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PREPARATIVE COUNTER-CURRENT CHROMATOGRAPHY WITH A ROTATING COIL ASSEMBLY

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SUMMARY

We have designed a simple bench top model of a counter-current chromatograph which performs efficient preparative separations without the use of solid supports. The stationary phase is retained by gravity in a large diameter coil which rotates to promote efficient mixing of the two phases. Continuous elution of the mobile phase is accomplished without the use of rotating seals. We demonstrated the efficiency of the system by separating gram quantities of dinitrophenyl amino acids. The design and construction of the apparatus should permit easy increases in scale for industrial applications.

INTRODUCTION

In the past, several devices for performing preparative-scale counter-current chromatography have been developed[1–10]. Among those schemes the most efficient separations have been obtained from schemes which employ a rotating coiled column in an acceleration field[2–10]. Efforts have been successfully made to eliminate the use of rotating seals in utilizing the horizontal flow-through coil planet centrifuges[4–10]. However, these devices hold a preparative column assembly on one side of the rotary arm and, therefore, application of a large preparative column requires a fair amount of laboratory space.

This paper describes a new preparative counter-current chromatographic scheme which compactly holds a large coil assembly at the center of the apparatus and is amenable for further scaling-up of the sample loading capacity for industrial separations. The partition capability of the present scheme was demonstrated by the separation of a set of dinitrophenyl (DNP) amino acids with a two-phase solvent system composed of chloroform–acetic acid–0.1 *N* hydrochloric acid at a volume ratio of 2:2:1.

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PRINCIPLE AND DESIGN OF THE APPARATUS

The present scheme uses a coiled tube which slowly rotates around its horizontal axis with respect to the gravitational field. Particles introduced in such a coil move toward one end of the coil. This end is defined as the head and the other end, the tail of the coil. A two-phase solvent system confined in this rotating coil distributes itself in such a way that nearly equal volumes of the two phases occupy each helical turn while any excess of either phase remains at the tail. This hydrodynamic behaviour of the solvents allows elution of either phase through the head while retaining a large amount of the stationary phase in each turn of the coil. Consequently, solutes introduced through the head of the coil are subjected to an efficient partition process between the mobile and stationary phases in each turn of the coil and are eluted through the tail in the order of their partition coefficients as in liquid chromatography but in the absence of solid supports.

Applications of this scheme requires a flow-through mechanism to elute the solvent through the rotating coil. The simple rotary coil assembly introduced here is equipped with a rotary-seal-free flow-through system which eliminates various complications such as leakage, corrosion and contamination caused by the use of rotating seals.

Fig. 1 shows a simplified cross-sectional view through the central axis of the apparatus. The motor drives a rotary frame which consists of three aluminum arms rigidly bridged together with links. The frame holds two rotary elements, the countershaft and the centrally located column holder assembly. The countershaft is equipped with a toothed pulley at one end and a plastic gear at the other end. The pulley of the countershaft is coupled with a toothed belt to an identical stationary pulley mounted on the stationary wall member of the apparatus. This coupling causes a counter-rotation of the countershaft on the rotary frame. This motion is further

