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PREPARATIVE COUNTER-CURRENT CHROMATOGRAPHY WITH A SLOWLY ROTATING GLASS COIL

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SUMMARY

An attempt to scale-up the preparative capacity of counter-current chromatography was successfully made by the use of a large-bore glass coiled column slowly rotating in the gravitational field. The capability of the present scheme was demonstrated in separations of dinitrophenyl amino acids on a two-phase solvent system composed of chloroform–acetic acid–0.1 *N* hydrochloric acid (2:2:1). The methods enables efficient separations of 600-mg samples in 10 h with a good recovery. The present scheme is amenable to be further scaled-up by the use of longer and/or larger-diameter columns.

INTRODUCTION

In the past various schemes of counter-current chromatography have been developed to perform highly efficient solute partitioning in the absence of solid supports[1–9]. Some of these schemes[4–8] are suitable for separating a relatively large quantity of samples and amenable to be further scaled-up by using coiled separation columns of a larger diameter.

The present paper introduces a preparative-scale counter-current chromatography which uses a large-bore glass coil slowly rotating in a gravitational field. A planetary motion of the horizontal flow-through coil planet centrifuge[6,7] has been adapted to eliminate the use of the rotating seals which would become a potential source of complications such as leakage, corrosion and contamination. A series of preliminary experiments has been performed to study the partition capabilities of the present method with a two-phase solvent system composed of chloroform–acetic acid–0.1 *N* hydrochloric acid (2:2:1) and a set of dinitrophenyl (DNP) amino acids as test samples. In these studies, the degree of stationary phase retention and partition efficiency were investigated with a short coiled column under a wide range of revolutional speeds and flow-rates. The preparative capability of the method was then demonstrated with a long column under the optimum operational conditions determined by the preliminary experiments.

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PRINCIPLE

The principle of counter-current chromatography with a slowly rotating coiled tube has been reported earlier[1,4,5]. Below, a brief description is given to the mechanism involved in the present method.

When a coiled tube filled with a liquid is rotated around its horizontally oriented axis in the gravitational field, particles suspended in the liquid move toward one end of the coil. This end is called the head and the other end, the tail of the coil. When the coil contains two immiscible liquid phases, the rotation soon establishes what is called a hydrodynamic equilibrium state. In this state the two phases are distributed in the coil in such a way that each phase occupies nearly equal space in each turn of the coil and any excess of either phase remains at the tail end of the coil while the two phases are constantly mixed by the rotation of the coil. This hydrodynamic behavior of the two phases can be efficiently used for solute partitioning. When the mobile phase (either upper or lower phase) is introduced at the head end of the coil to disrupt the hydrodynamic equilibrium state, the two phases quickly react to reestablish the original distribution pattern. This results in a counter-current flow of the phases in the coil: the stationary phase moves back toward the head end of the coil while the introduced excess amount of the mobile phase travels toward the tail. Consequently, the solutes introduced locally at the head end of the coil are subjected to an efficient partition process in each turn of the coil and separated according to their partition coefficients as in liquid chromatography but in the absence of solid supports.

EXPERIMENTAL

Apparatus

Fig. 1 shows the overall view of the apparatus. The motor (ElectroCraft Corp., MN, U.S.A.) drives the rotary frame, consisting of a pair of aluminium plates and links, around the horizontal stationary pipe mounted on the central axis of the apparatus. The rotary frame holds a pair of rotary shafts symmetrically spaced at 15 cm from the central axis of the apparatus. Each rotary shaft is equipped with a planetary gear which is engaged to an identical stationary sun gear mounted around the central stationary pipe. This gear coupling produces a synchronous planetary motion of each rotary shaft, *i.e.*, the rotation about its own axis and revolution around the central axis of the apparatus at the same angular velocity in the same directions. This synchronous planetary motion of the rotary shaft eliminates the need for the rotating seal as reported earlier[6,7]. In order to provide mechanical stability, a short coupling pipe was coaxially mounted on the free end of the rotary frame and the other end of the coupling pipe was supported by the stationary wall member of the apparatus through a ball bearing.

