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# CROSS-AXIS SYNCHRONOUS FLOW-THROUGH COIL PLANET CENTRIFUGE FOR LARGE-SCALE PREPARATIVE COUNTER-CURRENT CHROMATOGRAPHY

## III. PERFORMANCE OF LARGE-BORE COILS IN SLOW PLANETARY MOTION

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SUMMARY

A preparative capability of the present cross-axis synchronous flow-through coil planet centrifuge was demonstrated with 0.5 cm I.D. multilayer coils. Results of the model studies with short coils indicated that the optimal separations are obtained at low revolutional speeds of 100–200 rpm in both central and lateral coil positions. Preparative separations were successfully performed on 2.5–10 g quantities of test samples in a pair of multilayer coils connected in series with a total capacity of 2.5 l. The sample loading capacity will be scaled up in several folds by increasing the column width.

## INTRODUCTION

In the foregoing articles (Parts I[1] and II[2]), the capability of the cross-axis synchronous flow-through coil planet centrifuge (X-axis CPC) has been demonstrated in preparative counter-current chromatography (CCC) of dinitrophenyl (DNP)-amino acids and dipeptides with paired multilayer coils of 2.6 mm I.D. rotated at 450–500 rpm. The use of relatively high centrifugal force field (*ca.* 50 *g*), however, limits the use of longer columns for further scaling up the sample loading capacity of the system due to mechanical strain of the planetary centrifuge system. This problem may be solved by utilizing a large-bore separation column which shifts the optimal revolutional speed down to a few hundred rpm range and, therefore, permits safe operation of a much larger separation column.

The present paper describes preliminary studies on performance of a large-bore column of 5 mm I.D. Model studies were performed with single-layer short coils to

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investigate both retention of the stationary phase and partition efficiency for determination of the optimal operational conditions. The preparative capability of the present method was demonstrated on multigram separations of test samples with paired multilayer coils with a total capacity of 2.5 l.

## EXPERIMENTAL

### *Apparatus*

The present studies were performed with the X-axis CPC with a 20-cm revolutional radius which has been used in the previous studies. The design of the apparatus is described in detail in Part I[1].

Two types of coiled columns were tested: short coils for model studies on retention of the stationary phase and partition efficiency, and a pair of multilayer coils for preparative-scale separations. The short coil was prepared from a 4.2 m length of 5.5 mm I.D. fluorinated ethylene propylene (FEP) tubing (Galtek Corporation, Jonathan Industrial Center, Chaska, MN, U.S.A.) by winding it onto a holder hub of 15 cm diameter to form a single-layer coil. The coil was mounted on the holder at two locations, at the center of the holder ($l = 0$ cm) and at 9 cm on the left of the center ($l = -9$ cm). The total capacity of the short coil measured about 110 ml. Each multilayer coil was prepared from 5 mm I.D. polytetrafluoroethylene (PTFE) tubing (Zeus Industrial Products, Raritan, NJ, U.S.A.) by winding it onto a spool-shaped holder measuring 5 cm in width between the flanges and 10 cm in hub diameter. It consisted of 13 layers with 9–10 coils in each layer (total 120 coils) with a capacity of

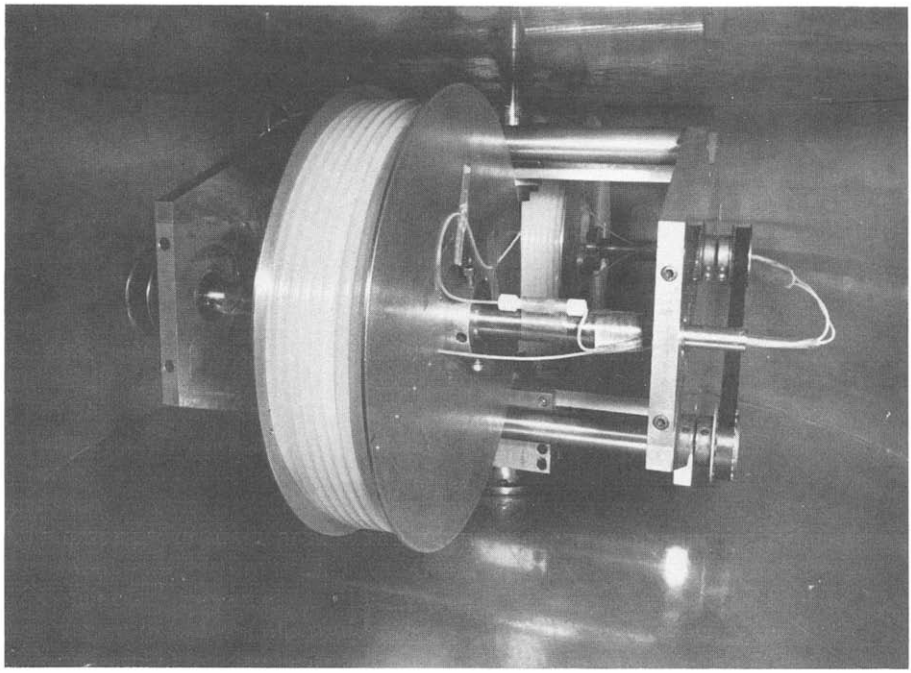


Fig. 1. Photograph of the X-axis CPC equipped with a pair of multilayer coils in the central position.

