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POSSIBILITIES FOR OPTIMIZATION OF OLIGOMER SEPARATION WITH SUPERCRITICAL FLUID CHROMATOGRAPHY

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SUMMARY

Based on the dependence of elution on the pressure and temperature under supercritical conditions, strategies are developed for improving oligomer separation by supercritical fluid chromatography. Temperature programming is shown to be applicable if the appropriate temperature region is chosen. Optimization strategies are also given, both for pressure-programmed separations and for separations with gradient elution. In particular, multiple gradient techniques are demonstrated to be capable of enhancing the efficiency of oligomer separations.

INTRODUCTION

In 1962, supercritical fluids were introduced as mobile phases in chromatography by Klesper et al.[1]. Seven years later, Jentoft and Gouw[2] reported the separation of styrene oligomers by means of supercritical fluid chromatography (SFC). Since then, SFC has become a valuable tool for the separation of oligomers. Most frequently, the separations of styrene[2-10] and related vinyl arene[11-13] oligomers have been studied, but a variety of other oligomers has been chromatographed, among them oligomers from butadiene[14], chlorotrifluoroethylene[15] and an α-olefin[15], as well as oligoethers[3,15-20] and oligosiloxanes[15,18,21-23].

The applicability of SFC to the separation of homologous series with members of widely differing molecular weights, as encountered in oligomer samples, is mainly due to two reasons: first, diffusion coefficients are considerably higher in supercritical media than in liquids, and secondly, the eluent strength can be varied considerably by changing the temperature, pressure and eluent composition.

In order to achieve separations over wide molecular weight ranges the application of gradient techniques is required. Since with supercritical gases an increase in pressure yields an increase in the eluent strength of the gas, capacity ratios are found to decrease with increasing pressure of a supercritical mobile phase[10,24-27]. This effect has been used for pressure or density gradient separations in SFC. Pressure gradients can be obtained by two different methods. First, the flow resistance downstream of the separation column can be increased at a constant pumping rate of the mobile phase as a liquid. The mass flow of the mobile phase is kept constant, and this leads to a decreasing linear velocity of the supercritical mobile phase within the

column at increasing pressure and density. This procedure has been used, *e.g.*, for separations of oligostyrenes[4,6,28]. In the second method, the flow-rate of the liquid mobile phase is increased at a constant flow resistance. The mass flow is thereby increased, thus compensating to some extent for the decrease in the linear velocity in the column. This technique has been applied especially in capillary or microbore separations using syringe pumps[10,14,15].

A second gradient method in SFC, of eluent composition ("gradient elution"), has been used successfully[6-8,11-13,16,29]. The applicability of this gradient technique is due to the fact that, with compressed gases, solute transport occurs by molecular interaction of the mobile phase and the solute, analogous to chromatography with liquid eluents. Accordingly, increasing the amount of a component with higher solvent strength in a binary supercritical mobile phase accelerates the elution of the substrates.

Increasing the mobile phase density during an experiment by lowering the temperature (negative temperature programming) has been of limited interest so far. The application of this gradient technique to oligomer separations has been described as difficult to control[5] or as yielding unsatisfactory results[6].

We have attempted to improve the temperature, pressure and composition programming of oligomer separations by a more detailed insight into the retention and resolution in SFC. In this connection, the usefulness of binary and ternary, *i.e.*, combined programmes was also studied.

EXPERIMENTAL

The SFC apparatus consisted of a modified high-performance liquid chromatographic (HPLC) instrument (1084 B; Hewlett-Packard, Waldbronn, F.R.G.), described in detail previously[8]. Among the modifications were valves for setting the pressure levels at the outlet of the separation column and at the detector, and an additional loop injector (Rheodyne 7125; ERC, Alteglofsheim, F.R.G.). The valves were either of the needle type (30VM-4074; Autoclave Engineers, Wettenberg, F.R.G.) or of the back-pressure-regulating type (Tescom 26-3220-24-004; WSM, Eching, F.R.G.). Temperature control and programming was accomplished by a laboratory oven (UT-5042 EK; W. C. Heraeus, Hanau, F.R.G.), equipped with a temperature programmer (Kelvitron TPG-2, Heraeus), or by a GC oven (Carlo Erba Fractovap 4200; Erba Science, Hofheim/Ts., F.R.G.). The mobile-phase pretreatment and delivery was as described previously[11,13]. The stainless-steel separation columns (25 cm × 4.6 mm I.D.) were packed with LiChrosorb Si 60, 10 μm (Merck, Darmstadt, F.R.G.) by a slurry method[11]. Unless otherwise indicated, the flow-rate was 1 ml/min, measured at the pumps in the liquid state at ambient temperature.

