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SUPERCRITICAL FLUID CHROMATOGRAPHY WITH FUSED-SILICA PACKED COLUMNS

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SUMMARY

Supercritical fluid chromatography has been carried out with a 200- μm I.D. fused-silica packed column and initial results are presented. The advantage of using a small bore column over a wide bore column is the low flow-rate which allows direct heating of the mobile phase in the analytical column. Consequently, the instrumentation and operation of the chromatographic system are simplified. The separation ability of the system has been demonstrated for polystyrene oligomers using a silica gel column and *n*-hexane-ethanol as the mobile phase. The effect of modifiers on retention is also described.

INTRODUCTION

Supercritical fluid chromatography (SFC) has attracted much attention, because of the high efficiency and ability to separate relatively non-volatile, thermally unstable and high-molecular-weight solutes which cannot be analyzed by gas chromatography. Since the introduction of SFC by Klesper *et al.*¹, various types of columns have been employed.

A long, wide bore column packed with large particles was initially employed, requiring long analysis times even at relatively high flow-rates². Moreover, a complicated chromatographic system was needed to attain supercritical conditions at such flow-rates²⁻⁴. This may be one of the reasons for the slower development of SFC compared to high-performance liquid chromatography (HPLC). Recently, a conventional HPLC column packed with small particles⁵ and an open tubular column^{6,7} have been introduced into SFC. One advantage for using packed columns is that many types of packing materials developed for HPLC are available. On the other hand, a very high efficiency in terms of the number of theoretical plates can be generated with a longer open tubular column compared to a packed column, because of its small pressure drop^{8,9}. Low flow-rates are also characteristic of open tubular columns.

In this work we describe initial results with a small bore fused-silica packed column for SFC. The flow-rate is of the order of a few $\mu\text{l}/\text{min}$. The use of this column allows direct heating of the mobile phase in the analytical column without a thermal

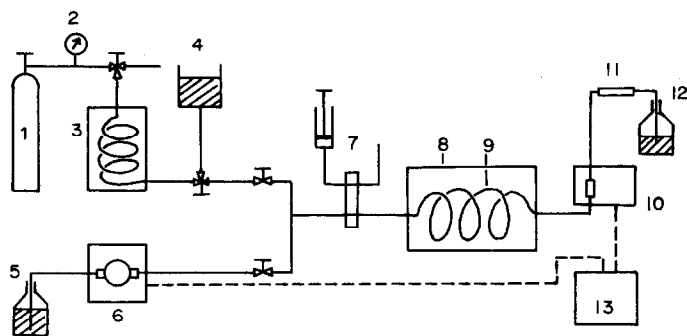


Fig. 1. Schematic diagram of the supercritical fluid chromatographic system. 1 = Nitrogen cylinder; 2 = pressure gauge; 3 = pump A; 4 and 5 = solvent reservoirs; 6 = pump B; 7 = micro valve injector; 8 = oven; 9 = column; 10 = UV detector; 11 = pressure restrictor; 12 = drain; 13 = recorder.

conditioning tube. Therefore, the instrumentation and operation of the SFC system can be simplified compared to that in earlier work.

EXPERIMENTAL

The chromatographic system shown in Fig. 1 comprised two pumps (Dupont LC-840, pump A; Jasco Twinkle, pump B), a Jasco micro valve injector (internal sample volume $0.1 \mu\text{l}$), a Shimadzu GC-5A gas chromatograph oven and a Jasco UVIDEC 100-III UV spectrophotometer. The mobile phase at room temperature was delivered to a fused-silica column ($58 \text{ cm} \times 200 \mu\text{m}$ I.D.) packed with silica gel (Develosil 100-10), which was placed in the oven. The column was prepared by means of the slurry method as previously reported¹⁰. Both sample injection and detection were carried out at room temperature. Pressure restriction was accomplished by connecting a short length of packed capillary to the detector outlet. A UV flow cell with an effective volume of about 20 nl was prepared from $100\text{-}\mu\text{m}$ I.D. fused silica. The flow-rate was measured by connecting a PTFE tube to the restrictor.

Constant pressure operation was carried out using pump A, which utilizes nitrogen pressure. This mode is similar to that employed Sie and Rijnders¹¹. The pressure was programmed by delivering the mobile phase from pump B to pump A after setting the pressure of pump A. A very smooth programme which was slightly concave rather than linear was employed. The rate of programming was adjusted by changing the flow-rate of pump B and/or the amount of mobile phase stored in pump A.

