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NEW HORIZONTAL FLOW-THROUGH COIL PLANET CENTRIFUGE FOR COUNTER-CURRENT CHROMATOGRAPHY

II. THE APPARATUS AND ITS PARTITION CAPABILITIES

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SUMMARY

The new horizontal flow-through coil planet centrifuge allows continuous elution through a pair of coiled separation columns without the use of rotating seals. Design of the apparatus enables each column to undergo a specific mode of synchronous planetary motion which produces a characteristic pattern of the acceleration field as described in Part I. Using typical two-phase solvent systems, retention of the stationary phase and partition efficiency were studied for each column under various revolutional speeds and flow-rates. The results indicate that one column enables preparative-scale separations and the other, analytical-scale separations both with a high partition efficiency comparable to that obtained in refined liquid chromatography.

INTRODUCTION

The new scheme of the horizontal flow-through coil planet centrifuge has been introduced for performing counter-current chromatography. The apparatus is equipped with a pair of coiled separation columns, each having a specific mode of synchronous planetary motion. The rotating-seal-free flow-through mechanism of the scheme and the characteristic acceleration field produced by these planetary motions were described in Part I[1]. This paper deals with the design of the prototype and studies on the partition capabilities of the apparatus. Two elementary requirements for solute partitioning, *i.e.*, retention of the stationary phase and partition efficiency, were investigated with short coiled columns under various revolutional speeds and flow-rates.

APPARATUS

The prototype of the new horizontal flow-through coil planet centrifuge has been designed according to the principle described in Part I[1]. Fig. 1 shows the design of the apparatus on a cross-sectional view through the central axis of the apparatus. The motor (ElectroCraft, Model E650) drives the rotary frame through

a pair of toothed pulleys and a toothed belt around the stationary pipe mounted on the central axis of the centrifuge. The rotary frame consists of a pair of rotary wings rigidly bridged with links and holds a pair of column holders symmetrically located 15 cm from the central axis of the apparatus. In order to introduce the desired planetary motion to each column holder, a set, consisting of the stationary pulley and gear, is coaxially mounted around the stationary pipe. The stationary pulley is directly coupled with a toothed belt to an identical pulley mounted on the pulley-side column holder (bottom) to produce a counter-rotation. The stationary gear is first engaged to an identical idler gear mounted around the link through a bearing. The idler gear is coaxially connected to an idler toothed pulley which in turn is coupled with a toothed belt to an identical pulley mounted on the gear-side column holder. Application of this idler arrangement eliminates the necessity of mounting a large-diameter gear on the gear-side column holder which would produce a heavy load on the bearings under a strong centrifugal-force field. The extra weight of the idler is conveniently counterbalanced by mounting a metal block of the same weight on the link located in the symmetrical position on the rotary frame. To stabilize the centrifuge system, a short coupling pipe is mounted coaxially on the left end of the rotary frame and supported by the stationary member of the apparatus with a bearing as illustrated.

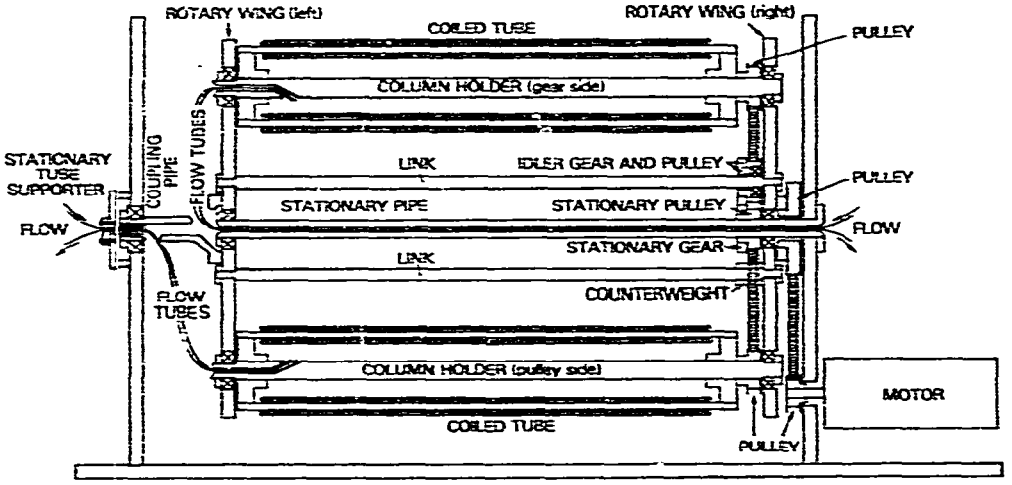

Fig. 1. Cross-sectional view of the apparatus.

The column is prepared by winding PTFE tubing (Zeus Industrial Products) onto a metal pipe to make a short column or column unit. The long column is made by connecting the desired number of column units, usually 10 units, in series in such a way that the tail end of the first column unit joins to the head end of the second column unit, the tail end of the second to the head end of the third, and so forth. The interconnections are made with standard PTFE tubing connectors and adaptors. The small-bore column for micro-scale separations can be prepared from one piece of tubing without interconnection between the column units. The typical column for large-scale separation is made of PTFE tubing, 2.6 mm I.D. and 0.5 mm wall

thickness wound around stainless-steel tubing with 1.25 cm O.D. and 1 mm wall thickness. The column for micro-scale separations is prepared from PTFE tubing, 0.55 mm I.D. and 0.3 mm wall thickness, coiled onto stainless-steel tubing with 0.68 cm O.D. and 1 mm wall thickness. The columns are symmetrically arranged around each column holder and tightly fixed with screws through a hole made at each end of the column unit core. When two different columns are mounted on the holders, the counter-weight should be added on the holder carrying the lighter column. Each column holder is equipped with two different levels to mount columns, one at 3.5 cm ($\beta^* = 0.23$) from the axis of the holder for large-bore columns and the other at 2.0 cm ($\beta = 0.13$) for small-bore columns.

