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TOROIDAL COIL PLANET CENTRIFUGE FOR COUNTER-CURRENT CHROMATOGRAPHY

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SUMMARY

A simple tabletop model of a versatile counter-current chromatographic system is introduced. The apparatus compactly holds a long coiled column around a drum-shaped holder. The acceleration produced by the synchronous planetary motion of the holder enables stable retention of the stationary phase in each helical turn of the coil while the mobile phase is continuously eluted through the column. The capability of the method was demonstrated on separations of DNP amino acids and oligopeptides using typical two-phase solvent systems. The present method will provide a universal application of solvent systems including aqueous–aqueous polymer phase systems used for partition of cell particles and macromolecules.

INTRODUCTION

The toroidal coil planet centrifuge[1] is one of a family of synchronous coil planet centrifuges that provide a particular mode of planetary motion to a coiled tube for performing counter-current chromatography. The greatest advantage of this present scheme over other counter-current chromatographic schemes[2–4] is that it has the capability of producing a strong centrifugal force field which provides a stable retention of the stationary phase for various two-phase solvent systems in a narrow-bore coiled tube. The apparatus holds a long narrow-bore coiled tube around its large diameter drum-shaped column holder in a coiled helix configuration. The holder undergoes a synchronous planetary motion of one rotation (around its own axis) during one revolution (around the central axis of the centrifuge) in the same directions. This planetary motion allows continuous elution of the solvent through the coiled separation column without the use of rotating seals[3]. Analysis of the acceleration field acting on the column shows that the acceleration vector is always directed inwardly from the periphery of the column holder to retain one of the phases stationary in each turn of the coil while the other phase is continuously eluted through the column. The acceleration vector also undulates in both magnitude and direction during each revolutional cycle to provide efficient mixing of the two phases. Consequently, solutes locally introduced at the inlet of the column are

subjected to an efficient partition process in each turn of the coil and are eluted out according to their partition coefficients as in liquid chromatography but in the absence of solid supports.

A brief introduction of the toroidal coil planet centrifuge has been given earlier[1]. In the present paper the principle of the method is elucidated by the aid of the analysis of acceleration and the performance characteristics of the apparatus is demonstrated by separations of dinitrophenyl (DNP) amino acids and oligopeptides using conventional two-phase solvent systems.

PRINCIPLE AND ANALYSIS OF ACCELERATION FIELD

Fig. 1A schematically illustrates the principle of the toroidal coil planet centrifuge. A large cylindrical coil holder is connected to the planetary gear which rolls around an identical stationary sun gear (shaded) mounted on the central axis of the centrifuge. With this gear arrangement the holder revolves around the central axis (axis of revolution) of the apparatus and simultaneously rotates about its own axis (axis of rotation) at the same angular velocity in the same direction. A pair of flow tubes from the coiled column first exits the holder at the axis of rotation, forms a loop to reach the axis of revolution, and then passes through the center of the stationary sun gear as illustrated in the figure. These tubes are tightly supported at the center of the sun gear. As reported earlier[3], the rolling action of the planetary gear around the stationary sun gear cancels out any revolutional effect, therefore the flow tubes are free of twisting at any arbitrary revolutional rate.

In order to comprehend the acceleration field produced in the present scheme, it is necessary to review the analysis previously made on the horizontal flow-through coil planet centrifuge[3,5]. This has a long, thin column holder but with the identical mode of planetary motion. For an analysis of acceleration, Fig. 1A may be reduced to a simple coordinate system shown in Fig. 1B where the axis of revolution is located at point 0. For the convenience of analysis, the coordinate system is selected in such a way that both the axis of rotation (Q_0) and the arbitrary point (P_0) on the holder start on the x-axis as illustrated. After time t, the location of the arbitrary point, P (x,y), is expressed by

$$x = R \cos \theta + r \cos 2\theta \tag{1}$$

$$y = R \sin \theta + r \sin 2\theta \tag{2}$$

where $R = \overline{Q_0}$, the radius of revolution; $r = \overline{PQ}$, the radius of rotation; $\theta = \omega t$; ω denotes the angular velocity of revolution. The magnitude of acceleration, α, is derived from these equations as

$$\alpha = \{(d^2x/dt^2)^2 + (d^2y/dt^2)^2\}^{\frac{1}{2}} = R\omega^2(1 + 16\beta^2 + 8\beta\cos\theta)^{\frac{1}{2}} \tag{3}$$

acting at the angle, γ_x, relative to the x-axis, *i.e.*,

$$\gamma_x = \pi + \tan^{-1}\{(d^2y/dt^2)/(d^2x/dt^2)\} = \pi + \tan^{-1}\frac{\sin\theta + 4\beta\sin 2\theta}{\cos\theta + 4\beta\cos 2\theta} \tag{4}$$

where $\beta = r/R$ provided $R \neq 0$.