RESULTS AND DISCUSSION

SFC system with fused-silica packed column

In this work the mobile phase at room temperature was delivered directly to the analytical column and then heated to the supercritical state as described above, while all the SFC systems reported previously have employed a thermal conditioning tube. Fig. 2 shows the separation of a synthetic mixture of twelve polycyclic aromatic hydrocarbons on the column ($58 \text{ cm} \times 0.2 \text{ mm}$ I.D.) packed with silica gel (Develosil

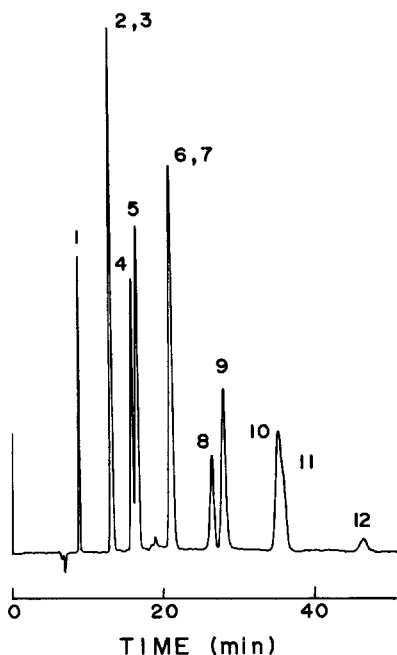


Fig. 2. Chromatogram of a synthetic mixture of polycyclic aromatic hydrocarbons. Peaks: 1 = naphthalene; 2 = phenanthrene; 3 = anthracene; 4 = fluoranthene; 5 = pyrene; 6 = triphenylene; 7 = benz[*a*]anthracene; 8 = benzo[*k*]fluoranthene; 9 = benz[*a*]pyrene; 10 = dibenz[*a,c*]anthracene; 11 = dibenz[*a,h*]anthracene; 12 = coronene. Column: 58 cm \times 0.2 mm I.D., Develosil 100-10 (10 μ m). Mobile phase: 10% ethanol in *n*-hexane. Temperature: 260°C. Inlet pressure: 40 atm. Detection wavelength: 254 nm.

100-10), using 10% ethanol in *n*-hexane as a mobile phase. Both the temperature and pressure were maintained constant. Most of the components are well resolved, although three pairs, *i.e.*, phenanthrene and anthracene, triphenylene and benz[*a*]anthracene and dibenz[*a,c*]anthracene and dibenz[*a,h*]anthracene, overlapped. Fig. 3 shows a Van Deemter plot for dibenz[*a,c*]anthracene. Here, the inlet pressure was kept constant and the resistance of the pressure restrictor changed. The minimum HETP was about 70 μ m, indicating that the number of theoretical plates was about 8000. Although this result indicates that the system is not optimized, it should be

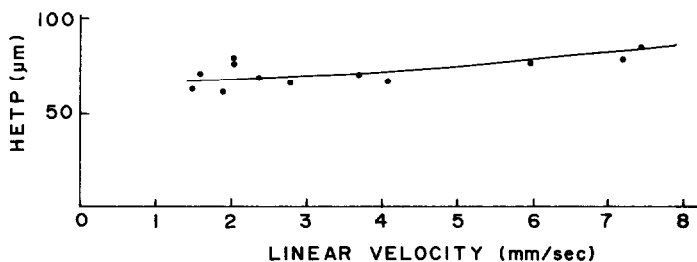


Fig. 3. Van Deemter plot for dibenz[*a,c*]anthracene. Inlet pressure: 50 atm. Other conditions as in Fig. 2.

noted that the HETP is relatively constant over a wide range of linear velocity.

On the basis of the above results, it may be concluded that the mobile phase can be heated effectively in the analytical column, as is the case with temperature-programmed elution in HPLC^{12,13}. Radial temperature gradients in such a small bore column seem to level out quickly. The system is also characterized by a very low flow-rate, typically a few $\mu\text{l}/\text{min}$. Therefore, the use of a small bore column simplified the instrumentation and operation of the SFC system, although extra-column band broadening has to be minimized.

Effects of temperature, pressure and modifier on retention

Separations of the mixture used in Fig. 2 were performed under the same conditions except for temperature. Below the critical temperature ($T_c = 234.2^\circ\text{C}$ for *n*-hexane) all the solutes were eluted together. This case may correspond to adsorption chromatography at elevated temperatures and with very strong mobile phases¹⁴. As the temperature was increased from T_c , the retention increased rapidly and then decreased slowly. The results can be accounted for by the change of the mobile phase density and the solute volatility as pointed out previously¹⁵. Further studies were not carried out.