The flow tubes from the column mounted on the pulley-side holder are first passed through the center hole of the holder and then led through a side hole of the coupling pipe to reach the stationary tube supporter extending from the stationary member of the centrifuge. On the gear-side column holder, flow tubes are similarly passed through the center hole of the holder and led through another side hole of the coupling pipe to enter the opening of the stationary pipe on the central axis of the centrifuge. These flow tubes are lubricated with silicone grease and protected by a piece of flexible plastic tubing at each supported portion to prevent direct contact against metal parts. If this protection is carefully followed, these tubes can maintain their function almost permanently.

Revolutional speed of the apparatus is continuously adjusted for 0 to 600 rpm with high stability and accuracy with a motor control unit (Motomatic Model E650M). The software required for the present apparatus including the elution pump, monitor and fraction collector can be chosen from those used for the conventional liquid chromatographic system. In the following studies, a Chromatronix Cheminert pump (Model 2) was employed for elution and LKB Uvicord III for monitoring the absorbance at 280 nm.

STUDIES ON RETENTION OF STATIONARY PHASE

Retention of the stationary phase in the coiled column is affected by various factors such as physical properties of the two-phase solvent system, column geometry, applied centrifugal-force field, and flow-rate of the mobile phase. Because of the complex hydrodynamic behavior of the two phases in the column, retention of the stationary phase under a given set of conditions is best determined by actual experimentation. While a great variety of two-phase solvent systems are available, those useful for practical separations can be classified into relatively few groups according to their common physical properties. Therefore, investigation on a typical phase system from each group will furnish useful data which can be applied to other phase systems within the same group. Among various physical properties of the solvent system, the interfacial tension plays the most critical role in retention of the stationary phase. For example, the combination of the use of a small-bore column and a high interfacial tension phase system tends to produce a plug flow.

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* As described in Part I, $\beta = r/R$ where r and R denote the radii of rotation and revolution of the point on the holder respectively. The value determines the pattern of the acceleration field acting on the gear-side column holder.

A combination of a large-bore column and a low interfacial tension phase system favors emulsification of the two phases. Both cases result in carry-over of the stationary phase. For successful separation, these complications must be eliminated by applying the optimum operational conditions predetermined by a series of preliminary tests.

In the present studies a set of typical two-phase solvent systems with a broad range of interfacial tension is selected to determine retention of the stationary phase in the column on each column holder under various revolutional speeds and flow-rates.

Method for determination of retention percentage. Two methods are employed. In both methods, the degree of stationary phase retention is expressed as percentage of the stationary phase volume retained in the column relative to the total capacity of the column.

In the first method, the column is filled with the stationary phase followed by pumping with the mobile phase under a given operational condition until the mobile phase appears through the outlet of the column. The eluate is collected into a graduated cylinder to measure the volume, V_s, of the stationary phase eluted through the column. The total column capacity, V_c, and the dead space volume in the flow tubes, V_f, are predetermined. Using these figures, the retention percentage is given by $100\ (V_c + V_f - V_s) / V_c$. In this method any amount of the mobile phase left in the space in the column and flow tubes would become a source of error in the measurement. Therefore, the contents of the column and the flow tubes should be completely replaced by the stationary phase before each experiment. This can be done by introducing a small amount of a solvent which is miscible with both phases before pumping the stationary phase into the column. This method is suitable for determination of retention percentage in small-bore columns.

The second method uses a colored indicator to observe the behavior of the stationary phase in a running column under stroboscopic illumination. The two-phase system is first equilibrated with a dye which is almost entirely partitioned to the stationary phase. In general, basic fuchsin or Sudan black B is useful for coloring non-aqueous phase and acid fuchsin, for the aqueous phase. The amount of these dyes should be minimized so that the color is just dense enough to observe the stationary phase while the interfacial tension of the solvent is not significantly altered. The coiled column is marked with a felt tip pen at every 10 turns to ease measurement by stroboscopic observation. The column is then entirely filled with the mobile phase followed by injection of the stationary phase of a known amount which occupies "A" turns of the coil. Then, the column is eluted with the mobile phase at a given flow-rate while the apparatus is spun at the desired revolutional speed. Elution of the mobile phase soon establishes a hydrodynamic equilibrium state of the two phases in the coiled column which is observed through the wall of the column by stroboscopic illumination. At this equilibrium state, the number of helical turns "B" containing the colored stationary phase is obtained. The retention percentage is given by $A/B \times 100$. The measurement can be repeated by changing the operational conditions to obtain a set of data until carry-over of the stationary phase occurs without replacing the column contents. This method also provides important information for the hydrodynamic behavior of the two-phase solvent system in the running column and is particularly suitable for large-bore columns.

Retention percentage for large-bore column. Using the second method described above, a series of experiments have been performed to measure retention percentage in a short coiled column prepared from 2.6 mm I.D. tubing (core: 1.25 cm O.D.) and consisting of 100 coil units with a total capacity of about 26 ml. The column was mounted on each holder at a location 3.5 cm from its axis or at $\beta = 0.23$. Several two-phase solvent systems were chosen for the present study as follows:

(1) *n*-butanol–acetic acid–water (4:1:5): low interfacial tension and high viscosity;

(2) chloroform–acetic acid–water (2:2:1): low interfacial tension and low viscosity;

(3) ethyl acetate–acetic acid–5% sodium chloride aqueous solution (10:11:9): medium interfacial tension;

(4) hexane–water: high interfacial tension.

Overall results of the experiments obtained with these two-phase solvent systems are summarized in Fig. 2. In each diagram retention percentage of the stationary phase is plotted against the revolutional speed in rpm. Several curves drawn in each diagram indicate the effect of flow-rates applied. The elution of the mobile phase is usually directed from the head to the tail end of the column but for the gear-side column the elution is also reversely applied from the tail to the head end of the coil. In general, retention of over 30% is considered to be satisfactory and that near 50% is ideal. In addition to the net amount of retention, the inclination of the curves should also be considered. When an operational condition is chosen at a portion of the curve showing a steep inclination, a slight shift of the revolutional speed will produce a considerable change in retention percentage. Therefore, for the reproducible results, it is desirable to select the operational conditions at or near horizontal portion of the curve.