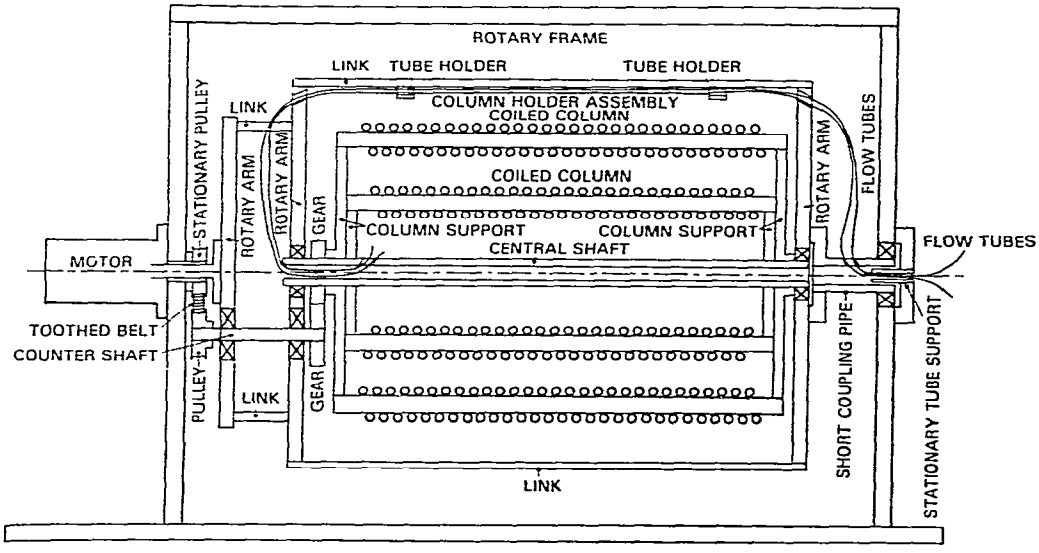

Fig. 1. A simplified cross-sectional view through the central axis of the apparatus.

conveyed to the central column holder assembly by 1:1 gear coupling. Consequently, the column holder assembly rotates around its own axis at a rate twice that of the rotary frame in the same direction. This particular design gives a great advantage in that the scheme allows the flow in and out of the rotating column without the use of rotating seals[11–13].

Separation columns used in the present studies consist of coiled glass tubes of 0.5 cm I.D., with different helical diameters (Kontes, Vineland, NJ, U.S.A.). One column has a 2.5-cm helical diameter with a 90-ml capacity and the other has a 1.25-cm helical diameter with a 45-ml capacity. Both columns contain approximately 50 helical turns. Each column is supported by a hollow aluminum core of the suitable diameter which is in turn mounted onto the column holder by a screw at each end. The column holder is equipped with two different levels for mounting columns, the first level being located 6.5 cm from the central axis of the apparatus and the second level, 13 cm from the same axis. A maximum of 30 columns can be mounted to the holder, 10 columns at the first level and 20 columns at the second level. The desired number of columns can be connected in series with a short piece of heat shrinkable PTFE tubing (Zeus Industrial Products, Raritan, NJ, U.S.A.) at each junction.

Flow tubes from the column are first led through the center hole of the column holder shaft, then passed through a pair of holes at the periphery of the rotary arms, and finally supported by a stationary tube support located at the central axis of the apparatus. These tubes are thoroughly lubricated with silicone grease and protected with a piece of plastic tubing to prevent contact with metal parts.

The rotational speed of the column assembly can be regulated up to 300 rpm. However, in the present studies, fragility of the glass column limits the maximum rate down to approximately 100 rpm. A Beckman Accu pump and Chromatronix pump are used to elute the solvents and an LKB Uvicord III to monitor the eluate at 280 nm.

EXPERIMENTAL

Preliminary studies on partition capability

The performance of the present counter-current chromatographic scheme was investigated by measuring the degree of stationary phase retention and partition efficiency. The two types of coils with 1.25 and 2.5 cm O.D. cores were tested, each mounted in both inner and outer positions of the column holder.

The degree of retention of the stationary phase in each column was measured with a two-phase solvent system composed of chloroform–acetic acid–water at a 2:2:1 volume ratio under various rotational speeds and flow-rates. The two-phase solvent system was first equilibrated in a separatory funnel at room temperature and separated before use. In each measurement the column was entirely filled with the mobile phase, either upper aqueous or lower non-aqueous phase. Then a given volume of the stationary phase which occupies "A" helical turns of the column was introduced through the head of the column. In order to visualize the stationary phase, a small amount of dye which favors partition to the stationary phase was dissolved in the stationary phase. Sudan III was used to color the non-aqueous phase and acid fuchsin to color the aqueous phase. Then the mobile phase was pumped through the head of the column while the column was rotated at a given rate. The two phases soon reached hydrodynamic equilibrium and the number of helical turns "B" containing the colored

stationary phase was read. The percentage retention relative to the total column capacity was obtained by the simple expression, $100A/B$. The measurement can be repeated by changing rotational speed or flow-rate without renewing the column contents until carryover of the stationary phase occurs.

