

Applications of semi-micro supercritical fluid chromatography with gradient elution to synthetic oligomer separation

MAKOTO TAKEUCHI* and TOSHINORI SAITO

New Project Development Office, JEOL Ltd., Musashino 3-1-2, Akishima City, Tokyo (Japan)

ABSTRACT

A semi-micro supercritical fluid chromatograph which is capable of using carbon dioxide as eluent and a modifier at any concentration, gradient elution and column temperature programming and capable of keeping the column back-pressure at any constant value independent of the eluent flow-rate was applied to oligomer separations. For the separation of low-molecular-weight polymers such as methyl methacrylate, polystyrene and polymethylphenylsiloxane, it was demonstrated that temperature-programmed elution was a valuable technique for the separation of higher-molecular-weight polymers or more polar polymers, and a gradient of eluent composition was useful for obtaining good resolution over the whole range of oligomers.

INTRODUCTION

For high-efficiency separations of polymers by supercritical fluid chromatography (SFC), the use of a gradient method is essential in order to obtain equally spaced peaks¹⁻³. There are three methods in gradient elution, two of them involving increasing the solvent strength with increasing mobile phase density, that is, by increasing the column pressure or by decreasing the column temperature, and the other involving changing the eluent composition.

The gradient method with a decreasing temperature gradient is a convenient method and wide applications are to be expected when the eluent composition can be changed and the back pressure can be set at will. However, for a first survey analysis of unknown polymers, the gradient method with changing composition is more powerful if a good solvent for the solute is used as a modifier; by increasing the concentration of the modifier up to 100% under extreme conditions, it is fairly certain that all the injected solute in the column will be removed out.

In this paper we consider elution with a composition gradient and a temperature gradient using a semi-micro packed column of silica-based ODS.

EXPERIMENTAL

Apparatus

Separations were performed using a prototype model JEOL JSF-8800 SFC instrument which is shown schematically in Fig. 1.

Two "intelligent cascade pumps"^{4,5} are provided; one is for delivery of carbon dioxide (CAP-G03) with help of a pump-head cooler (CAP-L02) and the other is for delivery of liquid organic solvent as modifier (CAP-C02) with help of a pressurized solvent reservoir (CAP-RP01). Liquid carbon dioxide delivered through a siphon equipped with a high-pressure cylinder (10 l, sufficient for 1 month's work) is supplied through a gas purification unit to the pump CAP-G03.

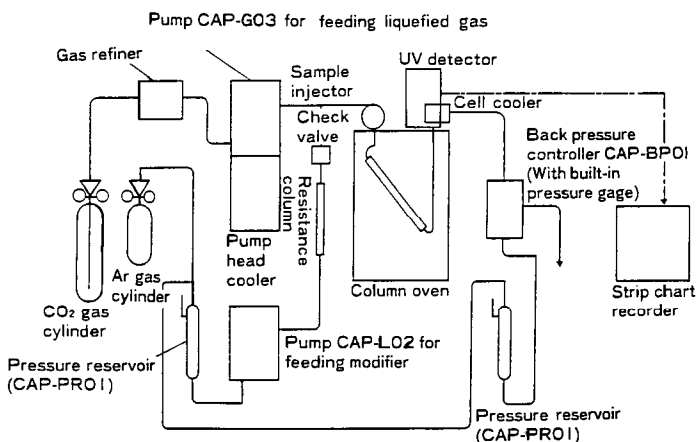


Fig. 1. Flow diagram of JSF-8800 supercritical fluid chromatograph. For explanation, see text.

The gas purification unit consisted of a serially connected silica column, an ODS column and a 0.01- μm particle filter. When "four nines" grade carbon dioxide was employed, this unit could be used for 4 months without a decrease in the purification power; to recover it from used columns, regeneration was possible by washing out the column with 500 ml of methanol delivered from a special valve mounted between the columns and the filter.