about 1250 ml. Two identical multilayer coils were connected in series and symmetrically mounted on the rotary frame to provide stable balance of the centrifuge system as shown in Fig. 1.

The thick-wall FEP tubing used for the short coil is transparent and resists flattening on coiling and, therefore, is ideal for model studies, whereas much lighter, less expensive PTFE tubing is more suitable in the practical use for fabrication of a long multilayer coil.

*Reagents*

Two-phase solvent systems were prepared from chloroform, *n*-butanol (both were glass-distilled chromatographic grade from Burdick & Jackson Labs., Muskegon, MI, U.S.A.); acetic acid (reagent grade from J. T. Baker, Phillipsburg, NJ, U.S.A.); 1 *M* hydrochloric acid (reagent grade from Sigma, St. Louis, MO, U.S.A.); and distilled water. The following compounds were used for test samples (all reagent grade from Sigma): $N^5$-2,4-DNP-L-ornithine (DNP-orn), N-2,4-DNP-L-aspartic acid (DNP-asp), N-2,4-DNP-DL-glutamic acid (DNP-glu), N,N-di(2,4-DNP)-L-cystine [diDNP-$(cys)_2$], N-2,4-DNP-$\beta$-alanine (DNP-$\beta$-ala), N-2,4-DNP-L-alanine (DNP-ala), N-2,4-DNP-L-proline (DNP-pro), N-2,4-DNP-L-leucine (DNP-leu), L-valyl-L-tyrosine (val-tyr), L-leucyl-L-tyrosine (leu-tyr) and L-tryptophyl-L-tyrosine (trp-tyr).

*Preparation of two-phase solvent systems and sample solutions*

Two different solvent systems were used: chloroform–acetic acid–0.1 *M* hydrochloric acid (2:2:1, v/v/v) for DNP-amino acid separations and *n*-butanol–acetic acid–water (4:1:5, v/v/v) for dipeptide separations. Each solvent system was thoroughly equilibrated in a separatory funnel at room temperature and the two phases separated shortly before use.

Sample solutions for DNP-amino acid separations were prepared as follows: For the preliminary studies with short coils, equal quantities of DNP-glu and DNP-ala were dissolved in the upper aqueous phase to make the concentration of each component 0.5 g%, and 0.5 ml of the sample solution was loaded in each experiment. For the large-scale preparative chromatography in the multilayer coils, mixtures of five DNP-amino acids with a total weight of 10 g were dissolved in 100 ml of the solvent mixture: The sample solution for the run with the upper phase mobile contained 1 g of DNP-orn, 2 g of DNP-asp, 2 g of DNP-glu, 1 g of diDNP-$(cys)_2$, and 4 g of DNP-ala, while that with the lower phase mobile contained 1 g of DNP-leu, 2 g of DNP-pro, 2 g of DNP-$\beta$-ala, 1 g of diDNP-$(cys)_2$, and 4 g of DNP-glu.

For the preliminary separations in short coils, the peptides were dissolved in the lower aqueous phase to make concentrations of val-tyr and trp-tyr 1 g% and 0.3 g%, respectively, and 0.5 ml of this solution were used for each run. For the preparative separations in the multilayer coils, 1 g of val-tyr, 1 g of leu-tyr, and 0.5 g of trp-tyr were dissolved in 200 ml of the solvent mixture.

*Model studies on stationary phase retention and partition efficiency in short coils*

Experiments were performed according to the procedure described in Part II[2]. For each experiment, the coil was first completely filled with the stationary phase. This was followed by injection of sample solution through the sample port. Then, the mobile phase was eluted through the coil at 120 ml/h while the apparatus was rotated

at the desired rpm value. The effluent from the outlet of the coil was continuously monitored with an LKB Uvicord S at 278 nm and fractionated into test tubes with an LKB fraction collector for later analysis. After the two peaks were eluted from the coil, the apparatus was stopped and the retention of the stationary phase was measured by collecting the column contents into a graduated cylinder. This was done by connecting the inlet of the column to a pressured nitrogen line, while the coil was slowly rotated at 100 to 200 rpm in a direction to make the outlet of the coil the head to facilitate emptying the column contents. The measurements were performed at various rpms, ranging from 0 to 300, using both the upper and the lower phases as the mobile phase in the central ($l$ = 0 cm) and the lateral ($l$ = −9 cm) coil positions.