The oligomer substrates were an oligostyrene sample (PS 800; Pressure Chemical Co., Pittsburgh, PA, U.S.A.) with a number-average molecular weight of 810 g/mol, and an oligo(2-vinylnaphthalene) sample obtained by anionic-initiated oligomerization, as described previously[11,12].

RESULTS AND DISCUSSION

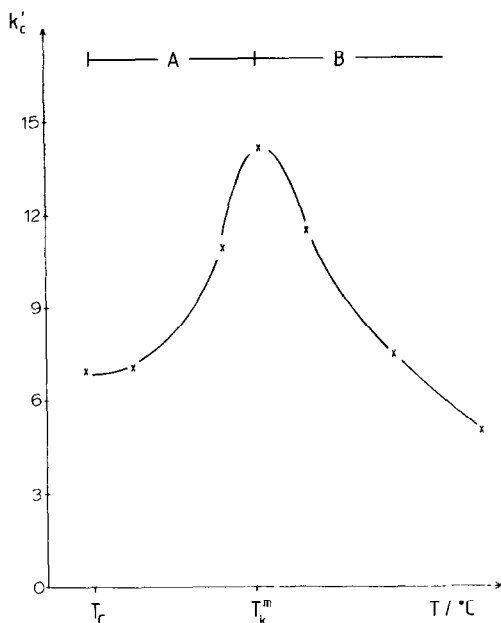From plots of the capacity ratio *versus* temperature, maxima are observed, as

Fig. 1. Dependence of the capacity ratio of chrysene on temperature at constant pressure. Mobile phase: carbon dioxide. Pressure: 250 bar. Stationary phase: LiChrosorb Si 60, 10 μm. Flow-rate: 1 ml/min. T_c = Critical temperature; T_k^m = temperature of the capacity ratio maximum. For temperature regions A and B, see text.

shown in Fig. 1 for the capacity ratio of chrysene, k_c', with carbon dioxide as the mobile phase. The general shape of such curves has been found to be independent of the type of the mobile[30,31] or stationary phase[25,31−33] and can be explained on a thermodynamic basis[32]. The occurrence of capacity ratio maxima is responsible for the failure of temperature-programmed separations: a negative temperature gradient, i.e., decreasing temperature, speeds up the elution only in the temperature range designated "A" in Fig. 1, while at higher temperatures, i.e., in range "B", capacity ratios increase with decreasing temperature. The difference between the temperature at the capacity ratio maximum, T_k^m, and the critical temperature, T_c

$$\Delta T_k = T_k^m - T_c$$

is higher for low-boiling than for high-boiling eluents[31]. Therefore, as an example, negative temperature gradients will be more applicable with carbon dioxide than with pentane as a mobile phase. Using carbon dioxide as the mobile phase, a negative temperature gradient has been successfully applied to the separation of alkanes from dodecane to octadecane with even carbon atom numbers[34].

Fig. 2 shows a temperature-programmed separation of styrene oligomers with carbon dioxide–1,4-dioxane (7:3, v/v) as mobile phase at 250 bar. For this eluent at this pressure, a T_k^m value of 240°C was found, the critical temperature of the mixture being estimated to be 150°C (cf., Fig. 8). A temperature gradient from 240 to 150°C led to equally spaced elution of about eight oligomers. When the temperature was
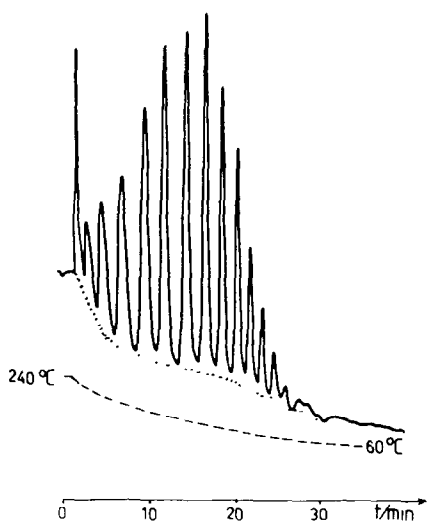
Fig. 2. Separation of styrene oligomers (PS 800) by means of negative temperature programming. Mobile phase: carbon dioxide–dioxane (7:3, v/v). Other conditions as in Fig. 1. The temperature gradient is shown; the baseline is represented by a dotted line.
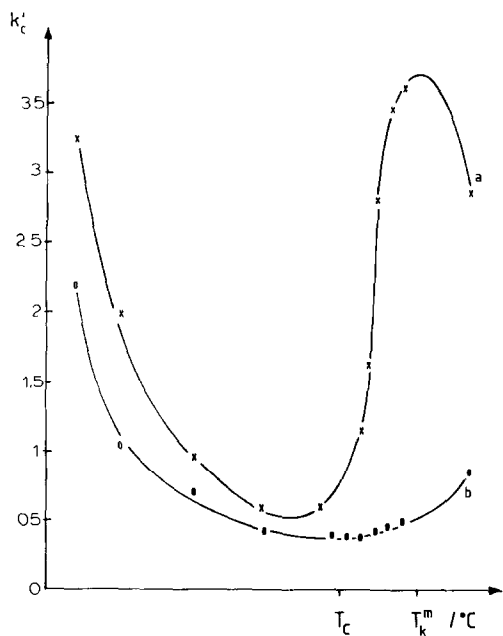

