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## REVIEW

# GRADIENT ELUTION IN SUPERCRITICAL FLUID CHROMATOGRAPHY

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## 1. INTRODUCTION

Supercritical fluid chromatography (SFC) has been well established as a method for separating substrates that consist of components with widely differing molecular weights. In most instances, this has been achieved by increasing the column pressure and, thereby, the mobile phase density during a run. As a unique feature of the supercritical state, the solvent strength of the eluent is closely related to its density<sup>1</sup>, which permits the application of pressure/density gradients. However, with increasing pressure, the effect on the supercritical fluid density decreases and finally approaches the low compressibility of liquids. Hence enhancing the solvent strength by means of increasing pressure will be limited. Moreover, with increasing density the self- and interdiffusion coefficients will decrease considerably, leading to a greater resistance to mass transfer and a decrease in analysis speed and efficiency.

It has therefore been of interest to increase the dissolution power of the supercritical mobile phase not by increasing the pressure but by the addition of a second component to the supercritical mobile phase. Of particular interest for substrates of widely differing solubility properties was thereby programming, *i.e.*, gradient elution. Addition of a second component, which possesses a higher dissolution ability for the substrate, is not necessarily connected with a change in the free volume of the mobile phase and will therefore less adversely affect the interdiffusion coefficient and the viscosity if the pressure is kept at sufficiently low levels.

This review attempts to cover the work reported in the literature on gradient elution in SFC up to the middle of 1986. No previous review on this subject has

appeared so far. Also included is work on retention, resolution and free volume on single mobile phases, which has a direct bearing on the understanding of gradient elution and gradient techniques in general. Applications of gradient elution are presented and strategies are discussed for optimization, particularly by the utilization of multiple gradients.

## 2. APPARATUS

Many types of apparatus have been proposed for SFC. For gradient elution, the independent and fully controllable metering of at least two streams of mobile phases is required. This requirement can be fulfilled by most commercial high-performance liquid chromatographic (HPLC) instruments today. As an example, a commercially available HPLC instrument (Hewlett-Packard Model 1084 B) capable of creating eluent gradients at a constant flow-rate was modified for SFC operation<sup>2,3</sup>. It is shown schematically in Fig. 1.

Eluents boiling below about 50°C are pre-pressurized in steel storage tanks (1) by applying a helium pressure. Alternatively, pre-pressurization can be accomplished by increasing the temperature of the storage tank above ambient, using an external heating tape. If one of the components of the mobile phase possesses a sufficiently high boiling point, it can also be fed without pre-pressurization from the glass bottles (2) originally provided with the HPLC instrument. The components are metered separately in the liquid state by the pumps of the Model 1084 B instrument (3) and pass consecutively the damping system combined with a device for measuring the feed rate (4), the mixing chamber (5), a filter (6) and the variable-volume injection system (7). For work with low-boiling eluents, a multiport loop-type valve (8) is used for injection instead of the variable-volume injector. The stream then enters the oven of the Model 1084 B instrument (9).

For SFC operation, the stream is usually led immediately out of the unheated oven to a larger external oven (10a) with forced air circulation (UT-5042 EK; Heraeus, Hanau, F.R.G.). This external oven can be heated to higher temperatures (300°C) and also accommodates larger columns. In addition, the oven can be tem-

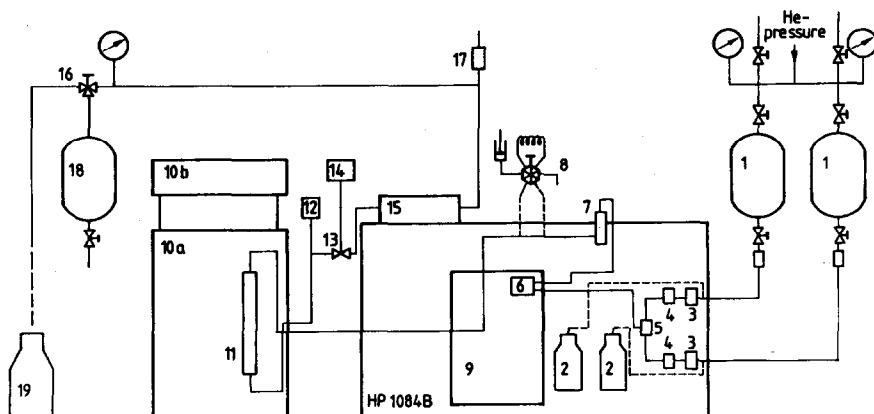


Fig. 1. Schematic diagram of SFC apparatus.

perature programmed by a programmer (10b) (Kelvitron TPG 2; Heraeus) equipped with a Pt-100 temperature sensor. The stream of mobile phase which is in the supercritical state within the oven passes through the separation column (11) and is allowed to liquefy after having left the oven. The pressure at the column end is monitored by an electric pressure transducer (12) (Siemens Messumformer Teleperm D, M 5644), while the pressure upstream of the column is measured by the pressure transducer of the Model 1084 B instrument itself. Control of the pressure at the column end is provided by a needle metering valve (13). For creating pressure gradients, the stem of this valve can either be connected to a motor which is controlled by a pressure feedback loop or more simply with the potentiometer axis of a "cut-a-curve" electromechanical time programmer (14). The eluate is then returned to the Model 1084 B instrument, specifically to its UV detector (15). The detector cell was modified to withstand higher pressures, as described in the literature<sup>4</sup>. An external metering valve (16) allows the pressure in the detector to be raised to a level that prevents the formation of gas in the mobile phase. To keep the pressure constant and to protect the detector, an adjustable spring-actuated safety valve is provided (17).

The eluent is finally collected in a pressure-resistant metal container as a liquid under its own vapour pressure or, with higher boiling eluents, in a glass bottle (19). The separation columns were made of stainless steel, length 25 cm and inner diameter 4.6 mm; they were packed with bare (unmodified) silica (LiChrosorb Si 100 or Si 60, 10  $\mu\text{m}$ ) using a slurry method<sup>5</sup>. To facilitate comparison, the total liquid flow-rate at the pumps was always set at 1 ml/min, unless indicated otherwise.

### 3. RETENTION WITH SINGLE SUPERCRITICAL ELUENTS

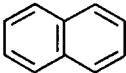
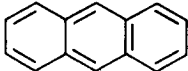
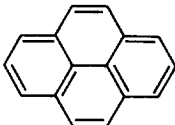
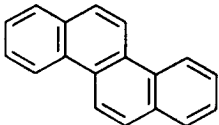
Scattered in the literature are some early results indicating that, at temperatures above critical, retention shows a complex behaviour. Thus, from various data<sup>6-11</sup>, maxima can be found for the capacity ratio,  $k'$ , in plots of  $k'$  versus temperature at constant pressure. Additionally,  $k'$  maxima can be obtained from plots of  $k'$  versus pressure at constant temperature, these maxima occurring at subcritical pressures<sup>12</sup>. To gain more insight into these phenomena, we started a series of experiments concerning the elution behaviour with different mobile phases which are summarized in Table 1. As the substrates for these studies, naphthalene, anthracene, pyrene and

TABLE 1  
PHYSICAL PROPERTIES OF ELUENTS

Eluent	$T_b$ ( $^{\circ}\text{C}$ )	$T_c$ ( $^{\circ}\text{C}$ )	$p_c$ (bar)	$\rho_c$ ( $\text{g cm}^{-3}$ )
Propane	-42.1	96.7	42.5	0.217
Isobutane	-11.7	135.0	36.5	0.221
<i>n</i> -Butane	-0.5	152.0	38.0	0.228
<i>n</i> -Pentane	36.1	196.5	33.7	0.232
<i>n</i> -Hexane	68.7	234.7	29.7	0.233
Dimethyl ether	-24.9	126.9	53.7	0.242
Diethyl ether	34.6	193.6	36.4	0.265
Carbon dioxide	-78.5 (s)*	31.0	73.8	0.468
1,4-Dioxane	101.4	313.9	52.1	0.370

\* Sublimation.