The partition efficiency of each column was evaluated with a two-phase solvent system composed of chloroform–acetic acid–0.1 *N* hydrochloric acid at a 2:2:1 volume ratio and a pair of DNP amino acids as test samples. The two-phase solvent system was equilibrated in a separatory funnel at room temperature and separated before use. The sample solution was prepared by dissolving N-DNP–DL-glutamic acid (DNP-glu) and N-2,4-DNP–L-alanine (DNP-ala) (Sigma, St. Louis, MO, U.S.A.) in the upper aqueous phase to obtain the 0.5 g% concentration of each component. In each separation the column was first filled with the stationary phase. This was followed by injection of 0.5 ml of the sample solution through the sample port located on the flow line between the pump and the inlet of the column. Then the mobile phase was pumped through the head of the column while the column was rotated at a given rate. The eluate through the outlet of the column was continuously monitored with an LKB Uvicord III at 280 nm. Separations were performed under a wide range of operational conditions, of rotational speeds (0–80 rpm) and flow-rates (120 and 240 ml/h), while both upper aqueous and lower non-aqueous phases were tested as the stationary phase.

Preparative counter-current chromatography with a long column

The preparative capability of the present scheme was examined with a long column consisting of 10 coils with 2.5 cm core O.D. connected in series (tail–head connection). The column consisted of nearly 500 helical turns with a total capacity of approximately 900 ml. It was symmetrically mounted on the outer positions of the column holder. The solvent system and the samples were the same as those used in the partition efficiency studies. The sample solution was prepared by dissolving 500 mg of each DNP amino acid for a total of 1 g in 30 ml of the solvent consisting of equal amounts of the upper and lower phases. 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 pumped through the head of the column at a rate of 120 ml/h while the column was rotated at the optimum rate determined by the preliminary studies. The eluates were collected with an LKB fraction collector to obtain a 12-ml fraction in each test tube. A 20-μl volume of each fraction was mixed with 3 ml of methanol to measure the absorbance at 430 nm with a Beckman DU spectrophotometer.

RESULTS AND DISCUSSION

The typical results of the retention studies are illustrated in Fig. 2 where the percentage retention of the stationary phase relative to the total column capacity is plotted against the rotational speed of the column. The several lines drawn in each diagram indicate the effect of different flow-rates. Retention near 50% is considered to be ideal while lower levels of retention can be suitable for separations if the inclination of the curve is near horizontal which insures a stable retention of the stationary phase upon fluctuation of the rotational speed.

Fig. 2 shows retention in the large coil (2.5 cm O.D. core) mounted in the outer position of the column holder for the lower non-aqueous phase (A) and the upper aqueous phase (B). As is clearly observed, the retention levels of the non-aqueous phase are substantially lower than those of the aqueous phase throughout the applied rotational speeds. This higher level of retention produced by the aqueous phase may be largely attributed to its greater affinity to the glass wall of the column. The effects of the flow-rate on the retention of the stationary phase are also clearly shown in these diagrams; the slower the flow, the higher the retention levels. Figs. 2C and D similarly