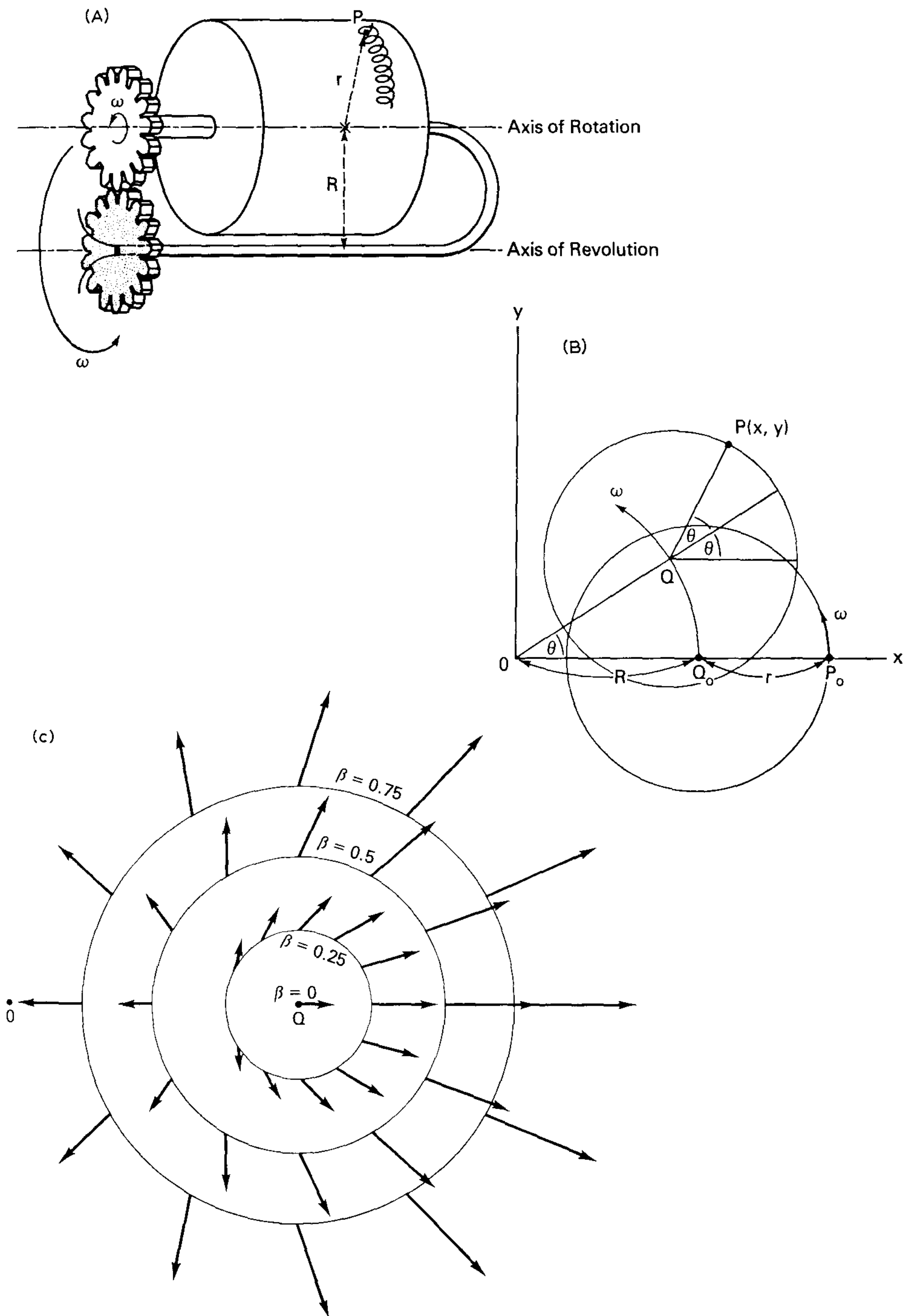


Fig. 1. (A), Principle of the toroidal coil planet centrifuge. (B), Coordinate system for analysis of acceleration. (C), Distribution pattern of the relative centrifugal force vector at various locations of the holder.

For ease of visualizing and further analyzing this undulating acceleration vector, the acting angle may be more conveniently expressed with respect to the rotating holder. Thus, the angle, γ, formed at the arbitrary point P relative to the rotational radius, PQ, is computed from eqn. 4 as

$$\gamma = \gamma_x - 2\theta - \pi = \tan^{-1}\{(-\sin\theta)/(4\beta + \cos\theta)\} \tag{5}$$

Using eqns. 3 and 5, the relative centrifugal vectors acting at various locations on the holder can be obtained and expressed as sets of arrows as shown in Fig. 1C.

Three circles centered at Q correspond to β values of 0.25, 0.5 and 0.75 as labelled while point Q, the center of rotation, is at $\beta = 0$. A set of arrows around each circle indicates the distribution pattern of the centrifugal force field at a given moment. It is important to note that the arbitrary point on the holder experiences these vectors in sequence during each revolutional cycle.

As clearly shown in the figure, when $\beta > 0.25$, the vector is always directed outwardly from the circle. The vector also undulates in its relative magnitude and direction according to the location on the holder at given β values. As the β value increases, the magnitude of the relative centrifugal force vector becomes greater while the amplitude of angular oscillation around the axis of rotation is reduced. This distribution pattern of the force vector provides a great advantage when the scheme is used for performing counter-current chromatography.