The separation column consists of ten units of glass coiled tube of (0.5 cm I.D., 2.5 cm core diameter) (Kontes, Vineland, NJ, U.S.A.) connected in series (head to tail connection) with PTFE heat shrinkable tubing (Zeus Industrial Products, Raritan, NJ, U.S.A.). The whole column has about 500 helical turns with a total capacity of approximately 900 ml. The column was symmetrically arranged around one of the rotary shafts by the aid of column supports. Counter-weight was applied on the other rotary shaft to balance the apparatus. The flow tubes from the column were first passed

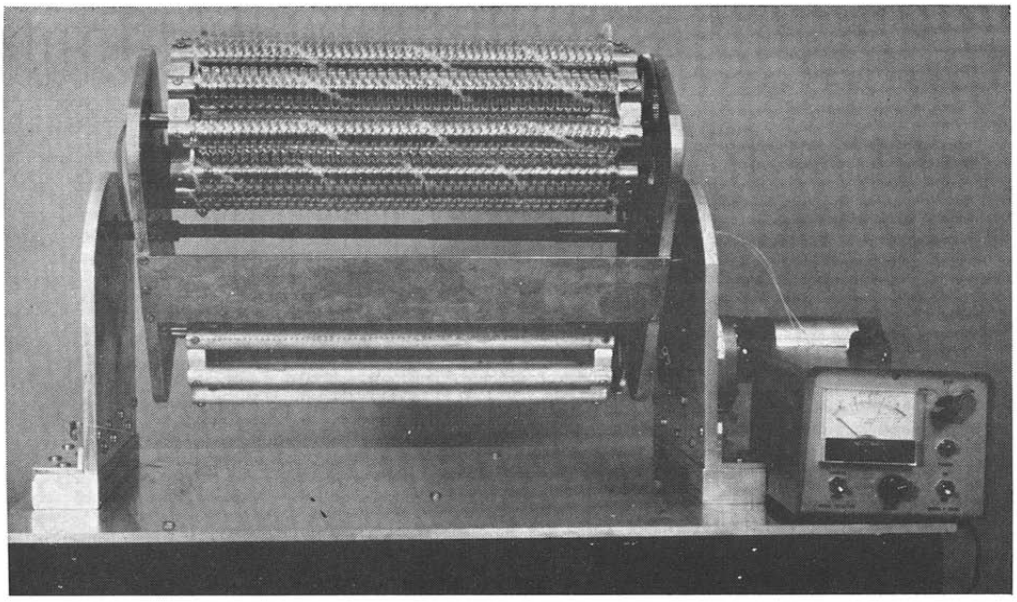

Fig. 1. Overall view of the apparatus.

through the center hole of the rotary shaft, led into the opening the central stationary pipe through a side hole of the short coupling pipe and tightly supported at the exit hole of the stationary pipe. These tubes were lubricated with silicone grease and protected by a piece of plastic tubing to prevent direct contact with metal parts.

The apparatus can be operated at uniform rates ranging from 0 to 100 rpm, the limiting factor being the fragility of the glass coil. A Chromatronix Cheminert Pump or Beckman Accu Pump was used to pump the solvents and an LKB Uvicord III to monitor the absorbance of eluate at 280 nm.

Preparation of two-phase solvent system and sample solution

All solvents and samples used in the present study were of reagent grades. The two-phase solvent system was prepared from chloroform (Burdick & Jackson Labs., Muskegon, MI, U.S.A.), glacial acetic acid (J. T. Baker, Phillipsburg, NJ, U.S.A.) and 0.1 *N* HCl at a volume ratio of 2:2:1. The solvent mixture was equilibrated in a separatory funnel at room temperature and separated before use. A pair of DNP-amino acids (Sigma, St. Louis, MO, U.S.A.), *i.e.*, 2,4,N-DNP-DL-glutamic acid and 2,4,N-L-alanine, was chosen as test samples, because of their suitable partition coefficients in the present solvent system. Sample solution was prepared by dissolving equal quantities of these samples in the stationary phase or in an equal amount of both phases to obtain the desirable concentrations of 0.5% and 1.0 g % for each component. The sample solutions were stored in the dark at 4°C.

Preliminary studies on retention of the stationary phase

The percentage of stationary phase volume retained in the rotating coiled column relative to the total column capacity was determined at various revolutional

speeds and flow-rates. Series of experiments were conducted with a single column unit which was marked by a felt tip pen at every five turns to ease the measurement. In order to visualize the stationary phase distributed in the rotating column, two-phase solvent system was equilibrated with a dye which favors partition in the stationary phase. Sudan III was used to color the lower non-aqueous phase and acidic fuchsin to color the upper aqueous phase. In each series of experiments, the column was first filled with the mobile phase followed by injection of a given amount of the colored stationary phase which occupies A turns of the coil. Then the mobile phase was pumped into the column while the column was rotated at a given revolutional speed. After the hydrodynamic equilibrium state was reached, the number of helical turns, B, containing the colored stationary phase was read. The retention percentage relative to the total column capacity was then obtained from an expression, $100A/B$. The experiment was continued by changing the flow-rate or revolutional speed without renewing the column contents.

