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Spiral column configuration for protein separation by high-speed countercurrent chromatography

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Abstract

Retention of the stationary phase of aqueous-aqueous polymer phase systems is improved by a spiral column configuration which utilizes the radially acting centrifugal force along the spiral pitch to retain the heavier phase in the outer portion and the lighter phase in the inner portion of the spiral channel. For the separation of proteins which has low mass transfer rates, the system needs further modification of the separation channel to interrupt the laminar flow and enhance mixing of the two phases. Two spiral column assemblies were developed, one using a disk with spiral grooves and the other, the spiral tube support which accommodates the multiple spiral layers made from a single piece of fluorinated plastic tubing. In the spiral disk assembly, the best protein separation is achieved by the mixer-settler system which vigorously mixes two phases by vibrating glass beads placed in every other section of barricaded spiral channel, while in the spiral tube assembly the partition efficiency of proteins is enhanced by compressing the tubing to interrupt the laminar flow of the mobile phase. In both systems protein samples were well resolved by choosing the suitable elution modes.

1. Introduction

Countercurrent chromatography (CCC) is a special form of liquid chromatography which uses no solid support in the separation column [1–6]. Although the system has an advantage of eliminating the undesirable irreversible adsorption of the sample onto the solid support, it must provide stable retention of the liquid stationary phase in the column. This requirement is achieved by generating an Archimedean screw effect on the coiled column which rotates in a centrifugal force field [2–4,7]. The system uses a unique type of flow-through centrifuge called the coil planet centrifuge (CPC) that produces planetary motion to the coiled column. In order to prevent leakage of solvent through a conventional rotary seal device, a series of seal-free planetary centrifuge systems has been introduced [8]. This paper will cover development of high-speed countercurrent chromatography and spiral column assembly for protein separation.

2. High-speed countercurrent chromatography (HSCCC)

2.1. A series of seal-free flow-through centrifuge systems

A series of seal-free centrifuge systems developed for performing CCC is illustrated in Fig. 1. They are divided into three groups as indicated at the top of the diagram. The synchronous systems (left) produce one rotation of the coil around its own axis per one revolution around the central axis of the centrifuge at the same angular velocity and the non-synchronous systems (right) give freely adjustable rotation-revolution ratios of the coil holder, while the non-planetary system (middle, bottom) is a transitional form of these two planetary systems. In all these systems the bundle of flow tubes from the rotating column holder is tightly supported at

the top of the centrifuge. In the type I CPC (left top), the vertical column holder rotates around its own axis while revolving around the axis of the centrifuge at the same angular velocity but in the opposite direction (it is identical to the motion of the vortex mixer). This counter-rotation of the coil holder prevents the tube bundle from twisting. This simple twist-free principle can be similarly applied to other synchronous systems of tilted (type I – L & I - X hybrids), horizontal (types L, X & L- X hybrid), dipping (type L-J & X – J hybrids) and inverted (type J) orientations of the coil holder. When the coil holder of type I is shifted toward the central axis of the centrifuge as indicated by an arrow, the coil holder becomes stationary because the reversed rotation of the coil holder cancels out the revolution effect. However, when this same manipulation is applied to the type J synchronous system, the result is radically different. Because in this system rotation and revolution of the coil holder are in the same direction, these two motions are added so that the coil holder rotates at the doubled speed around the axis of the centrifuge as in the conventional flow-through centrifuge system but without need for the rotary seal [9,10]. This non-planetary system provides a base for the non-synchronous systems. On the base of the non-planetary system, the coil holder is again shifted toward the periphery to undergo synchronous planetary motion as shown in the diagram. Since the revolution speed of the column holder is the sum of the synchronous rotation of the holder and the revolution rate of the base, the rotation-revolution ratio of the holder becomes freely adjustable.

In the past, many of these seal-free centrifuge systems have been built to examine their capability to perform CCC by mounting a coiled separation column around the periphery of the holder. Although most of these systems could produce efficient separations of samples with a variety of organic-aqueous two-phase solvent systems, the retention of the stationary phase was limited to substantially below 50% of the total column capacity that was further reduced at a higher flow rate of the mobile phase. The retention level of the stationary phase is one of the most important parameters that determine the peak resolution in CCC [11]. However, this problem has been finally solved by an incidental observation of hydrodynamic motion of the two phases in a coaxially mounted multilayer coil around the holder hub in the type-j coil planet centrifuge as described below.

