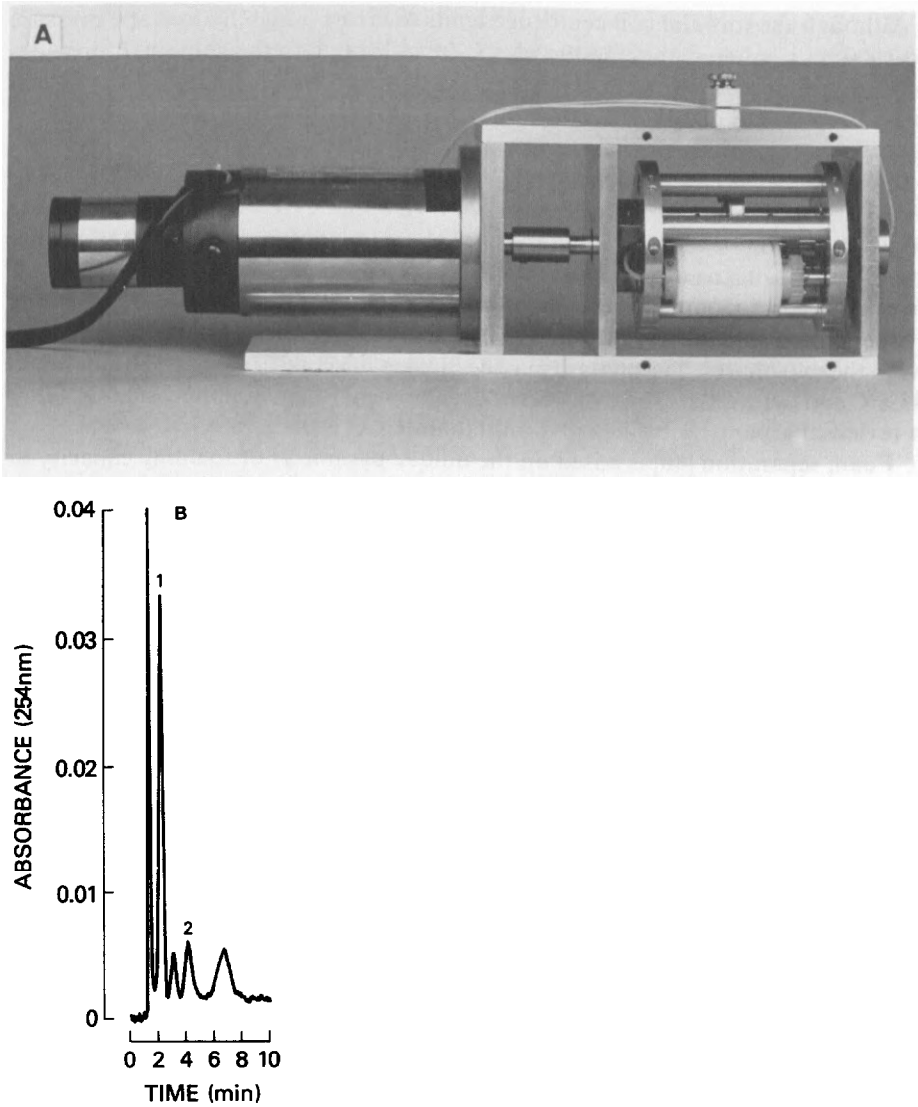

Fig. 13. (A) Analytical high-speed CCC centrifuge with 2.5 cm revolution radius. (B) Chromatogram of flavonoids obtained by the analytical high-speed CCC centrifuge. Peaks: 1 = isorhamnetin; 2 = quercetin. Conditions: apparatus, high-speed CCC centrifuge with 2.5 cm revolution radius, single multilayer coil, 0.85 mm I.D. and 8 ml capacity; sample, flavonoids from crude ethanol extract of *H. rhamnoides*, 210 μg; solvent system, chloroform–methanol–water (4:3:2, v/v/v); mobile phase, lower non-aqueous phase; flow-rate, 2 ml/min; revolution, 3500 rpm.

phase was pumped at a flow-rate of 1 ml/min while the column was subjected to a strong centrifugal force field (190 *g*) in order to retain a satisfactory volume of the stationary phase in the coil. The method produced rapid and efficient separations of an indole auxin mixture which were comparable to those obtained from the existing analytical high-speed CCC centrifuges in both partition efficiency and analysis time

[38]. Although the toroidal coil centrifuge tends to create a high hydrostatic pressure up to 1000 p.s.i., often damaging the plastic tubes leading to the column, it provides a stable tracing of the elution curves. It will be a better candidate for analytical CCC if its pressure problem can be overcome.