When a coiled tube is mounted around the holder, each helical turn of the column is exposed to the above centrifugal force field which separates two liquid phases in such a way that the heavier phase occupies the outer half and the lighter phase, the inner half of each coil unit. Angular undulation of the force vector efficiently mixes the interface and the two phases in each turn of the coil to reduce the mass transfer resistance. Consequently, solutes introduced locally at the inlet of the column are subjected to an efficient partition process and separated according to their relative partition coefficients as in liquid partition chromatography but without the use of solid supports.

The results of these analysis disclose a versatile feature of the present scheme. The use of two-phase solvent systems having extremely low interfacial tension requires a strong centrifugal force field combined with a small amplitude of angular oscillation to provide satisfactory retention of the stationary phase. Elution with a high interfacial tension phase system through a small-bore column also necessitates application of a strong centrifugal force field to overcome the tendency of plug flow which would cause carryover of the stationary phase. The operational conditions to meet such requirements can be attained in the present scheme simply by increasing the β value of the holder. Thus the present scheme permits universal application of the two-phase solvent systems including aqueous–aqueous polymer phase systems used for partition of macromolecules and cell particles[6].

The present scheme provides an additional advantage in its mechanical design. Because the large drum-shaped holder compactly holds a long coiled column, the length of the column holder shaft can be reduced so that the distortion of the shaft under a strong centrifugal force field is minimized. This allows operation at a high revolutional speed without imposing an excessive strain on the bearings supporting the column holder shaft. Furthermore, the effective force field acting on

the column mounted at the periphery of the holder is far greater than the force acting at the axis of the holder as illustrated in Fig. 1C. Thus, the present design is capable of producing a centrifugal force field of several hundred g which can retain even small cell particles suspended in a saline solution within the column.

Design of the apparatus

Fig. 2A shows a photograph of our prototype. The rotary frame consists of a pair of circular aluminum plates rigidly bridged with multiple aluminum links and is driven by a motor (ElectroCraft) around the stationary pipe mounted on the central axis of the centrifuge. The rotary frame holds a pair of cylindrical holders symmetrically spaced at a distance of 10 cm from the central axis of the apparatus. The holder having the coiled column is 15 cm in diameter ($\beta = 0.75$) while the other holder with a counterweight, 10 cm in diameter ($\beta = 0.5$). Each coil holder is equipped with a plastic gear (Winfred M. Berg, Inc.) which is engaged to an identical stationary gear mounted on the central stationary pipe. With this gear arrangement, each holder undergoes the desired planetary motion illustrated in Fig. 1A, *i.e.*, revolution around the central axis of the apparatus and rotation about its own axis at the same angular velocity in the same direction. In order to mechanically stabilize the centrifuge system, a short coupling pipe is coaxially mounted on the free end (right side) of the rotary frame while the other end of the coupling pipe is supported by the wall of the outside enclosure of the apparatus through a ball bearing.

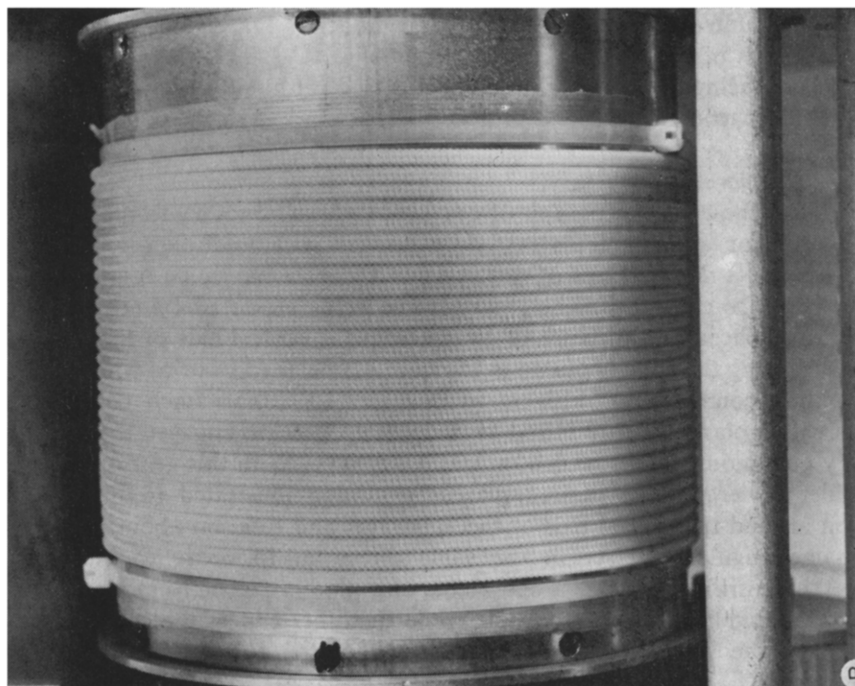
The coiled separation column is prepared by winding PTFE tubing (Zeus Industrial Products, Raritan, N.J., U.S.A.) onto a flexible core which is again coiled around the holder to form a coiled helix configuration as shown in Fig. 2B. A counterweight is applied to the other holder to balance the centrifuge. The flow tubes from the separation column are first passed through the center hole of the column holder shaft and then led into the coupling pipe through a side hole to enter the opening of the stationary pipe. The flow tubes pass through the central stationary pipe and emerge at the left side of the centrifuge enclosure. These flow tubes are thoroughly lubricated with silicone grease and also protected with a piece of plastic tubing at each supported portion to prevent direct contact against metal parts. When this protection is provided, the flow tubes can maintain their function almost permanently. The revolutional speed of the prototype is continuously adjustable up to 1000 rpm (450 *g*) with a Motomatic speed control unit (ElectroCraft).