2.2. Principle of HSCCC [4]

Fig. 2 shows the multilayer coil separation column and its planetary motion produced by the type-J synchronous CPC. When two immiscible liquid phases are enclosed in the coil, the planetary motion produced their rapid countercurrent movement resulting in complete separation of the two phases along the length of the coil, one phase (head phase) entirely occupying one end called the head and the other phase (tail phase) occupying the opposite end called the tail. (Here, the head-tail relationship refers to the Archimedean Screw effect in which all objects either heavier or lighter than the suspending medium in the coil are equally driven toward the head end of the coil.) This bilateral hydrodynamic distribution of the two phases can be effectively utilized for performing CCC in such a way that the tail phase is introduced through the head of the coil filled with the head phase or the head phase introduced through the tail of the coil filled with the tail phase. In either case, the system permits retention of a large amount of the stationary phase in the coil. The system also allows simultaneous injection of two phases through the respective end of the coil to perform dual CCC to separate the samples injected into the middle portion of the coil. This dual CCC has been successfully applied to foam CCC and dual CCC [4]

2.3. Hydrodynamic motion and distribution of two phases in the type-J CPC

Mathematical analysis of this planetary motion revealed a complex pattern of fluctuating centrifugal force field which varies according to the location of the point on the holder [4]. A series of experiments has been performed to study hydrodynamic behavior of two immiscible solvents in the coil undergoing the type-J planetary motion under stroboscopic illumination.

The results showed that a complex pattern of acceleration is created in the Fig. 2 coil arrangement associating an Archimedean screw force due to the density difference between the two liquid phases. As shown in Fig. 3, the two liquid phases contained in the tubing were strongly mixed when passing close to the central axis of revolution and completely decanted when passing far from the central axis as illustrated by Fig. 3 (top). This produced a succession of mixing and decantation moving zones inside the tubing as illustrated by Fig. 3 (bottom). It indicates that solutes present at any portion in the column are subjected to an efficient partition process of repetitive mixing and settling at an enormously high frequency of over 13 times per second at 800 rpm of column rotation that explains the high partition efficiency of the technique [3].

3. Problems for protein separation by HSCCC

Since 1980s, high-speed CCC has been successfully applied for separation of a variety of natural products using organic-aqueous two-phase solvent systems [12]. However, the method failed to separate macromolecules such as proteins using aqueous-aqueous polymer phase systems due to the following two major reasons: retention of the stationary phase and partition efficiency, as explained below.

3.1. Stationary phase retention of polymer phase systems in the multilayer coil in HSCCC

Retention of the stationary phase is one of the most important factors which determine the peak resolution in HSCCC [13]. As mentioned earlier, the method utilizes the Archimedean screw force to mix the two phases while retaining one of the phases as the stationary phase in the column. The amount of the stationary phase thus retained in the column is greatly affected by the physical properties of the two-phase solvent system such as viscosity, interfacial tension and difference in density between the phases. It was found that the retention of the stationary phase in the rotating column is highly correlated with the settling time of the two phases in a test tube which is also determined by the physical properties of the two phases including viscosity, interfacial tension and density difference between the two phases. This settling test is performed by introducing about 4 ml of two phases (2 ml each phase) into a capped test tube and gently inverting the tube 5 times to measure the time required to form a clear two layers [14]. Our experiments demonstrated that the two-phase solvent systems with the settling time shorter than 25 seconds can yield a sufficient level of stationary phase retention in the conventional multilayer coil of HSCCC while some polar solvent systems such as 1-butanol-acetic acid-water (4:1:5, v/v/v) with a longer settling time show low retention and can not be efficiently applied to HSCCC. The polymer phase systems used for separation of proteins possess high viscosity and low interfacial tension between the two phases hence require a long settling time of over one minute in the test tube indicating that they display low levels of the stationary phase retention in the ordinary multilayer coil.

However, this problem has been solved by modifying the column geometry of the conventional multilayer coil into the following two different spiral column designs: spiral disk assembly and spiral tube assembly. These spiral designs can generate a centrifugal force gradient along the radius of the spiral to retain the heavier phase in the periphery and the lighter phase in the proximal portion of the spiral channel to improve the retention of the stationary phase. Naturally, the effect of this centrifugal force gradient along the spiral radius is enhanced with the spiral pitch. Although in the past the spiral columns for HSCCC has been constructed simply by winding the tubing in a spiral fashion, the spiral pitch of these columns is limited to no more than the outer diameter of the tubing [15]. In order to create a high-pitch spiral column for HSCCC, a radical improvement of the column design is required.

