Journal of Chromatography, 214 (1981) 122–125
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,063

Note

Efficient preparative counter-current chromatography with a coil planet centrifuge

YOICHIRO ITO
Laboratory of Technical Development, National Heart, Lung and Blood Institute, Bethesda, MD 20205 (U.S.A.)
(Received June 2nd, 1981)

Partition efficiencies of preparative counter-current chromatographic schemes developed in the past[1–12] have been largely limited by the applicable flow-rates. High flow-rates tend to reduce the amount of stationary phase retained in the column and this in turn results in loss of both the peak resolution and the sample loading capacity of the system. The preparative counter-current chromatographic method described here has an extremely large capacity for retaining the stationary phase against high flow-rates of the mobile phase and, therefore, produces efficient separations in short periods of time.

PRINCIPLE

The scheme uses an intriguing hydrodynamic motion of two immiscible solvent phases in a rotating coiled tube. When a water-filled coiled tube is slowly rotated around its horizontally oriented axis, glass beads or air bubbles present in the coil move toward one end of the coil. This end is called the head and the other end, the tail of the coil. When such a coil is filled with two immiscible solvent phases, rotation of the coil distributes the two phases rather evenly in each helical turn while any excess of either phase remains at the tail end of the coil. This hydrodynamic equilibrium state of the two phases has been used for counter-current chromatography by introducing one of the phases through the head of the coil. This gives retention levels of the stationary phase relative to the total column capacity usually at less than 50% and the application of a higher flow-rate results in lower retention levels. It has been found, however, that a particular mode of planetary motion provided by the horizontal flow-through coil planet centrifuge could produce an uneven distribution pattern of the two phases and in some occasions the two phases are completely separated along the length of the coil with the lighter phase in the head end and the heavier phase in the tail end of the coil[13]. This particular hydrodynamic equilibrium pattern of the two phases can be efficiently utilized for counter-current chromatography. The coiled column is first filled with the upper stationary phase and then eluted with the lower mobile phase through the head of the coil while the apparatus is rotated at a given speed. As soon as the lower phase reaches the head of the coil, the two phases quickly establish a hydrodynamic equilibrium where the upper stationary phase dominates in volume in each helical turn. This gives a high level of stationary phase retention which

often exceeds 80% of the total column space. Once this hydrodynamic equilibrium is established, the introduced mobile phase moves towards the tail at an extremely high speed so that the retention level of the stationary phase becomes rather insensitive to the applied flow-rate of the mobile phase. Consequently, solutes introduced through the head of the coil are subjected to an efficient partition process between a large amount of the stationary phase and rapidly flowing mobile phase and separated according to their partition coefficients in a short period of time.

EXPERIMENTAL

Apparatus

Fig. 1 shows schematically a cross-sectional view through the central axis of the apparatus. The motor drives the rotary frame consisting of a pair of aluminum plates bridged with links (not shown in the figure) around the central stationary pipe (shaded). The rotary frame holds a pair of drum-shaped column holders each mounted on ball bearings symmetrically located at a distance of 10 cm from the central axis of the apparatus. Each column holder is equipped with a plastic planetary gear (Winfred M. Berg, Inc., New York, NY, U.S.A.) which is coupled to an identical stationary sun gear (shaded) rigidly mounted around the central stationary pipe. This gear arrangement produces a synchronous planetary motion of each column holder in such a way that it revolves around the central axis of the apparatus and simultaneously rotates about its own axis at the same angular velocity in the same direc-

