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INSTRUMENTATION FOR FAST MICRO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Instruments for fast micro high-performance liquid chromatography were assembled and their performance was evaluated in the reversed-phase mode. The system was operated at 300 kg/cm^2 with minimal band broadening, and the dependence of plate height on linear velocity was reduced by the use of small particle size phases.

INTRODUCTION

High-speed chromatographic separation is generally required for routine work that involves diagnosis, resolution of unstable chemical species or the kinetic study of reaction processes[1]. Although high-speed separation results in a large number of analyses in a given time, much large volumes of mobile phases are used than in conventional high-performance liquid chromatography (HPLC). This drawback can be overcome by micro-HPLC. However, efforts have been mostly devoted to the achievement of high resolution using a long column in micro-HPLC[2–7].

The aim of the work reported here was to assemble a micro-scale liquid chromatograph that could be operated with a short column at high pressure with minimal extra-column band broadening.

EXPERIMENTAL

A liquid chromatograph was constructed from a FAMILIC-300S pump (JASCO: Japan Spectroscopic, Tokyo, Japan), a micro valve injector ML-422 (0.02 μl; JASCO), a micro packed fused-silica column and a UV spectrophotometer UVIDEC-100II (JASCO) equipped with a modified flow cell (cell volume, 0.05 μl) as a detector.

Tube fittings and connecting tubing between each component for the high-pressure operation were carefully designed to minimize extra-column band broad-

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ening. The construction of the separation column is shown in Fig. 1. The fused-silica tubing (0.34 mm I.D. × 0.42 mm O.D.; Hewlett-Packard, Amstelveen, The Netherlands) was inserted into stainless-steel tubing (0.51 mm I.D. × 0.81 mm O.D.; Hakkoshoji, Tokyo, Japan) with an adhesive and fitted to 1/32 in. × 1/16 in. zero dead volume reducing union (Valco, Houston, TX, U.S.A.) before packing. The outlet of the fused-silica tubing was inserted in PTFE tubing (0.25 mm I.D. × 2 mm O.D.; Gasukuro Kogyo, Tokyo, Japan) and quartz wool was filled into the PTFE tubing as a filter. Packing materials were then slurried in acetonitrile or acetonitrile containing polyoxyethylene dodecyl ether (Kao Soap, Tokyo, Japan) and manually packed using a gas-tight syringe MS-GAN 025 (0.25 ml; Terumo, Tokyo, Japan). Silica ODS SC-01 (5 μm; JASCO), Develosil ODS-3 (3 μm; Nomurachemical, Seto-shi, Japan) and ODS-Hypersil (3 μm; Shandon, Cheshire, U.K.) were employed as packing materials. SC-01 is an irregular particle packing, the others are spherical particle packings. After packing, a tetrafluoroethylene filter Fluoropore FP-80 (0.80 μm pore diameter and 10 μm thickness; Sumitomo Electric, Osaka, Japan) was placed on the top of the column to prevent leakage of the packing materials. A Fluoropore filter of the right size (*ca.* 1/16 in.) was prepared in the laboratory from the commercially available one.

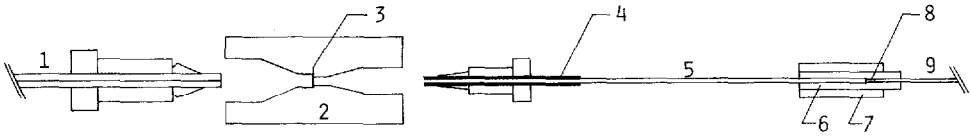

Fig. 1. Schematic diagram of the construction of the separation column and tube fittings. 1 = 57 μm I.D. × 1/16 in. O.D. (see text); 2 = 1/32 in. × 1/16 in. zero dead volume reducing union; 3 = fluoropore filter; 4 = stainless-steel tubing, 0.51 mm I.D. × 0.81 mm O.D.; 5 = fused-silica tubing, 0.34 mm I.D. × 0.42 mm O.D. (separation column); 6 = PTFE tubing, 0.25 mm I.D. × 2 mm O.D.; 7 = PTFE tubing, 2 mm I.D. × 4 mm O.D.; 8 = quartz wool; 9 = stainless-steel tubing, 50 μm I.D. × 0.30 mm O.D.

The flow cell of the detector was made of fused-silica tubing (0.26 mm I.D.) and connected to the outlet of the separation column by narrow-bore fused-silica tubing (10 cm × 55 μm I.D., 0.24 mm O.D.; Scientific Glass Engineering, Melbourne, Australia) and stainless-steel tubing (8 mm × 50 μm I.D., 0.30 mm O.D.; Erma Optical Works, Tokyo, Japan). The time constant of the detector was changed from 0.94 sec to 0.04 sec. Connecting tubing between the injector and the column was made of fused silica (6.3 cm × 57 μm I.D., 0.31 mm O.D.; Scientific Glass Engineering) inserted into stainless-steel tubing (6.3 cm × 0.5 mm I.D., 1/16 in. O.D.; Gasukuro Kogyo) with an adhesive to effect the ferrule connection with minimum dead volume. The total dead volume of the connecting system was *ca.* 0.5 μl.