3.2. Mass transfer rate of proteins through the interface of polymer phase systems

The partition efficiency in HSCCC highly depends on the mass transfer rate of analyte through the interface between the two phases. In order to measure the mass transfer rates of various analytes, we have constructed a simple rotary system as shown in Fig. 4A [16]. A set of test tubes is mounted around a motor shaft which is tilted at an angle of 18 degrees from the horizontal plain. The test is performed as follows: each test tube is filled with the equal volume of each phase of the polymer phase system composed of 12.5% (w/w) PEG1000 and 12.5% (w/w) K₂PO₄ in water. The test sample is then introduced into the lower phase followed by rotation of the tube at 30 rpm. This low speed rotation preserves the horizontal shape of the interface in the test tube while it constantly mixes the two phases to enhance mass transfer of the analyte through the interface. At regular time intervals, an aliquot of each phase is sampled from each phase to measure the concentration of analyte to calculate the rate of mass transfer through the interface. Suppose the analyte concentration in the lower phase is initially C₀ which decreases to C_t at time t, and the mass transfer coefficient, R, is proportional to the difference between C_t and C_∞ (equilibrium concentration of analyte in the lower phase), we get

$$-dC_t = (A/v)R(C_t - C_\infty)dt \quad (1)$$

where A is the interfacial area and V, the volume of the lower phase. Integrating Eq. 1 gives

$$-\ln[(C_t - C_\infty)/(C_0 - C_\infty)] = R(A/V)t \quad (2)$$

According to Eq 2 plotting $-\ln[(C_t - C_\infty)/(C_0 - C_\infty)]$ against time t should produce a straight line where R is computed from the slope provided that A/V is known.

Using a set of samples with a wide range of molecular size, the experiment was performed to obtain the slope of the curve given from Eq. 2. Fig. 4Ba shows the results of the experiment with 5 different samples with a wide range of molecular weight (Mw) including potassium dichromate (Mw 294), methylene blue (Mw 374), lysozyme (Mw 14,000), ovalbumin (Mw 45,000) and human serum albumin (Mw 68,000). In Fig. 4Ba five curves show different slopes of each analyte according to the molecular weight. The higher the molecular weight, the gentler the inclination of the slope. This relation is further expressed in Fig. 4Bb where mass transfer rate is plotted against the logarithm of the molecular weight of the analytes. Except for potassium dichromate, small inorganic molecule with high density, all organic analytes are arranged on a straight line according to their molecular weights.

The above studies clearly indicate that protein molecules have low mass transfer rates through the interface so that the partition efficiency of proteins in HSCCC would be highly dependent on the interfacial area of the two phases through which the mass transfer takes place. Although the area of interface for mass transfer could be increased by enhanced mixing of the two phases in the column to form a number of small droplets of one phase into the other, it may also tend to produce emulsification of the solvent causing a loss of stationary phase from the column. This challenging problem for protein separations to satisfy two mutually conflicting requirements of stable retention of the stationary phase and efficient mixing of the two phases in the polymer phase system has been finally solved by further modifying the spiral column geometry.

4. Spiral disk assembly

4.1. Standard column

Four different spiral disks were fabricated, two with a single spiral groove and the other two with 4 interwoven spiral grooves. Designs including the number of spiral grooves and their dimensions in these disks are summarized in Table 1 [17].

Fig. 5 shows photographs of single and multilayer spiral disk assemblies and their elements which improve the retention of the stationary phase of the conventional multilayer coil separation column. The designs of single-channel and four-channel spiral disks are illustrated in Figs. 6A and B, respectively. A series of studies with various two-phase solvent systems revealed that the 4-spiral disk can retain a satisfactory amount of the stationary phase for all solvent systems including viscous polymer phase systems [17].

In the separation of small molecules by the conventional multilayer coil, a satisfactory peak resolution is always attained from a high level of stationary phase retention. On the contrary the separation of the protein was found to be unsatisfactory in the spiral disks despite a satisfactory level of stationary phase retention [17]. As described earlier, this low efficiency of protein separations may be explained on the basis of their low mass transfer rates through the interface between the two phases. In order to improve the partition efficiency for protein samples with the polymer phase systems, therefore, mixing of the two phases should be enhanced to provide broad interfacial areas between the two phases.