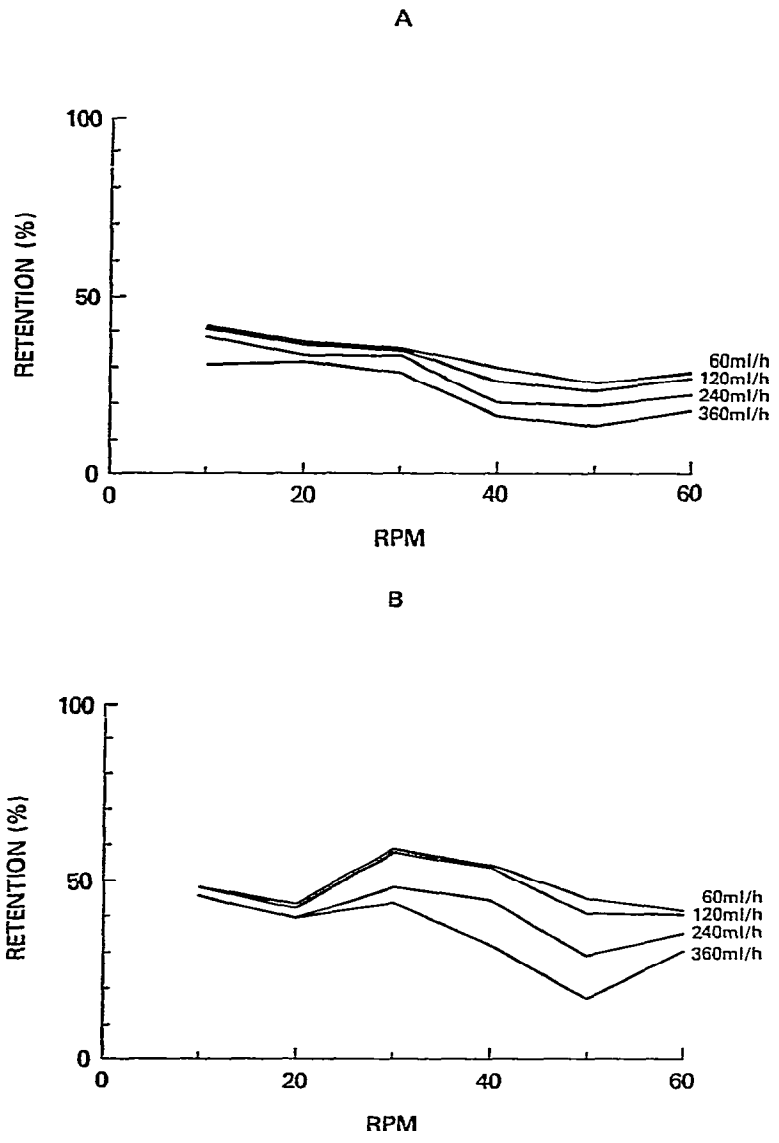

Fig. 2.