TABLE 2  
AROMATIC COMPOUNDS USED AS TEST SUBSTRATES

Compound	Structure	Formula	$M$ ( $\text{g mol}^{-1}$ )
Naphthalene		$\text{C}_{10}\text{H}_8$	128.17
Anthracene		$\text{C}_{14}\text{H}_{10}$	178.23
Pyrene		$\text{C}_{16}\text{H}_{10}$	202.26
Chrysene		$\text{C}_{18}\text{H}_{12}$	228.30

chrysene were chosen, as shown in Table 2<sup>13,14</sup>. These experiments were aimed at obtaining basic data for oligomer separations by means of gradient techniques. The substrate test mixture simulates a homologous series as encountered in oligomers and is easily obtained, in contrast to oligomer mixtures that are prepared or fractionated to contain only a few defined homologues. Thus, information should be obtainable with less experimental effort than with oligomer samples with a wider molecular weight distribution.

Figs. 2 and 3 show results obtained with dimethyl ether as the mobile phase<sup>13</sup>. From Fig. 2 it can clearly be seen that capacity ratio maxima are passed with increasing temperature, these maxima being located at temperatures not much above

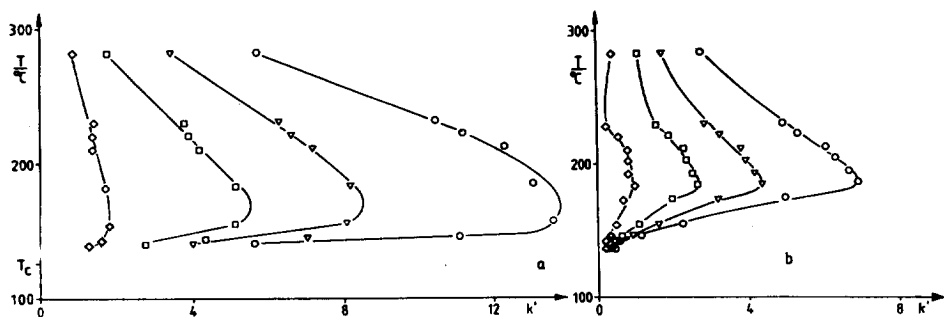


Fig. 2. Plots of temperature *versus* capacity ratio,  $k'$ , with dimethyl ether as the mobile phase at different column exit pressures,  $p_e$ : (a)  $p_e = 56$  bar; (b)  $p_e = 70$  bar. Substrates:  $\diamond$ , naphthalene;  $\square$ , anthracene;  $\nabla$ , pyrene;  $\triangle$ , pyrene;  $\circ$ , chrysene.

the critical temperature,  $T_c$ , and being more pronounced at lower pressure. The four chromatograms shown in Fig. 3 correspond to the data in Fig. 2b. The occurrence of capacity ratio and retention time maxima at a temperature of about 182°C is obvious.

A comparison of the chromatographic behaviour of chrysene with the four eluents propane, isobutane, *n*-butane and *n*-pentane by plots of capacity ratios *versus* temperature is shown in Fig. 4<sup>14,15</sup>. The temperatures involved range from ambient to 280°C, *i.e.*, from the liquid to the supercritical state. It is recognizable that at almost identical reduced pressures,  $p_r$ , and in a comparable pressure range, the maximum is most pronounced with the lowest alkane, propane, and is more pronounced with isobutane than *n*-butane. Additionally, all of these maxima occurred at temperatures above the critical temperatures. To summarize the effect of temperature at constant pressure:

(a) with eluents in the liquid state, the capacity ratios decrease with increasing temperature;

(b) after having passed the boiling temperature,  $T_b$ , (at subcritical pressures) or the critical temperature,  $T_c$ , (at supercritical pressures), the capacity ratios increase strongly, reach maxima and then decrease again<sup>13,15,16</sup>;

(c) this behaviour is the more pronounced the lower the pressure and the lower the critical temperature of the mobile phase;

(d) with increasing molecular weight of the substrate, the relative magnitude of the capacity ratio maximum increases and is slightly shifted to higher temperatures;

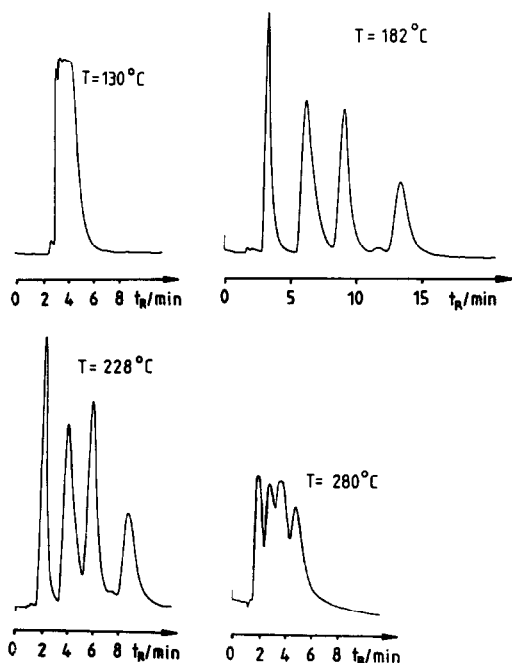


Fig. 3. Chromatograms of naphthalene, anthracene, pyrene and chrysene with dimethyl ether as the mobile phase at different temperatures: 130°C (slightly above  $T_c$ ); 182°C (maximum for average resolution  $\bar{R}$ ; *cf.*, Fig. 7); 228°C ( $\bar{R} = 1.5$ ); 280°C (highest temperature investigated).  $p_c = 70$  bar.