2.5. Dual counter-current chromatography

One of the most remarkable features of high-speed CCC is its ability to operate in a dual fashion where two immiscible phases undergo true counter-current movement through a narrow coiled tube. Appropriate solutes introduced in a sample at the middle portion of the coil, either batchwise or in a continuous mode, potentially can be separated and collected from each end according to their partition coefficients between the two phases. The method may be divided into two categories: liquid–liquid dual CCC and foam CCC. Since the recent development of liquid–liquid dual CCC has been reviewed separately by Lee [39] only foam CCC is elaborated here.

Foam separation [40] is based on the unique parameter of foaming capacity or foam affinity of samples in an aqueous solution. While it has great potential for application to biological samples, utility of the method has been extremely limited mainly due to its poor efficiency. In conventional instrumentation, foam separation was performed in a short column under unit gravity. Recently, we have developed a foam CCC centrifuge (scheme J synchronous planetary motion shown in Fig. 1) which provides a strong centrifugal force field to induce a rapid counter-current movement between the gas and liquid phases through a long narrow coiled tube [23]. The system conveniently utilizes the standard rotary-seal-free elution mechanism that permits the use of multiple flow tubes without risk of leakage and cross-contamination.

Fig. 14 illustrates a schematic layout of the foam CCC column equipped with five flow channels: the liquid is fed from the liquid feed line at the tail and collected from a liquid collection line at the head. Nitrogen gas is simultaneously introduced at the head and discharged through the foam collection line at the tail. The sample solution is introduced through the sample feed line at the middle portion of the coil either batchwise or in a continuous mode. A typical foam CCC column consists of 10 m × 2.6 mm I.D. PTFE tubing with a total capacity of about 50 ml. In the past the method was demonstrated in our laboratory in the separation of dyes with ionic surfactants [23,41,42], as well as separation of proteins in a phosphate buffer solution to minimize denaturation [23].

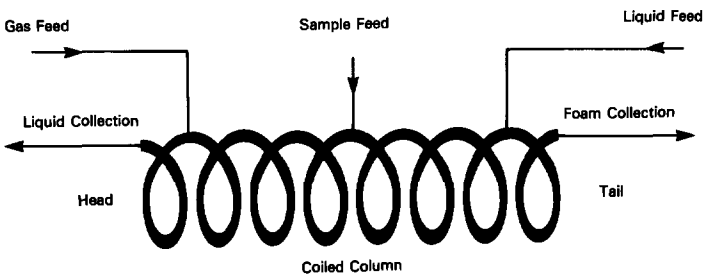

Fig. 14. Column design of foam CCC.

Recently, we have succeeded in separation of bacitracin components using only nitrogen gas and distilled water without the use of surfactants or any other additives [43,44]. Separation of the bacitracin components was initiated by introducing distilled water from the tail and nitrogen gas from the head at 80 psi while the column was rotated at 500 rpm. After a steady state counter-current equilibrium was established, the sample solution was injected through the sample feed line. During injection, the liquid flow was interrupted for about five minutes to allow the solutes to be distributed along the length of the coil according to their foaming capacity. Then, the pumping was resumed to collect the foam and liquid fractions from the respective terminals at 15-s intervals. These fractions were analyzed with high-performance liquid chromatography and the results are summarized in Fig. 15.