A Chromatronix Cheminert metering pump was used for elution of the solvent and an LKB Uvicord III for monitoring the absorbance at 280 nm.

Studies on partition capabilities of the apparatus

As in other counter-current chromatographic schemes, the performance of the present apparatus relies upon retention of the stationary phase and efficiency of solute partitioning between the two solvent phases in the coiled column. In order to determine the optimum operational conditions, a series of experiments were performed to study the degree of stationary phase retention and partition efficiency in short coiled columns under various revolutional speeds and flow-rates.

Two types of coiled columns were prepared each from a 7 m long, 0.55 mm I.D. PTFE tube by winding it onto a flexible nylon core of different diameters. One



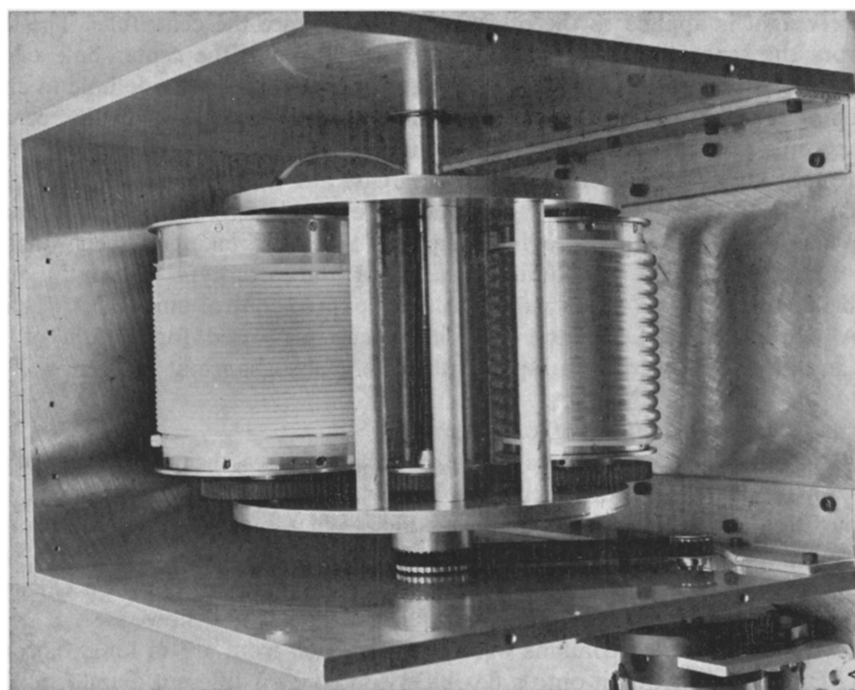


Fig. 2. (A), Overall view of the apparatus. (B), Coiled separation column mounted on the holder.

column had a core diameter of 5 mm and the other column, 1.5 mm. In both columns, the total capacity measured was approximately 2 ml. These columns were mounted on the column holder having β value of 0.75.

In the present study, two typical phase systems were selected, chloroform–acetic acid–0.1 *N* hydrochloric acid (2:2:1) for partition of DNP-amino acids and *n*-butanol–acetic acid–water (4:1:5) for partition of oligopeptides (figures in the parentheses indicate the volume ratios of the solvents). For each phase system a pair of samples with suitable partition coefficients were selected: N-DNP-L-glutamic acid and N-DNP-L-alanine for the chloroform phase system; L-valyl-L-tyrosine and L-tryptophyl-L-tyrosine for the *n*-butanol phase system. The DNP-amino acid sample mixture was prepared by dissolving each component in the aqueous phase at 0.5 g% and 10 μl of this solution was charged in each separation. The dipeptide sample mixture was similarly prepared to make final concentration of 1 g% for L-valyl-L-tyrosine and 0.3 g% for L-tryptophyl-L-tyrosine and 20 μl was applied in each run.

