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EFFECT OF THE PORE STRUCTURE OF SILICA GEL ON RETENTION BEHAVIOUR IN PRESSURE PROGRAMMING SUPERCRITICAL FLUID CHROMATOGRAPHY

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SUMMARY

The retention behaviour of polar and non-polar compounds on packed ODS-silica gel columns of various pore structures was investigated using pressure programming supercritical fluid chromatography (SFC) with carbon dioxide as a mobile phase. The pressure programming was useful in packed SFC as well as in capillary SFC, and polystyrene oligomers were separated efficiently according to their polymerization degree. The retention behaviour of polar compounds such as pyridine was affected by both the pore structure and the carbon content of the ODS-silica gel.

INTRODUCTION

In recent years, supercritical fluid chromatography (SFC) has been recognized as a powerful technique for the separation of non-volatile and thermally labile compounds such as oligomers and polyaromatic hydrocarbons not amenable to gas chromatography (GC)¹⁻⁴. The retention of a solute in SFC is controlled by the composition and the density of the mobile phase, the nature of the stationary phase and the temperature. Pressure-density programming is advantageous for retention control in SFC, somewhat analogous to temperature programming in GC and gradient elution in high-performance liquid chromatography (HPLC). Although pressure programming is common in capillary SFC using a small capillary tube as a flow restrictor at the outlet, it is not so common in packed column SFC because the use of a regulation valve or back-pressure valve is popular to maintain the entire eluent system at high pressure, and therefore it is necessary to adjust the valve automatically for the pressure control⁵⁻¹⁴.

In packed column SFC, the addition of polar modifiers to supercritical fluids has been reported by several investigators to improve the retention behaviour, because alkyl-bonded silica columns, typically ODS-silica gel for HPLC, give some problems due to the residual silanol groups which interact with polar compounds and cause undesirable tailing and irreversible adsorption on the columns; great efforts have been made to solve this problem¹⁵⁻²¹.

In this work the chromatographic behaviour of ODS-silica gels having various

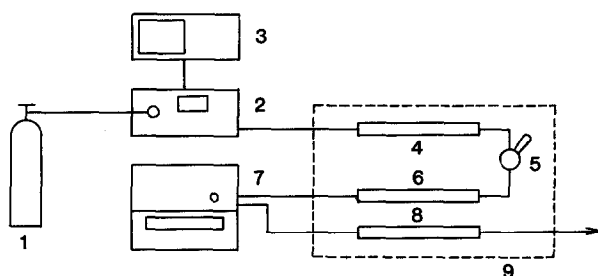


Fig. 1. Schematic flow diagram of the SFC apparatus. 1 = Carbon dioxide cylinder; 2 = pump; 3 = pump controller; 4 = precolumn; 5 = sample injector; 6 = separation column; 7 = UV detector; 8 = restrictor; 9 = oven.

pore structures was investigated by pressure programming SFC using carbon dioxide as the mobile phase without a modifier, to obtain basic knowledge for the development of completely inert SFC columns based on silica gel.

EXPERIMENTAL

Apparatus

The SFC system used is shown in Fig. 1. It consisted of a Shimadzu LC-6A pump the head of which was cooled, a Shimadzu LC-6A system controller for the pressure programming, a sample injector Model 7125 from Rheodyne with a 20- μ l sample loop, an UBILOG-60 UV detector from Oyo-Bunko Kiki, a capillary restrictor (20 cm \times 50 μ m I.D.) protected by a copper tube and a column oven from a Shimadzu LC-1 system.

Materials

The silica gels used for the preparation of ODS-silica gels were Super Micro Beads for HPLC (particle size 10 μ m, spherical) from Fuji-Davison Chemical and

TABLE I
PORE STRUCTURES AND ALKYL CONTENTS OF ODS-SILICA GELS

FD = Super Micro Bead from Fuji-Davison Chemical.