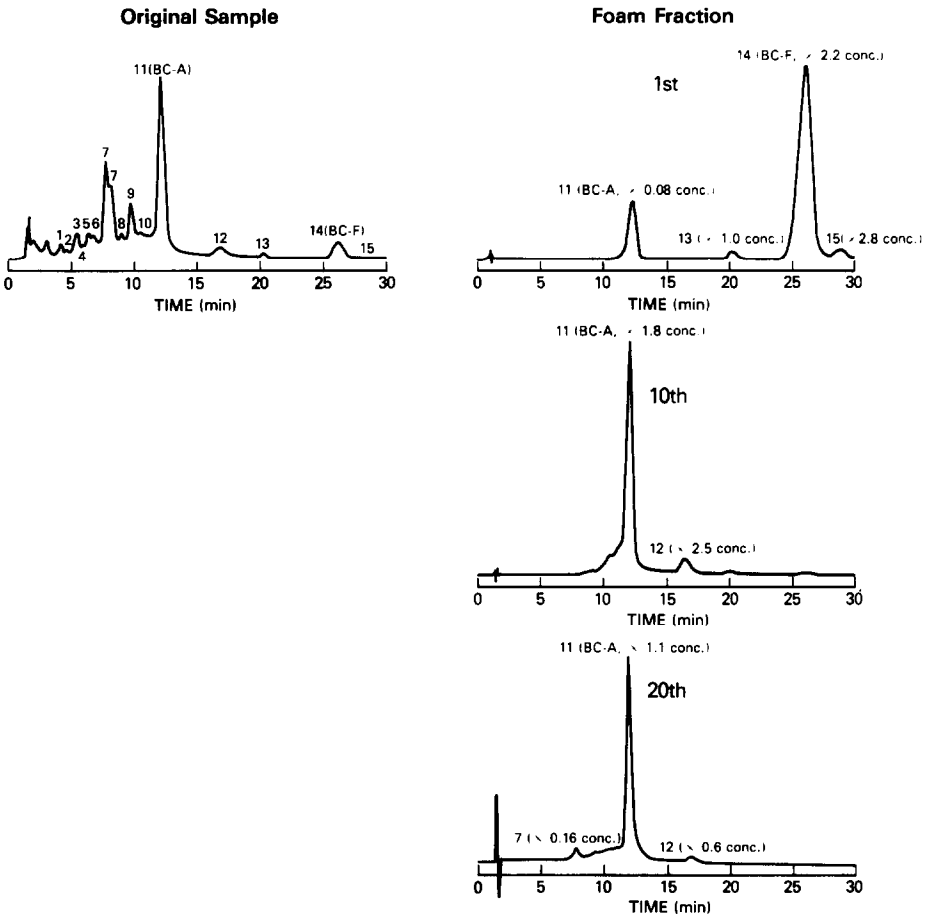

Fig. 15. Foam CCC of bacitracin. High-performance liquid chromatographic analysis of (left) the original sample and (right) the foam CCC fractions. High-performance liquid chromatography conditions: column, Capcell Pak C_{18}, 150 × 4.6 mm I.D., 5 μm; mobile phase, methanol–0.04 *M* Na_2HPO_4 (62:38), 1 ml/min; detection at 234 nm.

The chromatogram on the left was obtained from reversed-phase high-performance liquid chromatographic analysis of the original bacitracin sample which shows over 20 UV absorbing components. In this chromatogram, the most hydrophilic component eluted first and the remaining components followed in increasing order of hydrophobicity. Three chromatograms on the right were obtained from the foam fractions. In the first fraction, bacitracin F was enriched and in the 10th fraction, bacitracin A was almost completely separated from other components. At the 20th fraction, peak 7 appeared, while more polar components were not detected. The above results indicate that foam CCC separates the bacitracin components according to their hydrophobicity. On the other hand, in the liquid fractions the bacitracin components elute in decreasing order of polarity but with much less efficient separation than in the foam fractions [44].

More recently, this method has been successfully applied to continuous separation of bacitracin components. The results showed over one thousand-fold enrichment of foam active components as reported by Oka *et al.* [45].