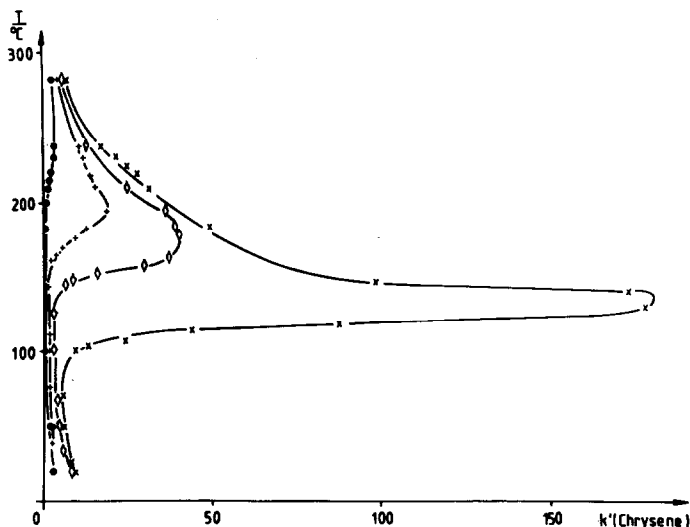


Fig. 4. Comparison of  $k'$  versus  $T$  plots for various alkanes. Substrate, chrysen. Eluents and pressures ( $p_c$ ): ●, *n*-pentane, 36 bar ( $p_r = 1.07$ ); +, *n*-butane, 39 bar ( $p_r = 1.03$ ); ◇, isobutane, 39 bar ( $p_r = 1.07$ ); ×, propane, 43 bar ( $p_r = 1.02$ ).

(e) a shift to slightly higher temperatures for the maxima is also observed at higher pressures.

We obtained analogous results with additional mobile phases (ethane,  $\text{CO}_2$ ,  $\text{N}_2\text{O}$ ,  $\text{CHF}_3$ ,  $\text{CClF}_3$ )<sup>17</sup>; with all of these mobile phases,  $k'$  maxima occurred at temperatures above  $T_b$  or  $T_c$ , the effect being more pronounced by the lower critical temperature of the mobile phase. In these studies only bare, unmodified silica was used as the stationary phase. Nevertheless, these effects appear not to be greatly dependent on the nature of the stationary phase, as the mentioned previous studies of other workers were performed in part on different stationary phases, and recently two other groups have reported similar results with capillary columns coated with polysiloxanes<sup>18,19</sup>.

Particularly detailed information was obtained from studies performed on *n*-pentane in the pressure and temperature ranges 30–75 bar and 25–300°C, respectively<sup>20</sup>. The number of data points was sufficient to form an “isocratic network”, and as an example the data for the chrysen capacity ratios are shown in a three-dimensional plot in Fig. 5. From such network plots, information about the influence of both temperature and pressure can be obtained for a given separation problem if merely some “anchoring” points are measured.

An explanation for this temperature dependence of the transport behaviour of the mobile phase may be found in two counteracting effects. The first is the decrease in density of the mobile phase on raising the temperature above  $T_c$ , which leads to a higher partition coefficient of the substrate and slower axial transport. The effect is related to the lower solubility of a pure substrate phase in a supercritical fluid of lower density. The second effect is that with increasing temperature the partition coefficient also tends to decrease. This is related to the usually observed decrease in absorptive power of a stationary phase, the increase in solubility of a pure substrate

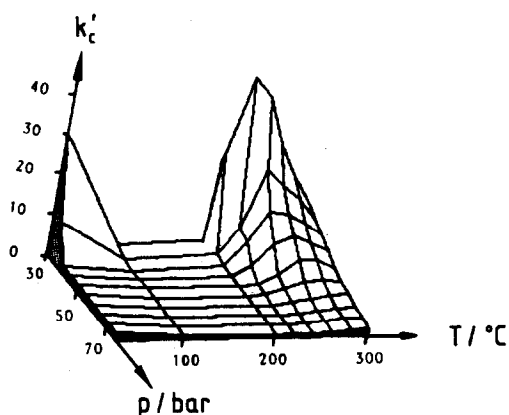


Fig. 5. Isocratic network plot of capacity ratios versus temperature and pressure. Substrate, chrysene (c).

in the mobile phase and the increase in vapour pressure of the substrate. Whereas the first effect, *i.e.*, the decrease in density, is particularly strong when the temperature is still close to  $T_c$ , the second effect becomes steadily more important as the temperature is raised. In consequence, the second effect becomes dominant relatively far from  $T_c$ .

The explanation given by Yonker *et al.*<sup>18</sup> is based on formal thermodynamic reasoning. They derived the following relationship between  $\ln k'$  and  $T$  at constant pressure:

$$\left(\frac{\partial \ln k'}{\partial T}\right)_p = \frac{1}{RT^2} \left[ \Delta \bar{H}_i^\infty(T_0) + \int_{T_0}^T \Delta \bar{C}_{p,i}^\infty dT \right] + \frac{1}{V_m^{\text{mob}}} \left( \frac{\partial V_m^{\text{mob}}}{\partial T} \right)_p \quad (1)$$

where  $\Delta \bar{H}_i^\infty$  is the enthalpy of transfer from the stationary to the mobile phase for a solute  $i$  at infinite dilution,  $\Delta \bar{C}_{p,i}^\infty$  the molar heat capacity at constant pressure for that transfer and  $V_m^{\text{mob}}$  the molar volume of the mobile phase. From this equation and choosing some fitting parameters, a good correlation was achieved with experimental results for chrysene with *n*-pentane at 36 bar. The interesting aspect of this thermodynamic model is the appropriate description of the chromatographic behaviour of a mobile phase in the liquid, supercritical and vapour state, and that it relates  $k'$  to density changes in the mobile phase.

#### 4. RESOLUTION WITH SINGLE MOBILE PHASES

As the retention data for the four substrates were determined simultaneously from chromatograms of the mixtures, information on changes in selectivity and in separation efficiency with differing chromatographic conditions was also provided by the studies. Impressions on this aspect can be obtained from Figs. 3 and 6, which show actual chromatograms. In Fig. 6, three chromatographic profiles have been added to the plots of elution volume,  $V_e$ , versus  $T$ . It can be seen that the peaks become narrower and less tailed when the temperature reaches the critical tempera-

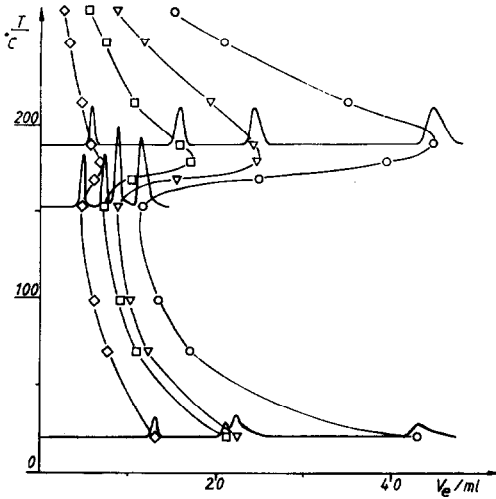


Fig. 6. Dependence of elution volume on temperature at constant pressure with *n*-butane as the eluent.  $p_e = 45$  bar. Symbols as in Fig. 2. Inserted are three chromatograms at  $T = 20, 155$  and  $190^\circ\text{C}$ .

ture of the eluent *n*-butane. Comparing the profiles at 20 and  $180^\circ\text{C}$ , which exhibit similar analysis times, the chromatographic resolution is obviously enhanced under supercritical conditions. This can be seen quantitatively from plots of the chromatographic resolution,  $R$ , calculated by eqn. 2 and averaged over the three peak pairs present:

$$R_i = \frac{t_{m+1} - t_m}{w'_{m+1} + w'_m} \cdot \sqrt{\ln 4} \quad (i = 1, 2, 3) \quad (2)$$