Preliminary studies on partition efficiency

Partition efficiency attainable by a single column unit was investigated by the aid of DNP-amino acid samples described earlier. The column was first filled with the stationary phase followed by the injection of 0.5-ml sample mixture containing each component at 0.5 g%. Then the mobile phase was pumped into the column at a given flow-rate while the column was rotated at a given revolutional speed. The eluate

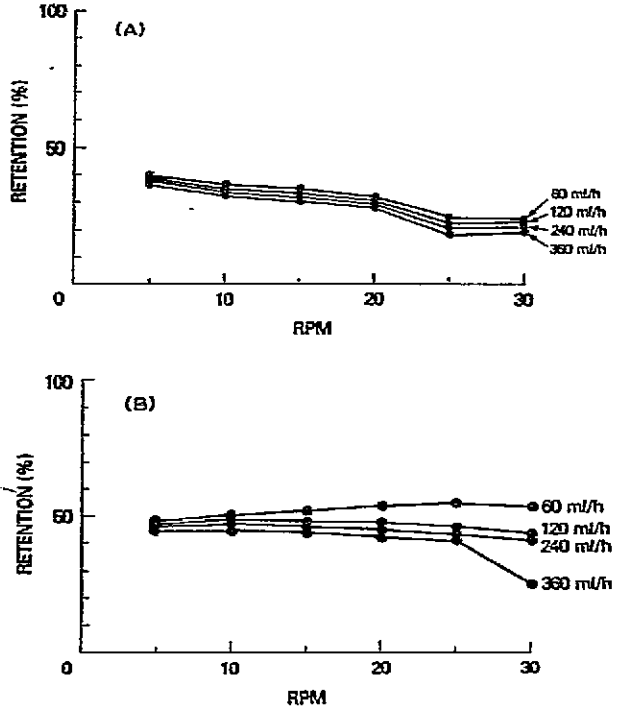

Fig. 2. Effects of revolutional speed and flow-rate on retention of non-aqueous (A) and aqueous stationary phases (B).

was continuously monitored with an LKB Uvicord III at 280 nm. The experiments were performed under a wide range of revolutional speeds and flow-rates and each phase was tested as the stationary phase.

Preparative-scale separations with a long coiled column

Large-scale preparative separations of the test samples were performed with a long column consisting of ten units of the short coiled column used in the preliminary experiments. The column was first filled with the stationary phase followed by sample charge. A sample size of 30 ml containing each component at 1 g% was used to demonstrate a preparative capability of the method. The mobile phase was then eluted through the column under the optimum operational conditions determined by the preliminary experiments. Alternatively, the column can be equilibrated with the two phases before sample injection. The eluate from the column was collected with a fraction collector to obtain a 12-ml fraction in each tube. A 50-μl volume of each fraction was mixed with 3 ml methanol for determination of absorbance at 280 nm with a Beckman DU spectrophotometer.

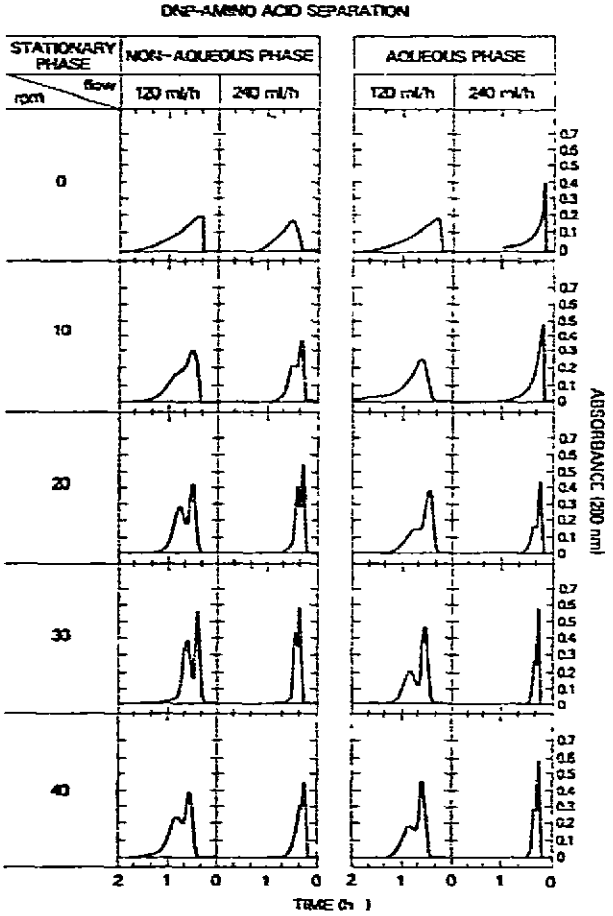

Fig. 3. Effects of revolutional speed and flow-rate on partition efficiency.