| | Specific surface area (m ² /g) | Pore volume (ml/g) | Pore diameter (Å) | ODS-silica gel | | | | | |
|---------|---|--------------------|-------------------|----------------|------|-------|------|-------|------|
| | | | | ODS-L | | ODS-M | | ODS-H | |
| | | | | %C | n* | %C | n* | %C | n* |
| FD-70 | 485 | 0.85 | 70 | — | — | — | — | 17.82 | 0.92 |
| FD-100 | 390 | 0.96 | 98 | 4.46 | 0.29 | 14.58 | 0.94 | 17.20 | 1.10 |
| FD-150 | 197 | 1.20 | 164 | 3.60 | 0.46 | 9.90 | 1.26 | 11.41 | 1.45 |
| FD-300 | 90 | 1.22 | 327 | 2.37 | 0.66 | 5.31 | 1.48 | 6.34 | 1.76 |
| FD-500 | 72 | 1.15 | 505 | 2.59 | 0.90 | 4.01 | 1.39 | 4.64 | 1.61 |
| FD-800 | 52 | 1.11 | 787 | 1.98 | 0.95 | 2.77 | 1.33 | 3.12 | 1.50 |
| FD-1000 | 32 | 1.00 | 1078 | 1.96 | 1.53 | 2.21 | 1.73 | 2.49 | 1.95 |

* Number of alkyl chains per 100 Å².

their properties are summarized in Table I along with the carbon contents and the number of alkyl chains per unit surface area (100 \AA^2). Silica particles of 10 \mu m in diameter were chosen instead of those of 5 \mu m because of the ease of column packing. Dimethyloctadecylchlorosilane was obtained from Shin-etsu Silicone Chemicals. Toluene from Wako Pure Chemical Industries was dried over calcium chloride before use as a solvent in the preparation of the ODS-silica gels. Polystyrene oligomers with average molecular weights of 300 (A-300) and 500 (A-500) used as samples were from Toyo Soda Manufacturing, and dissolved in hexane. As samples, pyridine from Kokusan Chemical Works and phenol from Nakarai Chemicals were dissolved in chloroform and methanol, respectively. Other reagents were of reagent grade and used without purification.

Preparation of ODS-silica gel packings

Silica gels summarized in Table I were modified with 2 mmol/g of dimethyloctadecylchlorosilane in toluene under reflux for 3 h with stirring. Three types of ODS-silica, that is low-carbon content (ODS-L), medium-carbon content (ODS-M) and high-carbon content (ODS-H), were prepared under different conditions such as with or without a catalyst. After cooling to room temperature, the ODS-silica gel was separated from the solvent by vacuum filtration using a sintered glass funnel, washed three times with benzene then, three times with dichloromethane and dried under vacuum at 110°C overnight. It was then subjected to elemental analysis (C, H, N) using a CHN Corder Type MT-3 from Yanagimoto. The ODS-silica gels were packed into a $250 \text{ mm} \times 4 \text{ mm}$ I.D. stainless-steel column with a slurry method using 2-propanol as a slurry solvent, and washed with methanol before attaching to the SFC apparatus.

RESULTS AND DISCUSSION

The solvating power of a supercritical fluid depends on the density and can be controlled as a function of pressure. Fig. 2 shows the effect of pressure programming (A) on the retention behaviour of a polystyrene oligomer compared with a constant-pressure mode of 230 kg/cm^2 (B) on a FD-300, ODS-H column. The pressure programming SFC was carried out by holding the pressure at 150 kg/cm^2 for 5 min after sample injection, followed by a linear pressure gradient up to 350 kg/cm^2 for 25 min and holding the pressure at 350 kg/cm^2 until all the sample had eluted. Styrene monomer was eluted first and other oligomers were eluted sequentially according to their polymerization degree. In the constant-pressure mode, the chromatographic peaks were broader and the sample retention increased as the polymerization degree of the oligomer increased. In the case of pressure programming, on the other hand, the peak shapes and the retention balance of the chromatogram were improved.

Three types of chromatogram of polystyrene oligomer obtained by the same pressure programming conditions as in Fig. 2A are shown in Fig. 3. The chromatograms were obtained on the high-carbon content ODS (ODS-H) silica gel columns prepared from FD-100, -300 and -1000. The separation profiles were better on the ODS-silica gels of smaller pore size and the samples were eluted more rapidly on the larger pore size columns. The poor shape of the later peaks was due to the presence of isomers in the peaks. Furthermore, the effect of the pore size of ODS-H silica gels