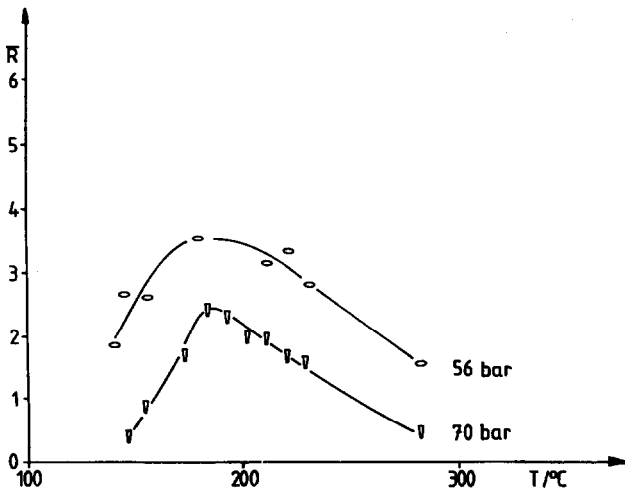


Fig. 7. Average resolution of PAHs in dimethyl ether at different temperatures and pressures,  $p_e$ , of 56 and 70 bar.



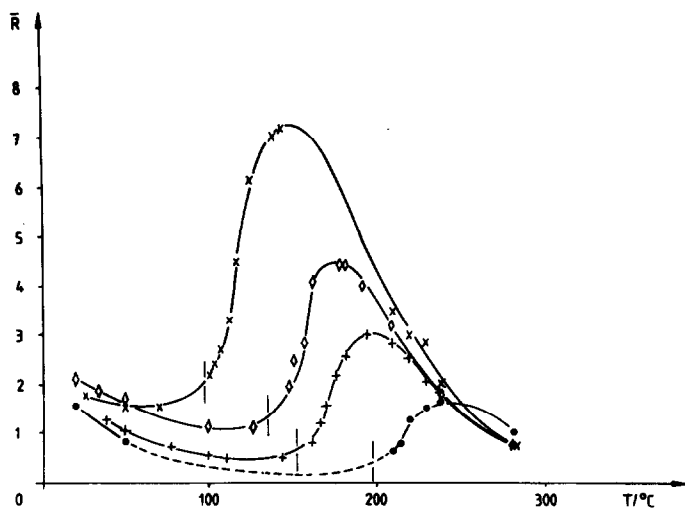


Fig. 8. Average resolution of PAHs for four alkanes as eluents. Symbols and pressures as in Fig. 4.

where  $R_i$  = resolution for the  $i$ th pair of peaks,  $t_m$  = retention time of the  $m$ th peak and  $w'_m$  = peak width at half-height. Fig. 7 shows the dependence of average resolution,  $\bar{R}$ , on temperature for dimethyl ether at two pressures. Fig. 8 shows plots of  $\bar{R}$  vs.  $T$  for the four alkane eluents at comparable reduced pressures. All the curves strongly resemble those for the capacity ratios. Again, maxima are formed that are more pronounced with lower pressures (Fig. 7) and lower critical temperature (Fig. 8) of the mobile phase, and that are located at lower temperatures with decreasing critical temperature of the eluent.

According to eqn. 3, the resolution  $R$  is determined by the capacity ratio  $k'$ , the selectivity  $\alpha$ , and the plate number  $n$ :

$$R = \frac{1}{4} \cdot \frac{k'}{1+k'} \cdot \frac{\alpha - 1}{\alpha} \cdot \sqrt{n} \quad (3)$$

As may be suspected from Fig. 6, where the peak shapes are improved near supercritical conditions, the resolution maxima are not only due to an increase in capacity ratios. To deduce the relative importance of  $k'$ ,  $\alpha$  and  $n$  for the formation of the  $\bar{R}$  maxima, plots of related terms, viz., the average capacity ratio term, the average selectivity term and the average value of the square root of the plate number (*cf.*, eqn. 3), were compared with a corresponding plot of the experimental  $\bar{R}$  for propane as the mobile phase<sup>15</sup>. From this comparison, a strong correlation was found between

$\sqrt{n}$  and  $\bar{R}$ , which could be shown to be almost linear. Contributions from the selectivity term were mainly due to selectivity changes for the peak pair anthracene-pyrene<sup>21</sup>.

Pressure effects on resolution can be seen from Fig. 9, which shows a plot of average resolution,  $\bar{R}$ , versus column end pressure,  $p_e$ , at four temperatures, obtained with  $n$ -pentane. At 50, 248 and 282°C, resolution maxima were found to exist between

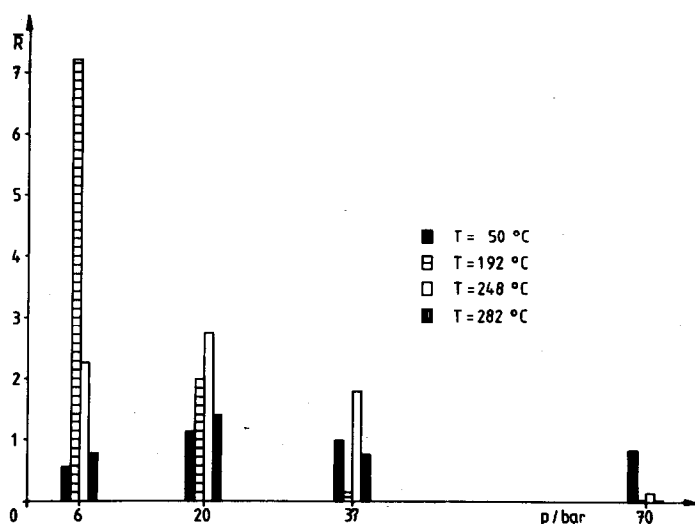


Fig. 9. Average resolution of polyaromatic hydrocarbons (PAHs; *cf.*, Fig. 2) versus column exit pressure with *n*-pentane as the mobile phase at four temperatures.

20 and 30 bar, whereas at 192°C, slightly below the critical temperature of *n*-pentane, no such maximum was seen, the highest  $\bar{R}$  being at the lowest pressure, *i.e.*, at 6 bar. It should be considered, however, that  $\bar{R}$  will depend on the linear velocity in the column, which is usually different with different pressure-temperature pairs, because the liquid flow-rate at the pumps was kept constant in these experiments. Fig. 10 shows in a three-dimensional plot the results from isocratic network studies<sup>20</sup>. By analogy with the effects on capacity ratios, resolution maxima are shifted to higher temperatures with increasing pressure. Such network plots can be used for optimizing a separation.