Fig. 4 shows the effect of pressure on retention. Here, the inlet pressure was increased using the same pressure restrictor. The results indicate a drastic decrease in retention with increasing pressure. The shape of the curves may reflect the density

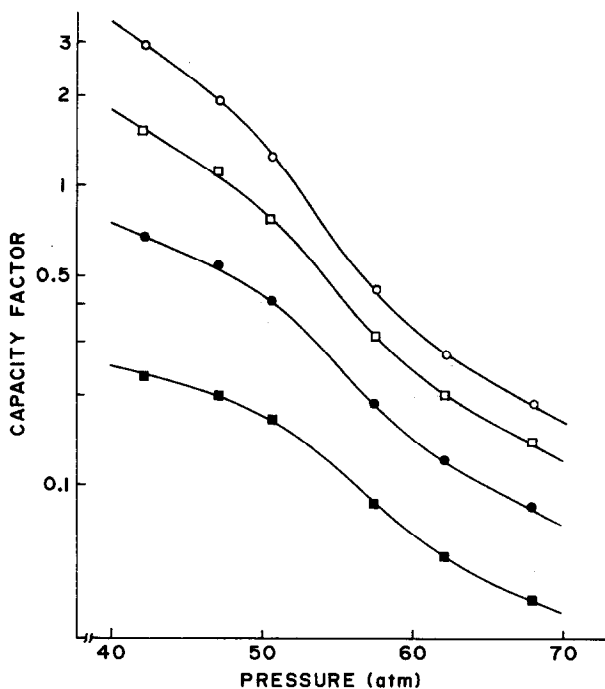


Fig. 4. Effect of pressure on retention. Solutes: ■, naphthalene; ●, anthracene; □, triphenylene; ○, dibenz[*a,c*]anthracene. Other conditions except for pressure as in Fig. 2.

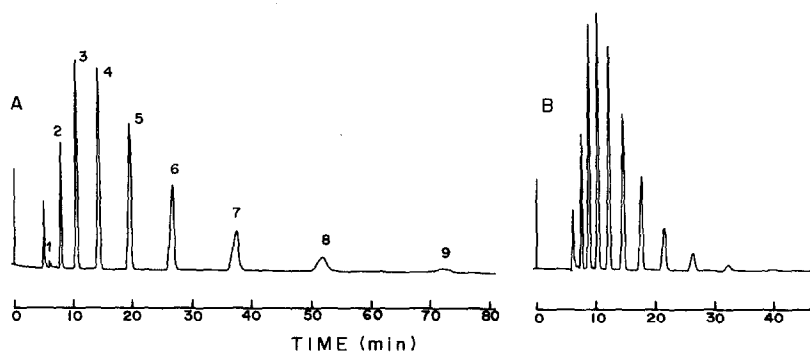


Fig. 5. Effect of modifier on the separation of polystyrene (MW 580). Column: 58 cm \times 0.2 mm I.D., Develosil 100-10 (10 μ m). Mobile phases: A, 10% ethanol; B, 10% isopropanol in *n*-hexane. Temperature: 260°C. Inlet pressure: 50 atm. Detection wavelength: 205 nm. The numerals on top of the peaks represent the degree of oligomerization *n*.

isotherm, *i.e.*, the portion indicating a rapid change in retention may correspond to the state near the critical point where the density changes quickly with pressure. Therefore, pressure programming may be most effective in this region for the separation of compounds with a wide range of capacity factors.

It has been suggested that many types of solvents can be used in SFC^{16,17}. In order to separate solutes with different polarities, an appropriate selection of the stationary and mobile phases is required in SFC as well as in HPLC. Here, the effect of modifier on retention was examined using a silica gel column and *n*-hexane as a base solvent. A polystyrenes mixture with an average molecular weight of (MW) of 580, $\text{CH}_3(\text{CH}_2)_3(\text{CH}_2\text{CHC}_6\text{H}_5)_n\text{H}$, (where *n* denotes the degree of oligomerization), was used as solute.

Alcohol has often been used as a modifier to reduce adsorption effects^{2,4}. Fig. 5 shows a comparison of the effects of ethanol and isopropanol on the separation of polystyrene oligomers. The retention increased with increasing concentration in both

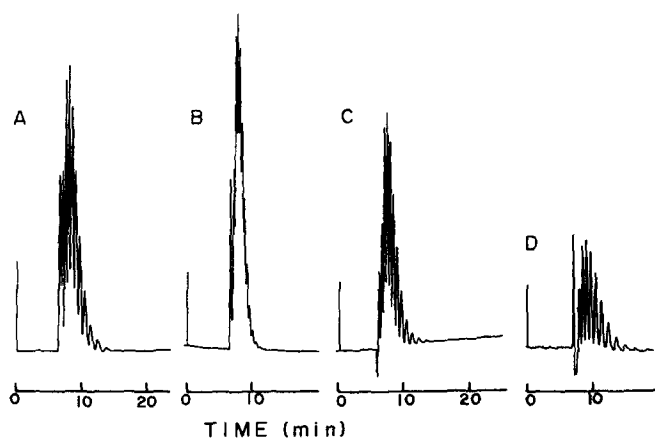


Fig. 6. Effect of modifier on the separation of polystyrene (MW 580). Mobile phases: A, no addition; B, dioxane; C, diisopropyl ether; D, ethyl acetate (each 10% in *n*-hexane). Other conditions as in Fig. 5.