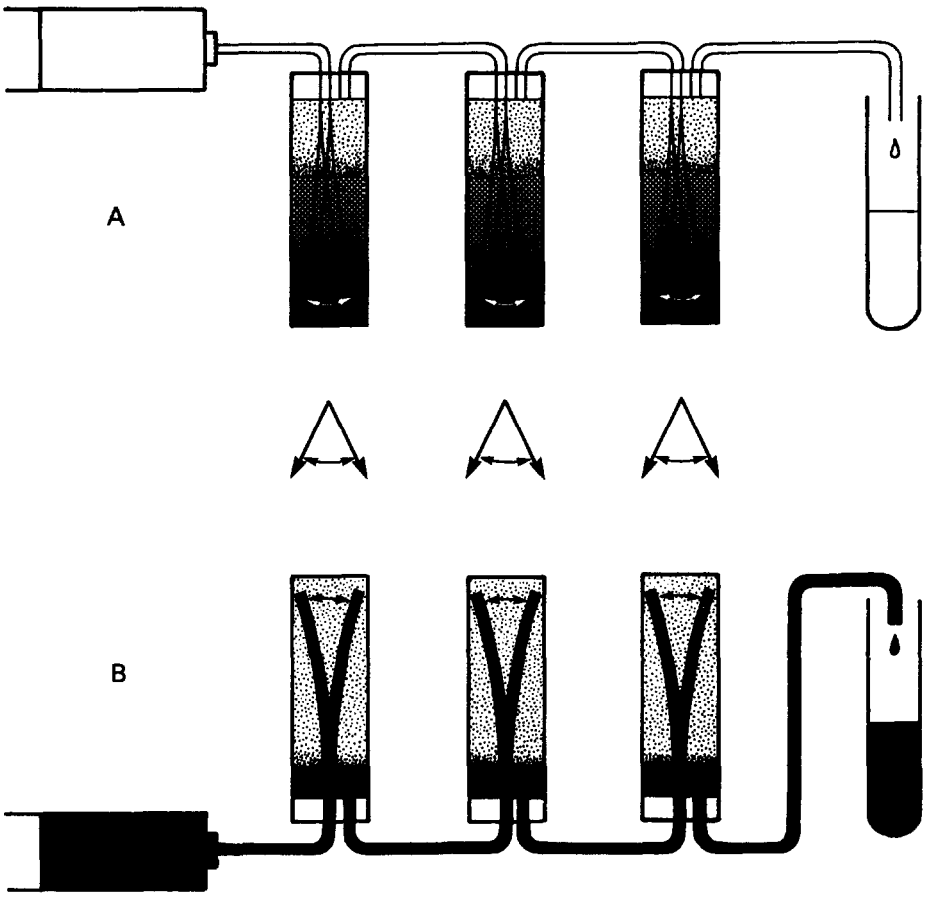

Fig. 16. Mechanism of multistage mixer-settler planetary centrifuge. A: Upper phase mobile; B: lower phase mobile.

2.6. Partition of macromolecules with polymer phase systems

Partition of macromolecules requires a special caution to prevent denaturation; the conventional organic-aqueous two-phase solvent systems are generally unsuited for this purpose [46]. The most commonly used solvent systems for partition of macromolecules contain one or more polymers (polyethyleneglycol, dextran, ficol, etc.) and various salts (sodium chloride, sodium or potassium phosphate, ammonium phosphate, etc.). These possess characteristic physical properties of high viscosity, low interfacial tension and relatively small density difference between the two phases [46]. Consequently, they require long settling times, and they have a strong tendency to emulsify. This causes a problem in maintaining stable retention of the stationary phase in the separation column with a continuous partitioning system like CCC. However, the above difficulty has been overcome by the use of various centrifugal CCC systems such as the angle rotor coil planet centrifuge [8], the elution centrifuge [10], the toroidal coil centrifuge [47,48], the toroidal coil planet centrifuge [49], the non-synchronous flow-through coil planet centrifuges [50–53], and the centrifugal droplet CCC apparatus (centrifugal partition chromatograph).