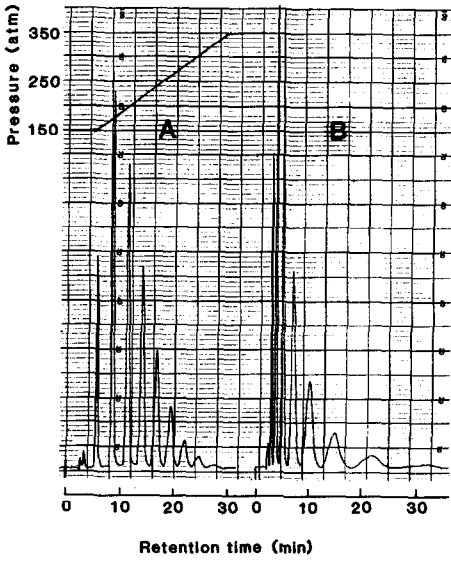


Fig. 2. Effect of pressure programming. Column: FD-300, ODS-H, 250 mm \times 4 mm I.D.; temperature; 50°C. Sample: polystyrene oligomer. (A) Pressure programming; (B) constant pressure, 230 kg/cm².

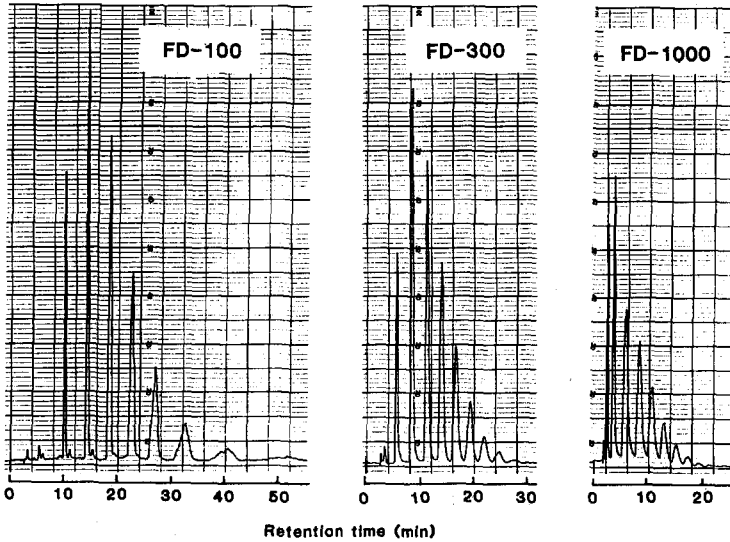


Fig. 3. Chromatograms of polystyrene oligomer on various ODS-silica gel columns. Column: ODS-H, 250 mm \times 4 mm I.D. Other conditions as in Fig. 2A.

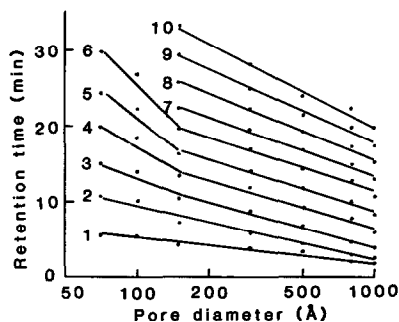


Fig. 4. Retention time of styrene oligomer vs. logarithm of pore diameter of ODS-silica gel. Conditions as in Fig. 3.

(logarithmic scale) on the retention behaviour of polystyrene oligomer under the same pressure programming conditions is shown in Fig. 4. The retention time of the sample increased as the polymerization degree increased, and decreased linearly as the pore diameter of the packings increased above the diameter of 150 Å. The retention behaviour of the styrene trimer ($n=3$) and heptamer ($n=7$), for instance, on the columns of different carbon contents of ODS-silica gel (ODS-L,M,H) is also shown in Fig. 5. The retention time of both samples decreased as the pore diameter of ODS-silica gel increased, and almost no dependence on the carbon content was observed. Therefore, the retention behaviour of non-polar compounds such as polystyrene oligomers, is suggested to be influenced by the pore structure much more