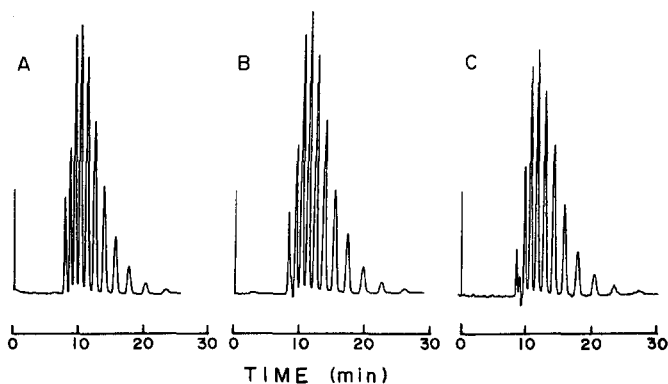


Fig. 7. Effect of tetrahydrofuran on the separation of polystyrene (\overline{MW} 580). Mobile phases: A, 5; B, 10; C, 20% THF in *n*-hexane, respectively. Other conditions as in Fig. 5.

cases, but the effect was larger with ethanol than isopropanol over the concentration range 0–20%.

Fig. 6 also shows the effect of modifier on retention. Here, dioxane, diisopropyl ether and ethyl acetate were used. Dioxane decreased the retention drastically^{18,19}, while ethylacetate increased the retention slightly. Diisopropyl ether had little effect on the separation compared to pure *n*-hexane. The effect of the concentration of tetrahydrofuran (THF) was also examined as shown in Fig. 7. Although THF is one of the best solvents to solubilize polystyrene, the retention was increased upon adding THF to *n*-hexane when compared with Fig. 6A. Fig. 7 also shows that the influence of THF on the retention is small; in practice retention times increased only slightly with increasing concentration. These results indicate that the nature of the super-

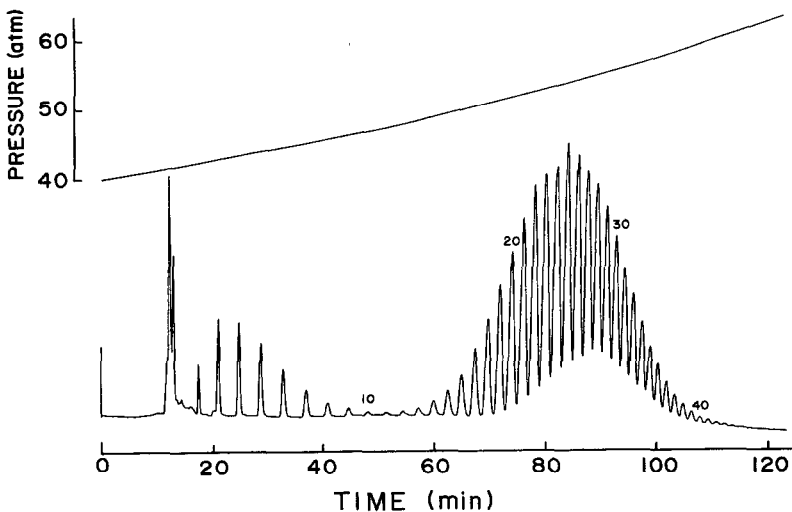


Fig. 8. Pressure-programmed elution of a mixture of polystyrenes of \overline{MW} 580 and 2900. Column: 58 cm \times 0.2 mm I.D., Devclosil 100-10 (10 μ m). Mobile phase: 5% ethanol in *n*-hexane. Temperature: 250°C. Detection wavelength: 205 nm.