Recently, two new CCC instruments have been introduced for partition of macromolecules with polymer phase systems. Fig. 16 shows the principle of the first scheme where a series of cylindrical partition units is interconnected with transfer tubes. The mobile phase is delivered through a long flexible mixer tube extending toward the bottom of the partition unit. Under a fluctuating centrifugal force field provided by a scheme J synchronous coil planet centrifuge (see Fig. 1), the mixer tube vibrates to mix the contents of each partition unit to promote the partition process. This mixing effect is reduced at the outlet of the partition unit where the mobile phase is separated and transferred to the next unit. Thus, the system can maintain a steady state hydrodynamic equilibrium between the mobile and stationary phases during the continuous elution. Consequently, the solutes locally introduced at the inlet of the column are subjected to an efficient partition process in each partition unit and separated according to their partition coefficients. Fig. 17 shows a cross-sectional sketch of the planetary centrifuge equipped with a multistep partition column assembly [54].

The second scheme utilizes a set of four multilayer coils which are serially connected with flow tubes and arranged around the holder shaft of the similar coil planet centrifuge as shown in Fig. 18 [55]. Because of the eccentric orientation of the multilayer coils relative to the column holder axis, each helical turn of the coil retains satisfactory amounts of the stationary phase while the planetary motion of the coil produces efficient mixing of the two phases. Although the present system is similar to the original horizontal flow-through coil planet centrifuge [11,12], the shortened column holder tolerates much higher revolution speed (1000 rpm) to produce more efficient mixing effect and at the same time provide better retention of the polymer phase systems.

The above CCC system has been used to separate cytochrome *c* and lysozyme in a polymer phase system composed of 12.5% polyethyleneglycol in 1 *M* potassium phosphate aqueous solution (Fig. 19). The first scheme (see above) is suitable for gram quantity separations and the second scheme for semipreparative separations ranging from micrograms to several hundred milligrams. An improved model of the eccentric multilayer coil planet centrifuge has recently become available from Peptide Tech-

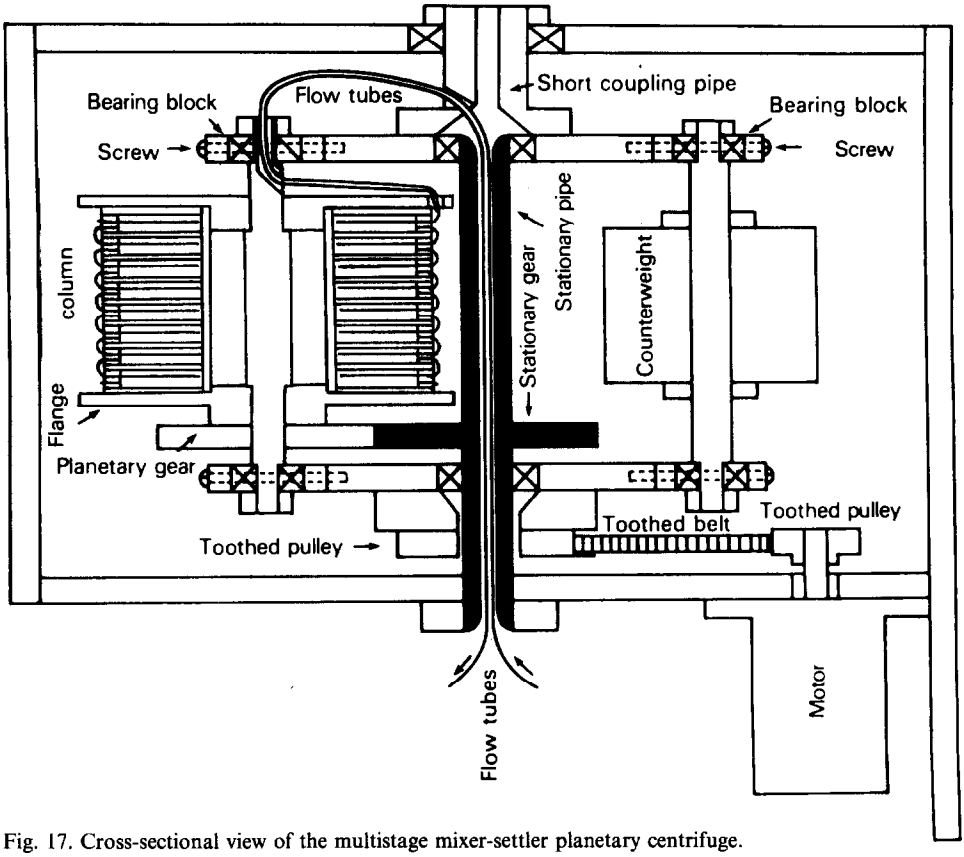

Fig. 17. Cross-sectional view of the multistage mixer-settler planetary centrifuge.

nologies Corporation (Washington, DC, U.S.A.) and Varex Corporation (Burtonsville, MD, U.S.A.) [56].