The temperature of the pump head of the CAP-G03 was kept constant between +3 and -3°C throughout. The mobile phase was carbon dioxide mixed with a second fluid as modifier. The composition of the mobile phase, expressed as the ratio of the carbon dioxide flow-rate to that of the second fluid, can be changed from 100% to 0%, but in most instances we kept the flow-rate of carbon dioxide constant while increasing that of the second fluid. This adjustment was easily made owing to the feature of the intelligent cascade pumps of flow-rate controllability independent of the loaded pressure and the compressibility of the fluid^{4,5}.

The two fluid lines were mixed at a T-union through a check valve for isolation of each mobile phase. A sample injector (Rheodyne 7520) with a volume of 0.2, 0.5 or 1 μl , depending on the size of the column used, was employed.

The columns employed were SFPak ODS 05S25 and 05M50 (JEOL), which were 250 mm \times 1.7 mm I.D. and 500 mm \times 1.0 mm I.D. semi-micro and micro columns packed with 5- μ m ODS.

A column oven which had the capability for regulated temperature programming within the range from 300°C to room temperature was fitted to an HP 5890 gas chromatograph (Hewlett-Packard) having no gas inlet and no detector, with necessary modifications for sample injection and a UV detector.

The UV detector (CAP-UV01) employed was equipped with a high-pressure cell for SFC (CAP-CC01) which had a 5-mm light path length and a 1- μ l cell volume. A thermo-electric cooling device was used to keep the carbon dioxide liquid.

To control the column bottom pressure, a back-pressure control unit (CAP-BP01) was employed at the outlet of the UV cell, consisting of a digital pressure-measuring device, an in-line filter and a constant-pressure release valve equipped with a temperature controller and a mechanical actuator. The constant-pressure release valve was provided with two lines, one as a pressure-released eluent line and the other as a solvent supply line which extended to the other pressurized solvent reservoir (CAP-RP01).

The solvent contained in the second reservoir should be a good solvent for the solute and miscible with the modifier, which was used for quickly restoring the initial operating conditions by flushing the residual solute from the column at the end of chromatographic run or for preventing precipitation of solute by flowing continuously throughout the running time, this procedure being necessary for stable operation of back-pressure regulation, especially when the solute was soluble in the supercritical phase but not in the modifier itself. The solvent reservoir (CAP-PR01) had a 70-ml capacity and three connection lines, for solvent, gas and drain in addition to the solvent outlet. The gas used was argon at about 7 kg/cm².

With a 0.22- μ m membrane filter provided in the solvent outlet of the reservoir, tedious processes such as degassing and filtering could be eliminated.

Mobile and stationary phases

Carbon dioxide was the main component of the eluent, to which a second fluid such as methanol, ethanol, *n*-hexane, dichloromethane, tetrahydrofuran, acetonitrile or hexafluoroisopropanol or their binary mixtures was added. The stationary phase was low-polarity end-capped ODS silica gel of particle diameter 5 μ m which had been developed for high-performance liquid chromatography (HPLC).

RESULTS AND DISCUSSION

As the experimental parameters of the system, the following four instrumental values can be selected independent of each other: flow-rate of carbon dioxide in the liquid phase, U_1 ; flow-rate of the second fluid (modifier), U_2 ; column temperature, T ; and back-pressure, P_b .

With column temperature-programmed elution (temperature gradient), T is changed from a certain value T_1 to a lower value T_2 at a rate $-\Delta T/\Delta t$, while the other parameters U_1 , U_2 and P_b are kept at constant. The primary effect of a temperature gradient on oligomer separation is to make the solvent strength increase with increasing mobile phase density according to the decrease in the column temperature.

The change in temperature leads to variations in the linear velocity, V , and the other characteristics of the solute, mobile phase and even the stationary phase, but these are minor changes in comparison with the primary effect in most instances.

In gradient elution, the solvent strength is increased with time by changing the composition of the eluent, which can be achieved by increasing U_2 and/or by decreasing U_1 , while T and P_b are kept constant. The former elution method in which U_2 is changed will be called the "modifier gradient" method.