4.2. Segmented spiral disks

Compared to the conventional multilayer coil, the spiral disk provides an advantage that the configuration of the channel can be modified and also suitable inserts may be placed into the channel to induce phase mixing. This possibility was first examined by placing short segments of PTFE tubing into the channel at regular intervals (Fig. 7A) to enhance the phase mixing by interrupting the laminar flow formation of the two phases. The results showed substantial improvement of partition efficiencies of protein separation as reported elsewhere [18]. Encouraged with this finding, two types of modified spiral disks were made, bead chain (Fig. 7B) and locular (Fig. 7C) [18] spiral disks. These disks clearly improved the peak resolution of proteins by yielding partial resolution of two peaks. However, when the partition efficiency obtained from the bead chain disk is computed from these separations, more than 40 compartments are needed to produce one theoretical plate indicating that there is much room to improve the separation by enhancing the mixing of the two phases.

4.3. Mixer-settler spiral disk [19,20]

The area of interface between the two phases in the separation channel may be enormously increased by actively mixing the two phases to form numerous small droplets of one phase into the other phase. This idea is tested by inserting glass beads into every other compartment of the locular disk (Fig. 7C). Another type of spiral disk called "barricaded disk" (Fig. 7D) was also designed to examine the above possibility. The mechanism of this mixer-settler CCC is illustrated in Fig. 8 where the upper diagram shows the portion of the locular channel with a glass bead. Fluctuation of the centrifugal force field produces vigorous agitation of the two phases by vibration of the glass beads in the mixing locule. In this scheme lower mobile phase entering into the mixing locule is mixed with the stationary upper phase and exit to the next empty locule (settling locule) for settling. Therefore, the system tends to gradually lose the stationary phase from the mixing locule. This problem is solved by the barricaded spiral disk shown in the lower diagram. In this barricaded disk, the two phases can freely undergo countercurrent through the opening at the top and bottom of each barricade hence the system can maintain stable retention of the stationary phase.

The composite diagram in Fig. 9 is to compare the partition efficiency produced by four different spiral channels illustrated on the top of each diagram. The diagram on the left shows the separation of protein with the standard spiral disk with 4 spiral channels (Column III) which gives almost no peak resolution. In the locular column with no glass bead (the second from the left), peak resolution is substantially improved showing partial resolution in all elution modes. But, the best separations are attained from two mixer-settler systems on the right. As expected, the barricaded disk shows a higher level of stationary phase retention than the locular disk and the best peak resolution at a flow rate of 0.5 ml/min.

Figs. 10A and B show mixer settler HSCCC separations of 5 protein samples by a multilayer spiral disk assembly consisting of 8 barricaded spiral disks each with a suitable polymer phase system [20]. In Fig. 10A, 4 proteins were eluted at partition efficiency of several hundred theoretical plates while the 5th protein is still retained in the column. In Fig. 10B all 5 proteins were eluted in 12 h. These chromatograms may represent the best protein separation so far achieved by countercurrent chromatography. One may question the possibility that vibrating glass beads might denature the protein molecules. In order to answer the above question, we have purified myrosinase from the water extract of kaiware daikon sprouts by mixer-settler CCC which yielded highly active enzyme fractions [21].

5. Spiral tube assembly

The spiral disk assembly described above requires the use of inert plastic disks precisely machined or molded to provide a complete seal to prevent interchannel leakage of the solvents. As an alternative system, the spiral tube assembly has been developed using a spiral tube support to accommodate multiple layers of a single piece of fluorinated plastic tubing [22]. This spiral column is less expensive than the spiral disks and eliminates a risk of leakage of the solvent through the seal. Fig. 11 shows a photograph of the spiral tube support fabricated in the NIH machine shop. It has four interwoven spiral grooves (5 cm deep, 2.8 mm wide) to accommodate a single piece of tubing to form multiple spiral layers through four radial transfer grooves.

5.1. Spiral column with modified tube configuration

Performance of the spiral tube assembly has been examined using a set of two-phase solvent systems with suitable samples. The results indicate that the separation of proteins gave low partition efficiency as expected from those from the spiral disk assembly. In order to improve the protein separation in the spiral tube assembly, the tubing configuration was modified by pressing with a pair of pliers perpendicularly at 1 cm intervals as shown in Fig. 12 [23]. This cross-pressed tubing substantially improved the peak resolution of proteins as well as that of dipeptide (Fig. 12). The partition efficiency of proteins has been further improved by compressing the four radial transfer grooves with a specially made tool with 2 or 4 extrusions which fit into the width of the groove. This process gives the following three beneficial effects for solute partitioning: first, the number of spiral layers accommodated in a given tube support is increased; second, the dead space in the transfer tube is reduced; and third, the laminar flow of the mobile phase is interrupted to enhance the partition process.