3. FUTURE DEVELOPMENT OF COUNTER-CURRENT CHROMATOGRAPHY

As discussed, recent work in our laboratory has been focused mainly on the synchronous coil planet centrifuge system. Various commercial models currently available have been successfully utilized for separation and purification of many natural and synthetic products [22].

Future advances in CCC technology may be expected from the development of the nonsynchronous coil planet centrifuge scheme (Fig. 1, right column) which has been neglected thus far due to its complex mechanical design. As described in the figure caption, the non-synchronous scheme has a unique feature in that the rotation and revolution rates of the coil are each independently adjustable. Consequently, choosing a suitable combination of slow coil rotation and high speed revolution will produce stable retention of the stationary phase for hydrophilic two-phase solvent systems such as aqueous–aqueous polymer phase systems used for partition of macromolecules and cell particles [46]. Although further development and refinement of the non-

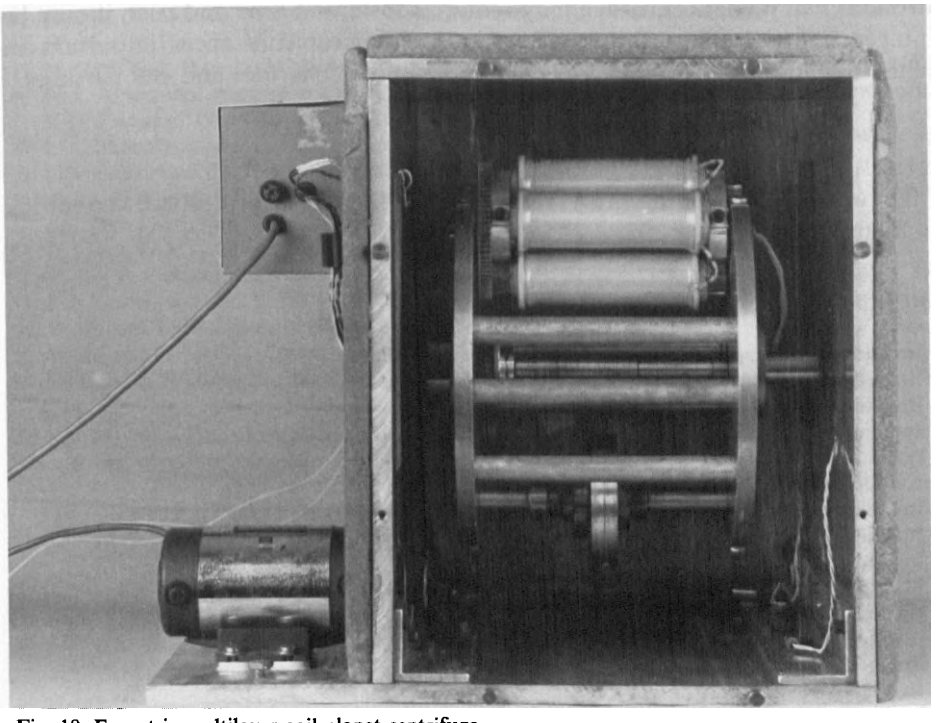

Fig. 18. Eccentric multilayer coil planet centrifuge.