Fig. 2 shows a typical example of temperature gradient elution. The sample was a non-ionic surfactant, Triton X-165 (octylphenylpolyoxyethylene). The experimental conditions were as follows; carbon dioxide, $U_1 = 150 \mu\text{l}/\text{min}$; modifier, ethanol, $U_2 = 25 \mu\text{l}/\text{min}$; $P_b = 169 \text{ kg}/\text{cm}^2$; $T_1 = 120^\circ\text{C}$; $T_2 = 70^\circ\text{C}$; rate, $-2.5^\circ\text{C}/\text{min}$; column, $500 \times 1.0 \text{ mm}$ I.D. ODS-5; detection, UV (210 nm); and sample size, $0.5 \mu\text{l}$.

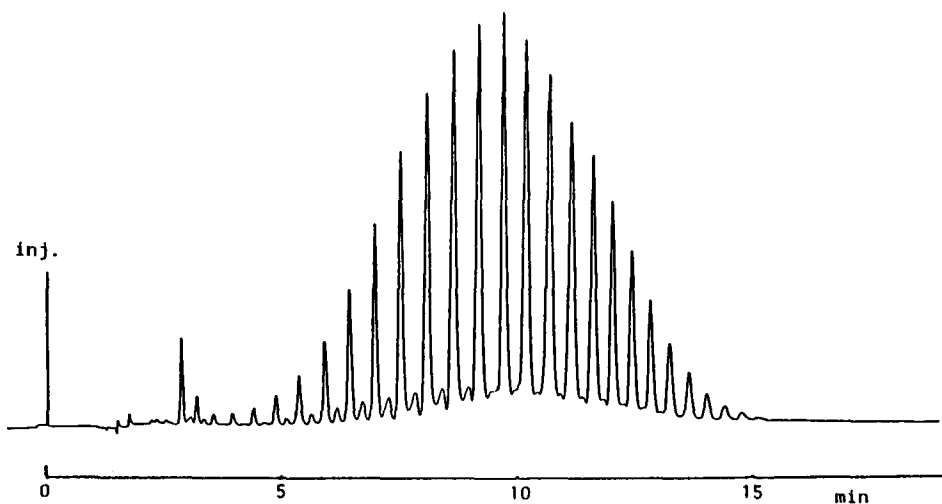
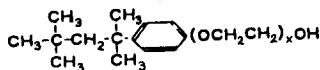


Fig. 2. SFC separation of octylphenylpolyoxyethylene (Triton X-165). Mobile phase, CO_2 at $150 \mu\text{l}/\text{min}$ and ethanol at $25 \mu\text{l}/\text{min}$; pressure at column outlet, P_b (initial) = $169 \text{ kg}/\text{cm}^2$; column temperature (initial), 120°C ; temperature gradient, $-2.5^\circ\text{C}/\text{min}$; column, $500 \times 1.0 \text{ mm}$ I.D. ODS-5; detection, UV (210 nm); sample size, $0.5 \mu\text{l}$.

Another example of temperature gradient elution applied to the analysis of methyl methacrylate (MMA) oligomer is shown in Fig. 3. The pressure dependence of the elution behaviour was also investigated on the same sample. Fig. 4 shows the change in retention time of fourteen arbitrarily selected peaks of oligomers *versus* the back-pressure change; these results were obtained from four successive chromatographic runs under the same conditions (except for the back-pressure change). The plots for thirteen of the peaks are parallel, which means that they were homologous linear-chain compounds of MMA. The line that was not parallel might indicate a different structure. This unknown peak was fractionated, then investigated with Fourier transform (FT)NMR, FT-IR and field desorption mass spectrometry