Retention curves obtained from the pulley-side column display a great variety in shape according to interfacial tension and viscosity of the two-phase solvent systems. For a low interfacial tension, high viscosity *n*-butanol system, retention declined sharply with the revolutional speed where stroboscopic observation revealed intensive emulsification of the phases in the coiled column. For other phase systems, retention curves follow a characteristic pattern with the rate of revolution. At a low revolutional speed around 200 rpm, the curves of non-aqueous stationary phases exhibit a sharp rise which becomes more pronounced in the solvent systems with higher interfacial tension. After this critical range of rpm, the retention becomes stable but the retention levels for the non-aqueous phase always exceed those for the aqueous phase in the same solvent system. These complex patterns of retention may be related to the various physical factors of the solvent system such as solvent-wall interaction, strong molecular cohesive forces and higher viscosity of the aqueous phase, etc. as discussed elsewhere[2].

On the other hand, the retention curves for the gear-side column follow a quite different pattern. In all solvent systems the curves smoothly approach the stable levels at a relatively low rpm and then display a long plateau up to the maximum revolutional speed of 600 rpm. These retention levels at the plateau are mostly within an ideal range for both non-aqueous and aqueous stationary phases. Furthermore, the reversed elution mode of the tail to head elution also gives similar retention levels where the same procedure for the pulley-side column results in

COLUMN HOLDER GEAR
Stationary Phase
NON-AQUEOUS PHASE
AQUEOUS PHASE
COLUMN HOLDER PULLEY
Stationary Phase
NON-AQUEOUS PHASE
AQUEOUS PHASE
TWO-PHASE SOLVENT SYSTEM
n-BuOH
CH3COOH
H2O
4 1 5
CHCl3
CH3COOH
H2O
2 2 1
HEAD - TAIL ELUTION
TAIL - HEAD ELUTION
RETENTION (%)
RPM

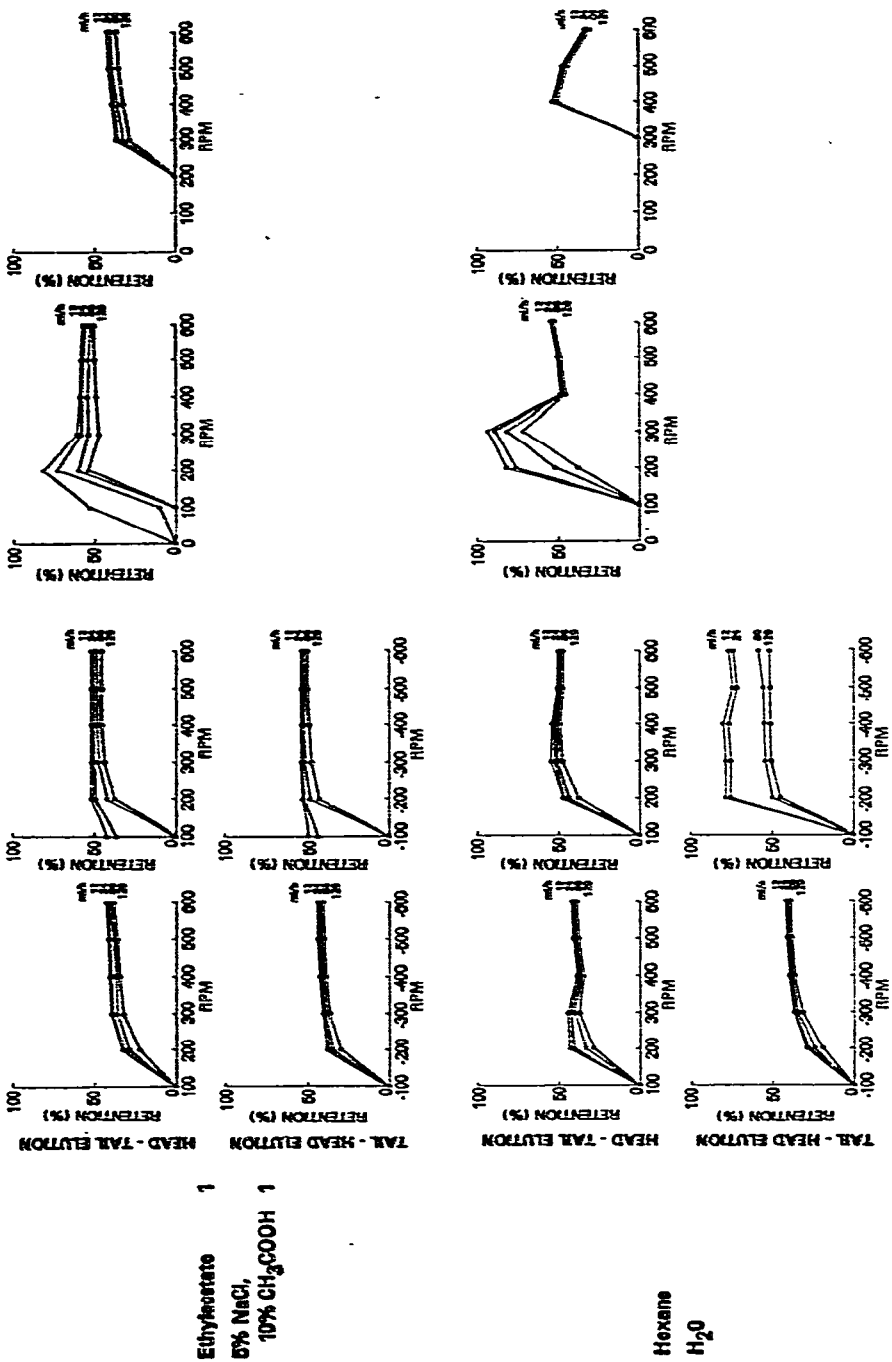

Fig. 2. Effects of revolutional speed and flow-rate on retention of the stationary phase in a large-bore column. Column: 2.6 mm I.D.