Fig. 13 illustrates the separation of the proteins with a spiral tube assembly consisting of 13 spiral layers of 1.35 mm ID PTFE tubing treated with both cross-pressing and radial groove compression [23]. The total capacity is about 85 ml. Using a two-phase solvent system composed of 12.5% (w/w) PEG and 12.5% (w/w) K_2HPO_4 in water, lysozyme and myoglobin were separated at flow rates from 0.5 ml/min to 2 ml/min under revolution speeds from 600 to 1,200 rpm. The peak resolution of proteins is steadily increased with the revolution speed and at 1,200 rpm two peaks are well resolved at a high flow rate of 2 ml/min in 90 min.

6. Conclusions

The partition efficiency of protein samples in HSCCC is improved by changing the column configuration from the conventional multilayer coil to the spiral column with high spiral pitch. A spiral column can be made in two different ways: spiral disk assembly and spiral tube assembly. In the spiral disk assembly mixer-settler CCC system is developed by inserting glass beads in every other section of the barricaded channel to enhance both the retention of the stationary phase and partition efficiency for protein separation. In the spiral tube assembly, using a spiral tube support a high-pitch multilayer spiral column is fabricated from a single piece of tubing to eliminate the risk of leakage of solvent from the junctions. Efficient protein separation is achieved by modifying the shape of tubing by cross-pressing at regular intervals and compressing four radial transfer tubes with a specially made simple tool. Both spiral columns yield high partition efficiency for protein separation with the polymer phase system.

References

1. Ito Y, Bowman RL. Countercurrent Chromatography: Liquid-liquid partition chromatography without solid support. *Science* 1970;167:281–283. [PubMed: 5409709]
2. Ito, Y. Principle and instrumentation of countercurrent chromatography. In: Mandava, NB.; Ito, Y., editors. *Countercurrent Chromatography: Theory and Practice*. Vol. Chapter 3. Marcel Dekker, Inc; New York: 1988. p. 79-442.
3. Walter, D. Conway, *Countercurrent chromatography: Apparatus, theory and applications*. VCH Publishers Inc; New York: 1990.
4. Ito, Y.; Conway, Walter D., editors. *High-Speed Countercurrent Chromatography Chemical Analysis Series*. Vol. 132. Wiley Interscience; 1996.
5. Thiébaud, JM Menet D., editor. *Countercurrent Chromatography, Chromatographic Series*. Vol. 82. Marcel Dekker, Inc; New York: 1999.
6. Berthod, A., editor. *Countercurrent Chromatography: The support-free liquid stationary phase*, Wilson and Wilson's *Comprehensive Analytical Chemistry*. Vol. XXXVIII. Elsevier; Amsterdam: 2002.
7. Ito Y. High-speed countercurrent chromatography. *CRC Crit Rev Anal Chem* 1986;17:65–143.
8. Ito, Y. Development of countercurrent chromatography (CCC) and Other Centrifugal Separation Methods in Century of Separation Science. Issaq, Haleem J., editor. Marcel Dekker; New York: 2001. p. 293-308.
9. Ito Y, Suaudeau J, Bowman RL. New flow-through centrifuge without rotating seals applied to plasmapheresis. *Science* 1975;189:999–1000. [PubMed: 1220011]
10. Ito, Y. Flow-Through Centrifuge Free of Rotating Seals Particularly for Plasmapheresis. U.S. Patent. 4,425,112. Jan 10. 1984
11. Conway WD, Ito Y. Resolution in countercurrent chromatography. *J Liq Chromatogr* 1985;8:2195–2207.
12. Ito Y. Golden rules and pitfalls in selecting optimum conditions for high-speed countercurrent chromatography (HSCCC). *J Chromatogr A* 2005;1065:145–168. [PubMed: 15782961]
13. Ito Y. Experimental observations of the hydrodynamic behavior of solvent systems in high-speed countercurrent chromatography: Part II. Phase distribution diagrams for helical and spiral columns. *J Chromatogr* 1984;301:387–403. [PubMed: 6501496]
14. Ito Y, Conway WD. Experimental observations of the hydrodynamic behavior of solvent systems in high-speed countercurrent chromatography: Part III. Effects of physical properties of the solvent systems and operating temperature on the distribution of two-phase solvent systems. *J Chromatogr* 1984;301:405–414. [PubMed: 6501497]
15. Guan YH, Smulders J, Fisher D, Sutherland IA. Spiral coils for counter-current chromatography using aqueous polymer two-phase systems. *J Chromatogr A* 2007;1151:115–120. [PubMed: 17239387]
16. Ito Y, Matsuda K, Ma Y, Qi L. Toroidal coil countercurrent chromatography: Mass transfer rates of proteins in polymer phase system. *J Chromatogr A* 1998;802:277–283. [PubMed: 9595695]