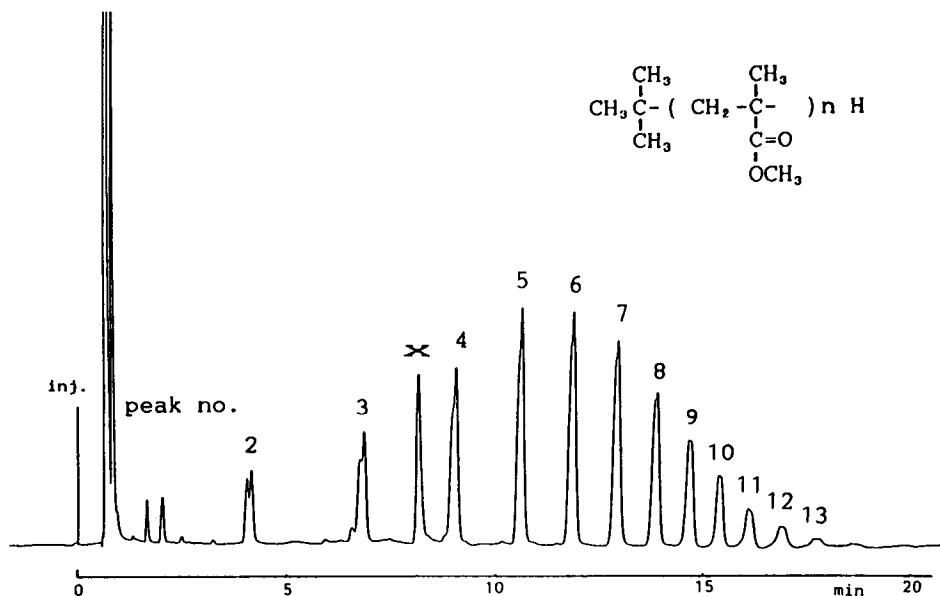


Fig. 3. SFC separation of MMA oligomers with temperature gradient elution. P_b (initial), 150 kg/cm²; eluent, carbon dioxide at 300 μ l/min and ethanol at 25 μ l/min; column temperature, $T_1 = 120^\circ\text{C}$, $T_2 = 70^\circ\text{C}$, rate = 3 $^\circ\text{C}/\text{min}$; detection, UV (210 nm).

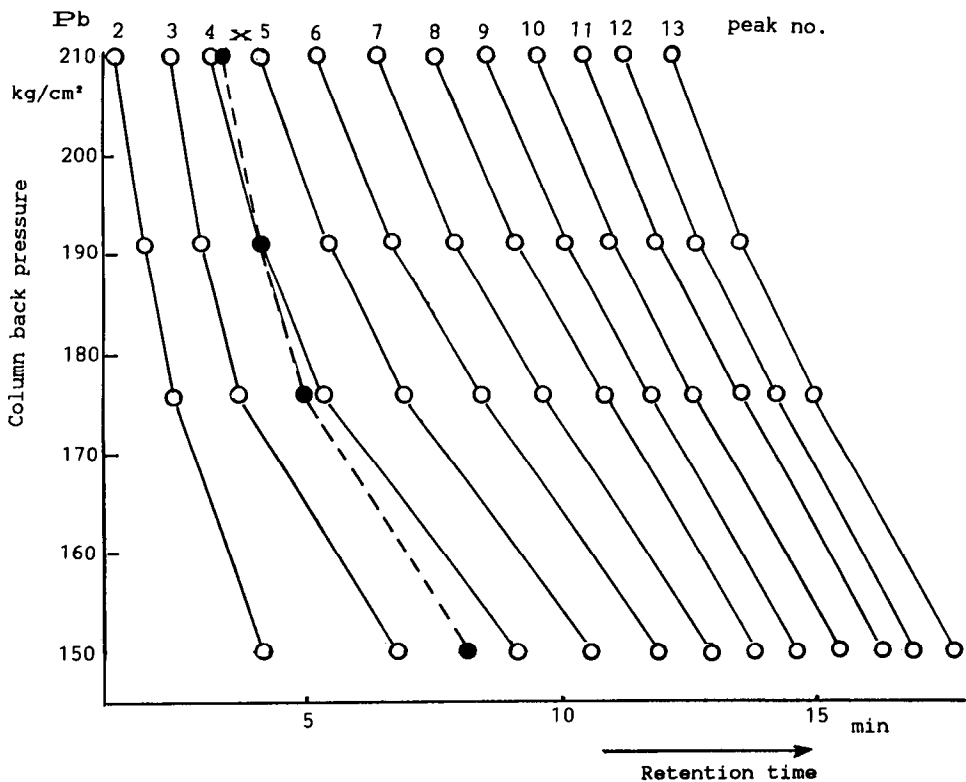


Fig. 4. Change in retention time of fourteen arbitrarily selected peaks in Fig. 3 depending on back-pressure, P_b . Other conditions as in Fig. 3.