The pump was generally operated in the constant-pressure mode because this gave a more stable flow-rate than the constant-flow mode. When necessary, the flow-rate of the mobile phase was determined by measuring the flow-rate of obtained effluent from the detector.

Reagents were obtained from Wako (Osaka, Japan), unless otherwise noted.

RESULTS AND DISCUSSION

HETP (height equivalent to a theoretical plate) values are inevitably increased by the high linear velocities, which is unsuitable for rapid resolution. However, we have found that the dependence of HETP on linear velocity is much reduced if fused-silica tubing is used as the column material in micro HPLC[8]: the maximum linear velocity was *ca.* 0.2 mm/sec using a 10-cm column packed with 5-μm particles and acetonitrile–water as the mobile phase, which was restricted by the low maximum pressure of operation (*ca.* 70 kg/cm^2). Fig. 2 shows the dependence of HETP on linear velocity and the time constant of the detector. Where the time constant is 0.04 sec, *ca.* 20 μm of HETP is obtained at *ca.* 1 cm/sec, which indicates that the peak width of a solute eluting in 60 sec is *ca.* 3 sec. This explains why the curve obtained with the large time constant (0.94 sec) deviates more from the one obtained with the smaller time constant at higher linear velocities. Divergence of values of HETP at lower linear velocities may originate from a difference between the efficiencies of the two columns. The minimum HETP value was *ca.* 8 μm.

Fig. 3 shows the effect of particle diameter on the relationship between HETP and linear velocity. The results suggest the use of 3-μm particles for fast micro-HPLC rather than 5-μm particles.

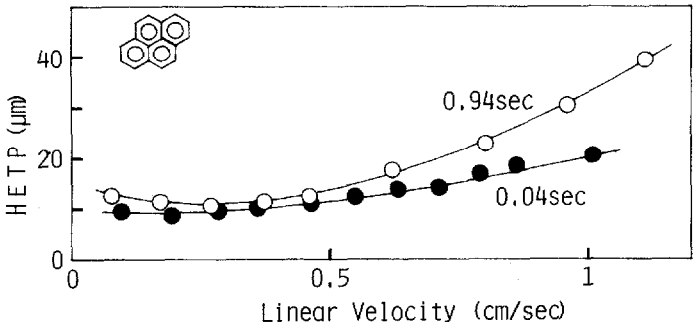

Fig. 2. Dependence of HETP on linear velocity and the time constant. Column, Develosil ODS-3, 10 cm × 0.34 mm I.D.; mobile phase, acetonitrile–water (7:3); sample, pyrene.

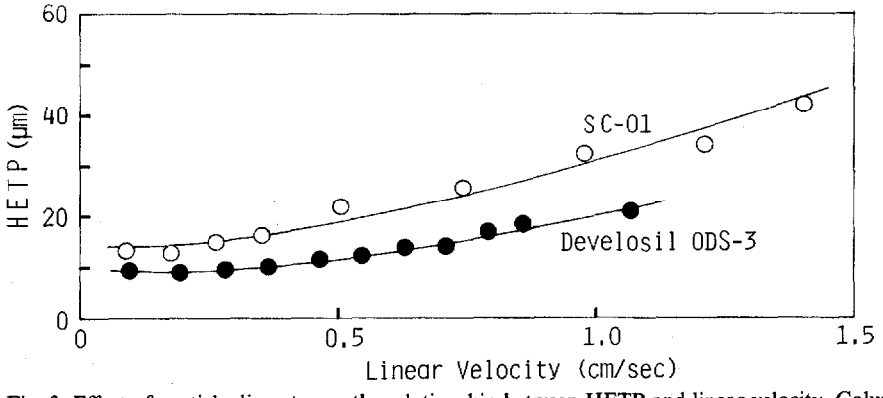

Fig. 3. Effect of particle diameter on the relationship between HETP and linear velocity. Column, 10 cm × 0.34 mm I.D.; mobile phase, acetonitrile–water (7:3). Sample, pyrene; time constant, 0.04 sec.

Fig. 4 demonstrates a separation of polynuclear aromatic hydrocarbons (PAHs) on a 10-cm column. Fourteen PAHs are separated in 4 min. The inlet pressure was 250 kg/cm^2 and the flow-rate was 27 μl/min.

Fig. 5 demonstrates a separation of eight PAHs on a 5-cm column in 45 sec. Short columns packed with 3-μm particles are favourable for rapid separations.

Fig. 6 shows a rapid separation (30 sec) of standard mixtures of caffeine, aspirin (Sigma, St. Louis, MO, U.S.A.) and phenacetin.