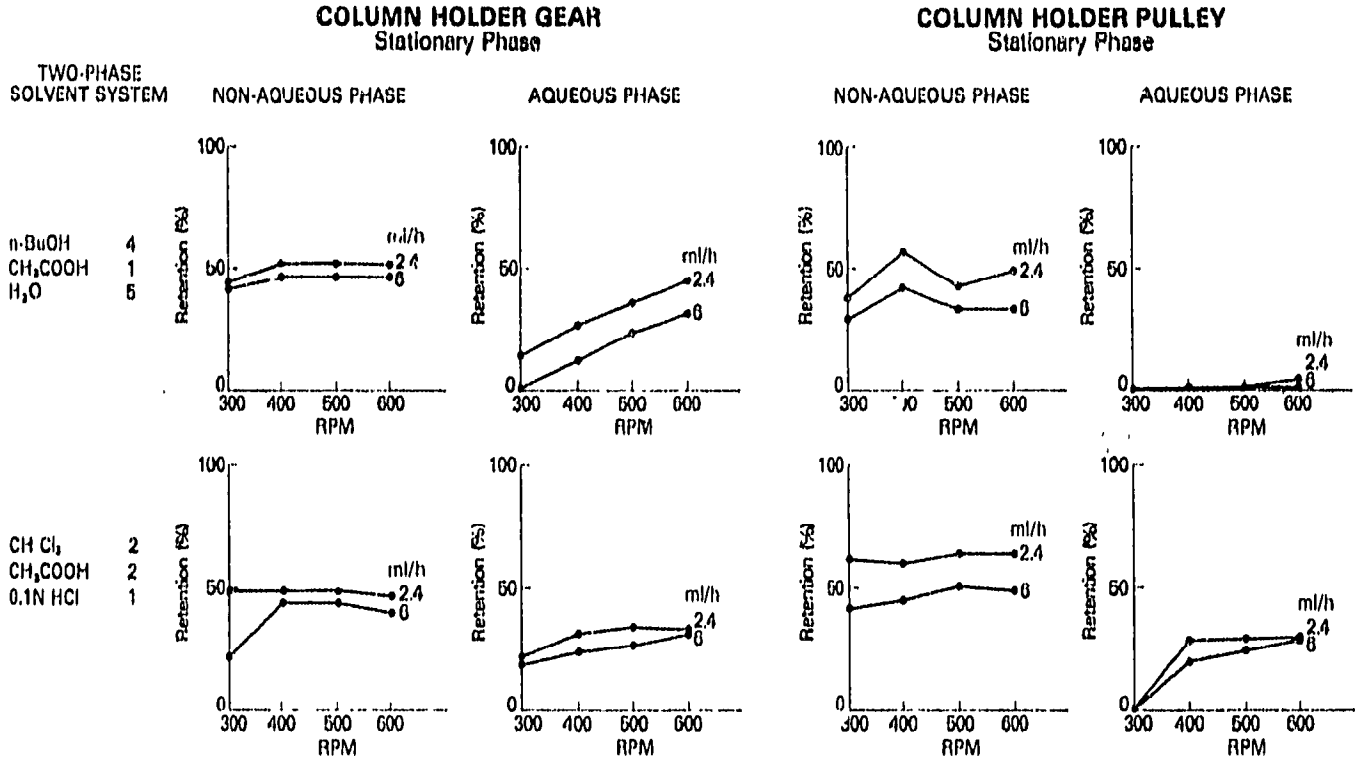

Fig. 3. Effects of revolutional speed and flow-rate on retention of the stationary phase in a small-bore column. Column: 0.55 mm I.D.

complete loss of the stationary phase above a relatively low rpm. Stroboscopic observation of the gear-side column reveals complete separation of the two phases in each turn of the coil with undulating interfaces swinging back and forth during each cycle of revolution. This finding is consistent with the results of analysis on the acceleration field described earlier[1]. Overall results indicate that for the use of a large-bore column the gear-side holder has greater advantages over the pulley-side holder in that satisfactory retention is available for a broad spectrum of solvent systems under a wide range of revolutional speeds and flow-rates.

Retention percentage for small-bore column. Retention of the stationary phase in small-bore coiled columns under a uniformly circulating acceleration field has been rather extensively studied with the flow-through coil planet centrifuge at a revolutional radius of 30 cm. In those previous studies, coiled columns with internal diameters of 1.2 mm, 0.85 mm, 0.55 mm, and 0.38 mm were tested for a variety of two-phase solvent systems[2]. The results indicated that the columns with over 0.55 mm I.D. gave a fair amount of retention in most of the solvent systems.

In the present studies, the 0.55 mm I.D. coiled column was tested for retention using two typical phase systems composed of chloroform–acetic acid–0.1 *N* hydrochloric acid (2:2:1) and *n*-butanol–acetic acid–water (4:1:5). The column was prepared by winding 0.55 mm I.D. PTFE tubing onto a stainless-steel pipe with 0.68 cm O.D. to make approximately 340 helical turns with a total capacity of about 2.4 ml. The column was mounted on each holder at a location 2 cm from its axis or at $\beta = 0.13$. The retention percentage was obtained by the first method previously described.

The results of the experiments are summarized in Fig. 3. In each diagram, retention percentage of the stationary phase is plotted against the applied revolutional speeds in rpm. Two curves drawn in each diagram indicate the data obtained under the flow-rates of 6 ml/h and 2.4 ml/h.

The overall results reveal characteristic features of the gear-side and pulley-side columns observed in the previous studies using the large-bore column. Compared with the previous data, the retention of the aqueous phases is much less than that of the non-aqueous phases especially in pulley-side column. This result suggests that in the small-bore column the effects of solvent-wall interaction on retention becomes more significant and the retention strongly favors the non-aqueous phase which has an affinity to the PTFE tube. In the *n*-butanol phase group, the pulley-side provides a satisfactory retention for the non-aqueous phases while retention for the aqueous phase requires a greater centrifugal-force field to reach a suitable level.

STUDIES ON PARTITION EFFICIENCY

When the coiled column enables retention of the stationary phase, elution of the mobile phase results in separation of the solutes according to their relative partition coefficients. However, the resolution of the solute peaks is affected by various factors such as geometry of the column, physical properties of the two phases, acting centrifugal-force field, and applied flow-rate. In order to achieve an efficient separation, the operational condition should provide broad interfacial area and/or efficient mixing of the two phases to minimize mass transfer resistance especially for viscous phase systems. On the other hand, excessive mixing of less viscous phase systems may

cause undesirable sample band spreading along the length of the coil to lower the peak resolution. Under a set of other operational conditions, revolutional speed determines the degree of mixing and therefore can be used as a parameter to optimize the operational conditions.