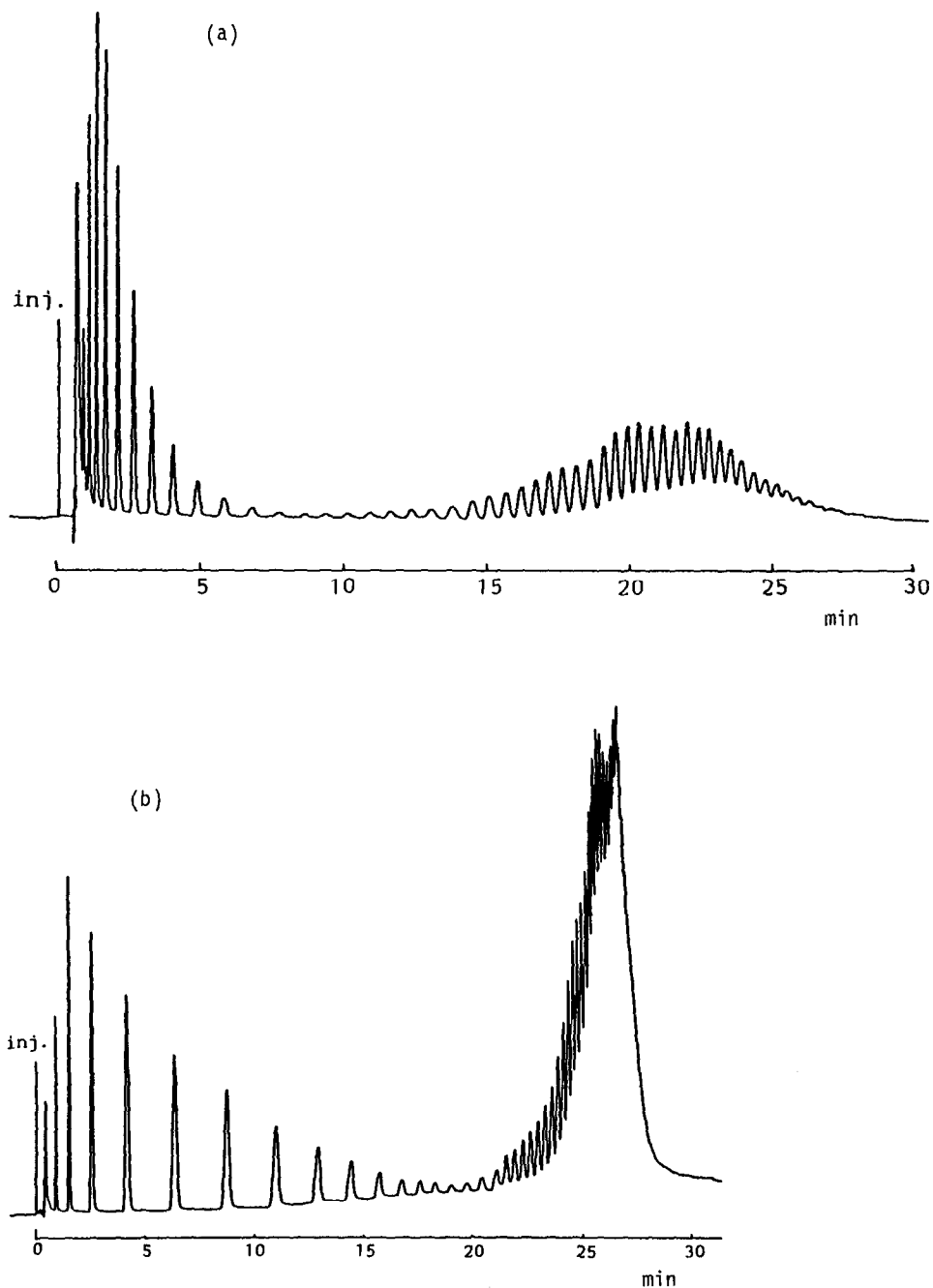


Fig. 5. SFC separation of styrene oligomers prepared by mixing two single dispersive oligomers, three parts of mean molecular weight (\bar{M}) = 600 and ten parts of \bar{M} = 4000. (a) With modifier gradient, carbon dioxide constant at 150 $\mu\text{l}/\text{min}$ and *n*-hexane increasing from 75 to 300 $\mu\text{l}/\text{min}$ in 30 min; (b) carbon dioxide gradient, decreasing from 300 to 100 $\mu\text{l}/\text{min}$ in 25 min, then kept constant, *n*-hexane increasing from 100 to 300 $\mu\text{l}/\text{min}$ in 25 min, then kept constant.

