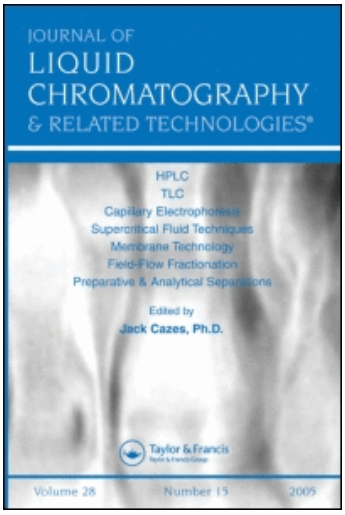

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Effects of Coriolis Force on Countercurrent Chromatography

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I. CCC INSTRUMENTATION

EFFECTS OF CORIOLIS FORCE ON COUNTERCURRENT CHROMATOGRAPHY

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ABSTRACT

During protein separation using the toroidal coil centrifuge, the partition efficiency was found to vary according to the direction of the elution through the coil. In most cases, eluting either the lighter phase toward the direction of rotation or the heavier phase in the opposite direction produced higher efficiencies. The cause of this unusual phenomenon may be attributed to the Coriolis force acting on the mobile phase in the rotating coil. Using a flow-through glass vial and colored mobile phases, the effects of the Coriolis force on moving droplets were observed under stroboscopic illumination. As expected, the path of the descending droplets (heavier phase) shifted against the direction of rotation whereas that of the ascending droplets (lighter phase) shifted toward the direction of rotation. The effect of this Coriolis flow on the partition efficiency was studied with a set of toroidal coils with various core diameters.

Overall results indicated that the best partition efficiency may be obtained when the Coriolis flow is nearly parallel to the toroidal coil segment where the partition process is taking place. The hydrodynamic effects of the Coriolis force on the mobile phase are discussed.

INTRODUCTION

Countercurrent chromatography (CCC) is known as liquid partition chromatography without a solid support.[1-5] It uses two mutually equilibrated immiscible solvents, one of which is permanently retained in the column while the other continuously passes through the stationary phase to elute the analyte. Consequently, the partition efficiency of CCC highly depends upon efficient phase mixing and formation of small droplets to minimize the mass transfer resistance.

One of the CCC schemes, the toroidal coil CCC,[1,6] uses a coiled column accommodated circularly around the periphery of the centrifuge bowl to form a dough-nut shaped configuration as indicated by its name (Fig. 1C). The rotation of the centrifuge bowl creates a radial centrifugal force field to retain one of the phases in each coiled turn while the other phase percolates through it.

Consequently, in this hydrostatic equilibrium the mixing of the two phases depends solely on the flow of the mobile phase, and the partition efficiency in a given column is strongly affected by the flowing pattern of the mobile phase as well as the size of its droplets generated in the stationary phase.

In the course of protein separation with a polymer phase system using the toroidal coil centrifuge, we have noticed a strange phenomenon: the sharpness of the elution peak varies according to the direction of the elution relative to the column rotation. More precisely, if the mobile phase is the lighter phase, eluting through the column toward the rotation of the column gave a sharper peak, and the reverse occurs if the mobile phase is the heavier phase.

This phenomenon may be explained on the basis of the effect of the Coriolis force which acts asymmetrically on the flowing mobile phase in the rotating toroidal coil. The present paper describes this unusual partitioning behavior of proteins in the toroidal coil together with our tentative explanation based on the Coriolis force effect.

