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SUPERCritical FLUID CHROMATOGRAPHY WITH MICRO PACKED COLUMNS AND CARBON DIOXIDE AS A MOBILE PHASE*

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SUMMARY

Supercritical fluid chromatography with micro packed fused-silica columns and carbon dioxide is investigated. The instruments were carefully constructed to operate under high pressures with minimum dead volume. Polynuclear aromatic hydrocarbons and styrene oligomers are rapidly separated on a micro column of 150 × 0.34 mm I.D.

INTRODUCTION

Supercritical fluid chromatography (SFC) was first demonstrated experimentally by Klesper *et al.*¹ and its superiority has recently been noted by many researchers using open-tubular columns²⁻⁵ or packed high-performance liquid chromatographic (HPLC) columns⁶. The principles, column performance, instrumentation and applications of SFC have been reviewed⁷⁻¹⁰.

Supercritical fluids have liquid-like densities, but lower viscosities and higher diffusivities, resulting in minimum plate heights at higher linear velocities compared with HPLC. *n*-Pentane and carbon dioxide have frequently been employed as the mobile phase, although various mobile phases can be employed to improve selectivity. The majority of previous studies have dealt with large particle column packings or open-tubular columns to minimize pressure drops across the column which would cause unusual peak broadening or distortion. Gere *et al.*⁶ found that the column pressure drop was not a problem within the constraints of their experimental conditions.

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This paper describes instrumentation for SFC using micro columns packed with fine particle packing materials. Carbon dioxide was selected as the mobile phase since it is non-toxic, inexpensive, is not flammable and has a low critical temperature (31°C).

EXPERIMENTAL

The chromatograph used was a micro high-performance liquid chromatograph¹¹ which had been modified to permit operation with carbon dioxide as the mobile phase (Fig. 1). A Familic-300S (JASCO: Japan Spectroscopic Co., Tokyo, Japan) was employed as the pump. A cylinder of carbon dioxide with a siphon supplied liquid carbon dioxide at ambient temperature to the pump through a 2- μm stainless-steel frit. The pump head was cooled with solid CO_2 , which improved the pumping efficiency. The Familic-300S pump was operated in the constant-pressure mode.

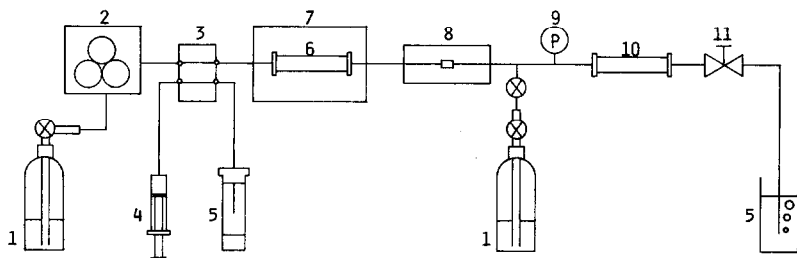


Fig. 1. Diagram of the apparatus for SFC. 1 = Carbon dioxide cylinder; 2 = pump (Familic-300S); 3 = micro valve injector; 4 = sample; 5 = waste reservoir or drain; 6 = separation column; 7 = oven; 8 = UV detector; 9 = pressure gauge; 10 = column for back pressure; 11 = metering valve.

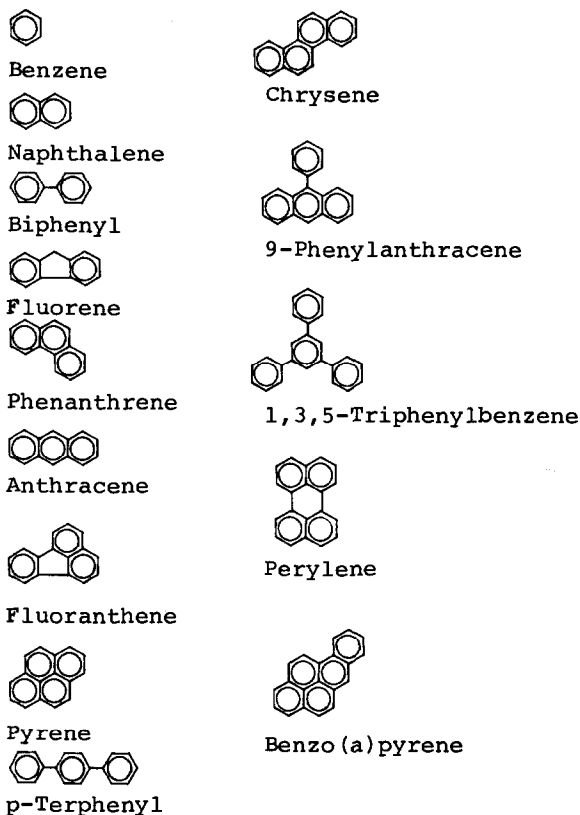
The sample was loaded by a micro valve injector ML-422 (JASCO), whose connecting parts were modified to minimize dead volume. The injector permitted loading of 0.02 μl of a sample solution with good reproducibility under high pressure, and withstood up to 350 kg/cm^2 .