(FD-MS) to determine its structure; these studies are still continuing, but the possibility was confirmed⁶. These results mean that temperature gradient elution has different features to pressure gradient elution, which is feasible for the pressure dependence analysis of oligomers consisting of components with different structures.

As shown in the above example, the temperature gradient method is useful and convenient for the analysis of oligomers that are non-polar or that have a molecular weight distribution that is not too large.

Unlike HPLC, in our SFC experiments column regeneration is rapid in most instances requiring only 3–5 min.

The flow-rates, U_1 and U_2 , and the back-pressure, P_b , were kept almost constant during temperature gradient elution. Only the density, ρ , and the linear velocity, v , of the eluent in the column were changed, but not in the cell of the UV detector, because the eluent was cooled quickly outside the oven. Consequently, the baseline movement was kept within a small range.

Fig. 5 shows two examples of composition gradient elution. The separation of styrene oligomers prepared by mixing two single dispersive oligomers, three parts of $\bar{M} = 600$ and ten parts of $\bar{M} = 4000$, were performed by using *n*-hexane containing 15% of ethanol as a modifier.

Fig. 5a shows the results of modifier gradient elution with the flow-rate of carbon dioxide being kept at constant at 150 $\mu\text{l}/\text{min}$ and that of the second fluid was increased from 150 to 300 $\mu\text{l}/\text{min}$, the overall flow-rate of the eluent thus increasing from 300 to 450 $\mu\text{l}/\text{min}$. Correspondingly, if the total flow-rate is kept constant, when U_2 is increased U_1 must be decreased to the same extent.

Fig. 5b shows the results of such gradient elution. The separation of the oligomer component of $\bar{M} = 600$ was good, but not that of the $\bar{M} = 4000$ component; however, the peak height was increased considerably compared with the results in Fig. 5a. As shown in the above example, for a first analytical survey of unknown polymers, this elution method is useful for confirming the maximum elution time of the highest retained component.

We confirmed that the effect of the modifier plays an important role in the high-speed elution of oligomers by maintaining a reasonably good resolution, as suggested by Smith *et al.*⁷ that several fold faster analyses are attainable in some instances than without a modifier. However, it has to be considered that the binary fluid mixture may remain as single phase at the temperature, pressure and composition used during separation. To investigate this aspect, we observed the retention behaviour of non-polar oligomers with a composition gradient at various temperatures over a wide range of compositions. As an example, the separation of methylphenylpolysiloxane oligomers, obtained at $T = 180, 150, 120, 90$ and 30°C , is shown in Fig. 6. The content of ethanol relative to the total volume changed from 0.2 to 0.499 (v/v) in 32 min. The initial back-pressure was kept at constant at *ca.* 212 kg/cm^2 and the pressure drops, ΔP , in the column were also observed.

The binary fluids are liquid at 30°C and supercritical at 180°C as is clear from the physical constants of each compound: ethanol, $T_c = 243^\circ\text{C}$, $P_c = 63 \text{ kg}/\text{cm}^2$; carbon dioxide, $T_c = 31^\circ\text{C}$, $P_c = 73 \text{ kg}/\text{cm}^2$. However, the properties under other conditions in the above experiments change from the supercritical to the subcritical liquid phase, depending on the increase in the component of high critical temperature. The chromatograms obtained here show that the mobile phase remained as a single phase