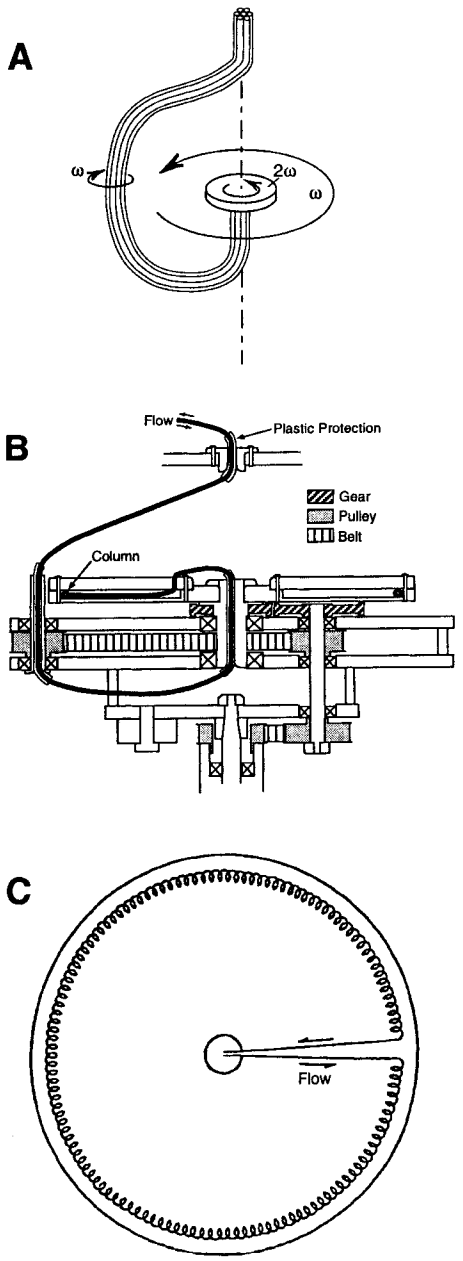

Figure 1. Mechanism and design of the seal-free flow-through centrifuge. A: motion of the bundle of flow tubes; B: design of the frame; C: typical toroidal coil configuration.

EXPERIMENTAL

Apparatus

A toroidal coil centrifuge used in the present studies is a commercial floor model (CRT 5000) in which the centrifuge head is modified in such a way that it permits continuous elution of the mobile phase without the rotary seal.[7] Fig. 1A shows the seal-free mechanism of the flow-through system. If the central bowl rotates about its own axis at 2 ω and the bundle of flow tubes revolves around the central axis at ω in the same direction, the tube bundle becomes twist-free provided that it counterrotates about its axis as indicated in the diagram. The actual design of the centrifuge frame is illustrated in Fig. 1B. The frame of the centrifuge consists of three horizontal plates which hold three rotary structures: centrifuge bowl, countershaft (right) and tube-supporting hollow shaft (left). The coupling of the lower pulley of the countershaft to the stationary pulley on the motor housing causes counterrotation of the countershaft with respect to the rotating frame. This motion is then conveyed to the central bowl by 1:1 gearing to double the angular velocity of the bowl. The motion of the countershaft is also transferred to the tube-supporting hollow shaft by means of the 1:1 gear ratio pulley coupling. Thus the system satisfies all the requirements indicated in Fig. 1A.

For the present study, several toroidal coils and a twisted column were prepared. A typical toroidal coil is schematically shown in Fig. 1C. The flat centrifuge bowl of 37cm diameter has an elected rim to support the toroidal coil at its periphery. Each coiled column was prepared by winding a single piece of PTFE (polytetrafluoroethylene) tubing (Zeus Industrial Products, Raritan, NJ, USA) of 1.07 mm ID and 20 m in length onto a flexible nylon pipe of various diameter, which was in turn placed at the periphery of the centrifuge bowl, making multiple spiral turns if necessary. The twisted column was prepared by folding the same tubing in two and twisting along its axis to form multiple pairs of twisted turns which gave a rope-like appearance. The twisted tubing was then wound around a spool-shaped holder which fitted into the centrifuge bowl. Various configurations and dimensions of the columns used in the present studies are summarized in Table 1.

Reagents

Polyethylene glycol (PEG) 1000, myoglobin (horse heart), lysozyme (chicken egg) and all dipeptide samples were purchased from Sigma Chemical Co., St Louis, MO, USA. Methyl *t*-butyl ether, 1-butanol, acetonitrile,

Table 1

Configurations and Dimensions of Toroidal Columns Used in the Present Studies

No.	Column	Core OD (mm)	Turns	Capacity (mL)	Pitch Angle* (mm/°)	Description
1	Twisted	0	2000	18	15/83.5	a pair of twisted tubing connected in series
2	Flattened	3.0	1390	12.8	2.6/13.2	tubing flattened because of small diameter core
3	Standard	3.0	1310	20	2.4/11.3	flattening prevented by filling water before
4	Standard	4.0	1200	15	2.2/8.9	slightly flattened
5	Double Coil	4.0	1210	20	4.4/17.4	double helix configuration
6	Standard	5.0	990	20	2.2/7.4	

* Angle against the radius. All columns were made from 20 m long, 1.07 mm ID, 2 mm OD PTFE tubing (standard wall 18) wound onto nylon pipe.

chloroform and trifluoroacetic acid (TFA) were all glass-distilled HPLC grade and obtained from Fisher Scientific Co., Fair Lawn, NJ, USA. Dibasic potassium phosphate was purchased from Mallinckrodt, Paris, KY, USA, and acetic acid from J.T. Baker Chemical Co., Phillipsburg, NJ, USA.