Fused-silica tubing was selected as the column material since it gave good results owing to its inert and smooth surface¹². For operation under high pressure, fused-silica tubing of 0.34 mm I.D. \times 0.42 mm O.D. (Hewlett-Packard, Avondale, PA, U.S.A.) was cemented into stainless steel tubing of 0.51 mm i.d. \times 0.81 mm O.D. (Hakkoshoji, Tokyo, Japan) with an epoxy-resin adhesive, Standard Araldite (CIBA-Geigy, Basle, Switzerland), which allowed high-pressure ferrule-type connection of the separation column. The construction of the separation column and tube fittings are shown in Fig. 2. The inlet of the column was connected to a 1/32 in. \times 1/16 in. zero-dead-volume reducing union (Valco, Houston, TX, U.S.A.) and to the micro valve injector through connecting tubing (57 μm I.D. \times 1/16 in. O.D.). Fused-silica tubing of 57 μm I.D. \times 0.31 mm O.D. was obtained from SGE (Melbourne, Australia) and stainless-steel tubing of 0.5 mm I.D. \times 1/16 in. O.D. from Gasukuro Kogyo (Tokyo, Japan). The former tubing was cemented into the latter with Standard Araldite, as was the separation column. The outlet of the column was fitted to a 1/32-in. zero-dead-volume union (Valco) and connected to the detector. Packing

(Hakkoshoji) was employed to protect curved parts of fused-silica tubing. The time constant of the detector was changed to 0.04 sec in order to permit rapid detection.

The second carbon dioxide cylinder located downstream of the separation column in Fig. 1 was employed for priming carbon dioxide into the flow line between the separation column and the drain. The above system permitted immediate increase of the outlet pressure. However, the second cylinder can be eliminated since the vapour pressure of carbon dioxide can be introduced from the first cylinder by use of a T-joint. Moreover, the outlet pressure could be equilibrated within 20 min without saturation of carbon dioxide vapour if tube fittings and connecting tubing with small dead volumes were employed. The outlet pressure was controlled by a packed column (3 μm , 230 \times 0.34 mm I.D.) and a metering valve 1315G4B (HOKE, Creskill, NJ, U.S.A.). It was monitored by a pressure gauge PG-M100 (JASCO).

Liquefied carbon dioxide used throughout the experiments was of ordinary food grade and supplied by Sogoyozai (Nagoya, Japan). Polynuclear aromatic hydrocarbons (PAHs; Scheme 1) were obtained from Wako (Osaka, Japan) or Tokyo Chemical Industry (Tokyo, Japan). Polystyrene A-500 was obtained from Toyo Soda Manufacturing (Tokyo, Japan). Other reagents were obtained from Wako, unless otherwise noted.



Scheme 1. Structures of PAHs.

RESULTS AND DISCUSSION

The diffusion coefficients in SFC are larger than those of conventional HPLC mobile phases. Thus, the minimum plate height can be attained at a higher linear velocity in SFC than in HPLC⁶. Fig. 4 demonstrates Van Deemter curves for 5- μm ODS columns using acetonitrile and water or carbon dioxide as the mobile phase.

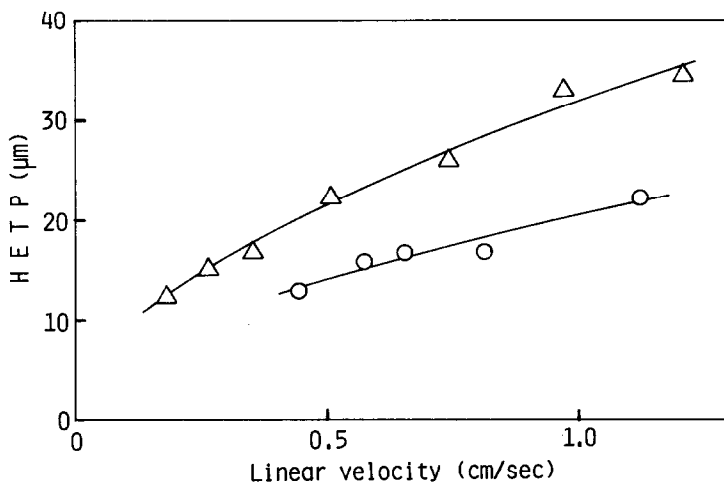


Fig. 4. Van Deemter curves for 5- μm ODS columns using acetonitrile-water (7:3) (Δ) and carbon dioxide (\circ) as mobile phases. Columns: silica ODS SC-01, 100 \times 0.34 mm I.D. (Δ); 150 \times 0.34 mm I.D. (\circ). Column temperatures: 25°C (Δ); 30°C (\circ). Sample: pyrene.