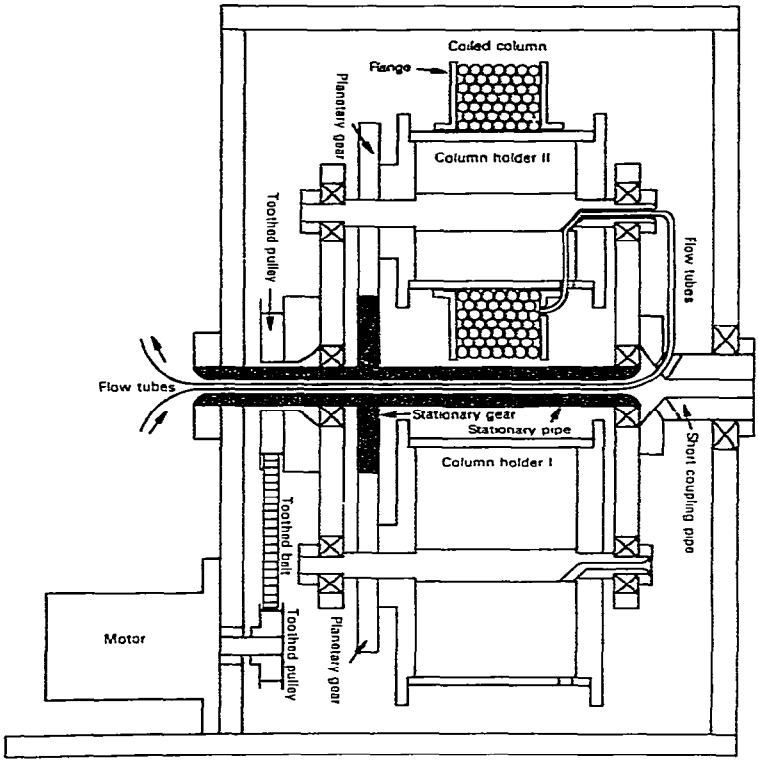

Fig. 1. Design of the apparatus.

tion. This particular mode of planetary motion of the column holder provides a desirable hydrodynamic equilibrium pattern of the two solvent phases in the coiled column and also renders a seal-free flow-through mechanism as described earlier[6]. In order to reinforce mechanical stability of the centrifuge system, the free end of the rotary frame is supported by a short coupling pipe which is mounted through ball bearing in the stationary wall member of the centrifuge.

The column was prepared from a 45 m × 1.65 mm I.D. PTFE tubing (Zeus Industrial Products, Raritan, NJ, U.S.A.) by winding it directly onto one of the holders (column holder II). The total column capacity was approximately 100 ml. A pair of aluminum flanges conveniently accomodates multiple layers of the tube as shown in the figure. A counter-weight was mounted on the other holder (column holder I) to balance the centrifuge system. The flow tubes from the coiled column were led through the central hole of the column holder shaft and then passed through the side-hole of the short coupling pipe to reach the opening of the central stationary pipe. These tubes were lubricated with silicone grease and protected with a piece of plastic tube at each supported portion to prevent direct contact with metal parts.

The revolutional speed of the apparatus is continuously adjustable up to 1000 rpm. Elution was performed with an ordinary metering pump and the eluate was continuously monitored with a UV monitor and/or fractionated with a fraction collector for later analysis.

Procedures

In order to demonstrate the capability of the present counter-current chromatographic scheme, a set of dinitrophenyl (DNP) amino acids (Sigma, St. Louis, MO, U.S.A.) was separated on a two-phase solvent system composed of chloroform–acetic acid–0.1 *N* hydrochloric acid (2:2:1). The two-phase solvent system was equilibrated in a separatory funnel at room temperature and separated before use. In each separation, the column was entirely filled with the upper aqueous stationary phase and 1 ml of the sample solution containing each component at 0.2–0.4 g% was injected through the sample port located on the flow line between the pump and the inlet of the coiled column. The apparatus was then rotated at 750 rpm and the lower non-aqueous mobile phase was eluted at a rate of 120 ml/h. The eluates were fractionated at 3-min intervals to collect 6 ml in each tube. Then, 1 ml 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

Fig. 2 shows a typical chromatogram obtained with the present scheme. Five DNP-amino acids were well resolved in symmetrical peaks and eluted out in 2.5 h. Partition efficiency expressed in terms of theoretical plates (T.P.) ranges from 350 T.P. for the first peak to 300 T.P. for the last peak. The stationary phase volume retained in the coiled column measured 70 ml which is equivalent to 70% of the total column capacity. The maximum pressure necessary to pump the mobile phase was 50 p.s.i. during separation. The sample size can be increased to 450 mg without detrimental effects on the peak resolution. The capacity of the scheme would be further increased by using a larger-diameter and/or longer column.