In each separation the column was first filled with the stationary phase followed by sample injection through the sample port. Then the column was eluted with the mobile phase while the apparatus was run at the desired revolutional speed. The eluate was continuously monitored through an LKB Uvicord III at 280 nm and then collected into a graduated cylinder to measure the volume of the stationary phase eluted from the column. The percentage retention of the stationary phase relative to the total column capacity can be obtained from the eluted stationary phase volume, V_s, the total column capacity, V_c, and the free space in the flow path, V_f, by

$$\text{Retention}\ (\%) = 100\,(V_c + V_f - V_s)/V_c \qquad (6)$$

Figs. 3A and B summarize the data for retention of the stationary phase under various operational conditions. In each diagram, the retention is plotted against the applied revolutional speed at two different flow-rates. The retention of near 50% is ideal but that over 30% is considered to be satisfactory if the inclination of the curve is relatively small. Operation at the near horizontal portion of the curve insures minimum carryover of the stationary phase even if there are shifts of revolutional speed. The overall results indicate that the satisfactory level of retention is obtained over a wide range of revolutional speeds and flow-rates for both solvent systems. The 5-mm core column gives a slightly higher level of retention than the 1.5-mm core column. Because of the solvent–wall interaction in a narrow-bore tube, the non-aqueous phase having an affinity to the PTFE tube gives substantially higher level of retention than the aqueous phase under a given set of operational conditions. The retention levels of the *n*-butanol phase system (Fig. 3B) is generally lower than those of chloroform phase system (Fig. 3A) due to its higher viscosity and the smaller difference in density between the two phases. Satisfactory retention levels obtained from these typical phase systems with contrasting physical properties suggest that the present method permits universal application of the two-phase solvent systems.

Fig. 4A summarizes the results of DNP-amino acid separations with both 5-mm and 1.5-mm core columns performed at revolutional speeds ranging between

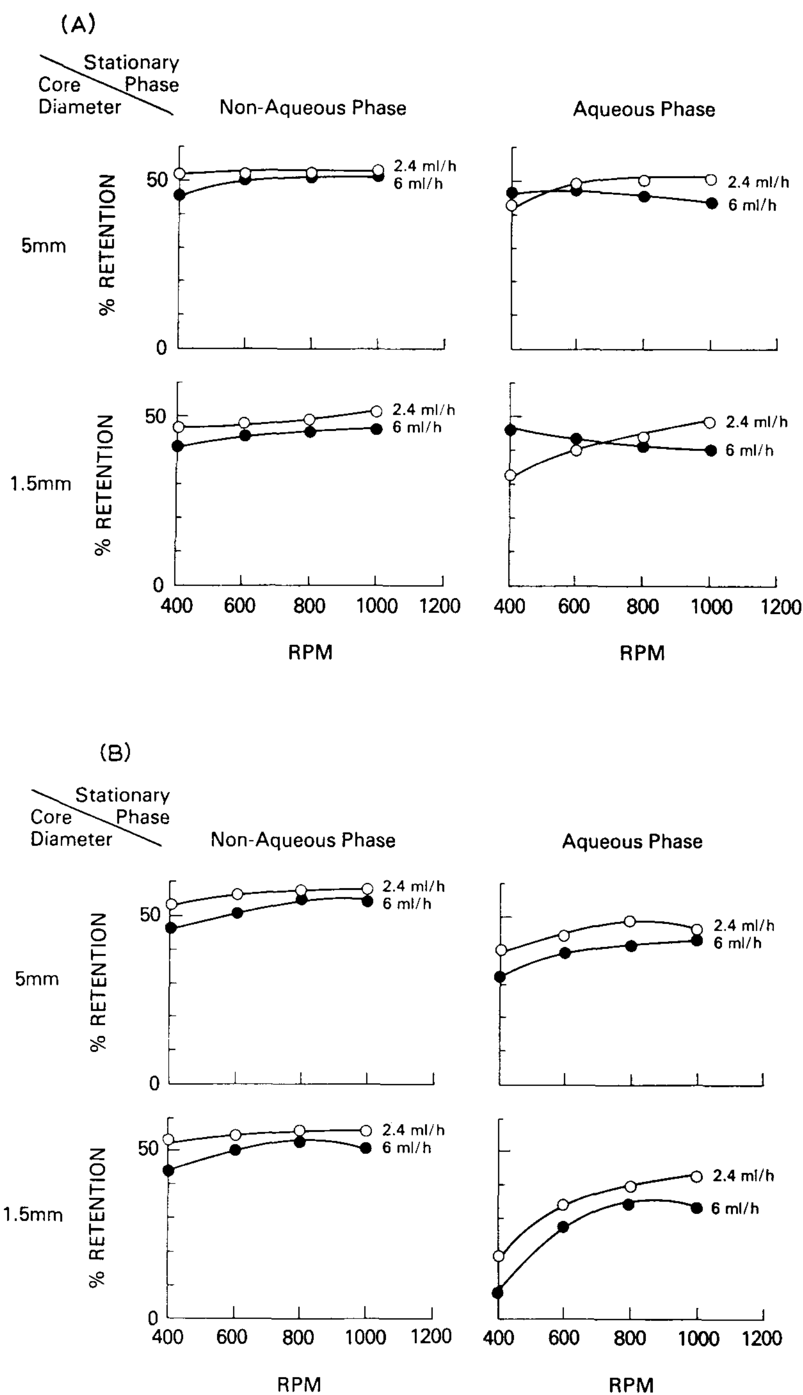


Fig. 3. (A), Retention of the stationary phase of the chloroform phase system in both 5-mm and 1.5-mm core columns. (B), Retention of the stationary phase of the *n*-butanol phase system in both 5-mm and 1.5-mm core columns.