In the present studies, two typical phase systems were selected for separation of samples with suitable partition coefficients, *i.e.*, chloroform–acetic acid–0.1 *N* hydrochloric acid (2:2:1) for separation of 2,4-dinitrophenyl (DNP)-DL-glutamic acid and DNP-L-alanine, and *n*-butanol–acetic acid–water (4:1:5) for separation of L-valyl-L-tyrosine and L-tryptophanyl-L-tyrosine. The DNP-amino acid sample mixture was prepared by dissolving each component at 0.5 g% in the stationary phase, 0.2 ml of the solution being applied for the large-bore column and 0.01 ml for the small-bore column. The peptide mixture was similarly prepared to obtain final concentrations of 1.0 g% for L-valyl-L-tyrosine and 0.3 g% for L-tryptophanyl-L-tyrosine, 0.2 ml of this solution being applied for the large-bore column and 0.05 ml for the small-bore column.

In each separation, the column was first filled with the stationary phase followed by sample injection through the sample port. Then the mobile phase was eluted at a given flow-rate while the apparatus was run at a desired revolutional speed. The eluate was continuously monitored through an LKB Uvicord III at 280 nm. From the resulted solute peaks recorded on the chart, the partition efficiency was obtained either by observing the relative peak resolution or in terms of theoretical plates (N) according to the conventional equation

$$N = (4R/w)^2 \tag{1}$$

where R and w denote the retention time and peak width respectively.

Partition efficiency of large-bore column. The column employed was identical to the large-bore column used for retention studies and consisted of 100 helical turns of 2.6 mm I.D. PTFE tubing coiled onto a 1.25 cm O.D. pipe with a total capacity of about 26 ml. The column was mounted on each column holder 3.5 cm from its axis or at $\beta = 0.23$, if not otherwise indicated.

Fig. 4 shows the results of DNP-amino acid separation on the chloroform–acetic acid–0.1 *N* hydrochloric acid (2:2:1) phase system obtained from both gear-side and pulley-side columns at a fixed flow-rate of 60 ml/h under various revolutional speeds ranging from 0 to 600 rpm. Each diagram indicates one experiment where the partition efficiency is estimated from resolution of the two peaks by measuring the relative height of the trough between the peaks.

The efficiency obtained from the gear-side column sharply increases with the revolutional speed up to 400 rpm. The peak resolution obtained by the head-tail elution mode is always better than that by the tail-head elution mode at a given rpm. The pulley-side column gives quite different results. The efficiency becomes maximum at relatively slow revolutional speeds of around 100 to 200 rpm where further increase of the revolutional speed results in a sharp decrease of the peak resolution. Also, in the pulley-side column, the results obtained by the stationary non-aqueous phase are much better than those obtained by the stationary aqueous phase, while the choice of the stationary phase makes little difference in resolution for the gear-side column. These results clearly indicate that the gear-side column is

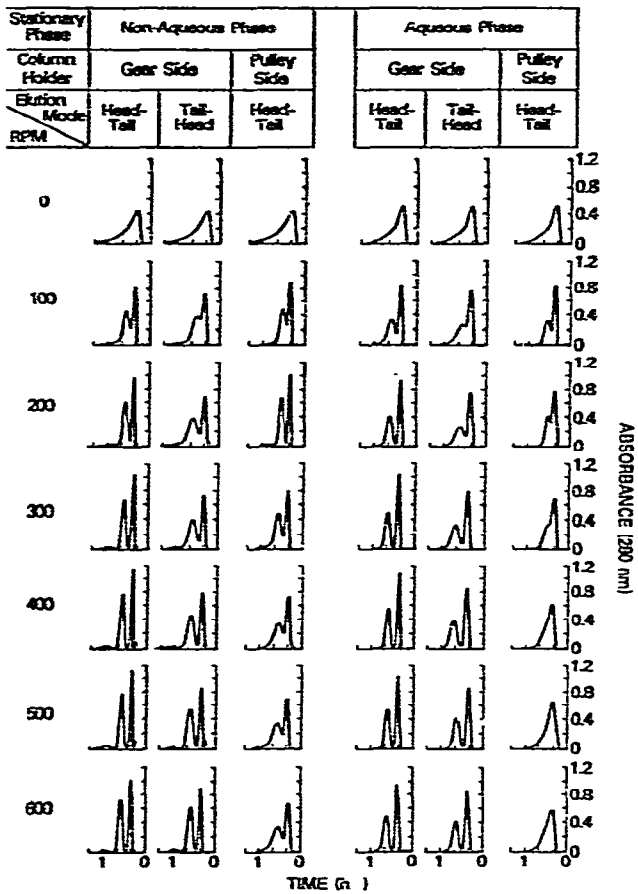

Fig. 4. Partition efficiency of large-bore column. DNP-amino acid separation with the gear-side and pulley-side columns.

much superior to the pulley-side column when a large-bore coiled tube is used.

Fig. 5 similarly shows effects of flow-rate and revolutional speed on DNP-amino acid separation with the same solvent system using the gear-side column. The data show that the best peak resolution is given by combination of revolutional speed and flow-rate. At a slow flow-rate of 24 ml/h, the highest resolution is found at 400 rpm for the non-aqueous stationary phase and at 200 rpm for the aqueous stationary phase. When the flow-rate of 120 ml/h is applied, the best results are given at around 500 rpm regardless of the choice of the stationary phase. The partition efficiency may also be expressed in terms of theoretical plates using eqn. 1. In this case the highest efficiency is given under the slow flow-rate of 24 ml/h while the time required to yield one theoretical plate is minimized at the highest flow-rate of 120 ml/h.