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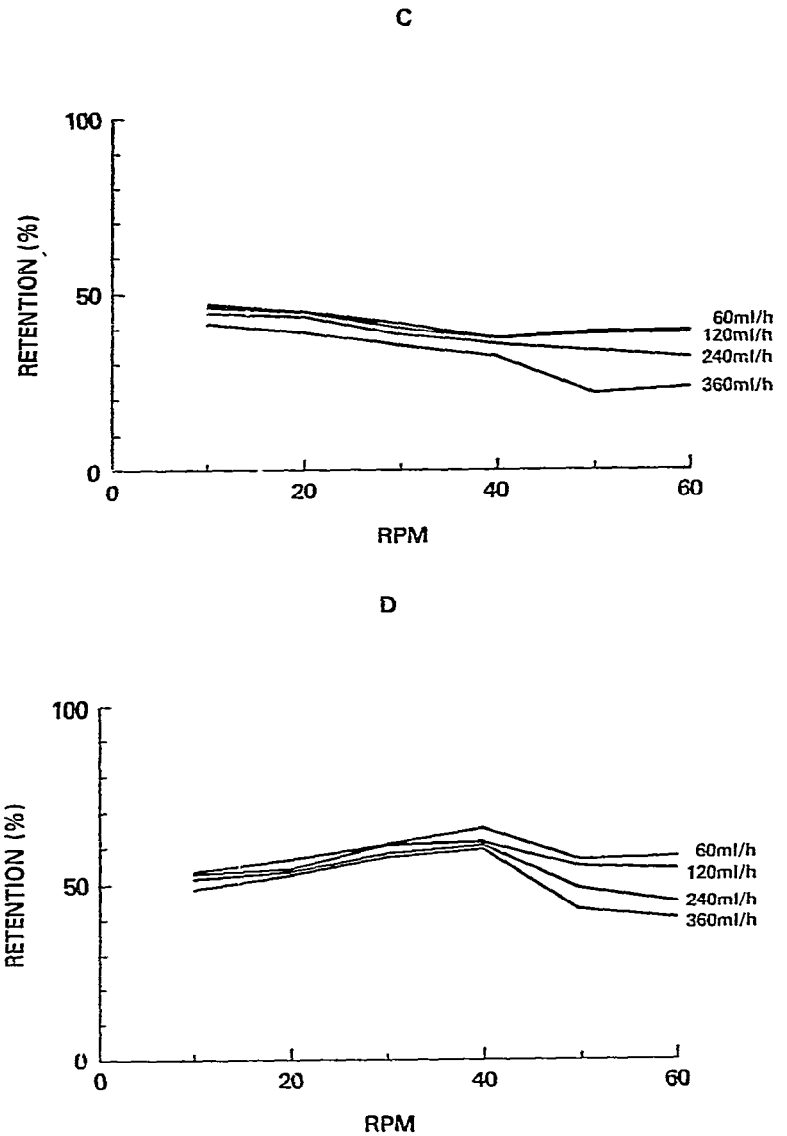

Fig. 2. The effect of rotational speed and flow-rate on stationary phase. Large coil in outside position: A, non-aqueous stationary phase; B, aqueous stationary phase. Small coil in outside position: C, non-aqueous stationary phase; D, aqueous stationary phase.

show the retention levels in the small coil (1.25 cm O.D. core) mounted in the outer position of the column holder for both stationary phases. The data clearly show that the retention levels produced by the small coil is substantially higher than those by the large coil for both non-aqueous and aqueous stationary phases. This may indicate that in the small core coil the linear velocity relative to the gravity becomes smaller resulting in less violent mixing of the two phases and, therefore, higher levels of phase retention at a given rotational speed occur.