As described in the foregoing articles (Parts I and II), performance of laterally mounted coils is affected by three factors, *i.e.*, the Archimedean screw effect represented by head-tail elution mode, planetary motion $P_I$ or $P_{II}$ (see Table I), and inward-outward elution mode. All possible combinations were examined at moderate revolutional speeds of 100 to 200 rpm to select the favorable experimental conditions which were further investigated at a broader range of revolutional speeds. The selected experimental conditions are summarized in Table I.

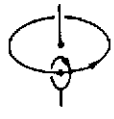
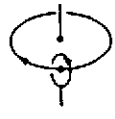
*Preparative separations with paired multilayer coils*

Preparative separations were performed with a pair of multilayer coils connected in series and mounted on the center of the holder shaft ($l$ = 0 cm), using the optimal experimental conditions determined by the preliminary model studies with short coils. The experiment was initiated by filling the entire column with the stationary phase. Air trapped in the column can be easily eliminated by rotating the column slowly in a direction to make the outlet of the column the head while pumping the stationary

TABLE I

SUMMARY OF EXPERIMENTAL CONDITIONS IN MODEL STUDIES WITH SHORT COILS

| *Coil position* | *Mobile phase* | *Head-tail elution mode* | *Planetary motion** | *Inward or outward elution mode (handedness)*** |
|---|---|---|---|---|
| Center ($l$ = 0 cm) | Upper phase | head→tail | | |
| | Lower phase | tail→head | | |
| Lateral ($l$ = −9 cm) | Upper phase | head→tail | $P_I$ | O (L) |
| | Upper phase | head→tail | $P_I$ | I (R) |
| | Upper phase | head→tail | $P_{II}$ | O (R) |
| | Upper phase | head→tail | $P_{II}$ | I (L) |
| | Lower phase | tail→head | $P_I$ | O (L) |
| | Lower phase | tail→head | $P_I$ | I (R) |
| | Lower phase | tail→head | $P_{II}$ | O (R) |
| | Lower phase | tail→head | $P_{II}$ | I (L) |

* Planetary motion: $P_I$:  ; $P_{II}$: 

** I = Inward elution; O = outward elution; L = left-handed; R = right-handed.

phase. During this filling process, stationary phase flowing from the outlet of the column can be recycled by adding it back to the reservoir, if desired. After completion of this procedure, sample solution was injected through the sample port. Then, the mobile phase was pumped into the coil in a proper elution mode at a flow-rate of 120 ml/h while the apparatus was rotated at the optimal revolutional speed. The effluent from the outlet of the column was continuously monitored with an LKB Uvicord S at 278 nm and then fractionated with an LKB fraction collector. After all peaks were eluted, the apparatus was stopped, and the column contents were pumped out by pressured nitrogen as described earlier. An aliquot of fractions was mixed with methanol and absorbance was determined with a Zeiss Model PM6 spectrophotometer: DNP-amino acids were analyzed at 430 nm and dipeptides at 280 nm as in the preliminary studies.

## RESULTS AND DISCUSSION

### *Retention of stationary phase and partition efficiency in short coils*

Fig. 2 illustrates a set of phase retention diagrams obtained from chloroform–acetic acid–0.1 *M* hydrochloric acid (2:2:1) (top) and *n*-butanol–acetic acid–water (4:1:5) (bottom) each with the upper (left) and the lower (right) phases used as the mobile phase. In each diagram, percent retention of the stationary phase relative to the total column capacity is plotted against the applied revolutional speed from 0 to 300 rpm. The data obtained from three different experimental conditions —the central coil position, and the lateral coil position with planetary motions $P_I$ and $P_{II}$ — are drawn with different symbols as indicated in the figure. The retention of over 50% is