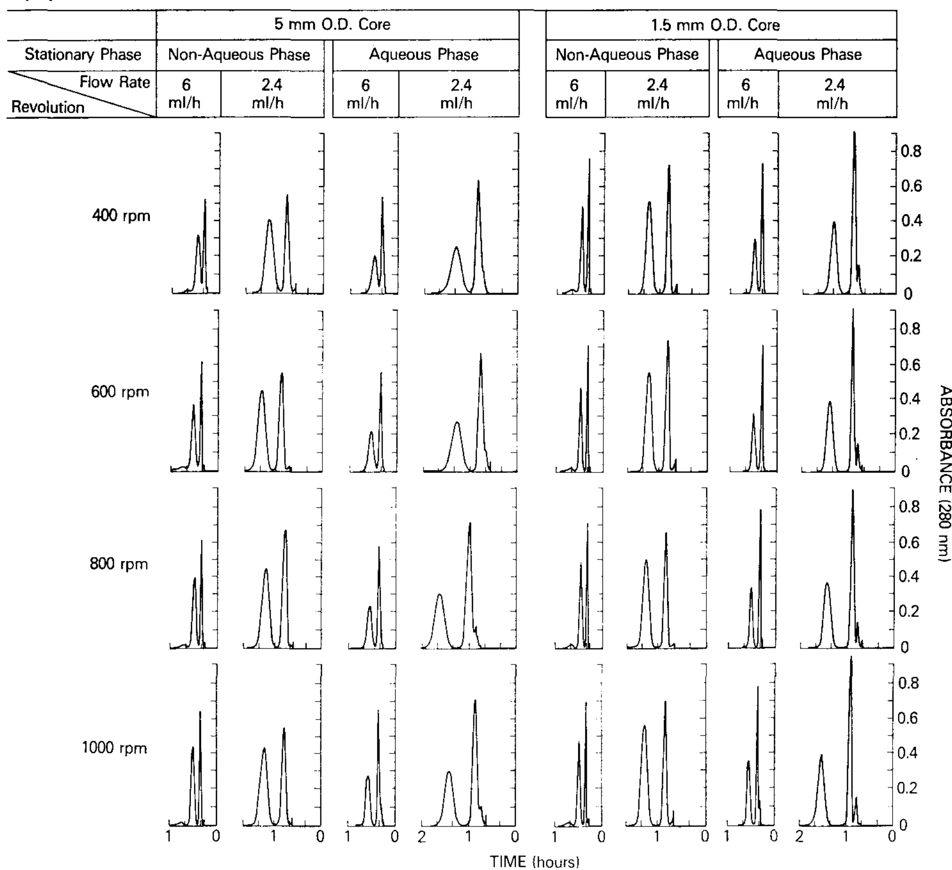


Fig. 4.

(Continued on p. 84)

400 and 1000 rpm. Flow-rates of 6 and 2.4 ml/h were applied using both non-aqueous and aqueous phases as the stationary phase. In each chromatogram, partition efficiency can be easily estimated by observing the degree of peak resolution and, if desired, expressed in terms of the number of theoretical plates using the conventional equation

$$N = (4R/w)^2 \tag{7}$$

where R denotes the retention time and w the peak width.

Overall results indicate that the 1.5-mm core column yields higher peak resolution than the 5-mm core column. Although good peak resolutions are given at 6 ml/h flow-rate in short periods of time, the highest partition efficiency is obtained at 2.4 ml/h flow-rate under revolutional speeds of 600 to 1000 rpm.

Fig. 4B similarly summarizes the results of the oligopeptide separation. Here again the peak resolution obtained by the 1.5-mm core column generally exceeds that obtained by the 5-mm core column. Poor peak resolution observed at 6 ml/h under