Fig. 6 shows the results of peptide separation with the *n*-butanol–acetic acid–water (4:1:5) phase system using the gear-side column. Overall results are found to resemble those obtained by DNP-amino acid separation with the chloroform phase

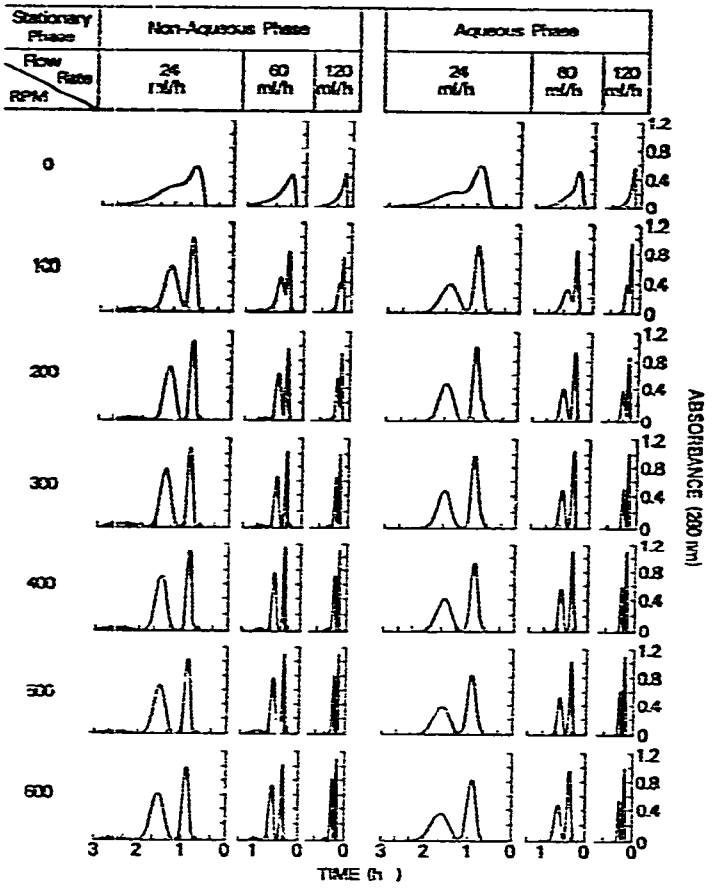

Fig. 5. Partition efficiency of large-bore column. DNP-amino acid separation with the gear-side column; effects of revolutional speed and flow-rate.

system. The partition efficiency increases with the revolutional speed up to 500 to 600 rpm in all groups. The inferior peak resolution at the highest flow-rate of 120 ml/h may be explained on the basis of high viscosity of the non-aqueous phase which increases mass transfer resistance.

Further experiments were performed to examine the effects of β values or the location of the column on the holder on separation of the same peptides with the *n*-butanol phase system. For this test the same column was mounted on the gear-side column holder 6 cm away from its axis or at $\beta = 0.40$ by applying a pair of extention blocks. The results of the experiments obtained by this modification are summarized in Fig. 7 where the separations obtained at $\beta = 0.40$ under 60 ml/h flow-rate are compared with those previously obtained at $\beta = 0.23$ under the otherwise identical conditions. These data clearly indicate that the column located on the holder at $\beta = 0.23$ yields a higher partition efficiency than that at $\beta = 0.40$ throughout all experimental conditions applied. This finding agrees with the results previously obtained in analysis of the acceleration field in that mixing of the two phases in the

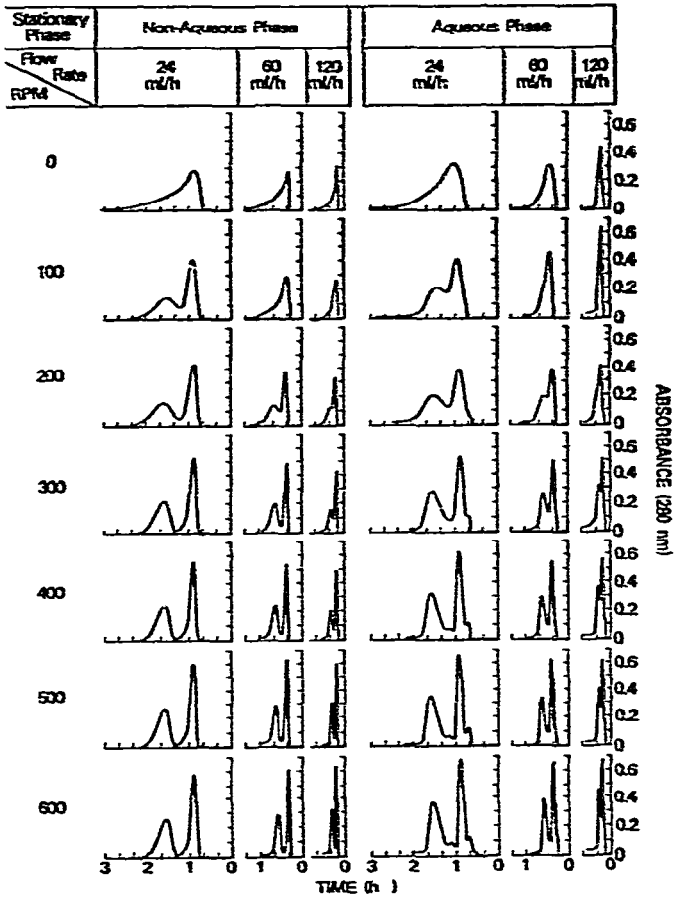

Fig. 6. Partition efficiency of large-bore column. Peptide separation with the gear-side column; effects of revolutional speed and flow-rate.

coiled column becomes less efficient on the gear-side holder as the β value increases. However, the advantages of applying large β values for the gear-side column should not be neglected for the application of polymer phase systems which have a great potential in partition of cells and macromolecules under mild environments[3]. Because of the aqueous–aqueous phase composition the polymer phase systems possess an extremely low interfacial tension; they tend to be emulsified under violently undulating centrifugal-force fields. With the use of large β values, satisfactory retention of the polymer phase systems may be feasible.