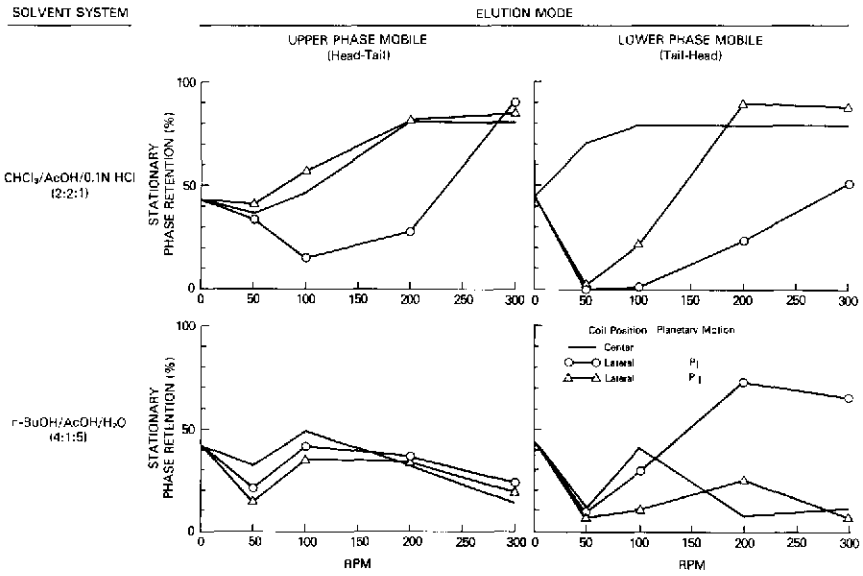



Fig. 2. Retention of stationary phase in short coils in the central and lateral positions. AcOH = Acetic acid; n-BuOH = *n*-butanol.

considered satisfactory, but over 30% may also be used for separation, if carryover of the stationary phase is minimum.

At 0 rpm, all groups yield similar retention at 40 to 45% simply by the effect of gravity. At a slow rotation of 50 rpm, most of the retention curves drop appreciably, some approaching the 0% line. Further increase of the revolutional speed to 100–200 rpm, however, results in a sharp increase of retention in all groups. Increasing the revolution above 200 rpm produces contrasted results between the two solvent systems: The retention of the chloroform phase system either continues to rise or forms a plateau, whereas that of the *n*-butanol phase system tends to decline.