Fig. 3. Dependence of the capacity ratio for chrysene on temperature. Mobile phase: pentane. Pressures: (a) 36 and (b) 70 bar. Other conditions as in Fig. 1.

subsequently kept constant at 150°C, the higher oligomers were eluted with ever increasing retention times. However, an extension of the gradient to temperatures below the critical temperature of the mixture, *i.e.*, down to 60°C, yielded a fair separation, the highest oligomers present, containing 16–18 monomer units, being eluted at about 90°C. Similarly, from data obtained with styrene oligomers in pentane at high pressures, it appears that the retention times of the higher oligomers decrease continuously upon decreasing the temperature from 300 to 100°C[35].

　　　Upon increasing the pressure, the capacity ratio maximum exemplified in Fig. 1 becomes less pronounced and finally disappears[31,36]. This is shown in Fig. 3 specifically for pentane as the mobile phase and pressures of 36 and 70 bar, *i.e.*, at reduced pressures, p_r, of 1.07 and 2.08. The disappearance of the maximum is due to the fact that, at higher pressures, the density varies less strongly with the temperature[32]. As Fig. 3 shows, the k' ratio, *i.e.*, the quotient of the k' values at the lower and at the higher pressure, is higher at the temperature of the capacity ratio maximum than at the critical temperature, the numerical values for the k'_c ratios being 7.2 at T_k^m and 1.9 at T_c. Additionally, selectivity maxima are observed at or near T_k^m for homologous substrates[35]. This suggests that pressure programming at the temperature T_k^m will maximize the separation efficiency of a pressure programme.
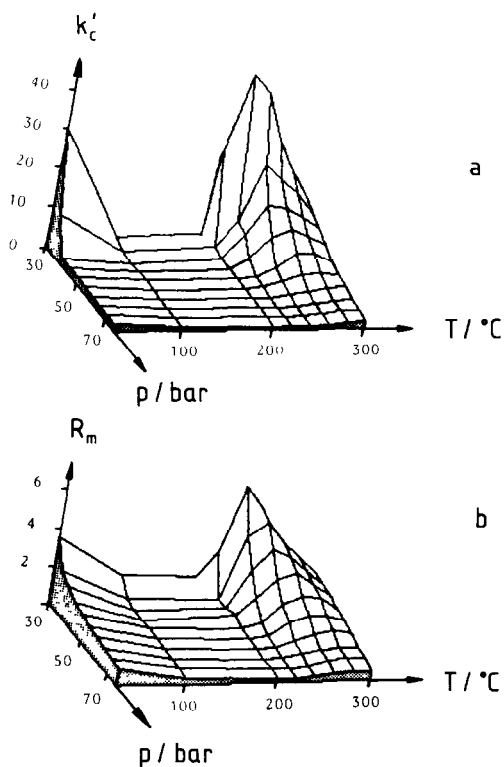

Fig. 4. Network plots of (a) the capacity ratio for chrysene and (b) the average resolution for the three adjacent peak pairs of a mixture of naphthalene, anthracene, pyrene and chrysene *versus* temperature and column outlet pressure Mobile phase: pentane. Stationary phase and flow-rate as in Fig. 1

