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Note

Use of a peristaltic pump for recycling chromatography with a micro-column

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Recycle chromatography is a useful technique for obtaining high resolution separation. Two methods have been described so far. One is the so-called dual column system[1] where the columns are used alternately by valve switching, the other is the closed-loop method using a reciprocating pump[2] where the eluent is held in a "collect" part of the system and is returned to the column by altering the pumping direction. In this paper, a new method using a peristaltic pump is examined for the closed-loop method in microcolumn high-performance liquid chromatography (HPLC).

In the peristaltic pump system, silicone, fluororubber or poly(vinyl chloride) tubing has generally been used. In such cases, there are many restrictions on the organic solvents that can be used. Recently, a peristaltic pump has been developed which makes it possible to use PTFE tubing. In this case, almost all kinds of organic solvents can be used. We have now applied the pump to the recycle chromatography in microcolumn HPLC.

EXPERIMENTAL

Apparatus and materials

A Peri Flon PF-7 peristaltic pump (Nippon Rikagaku Kikai, Tokyo, Japan) was used, with a PTFE tubing of 0.1 mm I.D. and 1.8 mm O.D. A SPD-2AM spectrophotometric detector (Shimazu, Kyoto, Japan) was used together with a laboratory-made micro flow-through cell for microcolumn HPLC. As a column, a fused-silica tubing of 0.2 mm I.D. was used, in which Spherisorb ODS (Phase Separation) of particle diameter 5 μm was packed by the slurry packing technique[3].

Procedure

A block diagram of the instrumental set-up is shown in Fig. 1. The fused-silica tubing 3 and PTFE tubing 4 were first disconnected, and during the column conditioning the tube 3 was placed in an eluent reservoir. After the conditioning, the fused-silica tubing was inserted into a sample reservoir and about 0.1 μl of the sample solution was drawn into the tube by turning on the motor switch of the roller on the pump for a few seconds. Then the fused-silica tubing was again inserted into the

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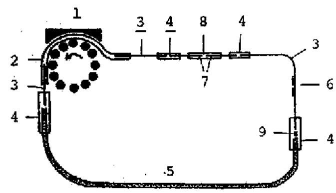

Fig. 1. Block diagram of the apparatus. 1 = Peristaltic pump; 2 = pump tubing of 0.1 mm I.D. and 1.8 mm O.D.; 3 = fused-silica tubing of 0.05 mm I.D.; 4 = PTFE connection tubing; 5 = microcolumn; 6 = micro flow-through cell; 7 = stainless-steel tubing of 0.2 mm I.D., 0.5 mm O.D. and length 15 mm; 8 = PTFE tubing of 0.4 mm I.D.; 9 = silica wool.

eluent reservoir and the chromatographic experiment was started. After placing the sample plug in the column, the fused-silica and PTFE tubings were connected and then the stainless-steel tubings 7 were deeply inserted into the PTFE tubing 8, as in Fig. 1. The insertion of the stainless-steel tubings into the PTFE tubing decreased the space in the PTFE tubing and consequently resulted in a slight back-pressure in the connecting tube between the flow-through cell and the top of the column; the development of bubbles in the flow-through cell was thus prevented.

RESULTS AND DISCUSSION

The flow-rate obtainable with a peristaltic pump can be changed by altering the I.D. of the pump tubing used or the speed of the roller on the pump. However, in order to minimize band broadening in the pump tubing, it is recommended to use as narrow an I.D. as possible, and the narrowest one currently available is 0.1 mm. On the other hand, the speed of roller should be as slow as practicable in order to reduce wear of the PTFE tubing. Thus there is an optimum flow-rate for each I.D. The value for the 0.1 mm I.D. tubing was about 1 μl/min; this was suitable for a 0.2 mm I.D. column, which packed with 5–10 μm particles showed the lowest height equivalent to a theoretical plate at that flow-rate.

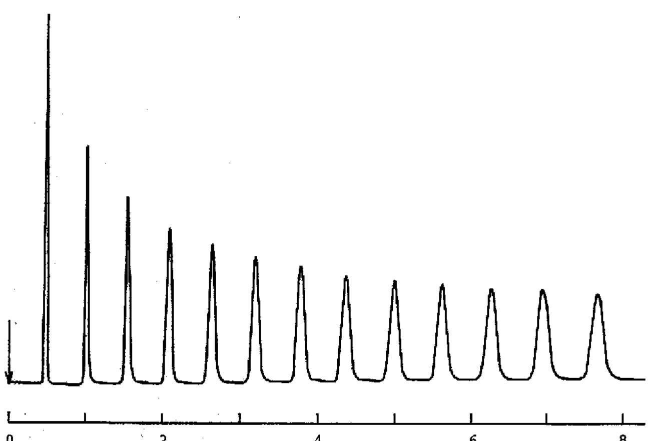

Fig. 2. An example of recycling by a closed-loop method using a peristaltic pump. Column: 20 m × 0.25 mm I.D. open-tubular column. Flow-rate: 30 μl/min. Sample: Co^{2+}. Detection: 600 nm. Eluent: water.

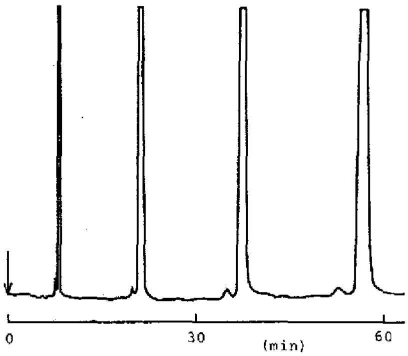

Fig. 3. Recycling chromatographic separation of an impurity in anthracene. Column: 5-μm ODS, 17 cm × 0.2 mm I.D. Eluent: 100% acetonitrile.

Before performing the practical separation, the possibility of recycling with the peristaltic pump was examined: a 20 m × 0.25 mm I.D. fused-silica tubing was connected instead of a real separation column and a flow-rate of 30 μl/min was applied by using a 0.5 mm I.D. PTFE pump tubing. The result is shown in Fig. 2, from which it is seen that a long period of recycling in microcolumn HPLC can be performed by using the Peri Flon peristaltic pump system.

Finally, a recycling chromatographic separation was examined with a column (17 cm × 0.2 mm I.D.) packed with a 5-μm ODS packing. The chromatogram obtained is shown in Fig. 3: an impurity in anthracene was successfully separated by recycling.

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