Observation of Coriolis Force Effect on Moving Droplets

The hydrodynamic effect of the Coriolis force was observed under stroboscopic illumination as follows: A glass vial (1.7mm in diameter and

45mm in length) equipped with an open top screw cap and a custom-made teflon plug was used. In order to introduce a continuous flow through the vial, a pair of PTFE tubes (0.85mm ID) (Zeus Industrial Products) was inserted through the teflon plug: One of the tubes ended near the top of the vial just a few mm below the plug while the other tube reached near the bottom of the vial. This tube arrangement allowed either the heavier mobile phase to flow continuously from the top toward the bottom of the vial (descending flow) or the lighter phase in the opposite direction (ascending flow).

The vial was first entirely filled with the stationary phase and securely mounted onto the periphery of the central bowl in such a way that the axis of the vial coincided with the radius of the bowl. A similar vial (without flow tubes), filled with water, was mounted symmetrically on the opposite side for counter-balancing.

The mobile phase was prepared by adding a suitable dye, such as Sudan III or Sudan black, for an organic phase and methylene blue for polymer phases to facilitate observation of the droplets under stroboscopic illumination. The experiment was performed by applying various rotation speeds ranging from 500 to 1000 rpm.

Solvent Systems for Partitioning Proteins and Peptides

Two different types of two-phase solvent systems were prepared: an aqueous-aqueous polymer phase system for protein partitioning, and two different organic-aqueous solvent systems both of which were used for separating dipeptide samples (control study). The polymer phase system consisted of 12.5%(w/w) polyethylene glycol and 12.5%(w/w) dibasic potassium phosphate in distilled water.

The organic-aqueous solvent systems for dipeptide separation were composed of methyl *t*-butyl ether/1-butanol/ acetonitrile/0.1% aqueous TFA (2:2:1:5, v/v) and 1-butanol/acetic acid/water (4:1:5, v/v). All solvent mixtures were thoroughly equilibrated in a separatory funnel at room temperature and the two phases were separated shortly before use.

Single Protein Partitioning for Investigation of Coriolis Effects

Several toroidal coils and a twisted column were evaluated for their partition efficiency with the polymer phase system using both myoglobin and lysozyme as test samples. In each experiment, the column was completely

filled with the stationary phase. This was followed by injection of 10mg of the protein sample through the sample port. Then, the column was rotated at an optimal rate usually at 1200 rpm while the mobile phase was eluted through the column at a flow rate of 0.2 mL/min. Both upper and lower phases were used as the mobile phase.

For the myoglobin sample, the heavier, phosphate-rich phase was used as the mobile phase and, for the lysozyme sample, the lighter PEG-rich phase was used as the mobile phase. In each sample, the experiment was repeated by switching the outlet and inlet of the column so as to compare the effect of the Coriolis force on the partition efficiency. The effluent from the outlet of the column was continuously monitored at 280 nm with a UV monitor (Uvicord S, LKB Instruments, Stockholm/Bromma, Sweden) and fractionated into test tubes at 2 min intervals (0.4mL/tube). After the peak was eluted, the centrifugation was stopped and the column contents were pushed out into a graduated cylinder by connecting the column inlet to a pressurized nitrogen cylinder. The retention of the stationary phase was calculated from the volume of the stationary phase recovered from the column.

Separation of Myoglobin and Lysozyme with a Polymer Phase System

A sample mixture containing myoglobin and lysozyme was separated using a toroidal coil of 1.07mm ID, 3 mm core diameter and 20mL capacity (see Table 1, column No. 3), with the polymer phase system under similar experimental conditions used in the single protein partitioning studies described above. From the obtained chromatograms, both theoretical plate numbers (TP) and peak resolution (R_s) were calculated according to the conventional formulae.