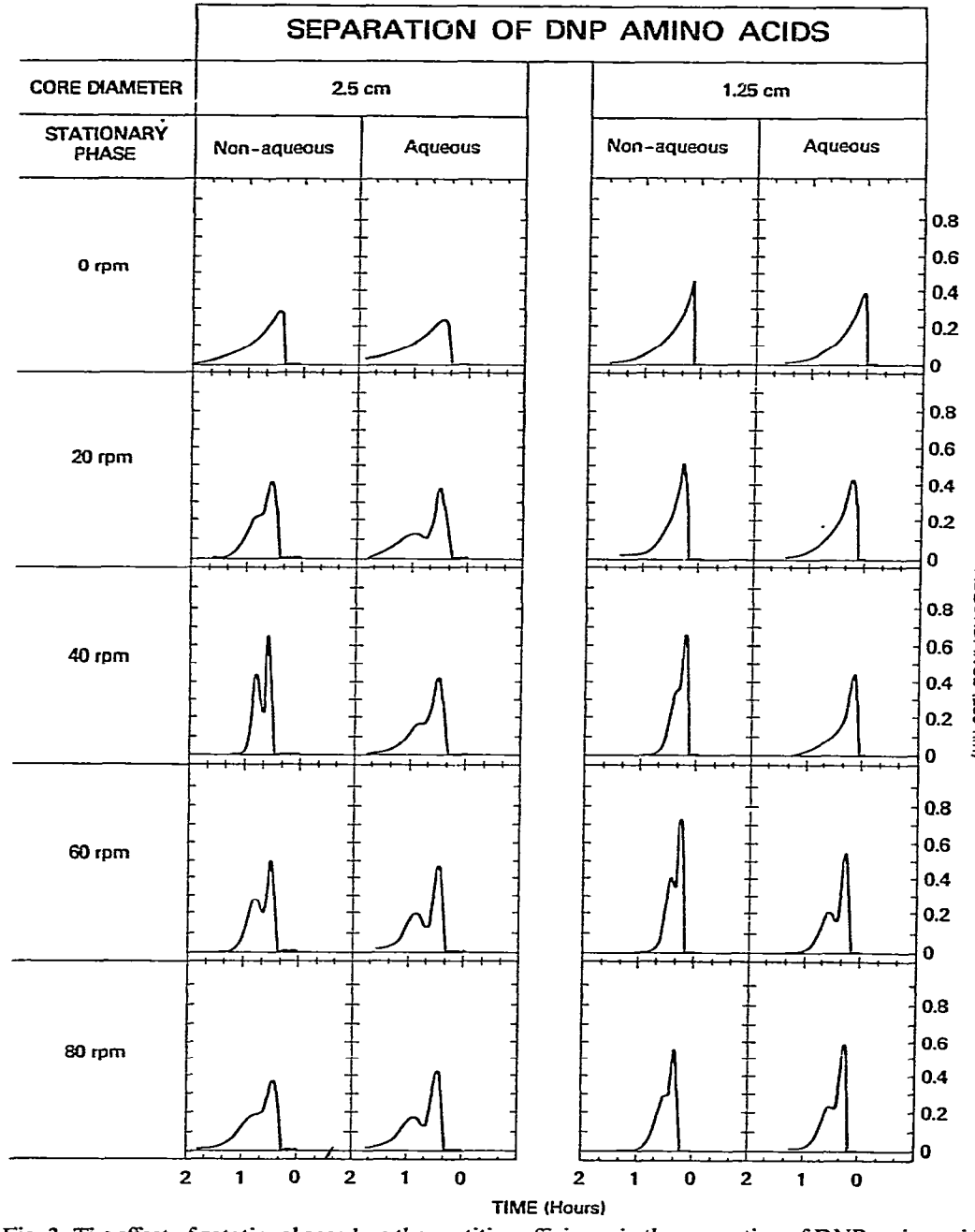

Fig. 3. The effect of rotational speed on the partition efficiency in the separation of DNP amino acids.

Data obtained with the same columns mounted in the inner position of the column holder gave similar results. Overall results indicate that satisfactory retention levels can be obtained with either type of coils under a wide range of rotational speeds and flow-rates.

Fig. 3. summarizes the results of DNP amino acid separation with a single coil mounted in the outer position of the column holder under a flow-rate of 120 ml/h. In each diagram, partition efficiency can be easily estimated by the resolution of the two peaks. In all groups the efficiency sharply increases as the rotational speed increases from 0 to 40–60 rpm, where the peak resolution becomes maximum. Further increase of the rotational speed to 80 rpm results in the loss of peak resolution. The optimum rotational speed thus ranges from 40 to 60 rpm for all groups. The highest peak resolu-