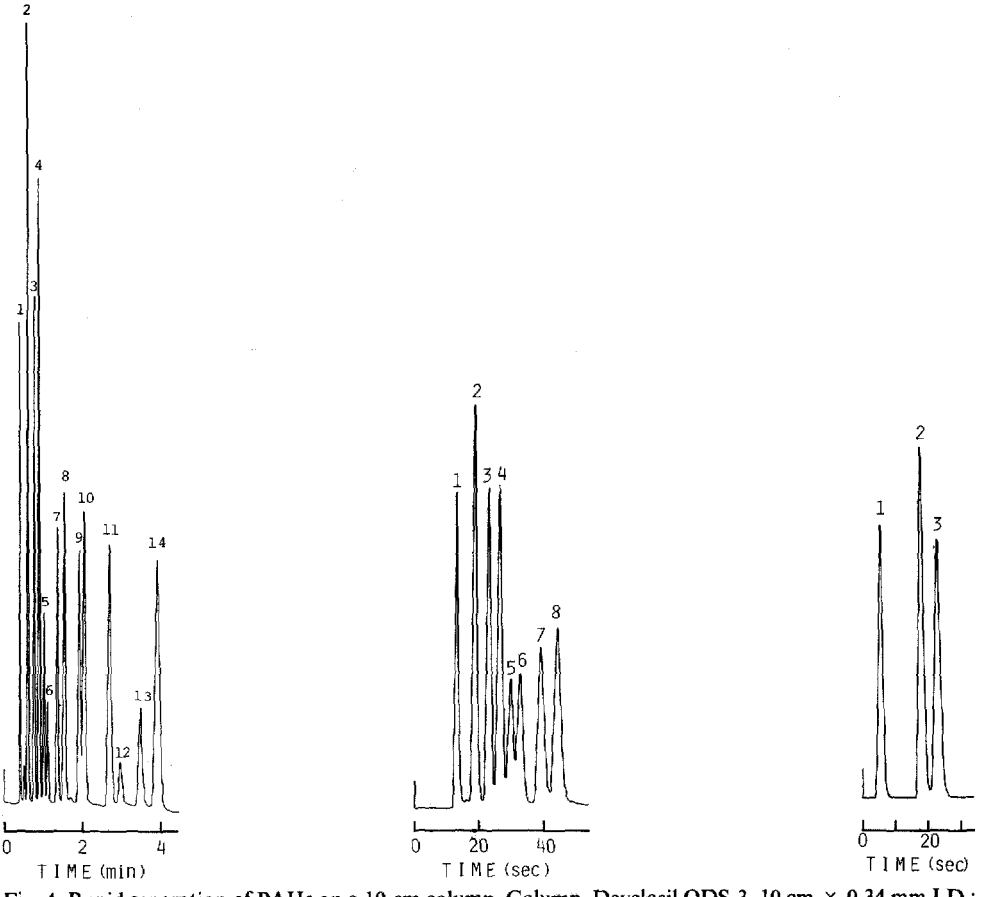

Fig. 4. Rapid separation of PAHs on a 10-cm column. Column, Develosil ODS-3, 10 cm × 0.34 mm I.D.; mobile phase, acetonitrile–water (7:3); inlet pressure, 250 kg/cm^2 (27 μl/min); samples: 1 = benzene; 2 = naphthalene; 3 = biphenyl; 4 = fluorene; 5 = phenanthrene; 6 = anthracene; 7 = fluoranthene; 8 = pyrene; 9 = *p*-terphenyl; 10 = 9-phenylanthracene; 11 = chrysene; 12 = perylene; 13 = benzo(*a*)pyrene; 14 = 1,3,5-triphenylbenzene. Wavelength of UV detection, 254 nm; time constant, 0.04 sec; column temperature, 25°C.

Fig. 5. Rapid separation of PAHs on a 5-cm column. Column, Develosil ODS-3, 5 cm × 0.34 mm I.D.; mobile phase, acetonitrile–water (7:3); inlet pressure, 150 kg/cm^2 (32 μl/min); samples as in Fig. 4; wavelength of UV detection, 254 nm; time constant, 0.04 sec; column temperature, 26°C.

Fig. 6. Separation of typical components in a pharmaceutical preparation. Column, ODS-Hypersil, 5 cm × 0.34 mm I.D.; mobile phase, acetonitrile–water–orthophosphate (18:81.9:0.1); inlet pressure, 300 kg/cm^2 (51 μl/min). Samples; 1 = caffeine; 2 = aspirin; 3 = phenacetin. Wavelength of UV detection, 225 nm; time constant, 0.04 sec; column temperature, 26°C.

CONCLUSION

Rapid separations were successfully performed on a micro-scale liquid chromatograph. The flow-rates used in micro-HPLC are much more smaller than those used in conventional HPLC, and are suitable for use in routine work.

REFERENCES

1 R. P. W. Scott, P. Kucera and M. Munroe, *J. Chromatogr.*, 186 (1979) 475.
2 P. Kucera and G. Manius, *J. Chromatogr.*, 216 (1981) 9.
3 F. J. Yang, *J. Chromatogr.*, 236 (1982) 265.
4 T. Takeuchi and D. Ishii, *J. Chromatogr.*, 238 (1982) 409.
5 D. Ishii and T. Takeuchi, *J. Chromatogr.*, 255 (1983) 349.
6 T. Takeuchi, D. Ishii and S. Mori, *J. Chromatogr.*, 257 (1983) 327.
7 Y. Hirata and K. Jinno, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 196.
8 T. Takeuchi and D. Ishii, *J. Chromatogr.*, 213 (1981) 25.