Effects of both temperature and pressure on the capacity ratio can be seen in the three-dimensional "network" plot shown in Fig. 4a. Such network plots have been obtained for pentane as the mobile phase and the substrates naphthalene, anthracene, pyrene and chrysene[37]. Two elevations are seen, one in the liquid state and a larger one in the fluid state above the critical temperature (T_c for pentane: 196.6°C). With increasing pressure, the latter k' elevation is observed to decrease, its crest moving to higher temperatures, and finally to disappear. Fig. 4b shows a corresponding network plot for the average resolution, R_m, calculated from the chromatograms of the four aromatic compounds as described previously[38]. Again, maxima are observed at temperatures above the critical temperature[30,31,35-37], and like the k' max-
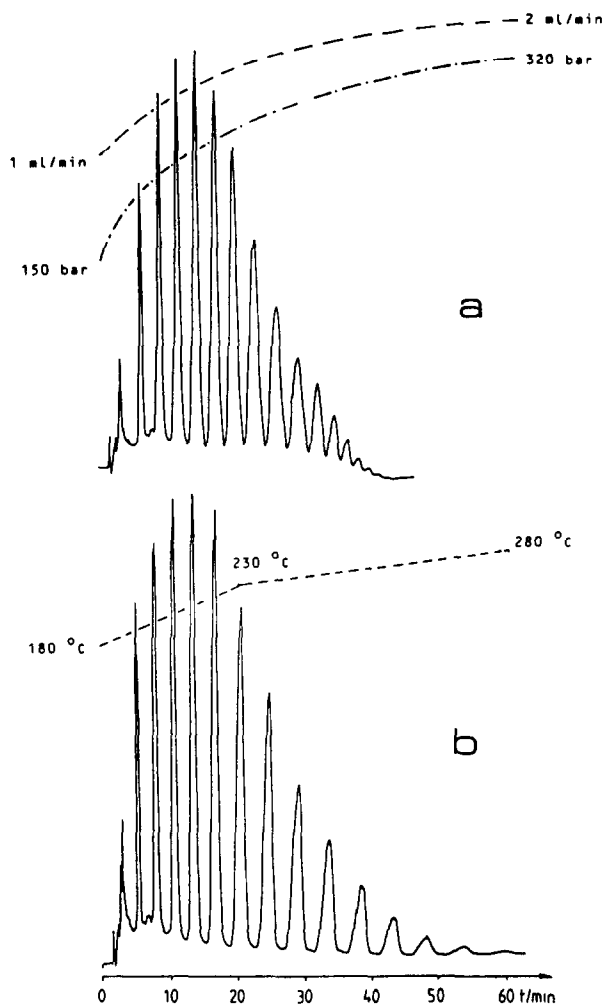

Fig. 5. Separations of styrene oligomers (PS 800) by means of (a) isothermal pressure programming at 180°C and (b) combined pressure and temperature programming. Mobile and stationary phases as in Fig. 2. The gradients are shown, the pressure and the flow programmes being valid for both (a) and (b).

ima, the R_m maxima decrease and are shifted to higher temperatures with increasing pressure.

In order to maintain high values for R_m, Fig. 4b suggests that the temperature be increased during a pressure-programmed separation according to the rise in T_R^m (temperature at the resolution maximum). The corresponding temperature gradient can be derived from three-dimensional plots of the type in Fig. 4b. The effect of such combined pressure and temperature gradients is demonstrated in Fig. 5, for separations of styrene oligomers with a carbon dioxide–dioxane mixture as the mobile phase. Although this mobile phase is different from that of Fig. 4, the general observation of increasing T_R^m with increasing pressure remains valid, also for binary eluents[39]. This will be addressed in more detail in a separate paper[40]. With the combined p/T programming (Fig. 5b), the separation efficiency is enhanced. For this example, the pressure gradient was generated by increasing the mass flow. Enhancement of separation efficiency is also obtained with combined p/T programming when the pressure gradient is obtained at a constant mass flow. This was demonstrated almost ten years ago by Klesper and Hartmann[4], who separated styrene oligomers with a mobile phase composed of 95% pentane and 5% methanol.

As mentioned above, besides pressure programming, gradients of eluent composition have been used in SFC. The application of this "gradient elution" technique to the separation of oligomers has been studied to some extent previously. In one of these studies[38] it was shown that an optimum pressure may exist for separating an oligomer sample by gradient elution; at this optimum pressure, a maximum overall resolution was obtained for the separation of an oligostyrene sample. Furthermore, it was demonstrated[11] that, by changing the column temperature, a separation could be adjusted to the complexity of the separation problem.