Separation of Dipeptides with Conventional Organic-Aqueous Solvent Systems

For the control studies, a set of dipeptides including tyrosyl-glycine, tyrosyl-alanine and tyrosyl-valine, was separated with two conventional organic-aqueous solvent systems by similar experimental procedures. The solvent systems used were 1-butanol/acetic acid/water (4:1:5, v/v) and methyl *t*-butyl ether/1-butanol/acetonitrile/0.1% aqueous TFA (2:2:1:5, v/v). For each solvent system the lower aqueous phases were used as the mobile phase, and the separation was repeated by switching the inlet and outlet of the column for elution.

Analysis of CCC Fractions

Collected CCC fractions of proteins were analyzed by adding 2 mL of distilled water to each tube and measuring the absorbance at 280 nm and 540 nm with a Zeiss spectrophotometer (Hanover, MD).

RESULTS

Stroboscopic Observation of Droplet Motion in a Rotating Vial

The effect of Coriolis force was demonstrated by a simple experiment using a flow through vial and various two-phase solvent systems. When the chloroform stained with Sudan III was introduced into the rotating vial filled with water, small chloroform droplets were clearly visible under stroboscopic illumination. The experiment was repeated by repositioning the vial by tilting it toward the action of the Coriolis force until the chloroform droplets directly hit the bottom of the vial. The magnitude of the Coriolis force was then determined by measuring the inclination of the vial against the radius of the centrifuge bowl.

As expected, the effect of the Coriolis force was clearly observed: The descending path of the chloroform droplets was shifted toward the direction opposite to the rotation of the centrifuge bowl. This shift of the droplet flow was enhanced by the higher rotational speed until the maximum rate of 1000 rpm was attained, where the shifted angle reached about 20 degrees against the radius of the centrifuge bowl.

The experiment was continued to test the ascending motion of the droplet using hexane and water, where the hexane stained with Sudan Black was introduced at the bottom of the vial previously filled with water. In this case, the motion of the droplets was shifted toward the direction of rotation. Similar experiments were performed using the polymer phase system composed of PEG1000 12.5%(w/w) and dibasic potassium phosphate 12.5%(w/w) in distilled water. Methylene blue was used to stain both lighter and heavier mobile phases. Although the dye was eventually partitioned into both phases, the initial motion of the droplets could be clearly observed for some period of time.

The polymer phases also exhibited a similar trend of Coriolis effects when observed with organic-aqueous solvent systems. These results are schematically illustrated in Fig. 2A.

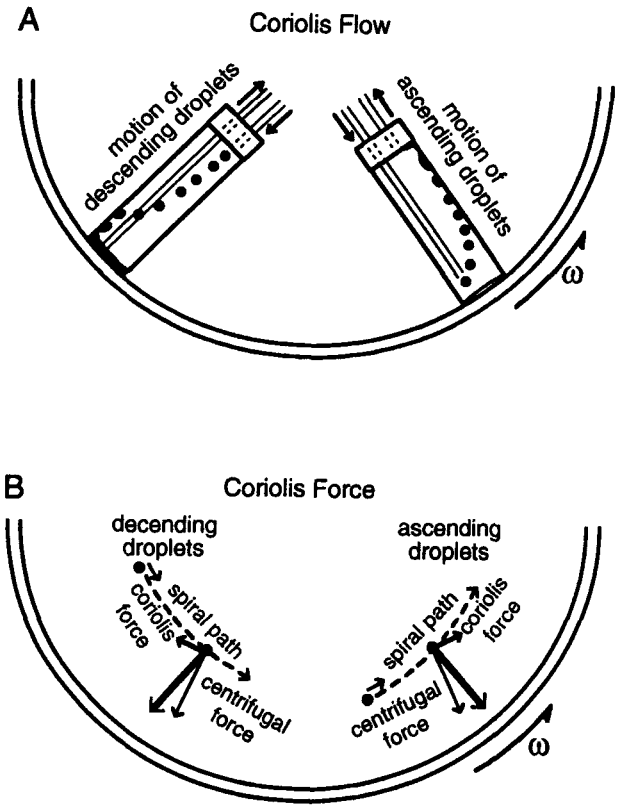

Figure 2. Coriolis effect on the droplet motion in the rotating vial. A: Observed droplet flow under stroboscopic illumination, (Left) "Coriolis flow" of the heavier phase, (right) "Coriolis flow" of the lighter phase. B: Mechanism of Coriolis effect, (Left) descending droplet; (right) ascending droplet.