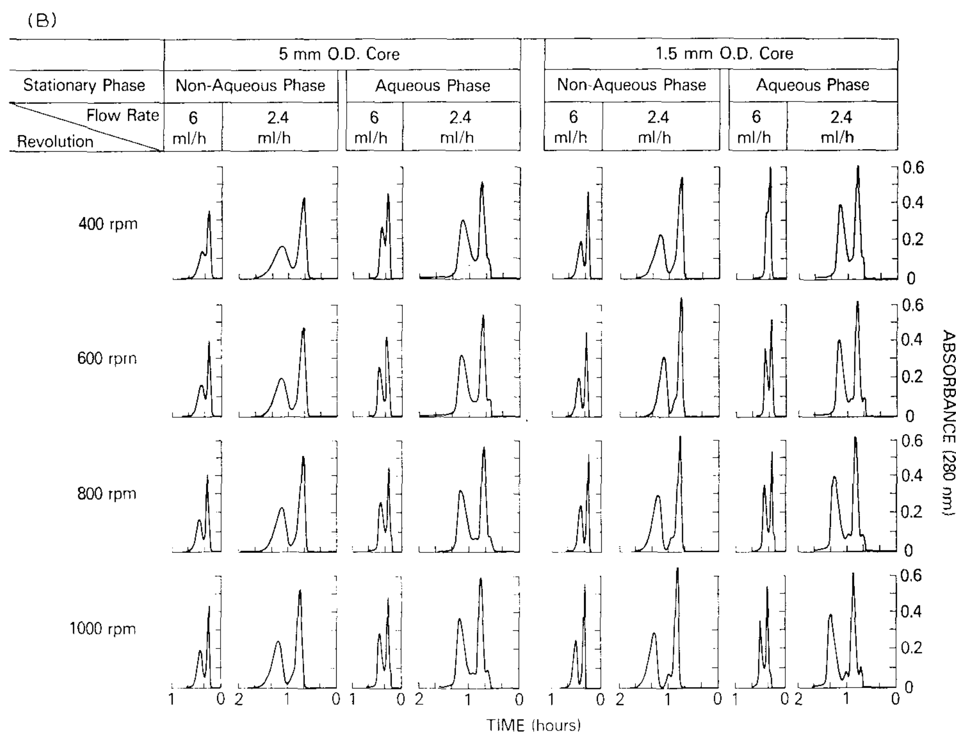


Fig. 4. (A), Effects of the revolutional speeds and flow-rates on the separations of DNP-amino acids with the chloroform phase system. (B), Effects of the revolutional speeds and flow-rates on the separations of dipeptides with the *n*-butanol phase system.

400 rpm was apparently caused by a low retention level of the stationary phase as seen in Fig. 3B. The best results are obtained by the 1.5-mm core column at 2.4 ml/h under 800 to 1000 rpm, where impurities present in the sample solution are partly resolved from the major peaks.

Separation of DNP-amino acids and peptides with a long column

The present method was applied for separation of DNP-amino acids and oligopeptides using a long coiled column. The separation column was prepared from a 50 m × 0.55 mm I.D. PTFE tube by winding it onto a 13 m × 1.5 mm O.D. nylon pipe to make approximately 8500 helical turns with a total capacity of about 18 ml. The column was mounted on the column holder with $\beta = 0.75$.

Suitable sets of samples were selected each from DNP-amino acids (Sigma, St. Louis, Mo., U.S.A.) and oligopeptides (Sigma) for separation with the two-phase solvent systems used in the previous experiments. The DNP-amino acid mixture was prepared by dissolving a set of samples in the aqueous phase to make a concentration of each component at 0.1 to 0.4 g%. The oligopeptide sample mixture was similarly prepared to bring the final concentration of each component at 0.05 to 0.6 g%.

In each separation, the column was first filled with the stationary phase and

50 μl of the above sample solution was injected through the sample port. Then the mobile phase was pumped into the column at a flow-rate of 2.4 ml/h while the apparatus was run at revolutional speeds ranging between 400 and 600 rpm. The eluate was continuously monitored with an LKB Uvicord III at 280 nm.

Fig. 5A shows a chromatogram obtained from a set of DNP amino acids by eluting with the aqueous phase under a revolutional speed of 400 rpm. All components were well resolved as symmetrical peaks and eluted out in 18 h. Fig. 5B shows a similar chromatogram obtained by elution with the non-aqueous phase. Partition efficiencies of these separations estimated from eqn. 7 range between 6000 and 2000 theoretical plates. Fig. 6A shows a chromatogram of oligopeptides obtained by eluting with the aqueous phase. Because of the non-linear distribution isotherm of

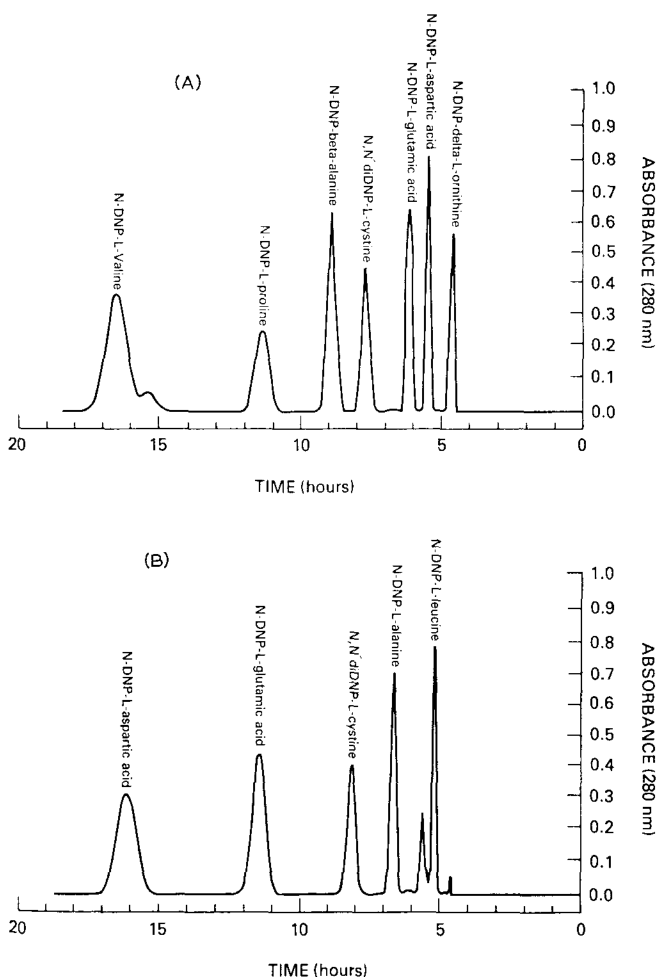


Fig. 5. Separations of DNP-amino acids with a long coiled column. Column: 0.55 mm I.D., 18 ml capacity, $\beta = 0.75$; solvent system: chloroform–acetic acid–0.1 N hydrochloric acid (2:2:1); sample volume: 50 μl; revolution: 400 rpm; flow-rate: 2.4 ml/h. (A), Chromatogram obtained by eluting with the aqueous phase. (B), Chromatogram obtained by eluting with the non-aqueous phase.