With the gradient elution technique, a combination is chosen of a poor solvent (eluent A) as the primary component and a good solvent (eluent B) as the secondary
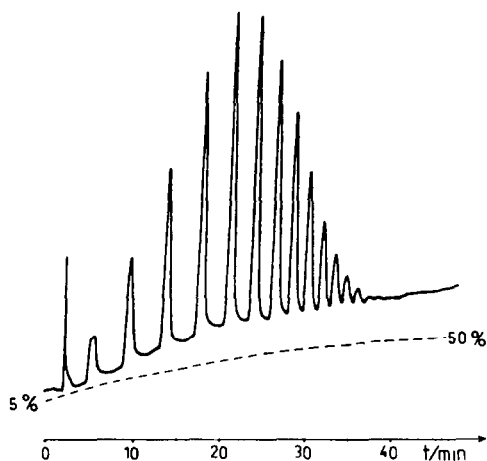

Fig. 6. Separation of styrene oligomers (PS 800) by gradient elution at isothermal and isobaric conditions. Mobile phase: carbon dioxide (A)–1,4-dioxane (B). Temperature: 135°C. Pressure: 275 bar. Stationary phase and flow-rate as in Fig. 2. The gradient is shown. The content of B in the mobile phase is based on volume, measured at the pumps in the liquid state.

component of the mobile phase, the amount of eluent B being increased during the separation. Because of its relatively high thermal stability, 1,4-dioxane has frequently been used as eluent B for the separation of oligomers from styrene and related monomers when the critical temperature of eluent A is high, such as with pentane[7,8,11-13,38]. However, low-boiling solvents can also be used as eluent A. Fig. 6 shows a separation of styrene oligomers with carbon dioxide as eluent A and 1,4-dioxane as eluent B under isothermal and isobaric conditions. Because of the low critical temperature of carbon dioxide, a secondary component can be chosen which is thermally less stable. Dichloromethane, having a critical temperature of 245°C, has been reported to be unsuitable as a mobile phase in SFC[41]. Nevertheless, we have used supercritical ethane–dichloromethane mixtures at 150°C without experiencing any problems from the low thermal stability of dichloromethane. For example, a separation of the styrene oligomers with the eluent ethane–dichloromethane is shown in Fig. 7.

For this separation, the flow resistor was kept constant during the separation, *i.e.*, the position of the needle valve downstream of the column was not changed during the experiment. This leads to an inherent pressure gradient if the viscosity of eluent B is higher than that of eluent A. Such an inherent pressure gradient enhances the effect of the composition gradient[8], and thus lower amounts of eluent B are needed for effecting the same separation as with constant pressure. For difficult-to-elute compounds it has sometimes been found necessary to apply a strong additional pressure gradient[11]. This can be accomplished by significantly increasing the flow-rate against a constant flow resistor.

With eluent mixtures, knowledge of the critical data for the mixtures is important. Unfortunately, experimental data are not available for many mixtures of
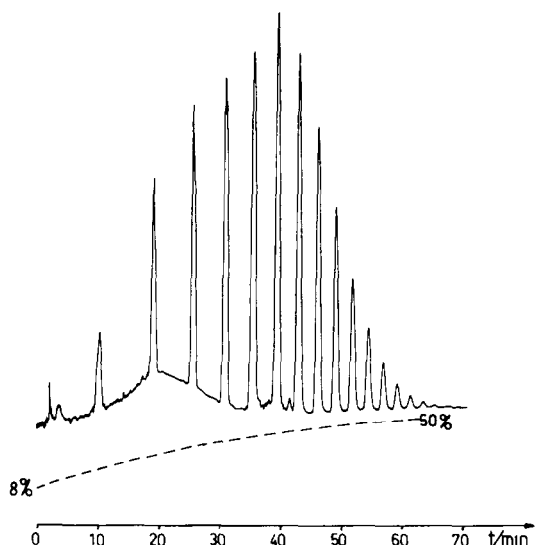