Studies on Partition Efficiency of Protein with a Polymer Phase System

Using various toroidal coils, the effect of the Coriolis force on the partition efficiency was studied using a polymer phase system composed of 12.5%(w/w) PEG 1000 and 12.5%(w/w) dibasic potassium phosphate and two protein samples. Myoglobin was eluted with the phosphate-rich lower mobile phase, and lysozyme with the upper PEG-rich mobile phase. The results of the experiments are summarized in Table 2.

Table 2

Effects of Coriolis Force on Partition Efficiencies of Proteins

Col. No.	Core (mm)	Capacity (mL)	Myoglobin Coriolis: parallel/crossing) TP	TP/mL	Ratio	Ret. %	Lysozyme (Coriolis: parallel/crossing) TP	TP/mL	Ratio	Ret. %
1	0	18	68/74	3.8/4.1	0.92	35/32	86/126	4.0/7.0	0.68	38/36
2*	3	13	107/117	8.4/9.1	0.91	29/26	150/178	11.7/13.9	0.84	35/35
3**	3	20	159/78	8.0/3.9	2.04	38/41	176/71	8.8/3.6	2.48	40/39
4	4	15	135/77	9.0/5.1	1.75	31/37	137/73	9.1/4.9	1.88	37/42
5***	4	20	110/85	5.5/4.3	1.29	35/35	137/86	7.2/4.5	1.59	43/43
6	5	20	101/69	5.1/3.5	1.46	37/37	134/86	6.7/4.3	1.56	40/42

* The tubing was flattened; ** flattening of the tubing was prevented by filling the tube with water before winding; *** winding a pair of tubing on core to increase the coil pitch. All columns are made from 20 m long, 1.07 mm ID (SW18) tubing wound onto nylon pipe. Solvent system: 12.5% (w/w) PEG 1000 - 12.5% (w/w) K_2HPO_4. Sample: each protein 10 mg in 1 mL stationary phase. Flow Rate: 0.2 mL/min; detection: 280 nm with PM6 spectrophotometer; revolution: 1200 rpm (seal-free centrifuge). See Table 1 for dimension and configuration of the column.

All columns were prepared from 1.07 mm ID PTFE tubing by twisting the pair or winding it onto nylon pipes with ODs ranging from 3 to 5 mm as indicated. A twisted pair listed at the top (No. 1) represents as an extreme case of a 0mm core OD. When a small core such as 3 mm OD was used, the tubing tended to be flattened (No. 2). This tube deformation was prevented by filling the tube with water and clamped at both ends before winding (No. 3). At 4 mm core, a pair of tubing was wound onto the core so that the pitch of the individual coil was doubled (No. 5). The dimensions of these columns are summarized in Table 1. In Table 2, the partition efficiency for each coil is expressed as a ratio of the theoretical plate number (TP) between the two elution modes, i.e., partitioning segment forming a less angle (parallel) to the Coriolis flow (Fig. 2) divided by that forming a greater angle (crossing) against the Coriolis flow.

For partitioning myoglobin with the lower phase mobile, the TP ratio of parallel/crossing increases as the core diameter decreases from 5 mm to 3 mm where it becomes maximum (2.04) in the non-flattened coil (No. 3). In the flattened coil wound onto 3 mm core (No. 2) and the twisted coil (No. 1), the Coriolis effect was reversed, i.e., the TP ratio becomes consistently less than 1 in both protein samples. The above relationship is also observed when lysozyme was partitioned with the upper phase mobile.

The ratio of the percentage retention (Ret. %) of the stationary phase was also listed in Table 2 for each separation. In most cases, the ratio is close to 1, indicating no significant effect on theoretical plate number.

Separation of Myoglobin and Lysozyme with Polymer Phase System

In order to study the Coriolis effect on peak resolution, two proteins were separated in the toroidal coil wound onto 3mm nylon core (see Table 1, column No. 3) which produced the greatest Coriolis effect on the single protein partitioning experiment (Table 2). The results are shown in Fig. 3 A & B.