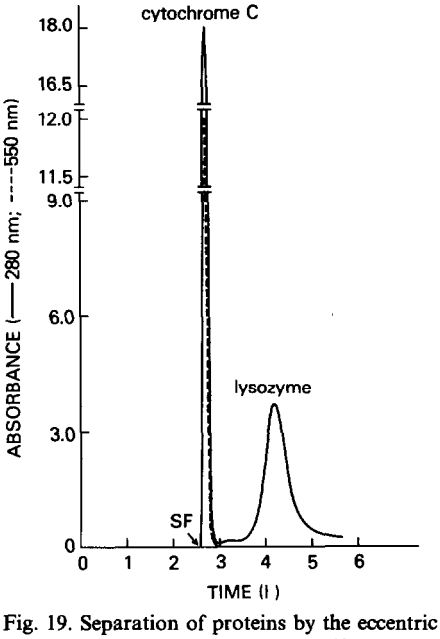

Fig. 19. Separation of proteins by the eccentric multilayer coil planet centrifuge. Conditions: apparatus, eccentric multilayer coil planet centrifuge with 10 cm revolution radius; a set of four multilayer coils were connected in series, 1.6 mm I.D. and 200 ml total capacity; sample, cytochrome *c* + lysozyme, each 100 mg; solvent system, 12.5% (w/w) polyethyleneglycol 1000 + 12.5% (w/w) anhydrous dibasic potassium phosphate in distilled water; mobile phase, lower phase; flow-rate, 1 ml/min; revolution, 800 rpm; SF = Solvent front.

synchronous coil planet centrifuge may require substantial time and cost, it may be advantageous for the separations of various biological substances important in biotechnology including proteins, nucleic acids, polysaccharides and cell particles.

4. ACKNOWLEDGEMENT

The author wishes to thank Dr. Henry M. Fales for his helpful advice and editing of the manuscript.

REFERENCES

1 Y. Ito and R. L. Bowman, *Science (Washington, D.C.)*, 167 (1970) 281.
2 Y. Ito and R. L. Bowman, *J. Chromatogr. Sci.*, 8 (1970) 315.
3 Y. Ito and R. L. Bowman, *Anal. Chem.*, 43 (1971) 69A.
4 T. Tanimura, J. J. Pisano, Y. Ito and R. L. Bowman, *Science (Washington, D.C.)*, 169 (1970) 54.
5 Y. Ito, in N. B. Mandeva and Y. Ito (Editors), *Countercurrent Chromatography: Theory and Practice*, Marcel Dekker, New York, 1988, Ch. 3, pp. 79–442.
6 Y. Ito and R. L. Bowman, *Science (Washington, D.C.)*, 173 (1971) 420.
7 Y. Ito and R. L. Bowman, *J. Chromatogr. Sci.*, 11 (1973) 284.
8 Y. Ito and R. L. Bowman, *Anal. Biochem.*, 65 (1975) 310.
9 Y. Ito, R. L. Bowman and F. W. Noble, *Anal. Biochem.*, 49 (1972) 1.
10 Y. Ito and R. L. Bowman, *Science (Washington, D.C.)*, 182 (1973) 391.
11 Y. Ito and R. L. Bowman, *Anal. Biochem.*, 82 (1977) 63.
12 Y. Ito and R. L. Bowman, *J. Chromatogr.*, 147 (1978) 221.
13 Y. Ito and R. Bhatnagar, *J. Liq. Chromatogr.*, 7 (1984) 257.
14 Y. Ito, *J. Chromatogr.*, 207 (1981) 161.
15 Y. Ito, *J. Chromatogr.*, 214 (1981) 122.
16 Y. Ito, J. Sandlin and W. G. Bowers, *J. Chromatogr.*, 244 (1982) 247.
17 Y. Ito, *J. Chromatogr.*, 358 (1986) 313.
18 Y. Ito, *J. Chromatogr.*, 358 (1986) 325.
19 Y. Ito, *Sep. Sci. Tech.*, 22 (1987) 1971.
20 Y. Ito, *Sep. Sci. Tech.*, 22 (1987) 1989.
21 Y. Ito, *J. Liq. Chromatogr.*, 11 (1988) 1.
22 Y. Ito, *CRC Crit. Rev. Anal. Chem.*, 17 (1986) 65.
23 Y. Ito, *J. Liq. Chromatogr.*, 8 (1985) 2131.
24 Y. Ito and T.-Y. Zhang, *J. Chromatogr.*, 449 (1988) 135.
25 Y. Ito and T.-Y. Zhang, *J. Chromatogr.*, 449 (1988) 153.
26 Y. Ito and T.-Y. Zhang, *J. Chromatogr.*, 455 (1988) 151.
27 Y. Ito, H. Oka and J. L. Slemp, *J. Chromatogr.*, 463 (1989) 305.
28 M. Bhatnagar, H. Oka and Y. Ito, *J. Chromatogr.*, 463 (1989) 317.
29 T.-Y. Zhang, Y.-W. Lee, Q.-C. Fang, C. E. Cook, R. Xiao and Y. Ito, *J. Chromatogr.*, 454 (1988) 185.
30 Y. Ito and F. E. Chou, *J. Chromatogr.*, 454 (1988) 382.
31 Y. Ito, H. Oka and J. L. Slemp, *J. Chromatogr.*, 475 (1989) 219.
32 Y. Ito, H. Oka and Y.-W. Lee, *J. Chromatogr.*, 498 (1990) 169.
33 Y. Ito, H. Oka, E. Kitazume, M. Bhatnagar and Y.-W. Lee, *J. Liq. Chromatogr.*, 13 (1990) 2329.
34 H. Oka and Y. Ito, *J. Chromatogr.*, 475 (1989) 229.
35 Y. Ito and Y.-W. Lee, *J. Chromatogr.*, 391 (1987) 290.
36 Y.-W. Lee, R. D. Voyksner, Q.-C. Fang, C. E. Cook and Y. Ito, *J. Liq. Chromatogr.*, 11 (1988) 153.
37 H. Oka, F. Oka and Y. Ito, *J. Chromatogr.*, 479 (1989) 53.
38 H. Oka, Y. Ikai, N. Kawamura, M. Yamada, K. Harada, M. Suzuki, F. E. Chou, Y.-W. Lee and Y. Ito, *J. Liq. Chromatogr.*, 13 (1990) 2301.
39 Y.-W. Lee, *J. Chromatogr.*, 538 (1991) 37.
40 P. Somasundaran, *Sep. Purif. Met.*, 1 (1972) 117.
41 M. Bhatnagar and Y. Ito, *J. Liq. Chromatogr.*, 11 (1988) 21.
42 Y. Ito, *J. Chromatogr.*, 403 (1987) 77.