In addition to the effect of pressure and temperature on  $k'$  and  $R$ , the effect of the linear mobile phase velocity on the chromatographic performance has been studied, using dimethyl ether as the mobile phase<sup>13</sup>. For evaluating the chromatograms, the resolution function  $Q$  (eqn. 4) was used, which is also applicable to peaks with strong overlapping:

$$Q = \frac{\sum_i^j f_i/g_i(\Delta V_e)_i}{\sum_i (\Delta V_e)_i} \quad (4)$$

where  $f_i$  = average peak height of the  $i$ th peak pair,  $g_i$  = average peak depth (between peaks of the  $i$ th peak pair),  $(\Delta V_e)_i$  = difference in elution volumes of the  $i$ th peak pair,  $i = 1, 2, 3$  and  $j = 3$ . At small flow-rates,  $Q$  remained approximately constant (Fig. 11), but above a flow-rate of 1 ml/min the resolution decreased strongly. It is to be expected that results different from Fig. 11 will be obtained when the temperature (172°C) and pressure ( $p_e = 70$  bar) are significantly changed.

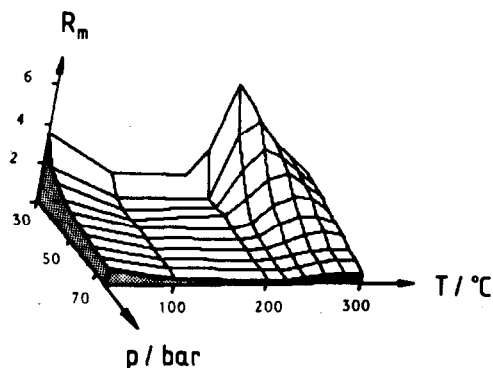


Fig. 10. Isocratic network plot of average resolution of PAHs *versus* temperature and pressure.

### 5. COMPARISON OF DIFFERENT MOBILE PHASES BASED ON FREE VOLUME

A comparison of mobile phases of different chemical nature has been made on the basis of the equal free volume<sup>22</sup>. The free volume of a mobile phase was calculated as a reduced parameter,  $f_r$ , from the density of the mobile phase and its density at a chosen standard state:

$$f_r = \frac{\rho - \rho_s}{\rho_s} = \frac{\rho}{\rho_s} - 1 \quad (5)$$

where  $\rho_s$  is the density with close packing of the molecules in the crystalline state under chosen standard conditions. The results are shown in Figs. 12 and 13, giving plots of chrysene capacity ratios and of selectivity between pyrene and chrysene *versus*

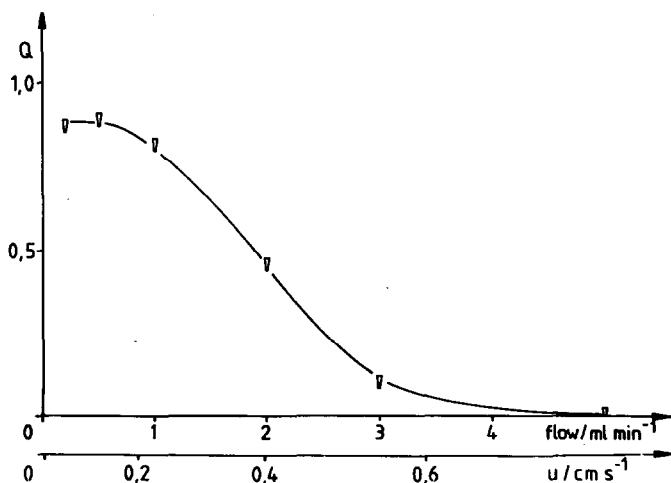


Fig. 11. Dependence of resolution function,  $Q$ , on the volume flow-rate in the liquid state at the pumps and on the linear velocity of the supercritical fluid in the column,  $u$ , for PAHs in dimethyl ether at  $172^\circ\text{C}$  and  $p_c = 70\text{ bar}$ .

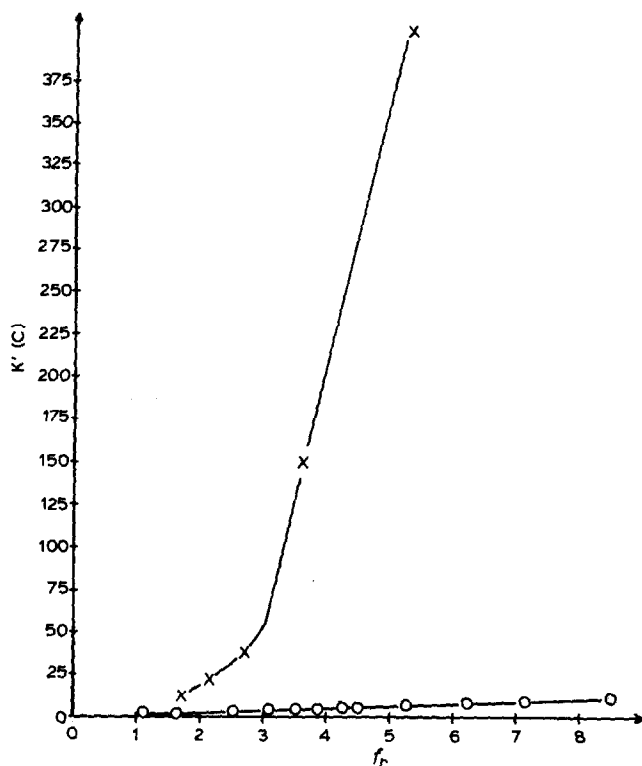


Fig. 12. Dependence of capacity ratio of chrysene (c) on free volume,  $f_r$ , in  $\text{CO}_2$  (x) and  $n$ -pentane (o) at a reduced temperature of  $T_r = 1.22$ .

the free volume, the mobile phases being carbon dioxide and  $n$ -pentane. It is observed that at equal  $f_r$  values, both the capacity ratio and selectivity are considerably higher with carbon dioxide than with  $n$ -pentane. This is probably connected with the lower solvent strength of carbon dioxide for the polycyclic aromatic hydrocarbons. It could prove to be a general relationship in SFC that the retention should be large in order to obtain a large  $\alpha$ .

The reason for using the free volume or, more generally, a reduced density, is that for a comparison of chemically different mobile phases, the density itself is less useful because the molecular weight does not have a primary influence on dissolution power, whereas the free volume does. The larger the space between the molecules of the mobile phase, the more the inherent interactions will be diminished. In addition to the free volume, the inherent interactions themselves, which are different for chemically different molecules, will be of primary importance. Therefore, comparison of  $k'$  and  $\alpha$  of different mobile phases at the same free volume will provide information about the inherent interactions.