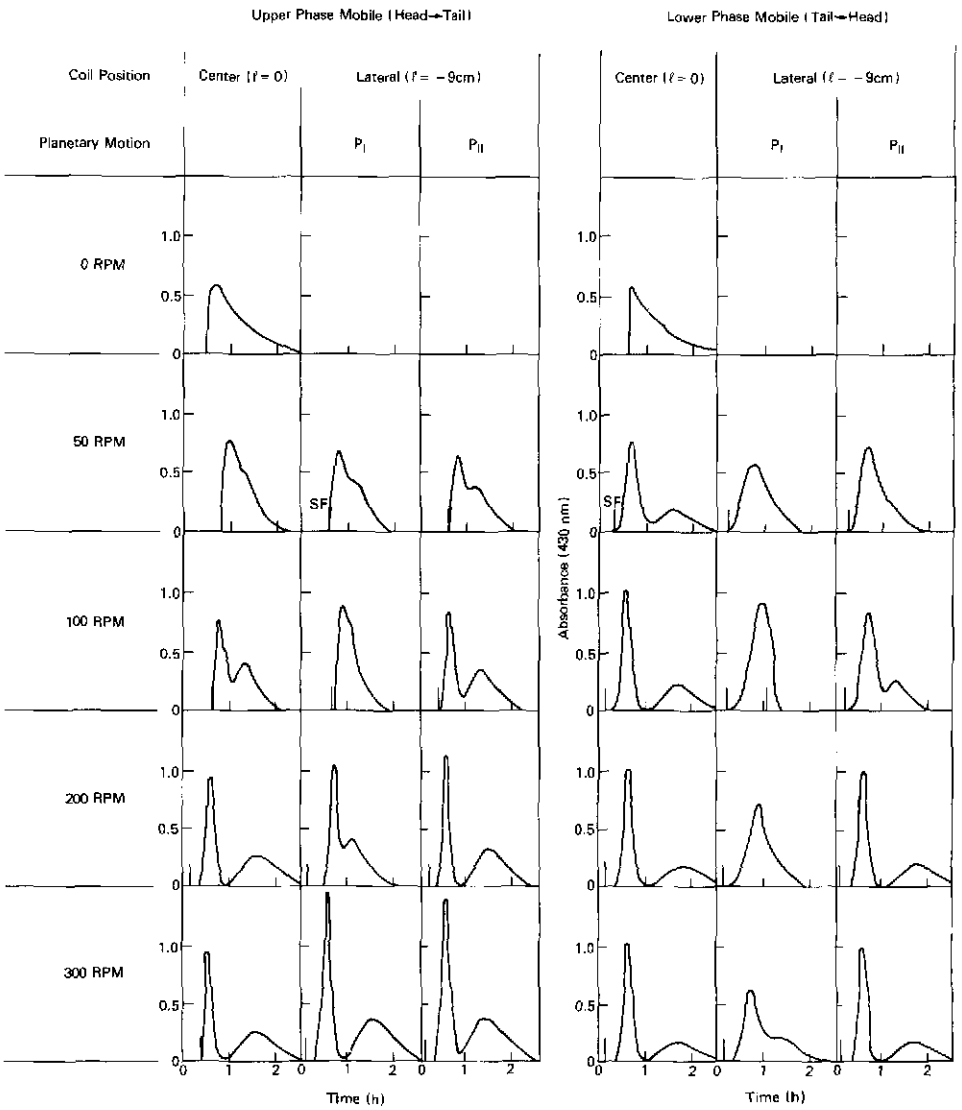



*Fig. 3. Effects of revolutional speed, column position and planetary motion on DNP-amino acid separation.*

The retention curves obtained from the central coil position are quite different from those obtained from the lateral coil position. Furthermore, retention curves obtained from the lateral coil position at the two planetary motions, $P_I$ and $P_{II}$, are mostly very different from each other: In the chloroform phase system, $P_{II}$ yields higher retention than $P_I$, whereas in the *n*-butanol phase system this relationship is reversed, $P_I$

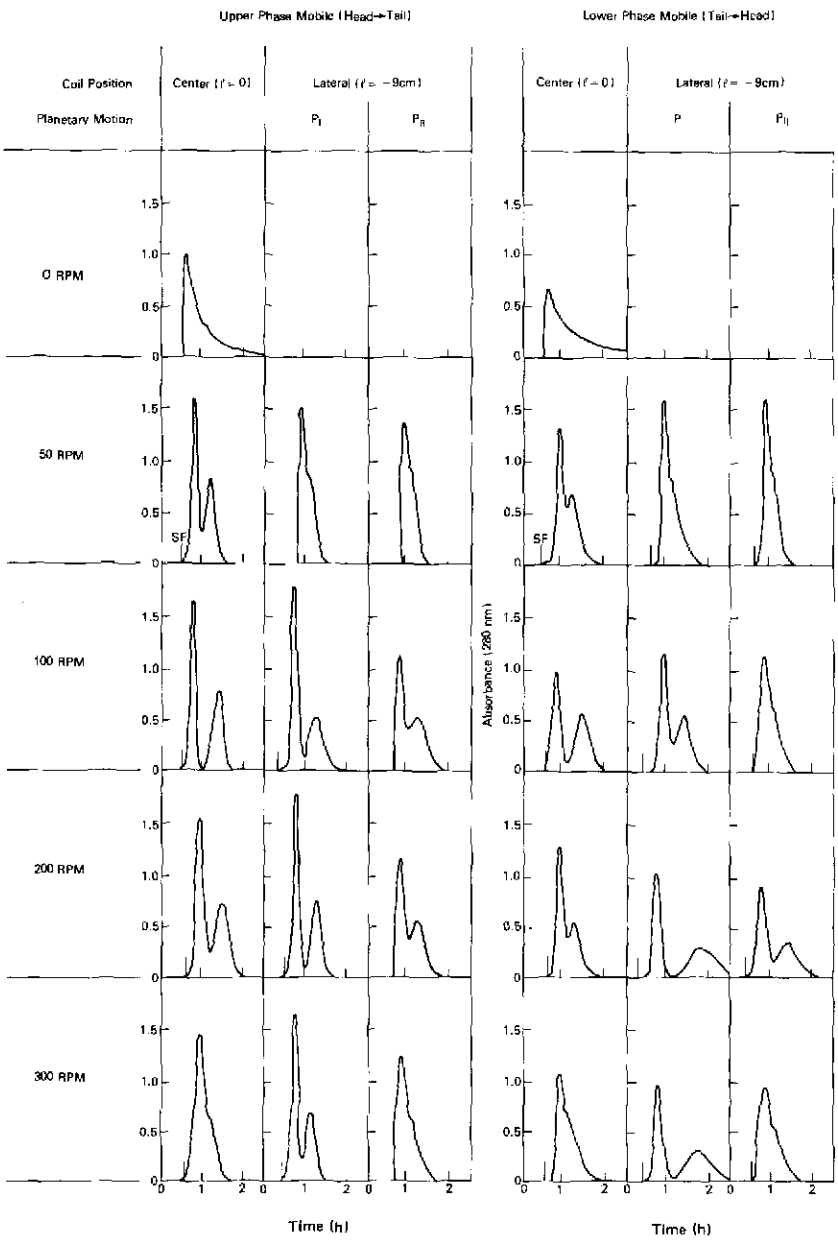



Fig. 4. Effects of revolutional speed, column position and planetary motion on dipeptide separation.

yielding better retention than $P_{II}$. Overall results indicated that satisfactory retention is available at relatively low revolutional speeds from 100 to 200 rpm in all groups.