Pyrene was chosen as a test solute. The experiment with carbon dioxide was carried out in the liquid phase region near the critical temperature (30°C). The plate height obtained with carbon dioxide is smaller than that with the acetonitrile-water mixture at the same linear velocity and the dependence of the plate height on the linear velocity in the former system is smaller than in the latter. Similar column efficiency was also attained in SFC region. The results indicate that carbon dioxide as a mobile phase is more suitable for rapid separation than conventional mobile phases. The inlet pressure was varied from 110 to 180 kg/cm² for the measurements with carbon dioxide in order to obtain different velocities; the k' (capacity factor) values slightly decreased when the inlet pressure exceeded 130 kg/cm², which may be due to the increase in density of carbon dioxide.

Physical parameters which affect the retention time have been investigated in SFC with carbon dioxide^{6,7} and it was found that the retention time strongly depends on the density of the mobile phase. The influence of column temperature on the capacity factor is shown in Fig. 5. The inlet pressure was kept at 110 kg/cm². The results can be explained by the change of the density.

Fig. 6 demonstrates the separation of PAHs on an ODS column. The inlet pressure was 150 kg/cm² and the column temperature was 35°C. The solute peak shapes are symmetric and no deterioration of column efficiency due to the pressure drop across the column was observed under our experimental conditions. The noise

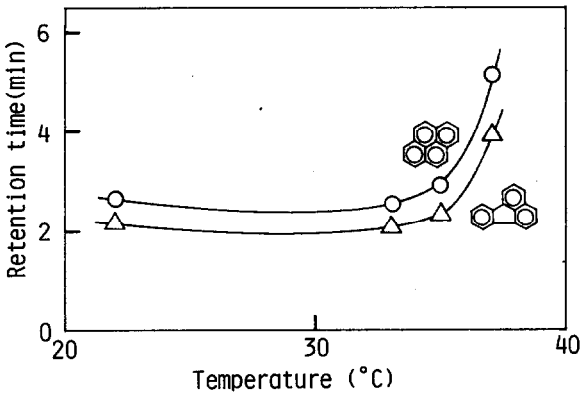


Fig. 5. Influence of column temperature on retention time. Column: silica ODS SC-01, 150 × 0.34 mm I.D. Mobile phase: carbon dioxide. Inlet pressure: 110 kg/cm².

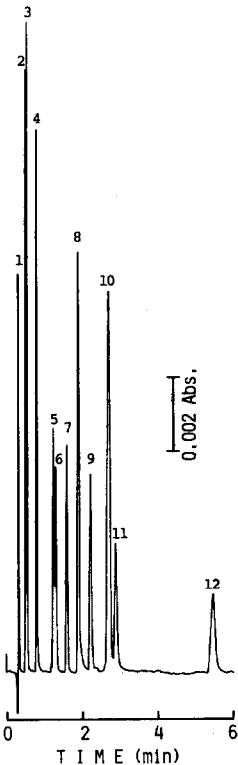


Fig. 6. Separation of PAHs on the ODS column. Column: silica ODS SC-01, 150 × 0.34 mm I.D. Mobile phase: carbon dioxide. Inlet pressure: 150 kg/cm². Column temperature: 35°C. Wavelength of UV detection: 245 nm. Samples: 0.02 μl of acetonitrile solution containing benzene (1), naphthalene (2), biphenyl (3), fluorene (4), phenanthrene (5), anthracene (6), *p*-terphenyl (7), 9-phenylanthracene (8), fluoranthene (9), 1,3,5-triphenylbenzene (10), pyrene (11) and chrysene (12).

level of the baseline is estimated to be 10^{-4} absorbance unit, which is better than that in previous work⁶. The improved results may be due to the difference in the flow cell structure. Effects of changes in density in the flow cell due to the pumping pulsation may be smaller for detection with the cross flow cell shown in Fig. 3 than with a conventional parallel flow cell. The sensitivity of detection was thus improved compared with the reported one⁶, e.g., the detection limit at a signal-to-noise ratio of 2:1 was 0.15 ng for naphthalene or 12 pg for anthracene.

Table I shows a comparison of the elution orders of PAHs in SFC, reversed-phase liquid chromatography (RPLC) and normal phase LC (NPLC). The structures of the PAHs examined are shown in Scheme 1. The elution order in SFC is primarily dependent on the ring number and rotatable solutes have a tendency to be eluted earlier than coplanar solutes with the same ring number, as shown in Table I. Similar behaviours are observed in NPLC using ODS as the stationary phase and isobutane as the mobile phase¹³. Although it is somewhat difficult to compare these data at different column temperatures, it may be true that silanol groups on the stationary phase significantly affect the retention of PAHs in SFC with carbon dioxide.