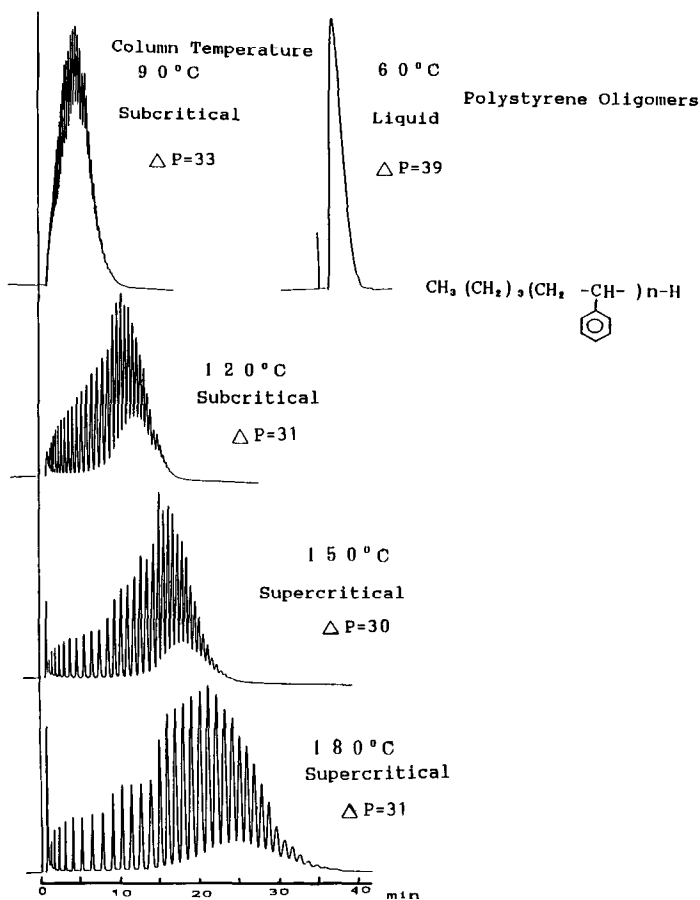


Fig. 6. SFC separation of methylphenylpolysiloxane oligomers with modifier gradient elution at 180, 150, 120, 90 and 30°C. Eluent, carbon dioxide at 300 $\mu\text{l}/\text{min}$, *n*-hexane increasing from 75 to 299 $\mu\text{l}/\text{min}$ in 32 min. ΔP = pressure drop in the column.

under all conditions employed. Moreover, a significant change in the pressure drop in the column was not observed over the whole range of conditions examined here, which means that there was virtually no increase in viscosity.

A similar example for styrene oligomers is shown in Fig. 7, obtained using a ternary fluid system of carbon dioxide, *n*-hexane and ethanol, the composition of which was changed from 0.5:0.45:0.05 to 0.33:0.60:0.07. The eluent is apparently supercritical at 180°C and liquid at 60°C. The chromatograms obtained show that elution was performed gradually and continuously depending on the changes in temperature and composition. The pressure drop in the column again remained almost constant, with only a small increase at 90°C and 60°C.

Generally, the physical properties of binary fluids can be discussed on the basis of the pressure-temperature-composition (P-T-X) diagram⁷, but this is complicated when it is not known if the binary system employed belongs to Type I at the given temperature and pressure.