Chromatograms in Fig. 3A were obtained by eluting the sample mixture in such a way that the effective coil segments are near parallel (parallel mode) to the Coriolis flow (Fig. 2) using both phases as the mobile phase. The left chromatogram was obtained by eluting the heavier mobile phase through the column against the direction of coil rotation, and the right chromatogram by eluting the lighter mobile phase toward the direction of coil rotation. The peak resolution in these separations (R_s) was 0.96 for the heavier phase mobile and 0.88 for the lighter phase mobile as indicated in the diagram.

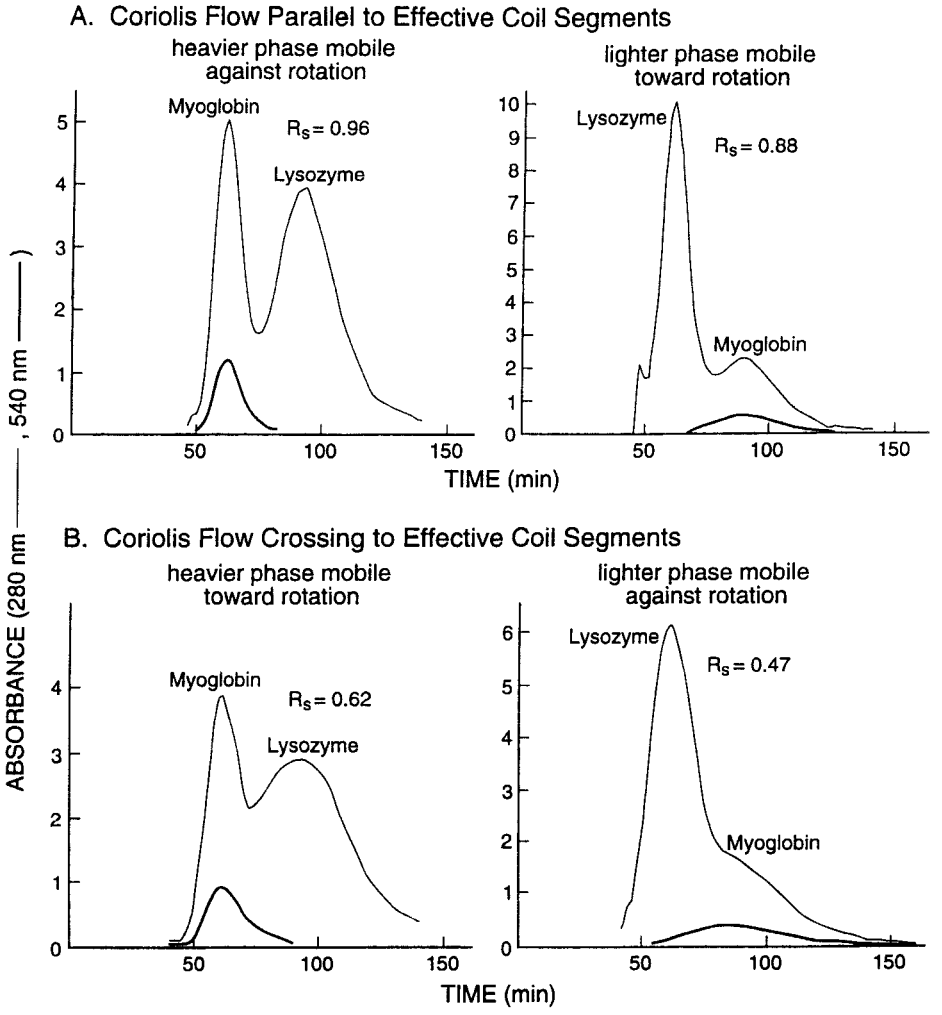

Figure 3. Protein separation with polymer phase systems by toroidal CCC. Experimental conditions are: Apparatus: seal-free toroidal coil centrifuge equipped with 37cm diameter bowl; column: toroidal coil of 1.07mm ID, 20 m long PTFE tubing wound around a 3 mm OD core (column No. 3, Table 1), 20 mL capacity; sample: myoglobin and lysozyme each 10 mg in 1 mL separation solvent (equal volumes of each phase); solvent system: 12.5%(w/w) PEG1000 and 12.5%(w/w) dibasic potassium phosphate in distilled water; mobile phase: heavier phase (A, left & B, left) and lighter phase (A, right & B, right); flow rate: 0.2 mL/min; elution direction: toward the rotation (A, right & B, left) and against the rotation (A, left & B, right); rotation: 1200 rpm.