## 6. RETENTION AND RESOLUTION WITH BINARY MOBILE PHASES

In recent years, studies have been extended to mixed mobile phases. As ex-

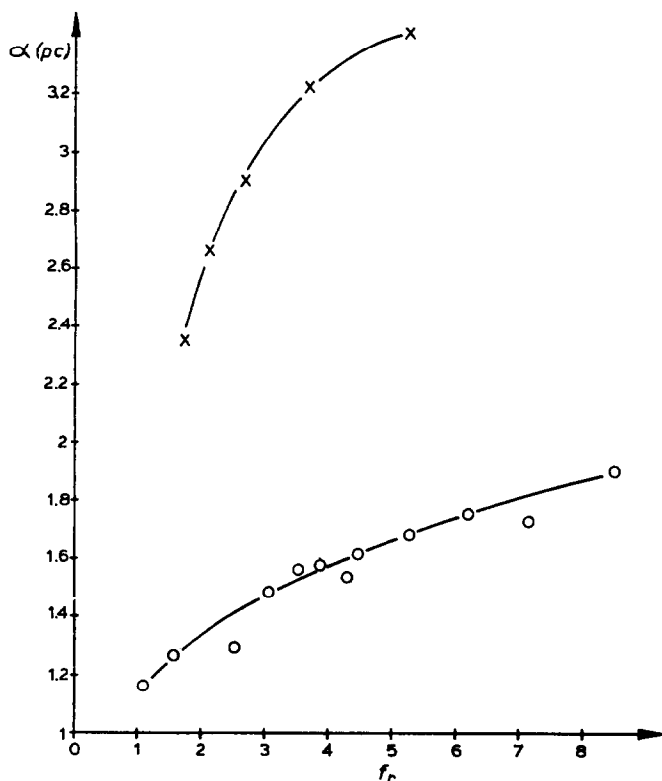


Fig. 13. Dependence of selectivity,  $\alpha$ , between pyrene (p) and chrysene (c) on free volume,  $f_r$ , in  $\text{CO}_2$  (x) and  $n$ -pentane (O) at a reduced temperature of  $T_r = 1.22$ .

pected, the capacity ratios decreased when the amount of a second component with higher solvent strength was increased, all other conditions being the same. Also,  $k'$  and  $R$  maxima were found, as with single mobile phases. They depended on temperature in a similar manner as with one-component mobile phases. Fig. 14 shows the temperatures of the resolution maxima as a function of mobile phase composition, with the column pressure being the parameter. The primary component of the eluent mixture was carbon dioxide and the secondary component of higher solvent power was 1,4-dioxane. As will be demonstrated subsequently, such graphs can be used for optimizing gradient separations<sup>23</sup>.

As already pointed out, gradient techniques are particularly useful for substrates whose individual components differ widely in solubility and/or adsorption. Because oligomers are usually composed of individual species of widely differing molecular weight, oligomer separation has become a domain of SFC since the work of Jentoft and Gouw<sup>24</sup>, who separated styrene oligomers by means of a pressure gradient. Pressure gradients, being a unique procedure with SFC, were the only gradient procedure applied until 1981, when we were able to demonstrate that gradients of eluent composition were applicable just as in HPLC<sup>2,25</sup>. This parallel is not surprising, as both chromatographic procedures are based on the same transport principle: owing to the dense nature of the mobile phase, substrate molecules are trans-

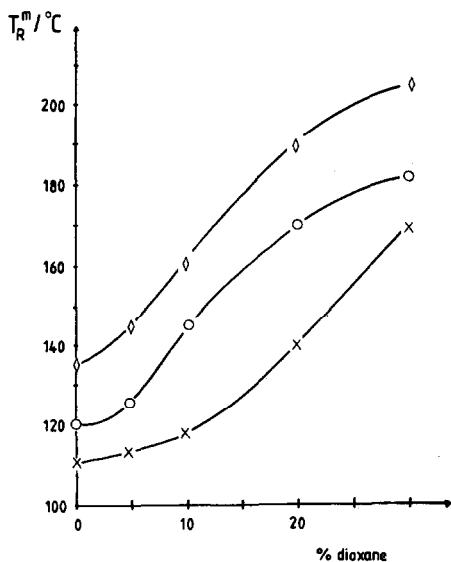


Fig. 14. Dependence of the temperature of the resolution maxima,  $T_R^m$ , on eluent composition and column exit pressure. Substrates, PAHs. Eluent,  $\text{CO}_2$ -1,4-dioxane. Key to symbols:  $\diamond$  = 250 bar;  $\circ$  = 200 bar;  $\times$  = 150 bar.

ported along the separation column by interactions between substrate molecules and the mobile phase, *i.e.*, solubility, and not, or not to the same extent as in gas chromatography, by vapour pressure.

For the separation of oligomers of styrene and related monomers, the following eluent compositions have been found to be suitable: *n*-pentane-cyclohexane<sup>2,3</sup>, diethyl ether-1,4-dioxane<sup>3</sup>, *n*-pentane-dioxane<sup>3,5,25,26</sup> and propane, *n*-butane or *n*-

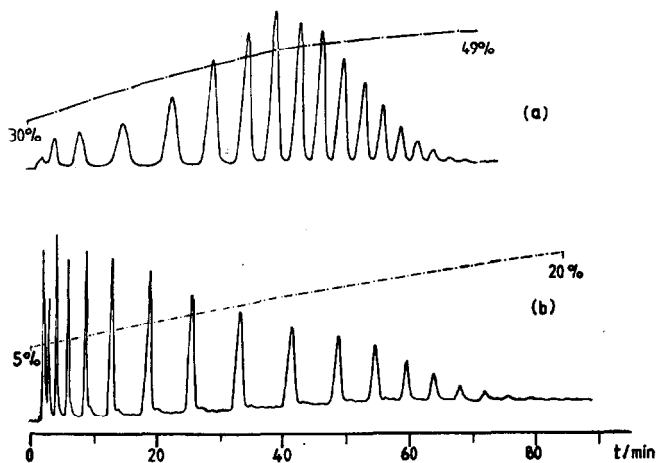


Fig. 15. Chromatograms of styrene oligomers with (a) propane-1,4-dioxane and (b) hexane-1,4-dioxane as eluents. Broken lines, content of 1,4-dioxane in the eluent mixture (v/v). Column temperatures: (a) 240°C; (b) 275°C. Column exit pressures at the start of the chromatograms: (a) 80 bar; (b) 41 bar.

hexane-dioxane<sup>3</sup>. In these eluent systems, cyclohexane or dioxane is the component with a higher solvent strength for the vinyl arene oligomers and, thus, increasing the concentration of either cyclohexane or dioxane permits the elution of higher molecular weight species. Figs. 15 and 16 show oligostyrene separations using *n*-alkane-dioxane mixtures<sup>3</sup>. The eluent gradient is given with the chromatograms. In Fig. 15a the binary mobile phase propane-dioxane and in Fig. 15b *n*-hexane-dioxane were employed. It is straightforward to change the composition programme to expand or compress portions of the chromatograms as desired. In Fig. 15a the first portion of the chromatogram is expanded and in Fig. 15b it is primarily the middle part.

Additionally, the separation of oxyethylene oligomers has been demonstrated by Gere<sup>27</sup> using carbon dioxide-methanol as the mobile phase and a column packed with polystyrene. This eluent pair has also been used for separations of aromatic hydrocarbons by gradient elution<sup>28</sup>.

Of our eluent systems, the combination *n*-pentane-dioxane proved to be particularly useful. With styrene oligomers, separations of individual oligomers were obtained up to a molecular weight range of 5500 g/mol, and even polystyrene samples could be separated<sup>29</sup> with molecular weights up to 600 000 g/mol, as shown in Fig. 17. In the latter instance individual molecular species could no longer be separated, but different polystyrene samples could be resolved. The separation of polymers is, of course, limited by the requirement that the molecular weight distributions must not overlap. As expected, higher dioxane contents and higher pressures were needed in order to elute the polymer samples compared with the oligomer species. Fig. 17a shows the separation of polystyrene standards of narrow molecular weight distribution; Fig. 17b shows the chromatogram of an industrially prepared polystyrene with a broad molecular weight distribution.