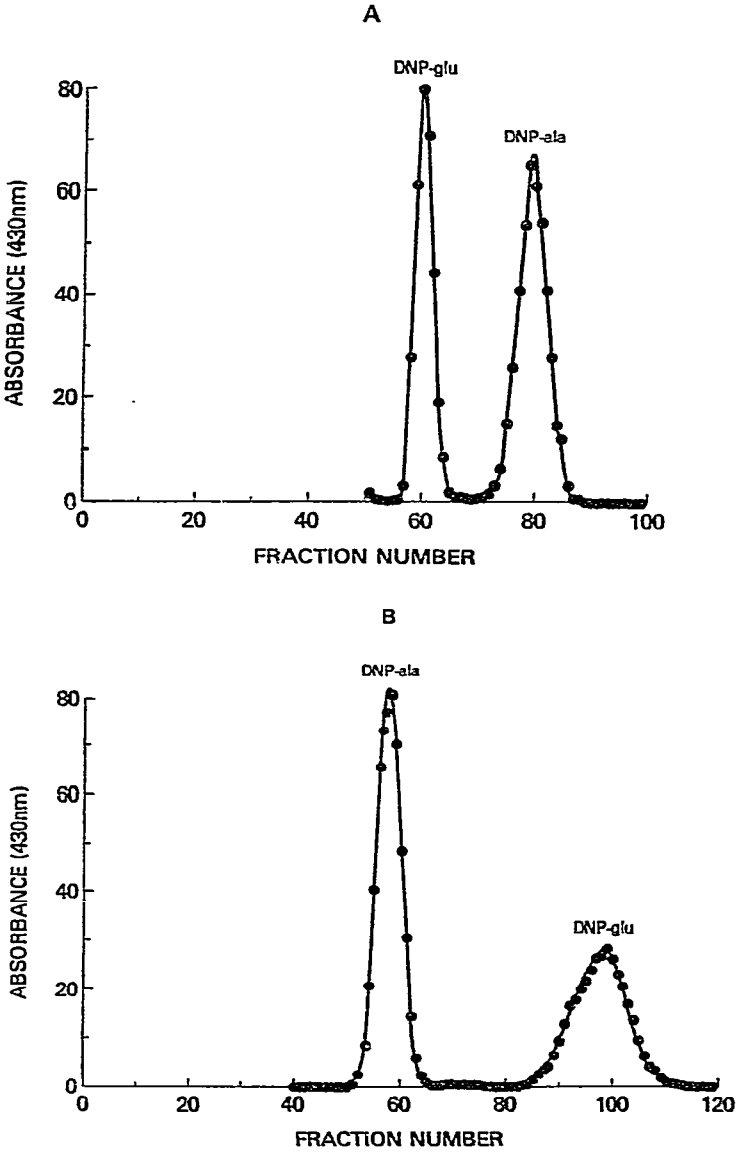

Fig. 4. Preparative-scale separations of DNP amino acids with a long coiled column. A, non-aqueous stationary phase; B, aqueous stationary phase.

tion is given by the large coil while the small coil could yield much higher resolution if two coils are connected to make the capacity equal to that of the single large coil. The results obtained with a higher flow-rate of 240 ml/h yielded less efficient separations in both small and large coils compared with those produced at 120 ml/h. The results obtained from the coils mounted in the inner position of the holder gave separations similar to those produced by respective coils mounted in the outer position of the column holder.

The preparative capability of the present counter-current chromatographic scheme was demonstrated by the separations of 1-g samples with a long column consisting of 10 large coils connected in series in the outside position. The separations were performed at a 120 ml/h flow-rate using both non-aqueous and aqueous stationary phases. Fig. 4A shows a chromatogram obtained at the optimum rotational speed of 40 rpm by using the non-aqueous phase as the stationary phase. The two DNP amino acids were completely resolved as symmetrical peaks and eluted out within 9 h. The partition efficiency calculated according to the standard formula[14] gives 1250 theoretical plates (T.P.) for the first peak and 880 T.P. for the second peak. Fig. 4B shows a similar chromatogram obtained at 60 rpm using the aqueous phase as the stationary phase. Because of higher aqueous phase retention, the peak resolution is much greater than that of the separation using the non-aqueous phase as stationary. The partition efficiency in the latter separation gives lower figures of 1000 T.P. for the first peak and 830 T.P. for the second peak.

The present scheme enables preparative-scale separation with a simple, compact apparatus. Separations are performed without the presence of solid supports and, therefore, complications such as sample loss, contamination and tailing of the solute peaks are minimized. The scheme yields high partition efficiency comparable to liquid chromatography while retaining high reproducibility and predictability inherent in the Craig counter-current distribution method. Because of its simplicity and mechanical stability, the present scheme can be further scaled up for large-scale industrial separations.

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