TABLE I
COMPARISON OF ELUTION ORDERS

Compound	SFC* (carbon dioxide; silica ODS SC-01; 35°C)	RPLC (acetonitrile-water 7:3; silica ODS SC-01; 24°C)	NPLC (isobutane; Develosil ODS-5; 23°C)	NPLC (isobutane; Develosil 100- 23°C)
Benzene	1	1	1	1
Naphthalene	2	2	2	2
Biphenyl	3	3	2	3
Fluorene	4	4	4	4
Phenanthrene	5	5	8	6
Anthracene	6	6	9	5
Fluoranthene	9	7	10	8
Pyrene	11	8	11	7
p-Terphenyl	7	9	6	12
Chrysene	12	10	12	10
9-Phenylanthracene	8	11	6	9
1,3,5-Triphenylbenzene	10	12	5	14
Perylene	R**	13	13	13
Benzo[a]pyrene	R	14	13	11

* Inlet pressure: 150 kg/cm².

** Strongly retained.

Fig. 7 shows the separation of PAHs on the NH₂ column. The PAHs exhibited similar retention behaviours as the ODS column. The sample was dissolved in methanol since use of acetonitrile resulted in peak broadening.

The separation of styrene oligomers on the ODS column is shown in Fig. 8. Although the detection wavelength was 205 nm, a very smooth baseline was observed, due to the transparency of carbon dioxide. It should be noted that diastereoisomers are also separated on the chromatogram.

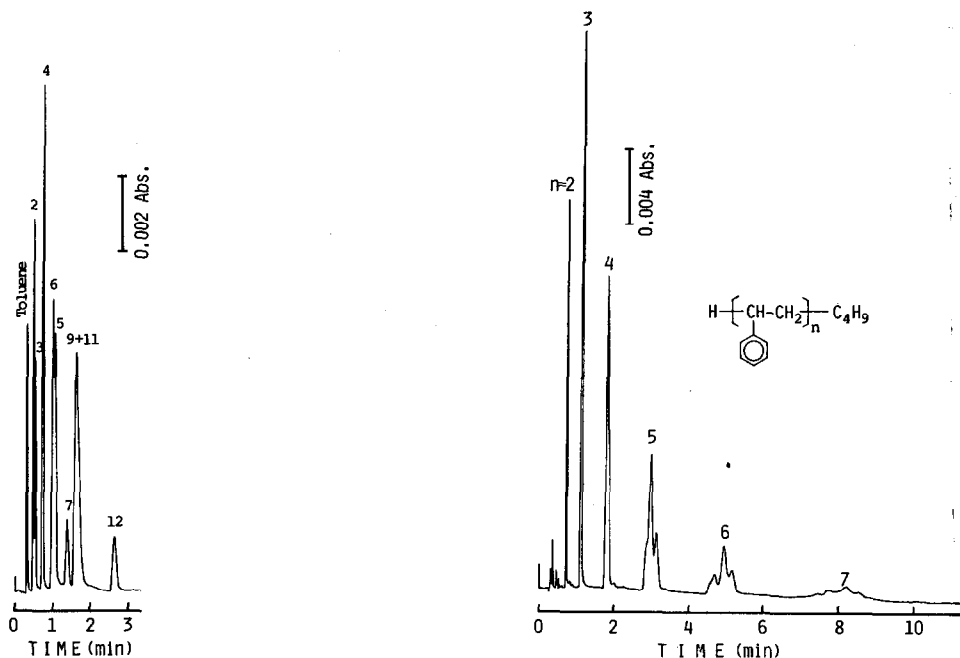


Fig. 7. Separations of PAHs on the NH₂ column. Column: Nucleosil 5NH₂, 150 × 0.34 mm I.D. Mobile phase: carbon dioxide. Inlet pressure: 170 kg/cm². Column temperature: 35°C. Wavelength of UV detection: 245 nm. Sample: see Fig. 6.

Fig. 8. Separation of styrene oligomers. Column: silica ODS SC-01, 150 × 0.34 mm I.D. Mobile phase: carbon dioxide. Inlet pressure: 150 kg/cm². Column temperature: 34.5°C. Wavelength of UV detection: 205 nm. Sample: Polystyrene A-500.

CONCLUSION

SFC with micro packed fused-silica columns and carbon dioxide was carried out and applied to the separation of PAHs or styrene oligomers. Rapid separations could be performed owing to the higher diffusivity of the mobile phase. A gradient elution method or a way of incorporating a modifier with carbon dioxide should be developed for microscale SFC so that various solutes may be subjected to SFC separations.

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