During an eluent composition programme the column pressure increases when

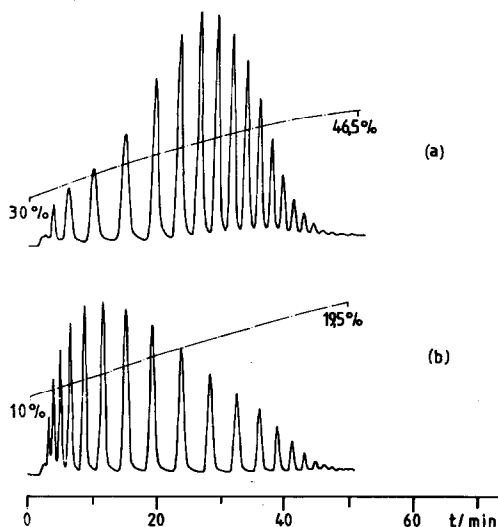


Fig. 16. Chromatograms of styrene oligomers with (a) *n*-butane-1,4-dioxane and (b) *n*-pentane-1,4-dioxane as eluents. Broken line, content of 1,4-dioxane in the eluent mixture (v/v). Column temperature, 240°C. Column exit pressures at the start of the chromatograms: (a) 69 bar; (b) 50 bar.

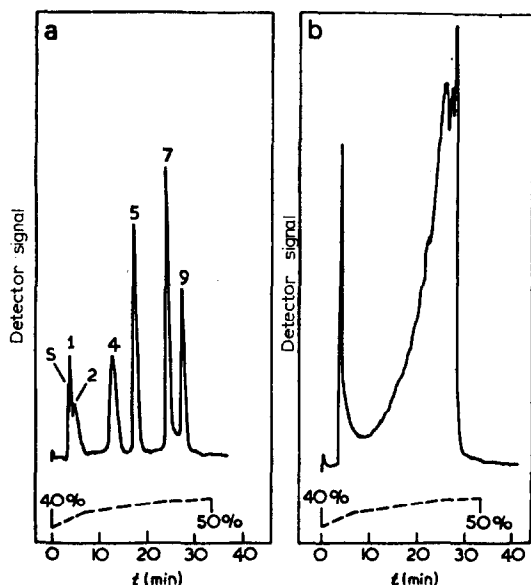


Fig. 17. (a) Chromatogram of a polystyrene mixture. S = Sample solvent; 1 = polystyrene sample (PS) with number-average molecular weight  $\bar{M}_n = 3600$  g/mol; 2 = PS with weight-average molecular weight  $\bar{M}_w = 10000$  g/mol; 4 = PS with  $\bar{M}_w = 93000$  g/mol; 7 = PS with  $\bar{M}_w = 254000$  g/mol; 9 = PS with  $\bar{M}_w = 600000$  g/mol.  $T = 250^\circ\text{C}$ . At the start of the chromatogram,  $p_e = 185$  bar. (b) Chromatogram of an industrial polystyrene sample.  $T = 250^\circ\text{C}$ . At the start of the chromatogram,  $p_e = 182$  bar.

the secondary component has a higher viscosity than the primary component and if the exit metering valve, *i.e.*, the flow resistance, is kept at a fixed setting. Such a pressure increase enhances the effect of the eluent composition gradient; the main effect, however, is due to the increasing content of the second component<sup>3</sup>.

Fig. 18 shows chromatograms of an anionically prepared sample of 2-vinylnaphthalene oligomers at two different temperatures, the mobile phase being *n*-pentane-dioxane. The quality of this separation made it obvious that at least three homologous series were obtained with the anionic procedure applied for the oligomerization reaction<sup>5</sup>, and a comparison could be made between radical and anionic oligomerizations by means of SFC<sup>26</sup>. The eluent composition gradient applied for these separations was formed by linearly increasing the ratio  $Q_B$ :

$$Q_B = \frac{P_B}{1 - P_B} \quad (6)$$

where  $P_B$  is the volume fraction of eluent component B, measured at the pumps in the liquid state.

The dependence of SFC retention on the eluent composition, pressure and temperature permits the application of coupled gradient techniques. Thus N-vinylcarbazole oligomers, which were found to be difficult to elute, were successfully separated by combining eluent composition and pressure programmes<sup>5,30</sup>. On the other hand, a temperature gradient can be superimposed on a pressure gradient or on a gradient of eluent composition in order to enhance the resolution. This corresponds



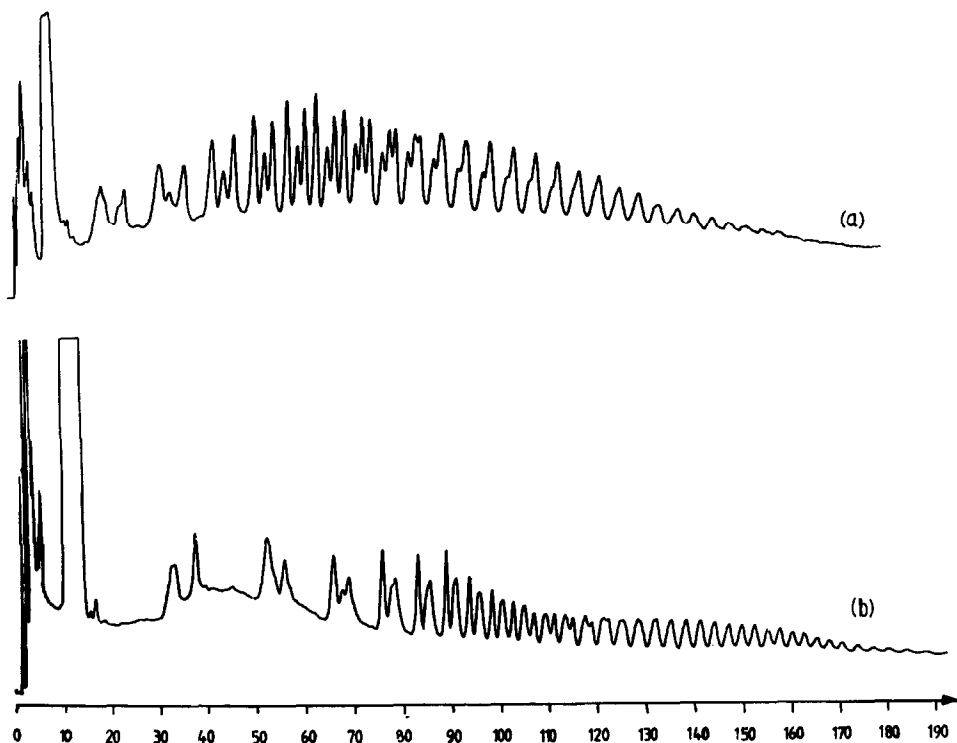


Fig. 18. Chromatograms of 2-vinylnaphthalene oligomers. Temperature: (a) 240°C; (b) 270°C. Column inlet pressures at the start of the chromatograms: (a) 52 bar; (b) 51 bar. Gradients, (a) 5–40% (v/v) 1,4-dioxane in *n*-pentane within 160 min; (b) 5–50% (v/v) 1,4-dioxane in *n*-pentane within 160 min; both gradients according to eqn. 6.

to Section 4, where it was pointed out that resolution shows maximum values at distinct temperatures, which depend on pressure and eluent composition. Thus, to retain high resolution throughout a gradient separation, the column temperature must be changed during the run. In fact, it was shown about 10 years ago that combining pressure and temperature gradients can improve a separation<sup>31</sup>.