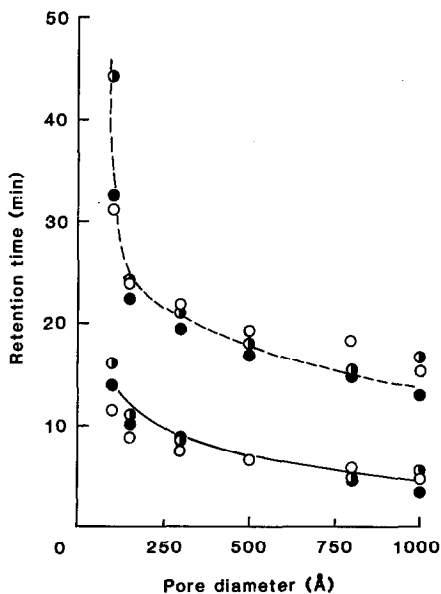


Fig. 5. Effect of the carbon content of ODS-silica gel on retention behaviour of polystyrene oligomer. ○, ODS-L; ◐, ODS-M; ●, ODS-H. —, Styrene trimer ($n=3$); - - -, styrene heptamer ($n=7$). Other conditions as in Fig. 2A.

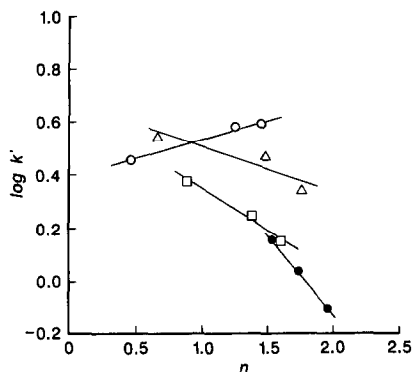


Fig. 6. Dependence of $\log k'$ for styrene pentamer ($n=5$) on the number of alkyl chains per 100 \AA^2 in various pore sizes of ODS-silica gel columns. \circ , FD-150; \triangle , FD-300; \square , FD-500; \bullet , FD-1000. Conditions: column, $250 \text{ mm} \times 4 \text{ mm}$ I.D.; column temperature, 50°C ; pressure, 230 kg/cm^2 .

than the hydrophobicity or the remaining silanol groups on the ODS-silica gel packings. The dependence of $\log k'$ for styrene pentamer ($n=5$) on the number of alkyl chains per 100 \AA^2 in various pore sizes of ODS-silica gel under constant pressure is shown in Fig. 6. The results provide evidence in support of the suggestion above and are inconsistent with those of reversed-phase liquid chromatography.

The residual silanol groups on chemically bonded silica gel tend to interact with polar compounds and give some problems such as irreversible adsorption or non-Gaussian peak shapes. The retention behaviour of polar compounds, for example pyridine and phenol on different carbon contents of ODS-silica gel was investigated in terms of the pore structure of the silica gel using a constant pressure of 230 kg/cm^2 at 50°C as shown in Figs. 7 and 8, respectively. The retention behaviour

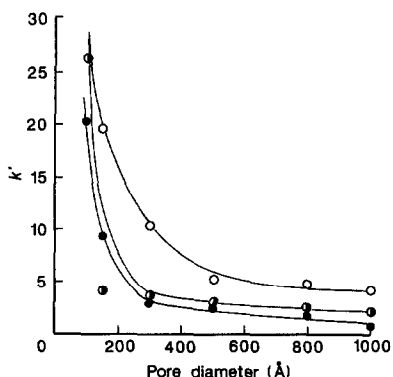


Fig. 7. Retention behaviour of pyridine on various ODS-silica gel columns: \circ , ODS-L; \bullet , ODS-M; \bullet , ODS-H. Other conditions as in Fig. 6.

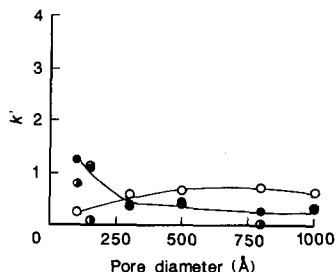


Fig. 8. Retention behaviour of phenol on various ODS-silica gel columns: \circ , ODS-L; \bullet , ODS-M; \bullet , ODS-H. Other conditions as in Fig. 7.

of pyridine was affected by both the pore structure and carbon content of the ODS-silica gel. This is presumably because more silanol groups remain on the ODS-silica gel packings having smaller pore sizes, that is larger specific surface areas, and also on those containing less bonded octadecyl. On the contrary, the retention behaviour of phenol was not affected by the pore structure or the carbon content of ODS-silica gel packings, presumably because phenol and silanol groups are both acidic and their interaction is much less than that of pyridine and silanol groups.

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