RESULTS AND DISCUSSION

Fig. 2 summarizes the results of the preliminary studies on the retention of the stationary phase. In each diagram, the retention percentage of the stationary phase relative to the total column capacity is plotted against the applied revolutional speed, where several lines indicate the effects of different flow-rates. Retention of near 50% is ideal and that over 30% is considered to be satisfactory although a lesser degree of retention can also be applied for separation. The results obtained with the stationary non-aqueous phase (A) show a satisfactory level of retention up to 20 rpm. The results with the stationary aqueous phase (B) shows ideal levels of retention in a wide range of revolutional speeds and flow-rates. The excellent retention of the aqueous phase may be caused by the affinity of the aqueous phase to the glass tube wall.

Fig. 3 summarizes the results obtained by the preliminary studies on the partition efficiency. The experiments were performed under a wide range of revolutional speeds from 0 to 40 rpm at the flow-rates of 120 ml/h and 240 ml/h where each phase was tested as the stationary phase. In each chart partition efficiency can be estimated from the resolution of the two peaks. The partition efficiency sharply increases with he increase in revolutional speed from 0 to 20–30 rpm, where the peak resolution

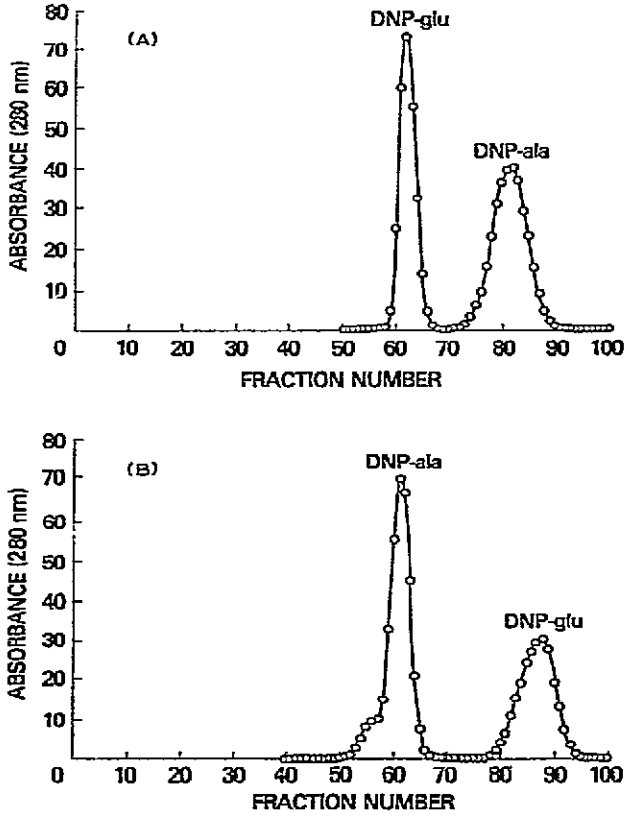

Fig. 4. Counter-current chromatograms of DNP-amino acids with a long column and non-aqueous (A) and aqueous stationary phases (B).

becomes maximum for both stationary phase groups. Further increase of the revolutional speed results in loss of peak resolution. The overall results indicate that the separation can be attained with either phase as the stationary phase.

The preparative capability of the present method was demonstrated in the separation of the DNP-amino acids with a long column consisting of ten units, each identical to that used in the preliminary studies. Fig. 4 shows preparative separations of a 600-mg sample mixture dissolved in 30 ml solvent. Both non-aqueous (A) and aqueous (B) phases were used as the stationary phase. Under the optimum operational conditions of a 120 ml/h flow-rate and 30 rpm, the peaks were well resolved and eluted out within 10 h. In both separations recovery of sample was found to be near 100%.

The present method is capable of separations with a sample size somewhat comparable to that in Craig apparatus. This preparative capacity may be further increased by the use of larger-diameter and/or longer columns. Compared with the counter-current distribution method, the present method yields higher partition efficiencies in shorter periods of time. In addition the apparatus is simple, compact and relatively inexpensive. The method will be useful in preparative-scale separations in research laboratories.

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