43 H. Oka, K. Harada, M. Suzuki, H. Nakazawa and Y. Ito, *Anal. Chem.*, 61 (1989) 1998.
44 H. Oka, K. Harada, M. Suzuki, H. Nakazawa and Y. Ito, *J. Chromatogr.*, 482 (1989) 197.
45 H. Oka, K.-I. Harada, M. Suzuki, H. Nakazawa and Y. Ito, *J. Chromatogr.*, 538 (1991) 213.
46 P. Å. Albertsson, *Partition of Cell Particles and Macromolecules*, Wiley-Interscience, New York, 1986.
47 S. D. Flanagan, G. Johansson, B. Yost, Y. Ito and I. A. Sutherland, *J. Liq. Chromatogr.*, 7 (1984) 385.
48 I. A. Sutherland and Y. Ito, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 1 (1978) 171.
49 I. A. Sutherland, D. Heywood-Waddington and T. J. Peters, *J. Liq. Chromatogr.*, 12 (1985) 2315.
50 Y. Ito, G. T. Bramblett, R. Bhatnagar, M. Huberman, L. Leive, L. M. Cullinane and W. Groves, *Sep. Sci. Tech.*, 18 (1983) 33.
51 L. Leive, L. M. Cullinane, Y. Ito and G. T. Bramblett, *J. Liq. Chromatogr.*, 7 (1984) 403.
52 Y. Ito, P. Carmeci, R. Bhatnagar, S. Leighton and R. Seldon, *Sep. Sci. Tech.*, 15 (1980) 1589.
53 I. A. Sutherland and Y. Ito, *Anal. Biochem.*, 108 (1980) 367.
54 Y. Ito and T.-Y. Zhang, *J. Chromatogr.*, 437 (1988) 121.
55 Y. Ito and H. Oka, *J. Chromatogr.*, 457 (1988) 393.
56 M. Knight, S. Gluch, K. Takahashi, T. T. Dang and R. A. Kahn, *J. Chromatogr.*, 538 (1991) 141.