Fig. 3 shows a set of chromatograms of DNP-amino acids obtained with a two-phase solvent system composed of chloroform–acetic acid–0.1 *M* hydrochloric

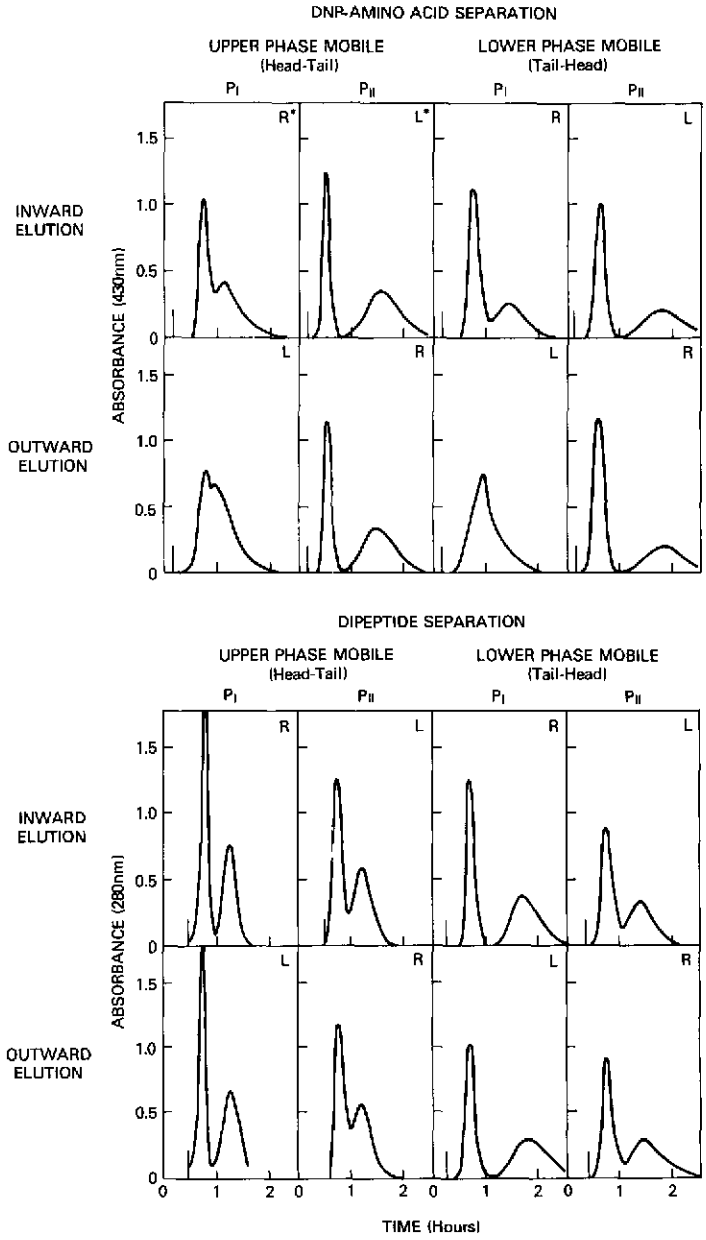



Fig. 5. Effects of inward or outward elution mode and planetary motion on partition efficiency.

acid (2:2:1) by using the upper (left panel) and the lower (right panel) phases as the mobile phase. These chromatograms are arranged according to the coil positions (top) and the applied revolutional speeds ranging from 0 to 300 rpm (left). Partition efficiency of each chromatogram is easily estimated from the degree of peak resolution by observing the height of the trough between the two peaks.

In the central coil position ($l = 0$ cm) (left column in each panel), the partition efficiency sharply increases with revolution up to 100–200 rpm where the two peaks are completely resolved. Further increase of revolutional speed to 300 rpm shows little improvement in peak resolution. In the lateral coil position ($l = -9$ cm), two planetary motions, $P_I$ and $P_{II}$, yield quite different results: $P_{II}$ produces high peak resolutions quite similar to those obtained from the central coil position, while $P_I$ gives much lower peak resolutions, especially when the lower phase is mobile (right panel).

Fig. 4 shows a set of chromatograms of dipeptides obtained with a two-phase solvent system composed of *n*-butanol–acetic acid–water (4:1:5). The chromatograms are arranged according to the format used in the foregoing DNP-amino acid separations. In the central coil position, partition efficiency rose with the increased revolution up to 100 rpm where the peak resolution becomes maximum. Further increase of revolutional speed results in decline of partition efficiency in both mobile phase groups. In the lateral coil position, planetary motion $P_I$ produces similar results, yielding the highest peak resolution at 200 rpm, while planetary motion $P_{II}$ gives much lower peak resolution.