17. Ito Y, Yang FQ, Fitze PE, Sullivan JV. Spiral disk assembly for high-speed countercurrent chromatography. *J Liq Chromatogr Rel Technol* 2003;26(9&10):1355–1372.
18. Ito Y, Yang FQ, Fitze P, Powell J, Ide D. Improved spiral disk assembly for high-speed countercurrent chromatography. *J Chromatogr A* 2003;1017:71–81. [PubMed: 14584692]
19. Ito Y, Qi L, Powell J, Sharpnack H, Metger H, Yost J, Cao X-L, Dong Y-M, Huo L-S, Zhu X, Li T. Mixer-settler countercurrent chromatography with a barricaded spiral disk assembly with glass beads. *J Chromatogr A* 2007;1151:108–114. [PubMed: 17316660]
20. Ito Y, Clary R, Sharpnack F, Metger H, Powell J. Mixer-settler counter-current chromatography with multiple spiral disk assembly. *J Chromatography A* 2007;1172:151–159.
21. Wade K, Fahey J, Ito Y. unpublished data.
22. Ito Y, Clary R, Powell J, Knight M, Finn TM. Spiral tube support for high-speed countercurrent chromatography. *J Liq Chromatogr Rel Technol* 2008;31:1346–1357.
23. Ito Y, Clary R, Powell J, Knight M, Finn MT. Improved spiral tube assembly for high-speed counter-current Chromatography. *J Chromatogr A* 2009;1216:4193–4200. [PubMed: 19062024]

Fig. 1.
Series of rotary-seal-free flow-through centrifuge systems.

Fig. 2.

Design of multilayer coil separation column and its type-J planetary motion. $\beta = r/R$ where r is the distance from the axis of rotation to the coil and R , the revolution radius or the distance between the axis of rotation and the axis of revolution.

Fig. 3. Distribution and motion of mixing zones in the spiral column undergoing type-J synchronous planetary motion observed under stroboscopic illumination.

Fig. 4.

Fig. 4A. Simple rotary system for studying mass transfer rate of solute through the interface of two-phase solvent system. a. Photograph and the apparatus, b. Interface and its rotary motion. Fig. 4B. Mass transfer rates of various test samples through the interface of two-phase solvent system. a. Mass transfer rate vis. time, b. mass transfer rate vis. molecular weight of the test samples.

Fig. 5. Photographs of spiral disk assemblies and their elements. Top row: a pair of metal flanges; middle row, left: spiral disk; middle row right: Teflon septum; bottom row left: single spiral disk assembly; bottom row right: multiple spiral disk assembly.

A

Original Spiral Disk (Single Channel)

Original Spiral Disk (4 channels)

Fig. 6. Original spiral disks. A. Single-spiral disk (spiral pitch 0.4 cm); B. Four-spiral disk (spiral pitch, 1.6 cm).

Spiral disk with tubing insert

Bead-Chain Spiral Disk

Locular spiral disk

Barricaded spiral disk

Fig. 7.

Photographs of four modified spiral disks. A. spiral disk with Teflon tubing insert; B. bead chain spiral disk; C. locular spiral disk; and D. barricaded spiral disk.

HYDRODYNAMIC MECHANISM IN MIXER-SETTLER SPIRAL CHANNEL

Fig. 8.
Mechanism of mixer-settler CCC.

Fig. 9.