Fig. 19 shows two oligostyrene separations by a combined eluent composition and pressure gradient<sup>23</sup>. One was obtained under isothermal (Fig. 19a) and the other under temperature-programmed (Fig. 19b) conditions. The temperature programme for Fig. 19b was determined using the graph shown in Fig. 14, *i.e.*, the temperatures needed for maximum resolution were taken from the  $T_R^m$  plots, and the resulting temperature programme was applied simultaneously with the eluent composition and pressure gradient. Comparison between the chromatograms shows that the distance and the resolution between the oligomer peaks are increased on superimposing a temperature programme.

Although the potential of multiple gradient techniques for SFC is just beginning to be developed, it can be anticipated that it will contribute to establish further supercritical fluid chromatography as a high-resolution separation method of special interest for the separation of complex mixtures.

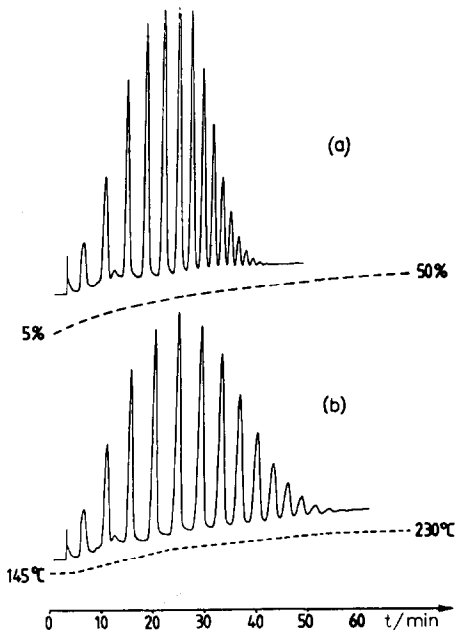


Fig. 19. Chromatograms of styrene oligomers: (a) by means of isothermal gradient elution at 145°C; (b) by binary composition-temperature programming. Mobile phase, CO<sub>2</sub>-1,4-dioxane. Pressure, 250 bar at the start and 295 bar at the end of the separations. Gradients are shown in the figure, the composition programme being valid for both (a) and (b).

## 7. SUMMARY

The dependence of retention and resolution in supercritical fluid chromatography on physical and chemical parameters is described. Both capacity ratios and resolution show maxima at temperatures above the critical temperature of the mobile phase. The temperatures at which these maxima occur depend on pressure, on the chemical composition of the eluent and on the molecular weight of the substrates. These results have been applied to improving gradient elutions and pressure gradient elutions by superimposing a temperature programme.

## 8. ACKNOWLEDGEMENTS

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## REFERENCES

- 1 J. C. Giddings, M. N. Myers, L. McLaren and R. A. Keller, *Science (Washington, D.C.)*, 162 (1968) 67.
- 2 F. P. Schmitz and E. Klesper, *Polym. Bull.*, 5 (1981) 603.
- 3 F. P. Schmitz, H. Hilgers and E. Klesper, *J. Chromatogr.*, 267 (1983) 267.
- 4 D. McManigill, R. Board and D. R. Gere, *Publication No. 43-5953-1647*, Hewlett-Packard, Avondale, PA, 1982, p. 21.
- 5 F. P. Schmitz, H. Hilgers, B. Lorenschat and E. Klesper, *J. Chromatogr.*, 346 (1985) 69.
- 6 S. T. Sie and G. W. A. Rijnders, *Sep. Sci.*, 2 (1967) 729.
- 7 S. T. Sie and G. W. A. Rijnders, *Sep. Sci.*, 2 (1967) 755.
- 8 M. Novotný, W. Bertsch and A. Zlatkis, *J. Chromatogr.*, 61 (1971) 17.
- 9 G. W. A. Rijnders, J. P. A. Bleumer and M. van Krevelen, *Usp. Khromatogr.*, (1972) 65; *C.A.*, 78 (1973) 37708y.
- 10 K. Fuzita, I. Shimokobe and F. Nakazima, *Nippon Kagaku Kaishi*, (1975) 1348; *C.A.*, 83 (1975) 188012w.
- 11 J. A. Nieman and L. B. Rogers, *Sep. Sci.*, 10 (1975) 517.
- 12 B. P. Semonian and L. B. Rogers, *J. Chromatogr. Sci.*, 16 (1978) 49.
- 13 D. Leyendecker, F. P. Schmitz and E. Klesper, *J. Chromatogr.*, 315 (1984) 19.
- 14 F. P. Schmitz, D. Leyendecker and E. Klesper, *Ber. Bunsenges. Phys. Chem.*, 88 (1984) 912.
- 15 D. Leyendecker, F. P. Schmitz, D. Leyendecker and E. Klesper, *J. Chromatogr.*, 321 (1985) 273.
- 16 F. P. Schmitz, H. Hilgers, D. Leyendecker, B. Lorenschat, U. Setzer and E. Klesper, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 590.
- 17 D. Leyendecker, *Thesis*, RTW Aachen, Aachen, 1986.
- 18 C. R. Yonker, B. W. Wright, R. C. Petersen and R. D. Smith, *J. Phys. Chem.*, 89 (1985) 5526.
- 19 T. L. Chester and D. P. Innis, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 561.
- 20 D. Leyendecker, D. Leyendecker, F. P. Schmitz and E. Klesper, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 566.
- 21 F. P. Schmitz, *J. Chromatogr.*, 356 (1986) 261.
- 22 E. Klesper, D. Leyendecker and F. P. Schmitz, *J. Chromatogr.*, 366 (1986) 235.
- 23 D. Leyendecker, D. Leyendecker, F. P. Schmitz and E. Klesper, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 525.
- 24 R. E. Jentoft and T. H. Gouw, *J. Polym. Sci., Polym. Lett. Ed.*, 7 (1969) 811.
- 25 F. P. Schmitz and E. Klesper, *Makromol. Chem., Rapid Commun.*, 2 (1981) 735.
- 26 F. P. Schmitz and H. Hilgers, *Makromol. Chem., Rapid Commun.*, 7 (1986) 59.
- 27 D. R. Gere, *Application Note 800-3*, Publication No. 43-5953-1690, Hewlett-Packard, Avondale, PA, 1983.
- 28 A. L. Blilie and T. Greibrokk, *J. Chromatogr.*, 349 (1985) 317.
- 29 F. P. Schmitz and E. Klesper, *Polym. Commun.*, 24 (1983) 142.
- 30 F. P. Schmitz, H. Hilgers and B. Gemmel, *J. Chromatogr.*, 371 (1986) 135.
- 31 E. Klesper and W. Hartmann, *Eur. Polym. J.*, 14 (1978) 77.