Fig. 3B shows similar chromatograms obtained by eluting the mobile phase in the opposite direction so that the effective coil segments form a greater angle (crossing mode) to the Coriolis flow. The left chromatogram was obtained by eluting the heavier phase through the coil toward the direction of rotation, and the right chromatogram by eluting the lighter phase against the direction of rotation. The peak resolution of these separations was 0.62 for the heavier phase mobile and 0.47 for the lighter phase mobile, both being much lower than those obtained by the parallel mode.

Control Studies on Coriolis Effect Using Organic-Aqueous Solvent Systems

Using a dipeptide mixture and organic-aqueous two-phase solvent systems, similar experiments were performed to investigate the effect of the Coriolis force on partition efficiency and peak resolution. However, these control studies yielded negative results. The chromatograms obtained by eluting the aqueous phase from either end of the column were identical. These results indicate that there is no detectable Coriolis effect on the partition efficiency for the organic-aqueous solvent systems selected for the present study.

DISCUSSION

While separating proteins with polymer phase system using a toroidal coil centrifuge, we have noticed a strange phenomenon that the partition efficiency showed a significant difference when the inlet and outlet of the toroidal coil were reversed under the otherwise identical experimental conditions. Since the design of the toroidal coil elution system is symmetrical, this difference must be caused by the effect of the Coriolis force asymmetrically acting on the flowing liquid in the rotating centrifuge. The Coriolis force, acting on an object moving on a rotating body such as the earth, was first analyzed and reported by the French mathematician, Gaspard G. Coriolis in 1835. Considering the slow rotation of the earth, the Coriolis force generated in the high-speed centrifuge system would play much more significant role in fluid dynamics.

The effect of the Coriolis force on the moving droplets was successfully detected by stroboscopic observation using a colored mobile phase as schematically illustrated in Fig. 2A. The experiment revealed that the path of the ascending droplets was shifted toward the direction of the rotation and that of the descending droplets toward the opposite direction, regardless of the nature of the applied solvent system.

The mechanism of this "Coriolis flow" is illustrated in Fig. 2B. When the droplet is subjected to the centrifugal force, it moves along a spiral path with respect to the outside observer: The descending droplet on the left is forming a downward spiral path and the ascending droplet on the right, an upward spiral path (dotted lines). Therefore, the net force generated by each motion is given as an outwardly directed vertical vector tangential to the spiral path as indicated as a thick arrow. In each case, this force can be divided into two components, i.e., the radially acting centrifugal force (long arrow) and the Coriolis force (short arrow) acting tangentially to the rotation as indicated in the diagram. Thus, the descending droplet is subjected to the Coriolis force acting toward left whereas the ascending droplet is subjected to the Coriolis force acting toward right. This explains the Coriolis flow illustrated in Fig. 2A. The actual path of the "Coriolis flow" is, however, hardly predictable because the phenomenon involves many parameters such as size, shape and relative density of the droplet, viscosity of the stationary phase as well as the acceleration field at every location on the path.

The effect of the Coriolis force on the partition efficiency of proteins in the polymer phase system was examined using a set of toroidal coils and a twisted column (Table 1) mounted on the seal-free centrifuge. As summarized in Table 2, the Coriolis effect was detected in most of the toroidal coils in both mobile phases by eluting the coil in both directions. The results clearly show an important fact: with few exceptions, the higher partition efficiency is produced when the partition process takes place in the toroidal coil segments nearly parallel to the "Coriolis flow" shown in Fig. 2A. This rule holds true in most of the toroidal coils with core diameters from 5 to 3 mm (No. 3, Table 1) which yielded the highest efficiency. On the other hand, the partition efficiencies of the extensively flattened coil with 3 mm core and the twisted column with a much greater helical pitch (see Table 1, No. 1 and No. 2) exhibited an opposite trend.