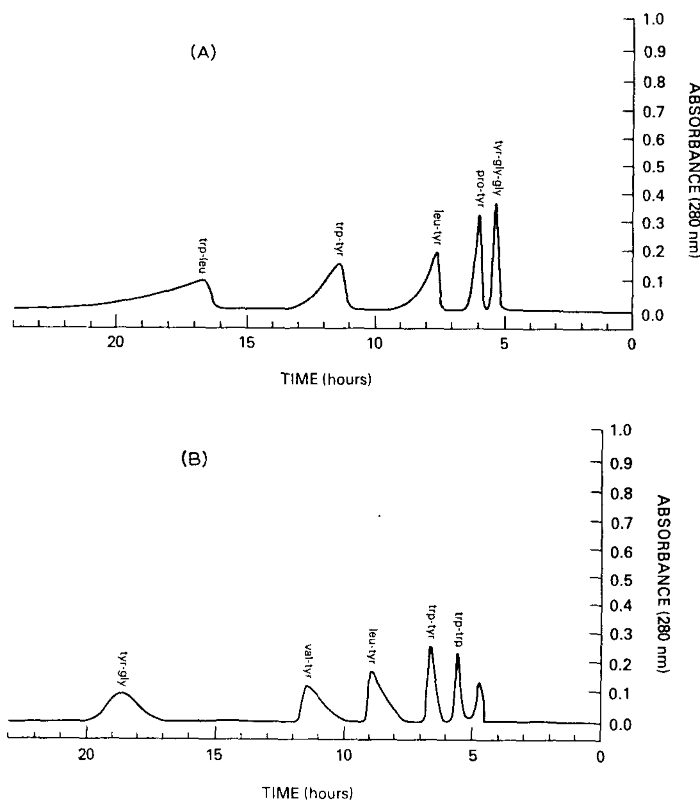


Fig. 6. Separations of oligopeptides with a long coiled column. Solvent system: *n*-butanol–acetic acid–water (4:1:5); revolution: 600 rpm; other conditions as in Fig. 5. (A), Chromatogram obtained by eluting with the aqueous phase. (B), Chromatogram obtained by eluting with the non-aqueous phase.

the samples in the *n*-butanol phase system, peaks show intensive skewing. The shape of this skewing is reversed when the samples are eluted with the non-aqueous phase as shown in Fig. 6B. The degree of skewing will be decreased as the sample concentration is reduced. As expected from the results of the previous studies, partition efficiency of the oligopeptide separation is lower than that in the DNP-amino acid separation mainly due to the higher viscosity of the normal butanol phase system. Partition efficiencies of these oligopeptide separations range between 3000 and 300 theoretical plates.

These chromatograms were obtained under the revolutional speeds considerably below the optimum ranges determined by the previous studies. The use of a long coiled column causes a pressure build-up in the column which could exceed the maximum capacity of the Chromatronix pump rated at 500 p.s.i. This limited the operation from 400 to 600 rpm to maintain a safety pressure range below 400 p.s.i. However, the PTFE tube used in the column can hold much higher pressures and, therefore, the use of a metering pump with a higher pressure capacity would yield substantially better resolution by allowing higher revolutional speeds.

CONCLUSIONS

The toroidal coil planet centrifuge is a compact tabletop model suitable for laboratory use. Because of the use of the unique pattern of the centrifugal force field together with the stable mechanical design, the method allows universal applications of two-phase solvent systems to yield highly efficient chromatographic separations of solutes which are comparable to those obtainable by a refined liquid chromatography. The method offers a great advantage over liquid chromatography in that the separation is performed under the absence of solid support, hence eliminates all complications caused by the adsorption effects such as a sample loss, contamination and tailing of the solute peaks.

The capability of the present method will be extended to partition of macromolecules and cells on aqueous–aqueous polymer phase systems[6] and also to the elutriation of cell particles with a physiological solution.

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REFERENCES

1 Y. Ito, *Anal. Biochem.*, in press.
2 Y. Ito, R. E. Hurst, R. L. Bowman and E. K. Achter, *Separ. Purif. Methods*, 3 (1974) 133.
3 Y. Ito and R. L. Bowman, *J. Chromatogr.*, 147 (1978) 221.
4 Y. Ito, *Anal. Biochem.*, 100 (1979) 271.
5 Y. Ito, *J. Chromatogr.*, 188 (1980) 33.
6 P. Å. Albertsson, *Partition of Cell Particles and Macromolecules*, Almqvist & Wiksell, Stockholm and Wiley, New York, 1971.