Effect of the inward or outward elution mode on the partition efficiency was investigated with right-handed (R) and left-handed (L) coils mounted in the lateral position ($l = -9$ cm). Fig. 5 shows a set of chromatograms of the test samples obtained at the optimum revolutional speeds, *i.e.*, 200 rpm for DNP-amino acid separations (upper panel) and 100 rpm for dipeptide separations (lower panel). In both samples, chromatograms obtained from the inward and outward elution modes under the otherwise identical experimental conditions are quite similar to each other compared with those obtained from a given inward or outward elution mode at the two different planetary motions, $P_I$ and $P_{II}$. These results clearly indicate that the mode of planetary motion produces much more profound effects on partition efficiency than the inward/outward elution mode.

The fact that the inward-outward elution mode produces no significant effect on the partition efficiency gives an important implication for the use of multilayer coils in the lateral location on the holder. The multilayer coil used in CCC consists of multiple coiled layers in which running direction of the coil along the axis of the holder reverses in each neighbouring layers. For example, if the first layer runs outward from the right toward the left, then the second layer runs inward from the left toward the right, and so forth. Thus, under a given experimental condition, each neighbouring coiled layer is subjected to opposite inward/outward elution modes, while other conditions such as the head-tail elution mode and planetary motion remain the same. Consequently, the above results ensure the efficient use of the multilayer coils in the lateral position provided that other experimental conditions are properly selected.

*Preparative separations with paired multilayer coils*

Capability of the present CCC system was demonstated on multigram separations of test samples with a pair of long multilayer coils with a total capacity of about

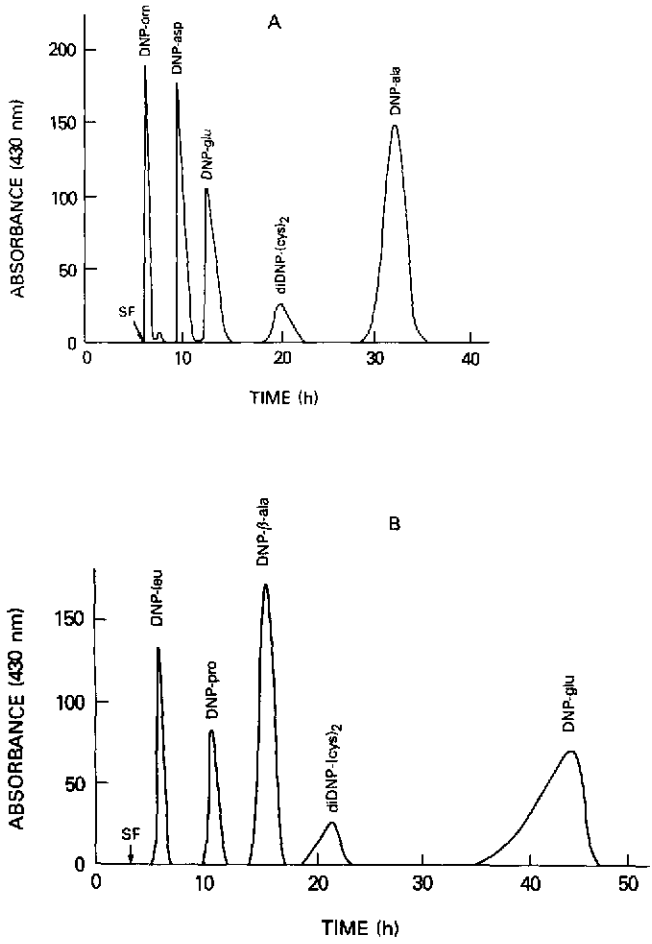



Fig. 6. Preparative separations of DNP-amino acids. Conditions: apparatus, X-axis CPC, 20 cm radius; column, paired multilayer coils, 5 mm I.D., 2.5 l capacity, $\beta$ = 0.25–0.6, central position; sample, DNP-amino acids, 10 g; solvent system, chloroform–acetic acid–0.1 *M* hydrochloric acid (2:2:1); mobile phase, upper aqueous phase (A) or lower non-aqueous phase (B); elution mode, head→tail (A) or tail→head (B); flow-rate, 120 ml/min; revolution, 200 rpm, $P_I$ (A) or $P_{II}$ (B); retention, 67% (A) or 84% (B). SF = Solvent front, $\beta$ = ratio of the coil radius to the radius of revolution.

2.5 l. The separations were performed under the optimal operational conditions determined in the preliminary model studies with short coils.