Here, it should be noted that the large-bore column mounted on the gear-side holder can retain a satisfactory amount of stationary phase, when the apparatus is rotated slowly. In this case the gravitational acceleration field establishes the hydrodynamic equilibrium state of the two phases in the coiled tube. Previous experiments have shown that the optimum revolutional speeds for such gravitational separations are 25 rpm for DNP-amino acids and 7.5 rpm for peptides using the respective two-phase solvent systems applied in the present studies[4,5]. Although peak resolutions obtained under the slow revolutional speeds are quite satisfactory, similar or even

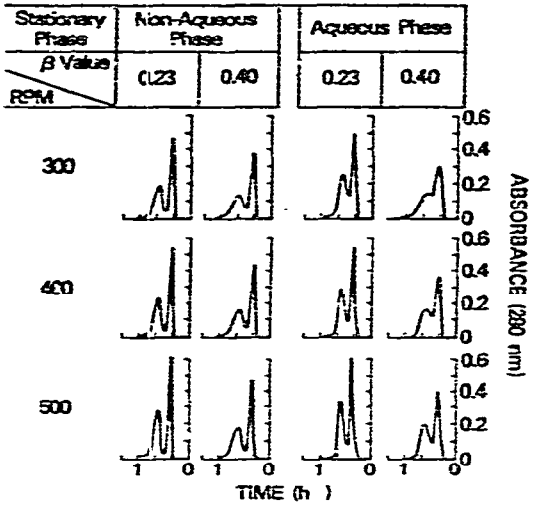

Fig. 7. Partition efficiency of large-bore column. Peptide separation with the gear-side column; effects of β values.

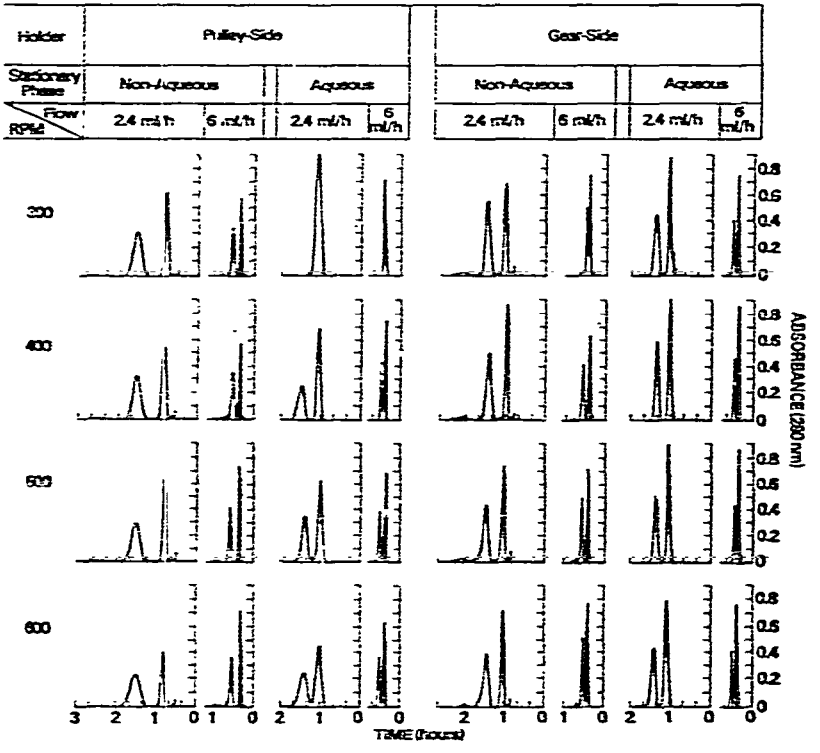

Fig. 8. Partition efficiency of small-bore column. DNP-amino acid separation; effects of revolutional speed and flow-rate. Column: 0.55 mm I.D.

superior results can be attained under faster revolutional speeds within much shorter elution times.

Partition efficiency of small-bore column. The small-bore column used in these studies is identical to that used in the retention studies. The column consisted of about 340 helical turns of a 0.55 mm I.D. PTFE tube coiled on 0.68 cm O.D. stainless-steel tubing with a total capacity of about 2.4 ml. The column was mounted on each column holder at a location of 2 cm from its axis or at $\beta = 0.13$.

Fig. 8 summarizes the results obtained by separation of the two DNP-amino acids on the chloroform phase system using combinations of various revolutional speeds and flow-rates. Overall results indicate that the pulley-side column yields more efficient separation especially when the non-aqueous phase is used as the stationary phase. Fig. 9 shows similar data obtained on separation of the two peptides on the *n*-butanol phase system. As expected from the results of the retention studies, no separation was observed in the pulley-side column when the aqueous phase was used as the stationary phase. Although the pulley-side yields slightly higher resolution at 2.4 ml/h of flow-rate with the stationary non-aqueous phase, the gear-side column gives more uniform results and permits the choice of the stationary phase.

COUNTER-CURRENT CHROMATOGRAPHY WITH LONG COILED COLUMNS

The foregoing studies on retention and partition efficiency with a single column unit provide optimum operational conditions which can be directly applied to a separation in a long column consisting of a desired number of the identical column units connected in series. In order to obtain successful separation, however, some precautions should be taken to avoid unnecessary sample band broadening which tends to occur in a long preparative column.

Theoretically, the partition efficiency should increase linearly with the length of the coiled column. However, our experiments have shown that the partition efficiency obtained with a long column is sometimes significantly lower than that estimated from the results obtained with the short column especially for a large-bore column. The loss of efficiency may be largely attributed to high pressure built up in the column which causes considerable expansion of the column and occasional surging of the solvent upon a slight shift of the revolutional speed. This detrimental effect, however, can be minimized by applying a narrow-bore tubing at each junction between the column units. A small-bore column has a relatively strong resistance against expansion and a very long column can be prepared from one piece of tubing without risk of losing peak resolution.

The choice of the solvent phases for preparation of the sample solution is important when a large sample volume is to be applied. If the sample contains solutes with high partition coefficients which favor the partition to the mobile phase, the sample should be dissolved in the stationary phase. In this way the mobile phase eluted through the column enables concentration of the solutes into sharp sample bands. Although other solutes with lower partition coefficients present in the sample solution may initially form broader sample bands, they are subjected to a longer partition process or greater dilution in the column and, therefore, the initial band width becomes less significant for the peak resolution.