Compared with droplet counter-current chromatography[1,2], the present method permits a much higher flow-rate (7.5 times) and yet yields substantially higher partition efficiency per unit length of column. Because of the extremely high retention levels of the stationary phase, the present scheme has a large sample loading capacity

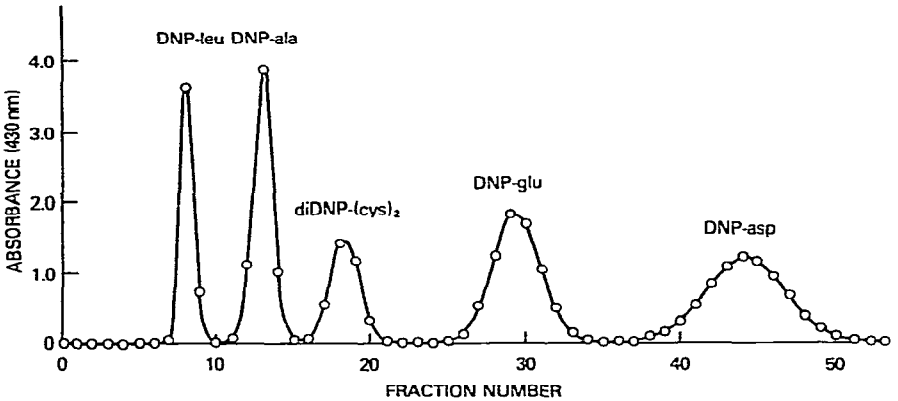

Fig. 2. Counter-current chromatogram of DNP-amino acids.

and yields a high peak resolution (equivalent to 1000 T.P.), compared to other counter-current chromatographic schemes[3-12] where retention of the stationary phase measures less than 50% of the total column capacity.

The high performance of the present scheme is largely attributed to its great capacity for retaining the stationary phase against a high flow-rate of the mobile phase. The results of our preliminary experiments with a variety of two-phase solvent systems have indicated that the retained stationary phase volume increases as the density difference of the two phases becomes greater. Therefore, the partition efficiency of some solvent pairs such as *n*-butanol–aqueous systems is greatly improved by adding salts to the system which increases the density of the aqueous phase. For example, two-phase solvent systems such as *n*-butanol–1 *M* ammonium formate (1:1) and *n*-butanol–1 *M* sodium chloride (1:1) are extremely useful for the separation of peptides. In these systems, the partition coefficients of samples are conveniently adjusted by adding a small amount of acid such as dichloroacetic acid, etc. Application of gradient or stepwise elution also becomes possible with these solvent systems.

The present unit is a compact table top model which has dimensions of 1 × 1 × 1.5 ft. Because of its simplicity in design, the apparatus is relatively inexpensive and may be constructed in a small machine shop without difficulty. The method is extremely versatile and would be very useful for preparative separation and purification of various biological materials.

REFERENCES

1 Y. Ito and R. L. Bowman, *J. Chromatogr. Sci.*, 8 (1970) 315.
2 T. Tanimura, J. J. Pisano, Y. Ito and R. L. Bowman, *Science*, 169 (1970) 54.
3 Y. Ito and R. L. Bowman, *Anal. Biochem.*, 78 (1977) 506.
4 Y. Ito and R. L. Bowman, *J. Chromatogr.*, 136 (1977) 189.
5 Y. Ito and R. L. Bowman, *Anal. Biochem.*, 82 (1977) 63.
6 Y. Ito and R. L. Bowman, *J. Chromatogr.*, 147 (1978) 221.
7 Y. Ito, *Anal. Biochem.*, 100 (1979) 271.
8 Y. Ito, *J. Chromatogr.*, 188 (1980) 33.
9 Y. Ito, *J. Chromatogr.*, 188 (1980) 43.
10 Y. Ito and G. J. Putterman, *J. Chromatogr.*, 193 (1980) 37.
11 Y. Ito, *J. Chromatogr.*, 196 (1980) 295.
12 Y. Ito and R. Bhatnagar, *J. Chromatogr.*, 207 (1981) 171.
13 Y. Ito, *J. Chromatogr.*, 207 (1981) 161.