Fig. 6 shows chromatograms of DNP-amino acids with a solvent system composed of chloroform–acetic acid–0.1 *M* hydrochloric acid (2:2:1) using the upper (Fig. 6A) and the lower (Fig. 6B) phases as the mobile phase. In each separation, a total of 10 g quantity samples was completely resolved into discrete peaks in 35 to 50 h. Partition efficiencies computed according to the conventional gas chromatographic formula, $N = (4t_R/W)^2$ ($N$ denotes the partition efficiency expressed in terms of theoretical plate number, $t_R$ the retention time of the peak maximum and $W$ the peak width expressed in the same unit as $t_R$), range from 400 to 1000 theoretical plates.

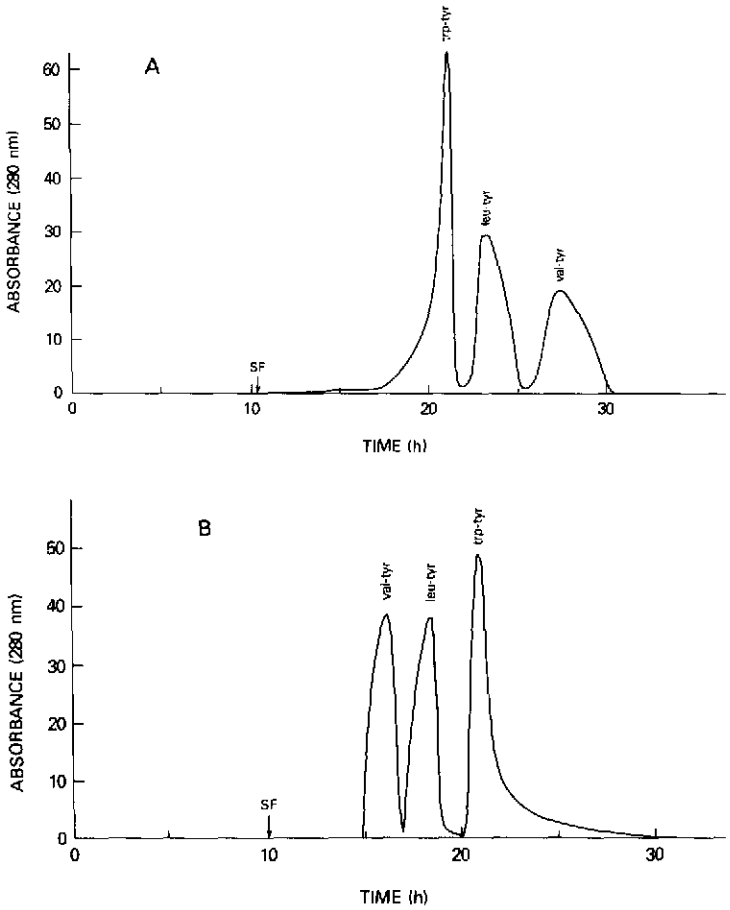



Fig. 7. Preparative separations of dipeptides. Conditions: apparatus, column and flow-rate as in Fig. 6; sample, dipeptides, 2.5 g; solvent system, *n*-butanol–acetic acid–water (4:1:5); mobile phase, upper non-aqueous phase (A) or lower aqueous phase (B); elution mode, head→tail (A) or tail→head (B), revolution, 100 rpm, $P_I$ (A) or $P_{II}$ (B); retention, 27% (A) or 33% (B).

Fig. 7 similarly illustrates chromatograms of dipeptides obtained with a two-phase solvent system composed of *n*-butanol–acetic acid–water (4:1:5) using the upper (Fig. 7A) and the lower (Fig. 7B) phases as the mobile phase. Non-linear isotherm of the peptides in the present solvent system produced skewed peaks, especially in trp-tyr, in both chromatograms. Nevertheless, all three components were fairly well resolved in 30 h.

The above results successfully demonstrate an excellent preparative capability of the present X-axis CPC equipped with 5 mm I.D. multilayer coils in the central coil position ($l = 0$ cm) at low revolutional speeds of 100–200 rpm. As indicated by the results obtained from the model studies with short coils, the lateral coil position ($l = -9$ cm) can also produce similar results at the same revolutional speed under the proper mode of planetary motion (see Figs. 3 and 4). This clearly indicates that the

column capacity, hence the sample loading capacity, of the present system can be increased in several folds simply by extending the width of the multilayer coils toward one side of the holder shaft. The results of present studies also strongly suggest that the use of large-bore coils would further decrease the optimal revolutional speed below 100 rpm, as in the case of the slowly rotating coil assembly reported earlier[3,4], thus facilitating the use of huge capacity multilayer coils for industrial-scale separations.

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