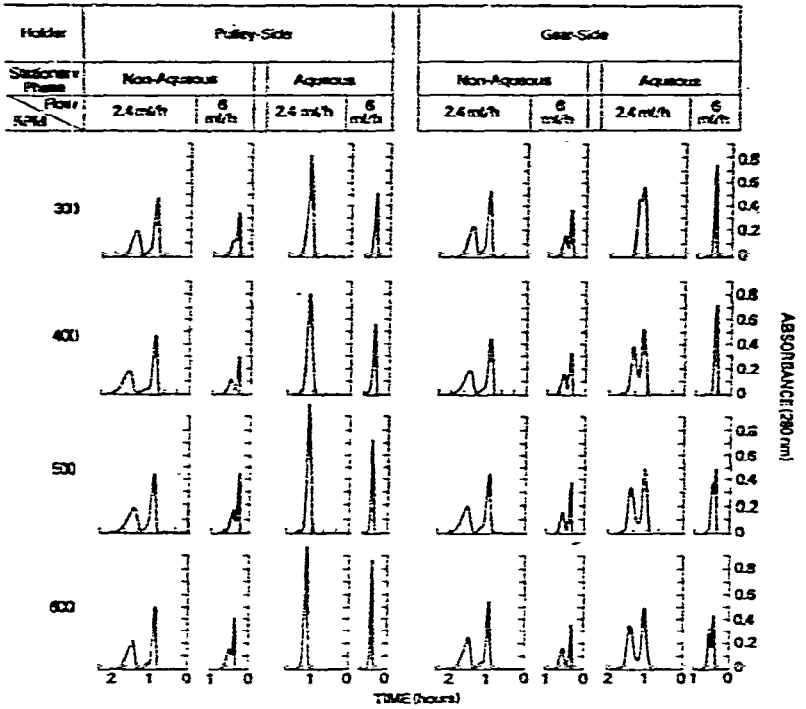

Fig. 9. Partition efficiency of small-bore columns. Peptide separation; effects of revolutional speed and flow-rate. Column. 0.55 mm I.D.

Taking these precautions, preparative-scale separations were performed with a large-bore column which is equivalent to 10 column units applied in the previous studies. The column was prepared by connecting the 10 column units in series with a piece of 10 cm long, 0.4 mm I.D. PTFE tubing at each junction between the column units. The column was arranged symmetrically around the gear-side column holder 3.5 cm away from its axis or $\beta = 0.23$. The two-phase solvent systems and samples were those used in the foregoing studies on partition efficiency. In each experiment the column was first filled with the stationary phase followed by sample injection through the sample port. Then the mobile phase was eluted at the indicated flow-rate while the apparatus was run at the optimum revolutional speed determined by the previous experiments. The eluate from the column was continuously monitored with an LKB Uvicord III at 280 nm through a 1.8 mm light path flow cell.

Fig. 10 shows chromatograms obtained with a long large-bore column on the gear-side holder using the samples and two-phase solvent systems indicated on the chart. In both DNP-amino acid and peptide separations, impurities originally present in the sample solutions were well separated out from the major peaks. The partition efficiencies calculated from these chromatograms according to eqn. 1 range between 950 and 1950 theoretical plates, showing an efficiency loss of 10 to 20% compared with the figures estimated from the results obtained with the single column unit. In these separations, the sample volume can be increased by diluting with the stationary phase up to 10 ml without significant loss of peak resolutions.

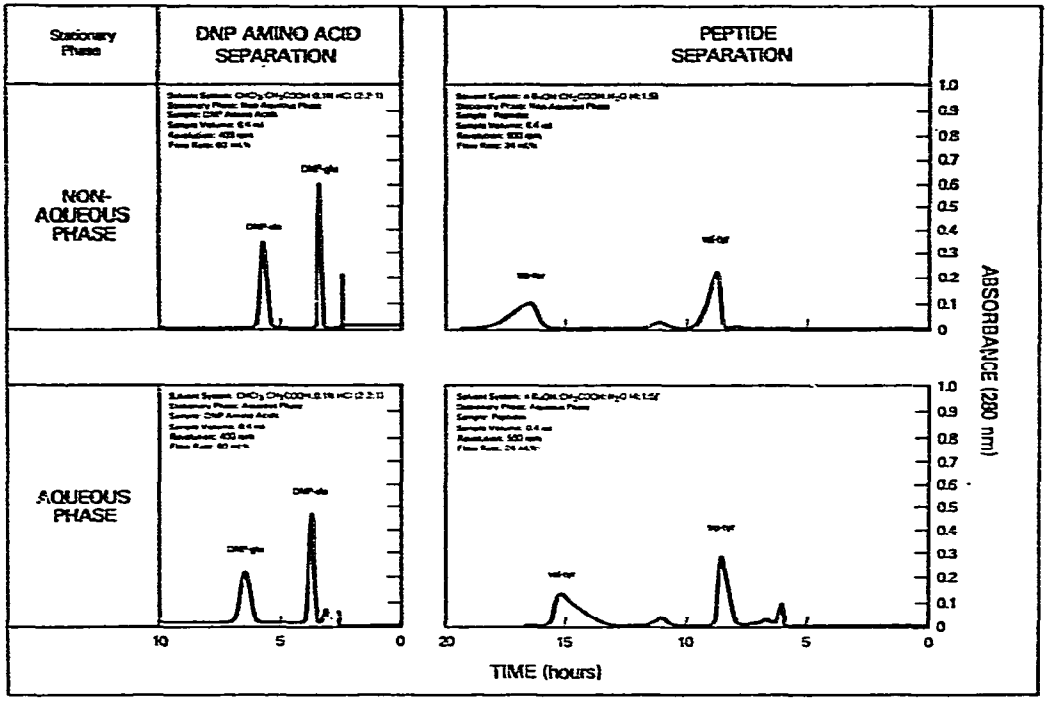

Fig. 10. Counter-current chromatograms with a long large-bore column.

CONCLUSION

The new horizontal flow-through coil planet centrifuge was successfully designed to allow continuous elution through a pair of coiled separation columns without the use of rotating seals. Each column undergoes the desired synchronous planetary motion to provide specific pattern of the acceleration field for performing counter-current chromatography in preparative or analytical scales. Investigation on retention of the stationary phase and partition efficiency with short columns indicated that a highly efficient separation can be attained in a wide range of revolutional speeds and flow-rates. These optimum operational conditions thus determined can be applied to long coiled columns to obtain the desired number of theoretical plates according to the relative partition coefficients of the aimed materials. Separations of various DNP-amino acids and peptides with the present apparatus will be presented in Part III.

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