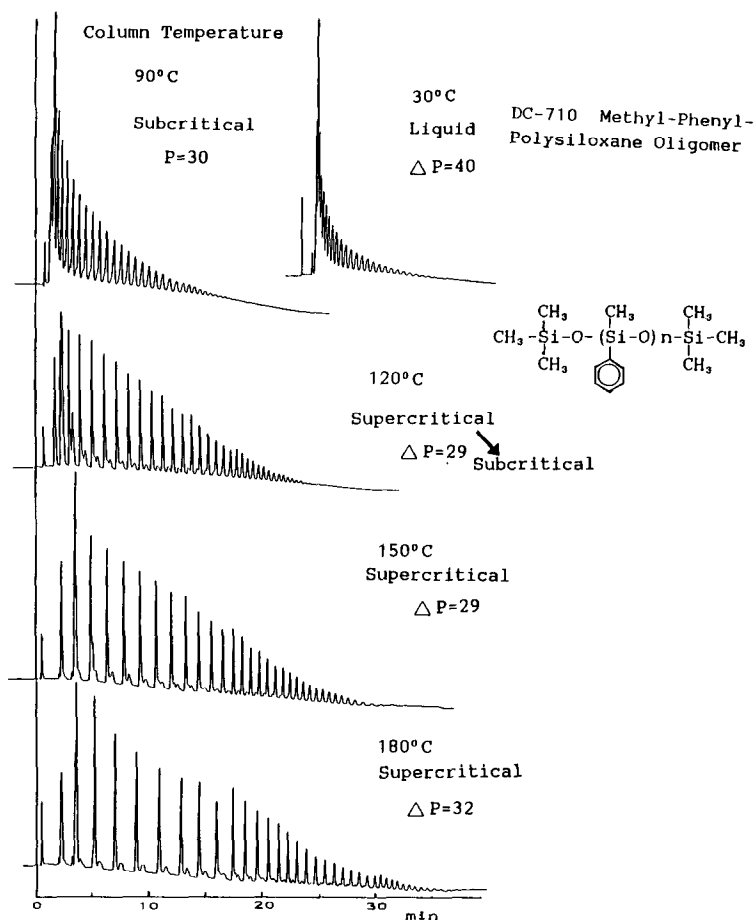


Fig. 7. SFC separation of styrene oligomers ($M = 2100$) with modifier gradient elution at 180, 150, 120, 90 and 60°C. Eluent, carbon dioxide at 150 $\mu\text{l}/\text{min}$, *n*-hexane-ethanol (85:15) increasing from 150 to 300 $\mu\text{l}/\text{min}$ in 30 min. ΔP = pressure drop in the column.

The confirmation method described in the above example was practical, useful and simple. Also, we can calculate a brief density, ρ , in the column as follows: the linear velocity, v , in the column is given by $(U_1 + U_2)/\rho$, and v is inversely proportional to the retention time, τ , of the void peak, hence ρ can be derived from the equation $\rho = k\tau(U_1 + U_2)$, where k is an instrumental factor that can be determined from the result of using a known fluid such as pure carbon dioxide.

CONCLUSION

A semi-micro packed column SFC instrument was used extensively for the separation of synthetic oligomers by using gradient elution. A temperature gradient is useful and convenient when the eluent composition and back-pressure can be selected freely, to obtain a stable baseline and a fairly high resolution chromatogram in a short

running time with rapid column regeneration. A composition gradient is more powerful for the elution of an unknown polymer in a first trial analysis. By performing separations of known oligomers with composition gradients at various temperatures and pressures, the properties of the binary or ternary fluids can be confirmed.

The system is promising for the fractionation of eluted components for subsequent structure determination by ^1H NMR, FT-IR, and FD-MS. Further expansion of applications can be expected by the use of different stationary phases and different modifiers.

REFERENCES

- 1 F. P. Schmitz and B. Gemmel, in M. Yoshioka, S. Parvez, T. Miazaki and H. Parvez (Editors), *Progress in HPLC*, Vol. 4, VSP, Utrecht, Tokyo, 1989, pp. 74–85.
- 2 E. Klesper and F. P. Schmitz, in C. M. White (Editor), *Modern Supercritical Fluid Chromatography*, Hüthig, Heidelberg, 1987, pp. 1–13.
- 3 R. D. Smith, H. T. Kalinoski and H. R. Udseth, *Mass Spectrom. Rev.*, 6 (1987) 445–496.
- 4 T. Saito and M. Takeuchi, in Yoshioka *et al.* (Editors), *Progress in HPLC*, Vol. 4, VSP, Utrecht, Tokyo, 1989, pp. 25–51.
- 5 T. Saito and M. Takeuchi, *JEOL News*, 23A, No. 2 (1987) 15–19.
- 6 K. Hatada, K. Ute, T. Nishimura, M. Takeuchi and T. Saito, *Polym. Bull.*, 23 (1990) 157–162.
- 7 R. D. Smith, B. W. Wright and H. T. Kalinoski, in M. Yoshioka, S. Parvez, T. Miazaki and H. Parvez (Editors), *Progress in HPLC*, Vol. 4, VSP, Utrecht, Tokyo, 1989, pp. 111–155.