Protein separation with four different spiral disks. Apparatus: type-J coil planet centrifuge with 10 revolution radius; column: Four different single spiral disks as indicated at the top of each diagram, from left to right: standard disk, locular disk, locular disk with glass beads, barricaded disk with glass beads; A. solvent system: 12.5% (w/w) PEG1000-12.5% (w/w) K_2HPO_4 in water; sample: myoglobin ($K=0.58$) and lysozyme ($K=1.69$) each 5 mg in 1 ml of upper phase; revolution speed: 800 rpm; detection: 280 nm.; flow rates, elution modes are indicated in the diagram. Elution modes: L-I-T : Lower phase introduced from the internal head end; L-I-H: Lower phase introduced from the internal tail end; U-O-T: upper phase introduced from the external tail end; U-O-H; upper phase introduced from the external head end.

Fig. 10. Preliminary separation of five standard proteins by mixer-settler multidisk spiral assembly. Apparatus: type-J coil planet centrifuge with 10 revolution radius; column: multidisk assembly

consisting of 8 barricaded four-channel spiral disks (2.8 mm wide 2 mm deep, glass bead placed in every other section) with 160 ml total capacity. A. Solvent system: 12.5% (w/w) PEG1000-12.5% (w/w) K_2HPO_4 in water; Mobile phase: lower phase; sample: cytochrome c ($K=0.02$), myoglobin ($K=0.58$), ovalbumin ($K=1.26$), lysozyme ($K=1.69$), and bovine serum albumin ($K=1.95$), each 5–6 mg in 1 ml of each phase; flow rate: 0.25 ml/min; revolution: 800 rpm; detection: 280 nm; retention of stationary phase: 52%, B. solvent system: PEG1000/ $K_2HPO_4/KH_2PO_4/H_2O$ (16:8.3:4.2:71.5, w/w); mobile phase: lower phase; cytochrome c (5 mg, $K=0.035$), human serum albumin (20 mg, $K=0.4$), β -lactoglobulin (20 mg, $K=0.69$), α -chymotrypsin (20 mg, $K=1.2$), trypsinogen (20 mg, $K=2.1$) in 2 ml of each phase; flow rate: 0.5 ml/min; revolution: 1000 rpm; retention of stationary phase: 53.6%.

Fig. 11. photographs of the spiral tube support with the transfer passage. The sharp turn at each terminal of the spiral grooves were rounded to prevent the kinking of tubing.

Fig. 12. Performance of spiral tube assembly with cross-pressed tubing in separation of protein and dipeptide samples.

Fig. 13.

Protein separation by cross-pressed and 4-transfer-channel compressed spiral tube assembly at various flow rates and revolution speeds. Experimental conditions are as follows: Apparatus: type-J coil planet centrifuge with 10 revolution radius; column: 1.35 mm I.D. PTFE cross-pressed spiral tube assembly consisting of 15 spiral layers with 85 ml capacity which was compressed along the four radial transfer grooves; sample: solvent system: 12.5%(w/w) PEG1000 and 12.5%(w/w)K₂HPO₄ in water; elution mode: L-I-T or lower phase introduced through the internal tail terminal of the spiral; sample: lysozyme and myoglobin, each 5 mg in 1 ml of upper phase; flow rates, revolution speeds, stationary phase retention, theoretical plate number (TP) and peak resolution (R_S) in each separation are all indicated in the diagram.

Table 1

Dimensions of four spiral channels used in the present studies.

Disk No.	number of spiral	width* (mm)	depth* (mm)	pitch (mm)	capacity (ml)
Column I	1	2.6	2.0	4	23
Column II	1	1.5	3.7	4	21
Column III	4	2.6	2.0	16	23
Column IV	4	1.5	3.7	16	21

* The width of the spiral groove becomes the depth of the channel and the depth becomes the width of the channel when assembled.

Table 2**TWO-PHASE SOLVENT SYSTEMS AND TEST SAMPLES**

Two-Phase Solvent Systems		Test Samples	
1-butanol	4	tryptophyl-tyrosine(trp-tyr)	0.5 mg
acetic acid	1	valyl-tyrosine(val-tyr)	2.0 mg
water	5		
PEG1000*	12.5%(w/w)	lysozyme (chicken egg)	10 mg
K ₂ HPO ₄	12.5%(w/w)	myoglobin (horse muscle)	10 mg
water	75.0%(w/w)		
dextranT500**	5% (w/w)		
PEG 8000	5% (w/w)	-	
water	90% (w/w)		

* PEG 1000: polyethyleneglycol with average molecular weight of 1,000

** dextranT500: dextran with average molecular weight of 500,000