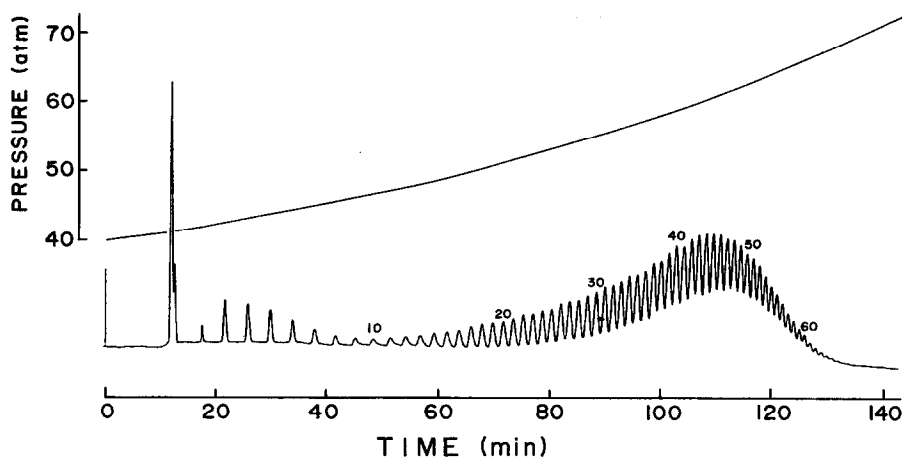


Fig. 9. Pressure-programmed elution of a mixture of polystyrenes of \overline{MW} 580 and 5700. Conditions as in Fig. 8.

critical fluid is considerably different from that of the normal liquid. Although a silica gel column was used exclusively here, further study suggested that an appropriate selection of stationary phase as well as mobile phases is required to attain optimum resolution of solutes of different polarities.

Separation of high-molecular-weight polystyrene oligomers

The above results showed that THF is a weaker solvent than *n*-hexane for the separation of polystyrene, and that injection of a large volume of THF has little effect on the resolution. Therefore, polystyrene samples were dissolved in THF and

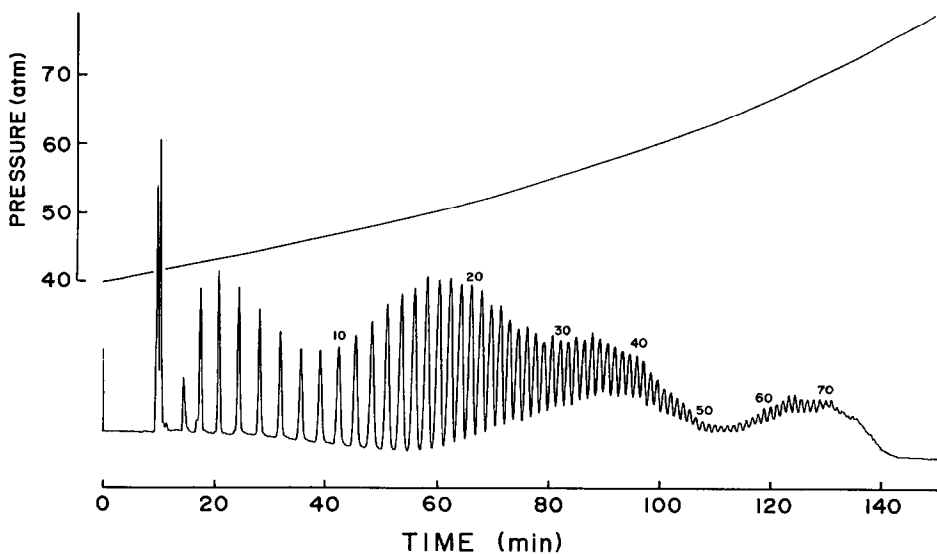


Fig. 10. Pressure-programmed elution of a mixture of polystyrenes of \overline{MW} 580, 2100, 4250 and 9000. Conditions as in Fig. 8.

diluted in *n*-hexane. Fig. 8 and 9 show chromatograms of polystyrenes of MW 2900 and 5700, respectively, where a polystyrene of MW 580 was spiked to determine the oligomerization degrees of individual oligomers. Pressure programming was carried out as described in the Experimental section. The programme was slightly concave, but very stable, and continuous programming could be accomplished with this system. The programming rate was adjusted as described. Any type of programme could be attained by adjusting the flow-rate of pump B.

Polystyrene has often been used as a standard to estimate the efficiency of SFC systems, because it contains a series of oligomers having a wide range of molecular weights. The highest degree of polymerization of polystyrene which has been resolved into individual oligomeric species is usually around 50^{20,21}. Here, oligomers with $n > 60$ were well resolved as shown in Fig. 9. Fig. 10 also shows a chromatogram of a mixture of polystyrene standards, *i.e.*, MW 580, 2100, 4250 and 9000. Individual oligomers with $n > 70$ are well resolved. Considering the moderate efficiency of the column used, that is, 8000 theoretical plates, the separation ability of the system could still be increased by further optimization.

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