From the above results, it may be assumed that the inclination of the effective segment of the toroidal coil relative to the "Coriolis flow" is a major factor in determining partition efficiency. Fig. 4 schematically illustrates the Coriolis effect on the mobile phase flow through the effective coil segments. When the partition takes place in the coil segment near parallel to the "Coriolis flow" (parallel mode, Fig. 4A), the mobile phase is dispersed into the stationary phase forming multiple droplets with a large interface area. In contrast, if the coil segment forms a significant angle to the "Coriolis flow" (crossing mode, Fig. 4B), the mobile phase tends to flow along the inner wall of the tube segments thus limiting the interface area available for mass transfer.

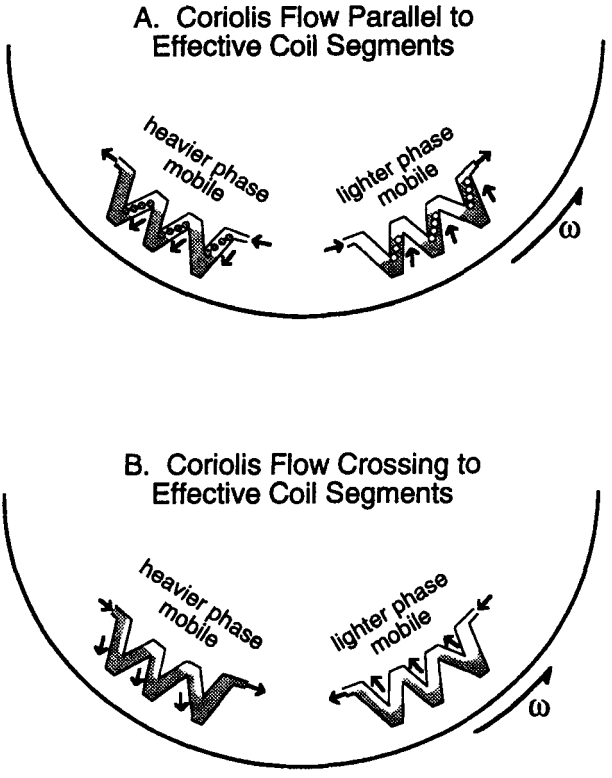

Figure 4. Flow patterns of the mobile phase caused by Coriolis force. A: Mobile phase flowing in the toroidal coil segments in near parallel orientation to the "Coriolis flow" direction, (left) the heavier phase mobile and (right) the lighter phase mobile; B: Mobile phase flowing in the toroidal coil segments in crossing orientation to the "Coriolis flow", (left) heavier phase mobile and (right) lighter phase mobile.

It is interesting to note that the above Coriolis effect was not observed in the separation of dipeptides with the conventional organic-aqueous solvent systems. This may be explained on the basis of the difference in physical properties between the conventional and polymer phase systems. The conventional solvent systems have relatively high interfacial tension, low viscosity and a large density difference between the two phases. Thus, in the toroidal coil, the mobile phase will form relatively large droplets in the stationary phase which quickly reach the other side of the coil segment. Therefore, under these conditions, the principal partition process in the toroidal coil may take place at a pair of interfaces present in each turn of the coil by the aid of a convection current of the stationary phase induced by the flow of the

mobile phase. However, the Coriolis force may produce some important effects on the partition process even with conventional solvent systems in the locular column configuration such as one used in centrifugal partition chromatography[8] where the width of the partition compartment is much greater than that of the toroidal coil used in the present studies.

CONCLUSIONS

In the toroidal coil CCC system, the partition efficiency of proteins with a polymer phase system varies significantly with the direction of elution: Eluting either the lighter phase along the direction of coil rotation or the heavier phase in the opposite direction produces substantially higher efficiency in terms of both theoretical plate number and peak resolution.

The mechanism of this strange phenomenon may be explained on the basis of Coriolis force: When the force acts along the effective segments of the toroidal coil, the mobile phase is dispersed into the stationary phase to provide a broad interface area enhancing mass transfer. When the force formes a large angle against the effective segments, the mobile phase tends to flow along the inner wall of the tubing with a limited interface area resulting in lower efficiency. This unique parameter may be effectively used for developing a new column design to improve the partition efficiency of toroidal coil CCC for protein separation.

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