Fig. 7. Separation of styrene oligomers (PS 800) by gradient elution under isothermal conditions. Mobile phase: ethane (A)–dichloromethane (B). Temperature: 150°C. Pressure: 210 bar at the start of the chromatogram and 230 bar at the end of the separation. The composition gradient is shown. Other conditions as in Fig. 6.
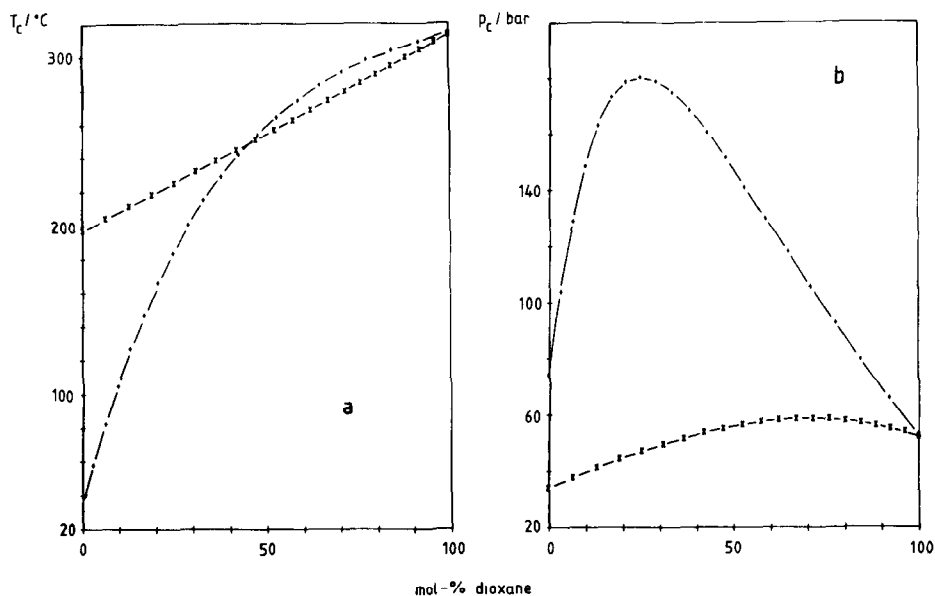
Fig. 8. Critical temperatures (a) and critical pressures (b) of two mixtures: x, pentane–1,4-dioxane; +, carbon dioxide–1,4-dioxane. Calculated according to published methods[42].
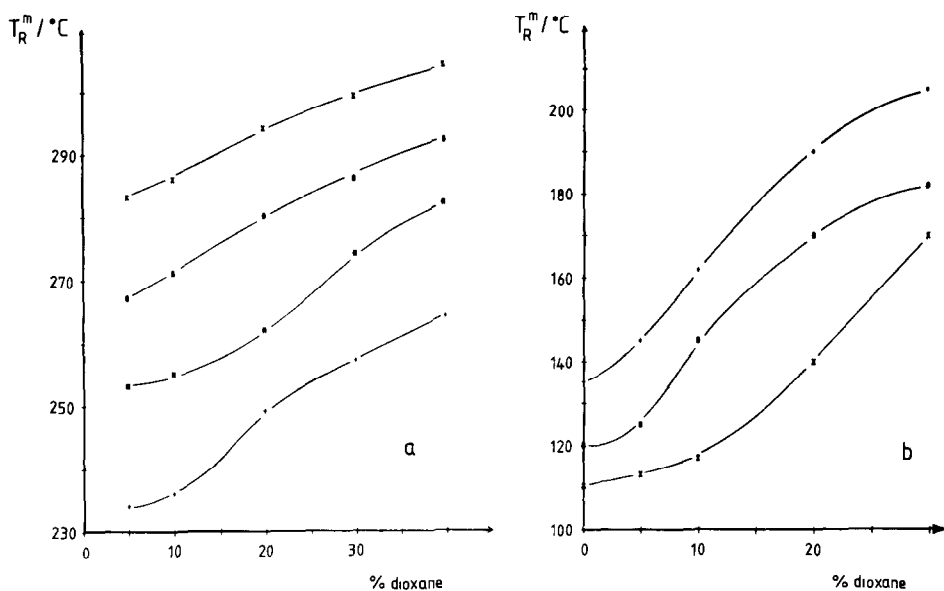

Fig. 9. Dependence of the temperature at the resolution maximum, T_R^m, on the amount of 1,4-dioxane in mixtures of (a) pentane–dioxane and (b) carbon dioxide–dioxane at different pressures. Stationary phase and flow-rate as in Fig. 1. Pressures: (a) ×, 60; ○, 50; #, 40; +, 30 bar; (b) +, 250; ○, 200; ×, 150 bar. Dioxane percentages are v/v for (a) and w/w for (b).
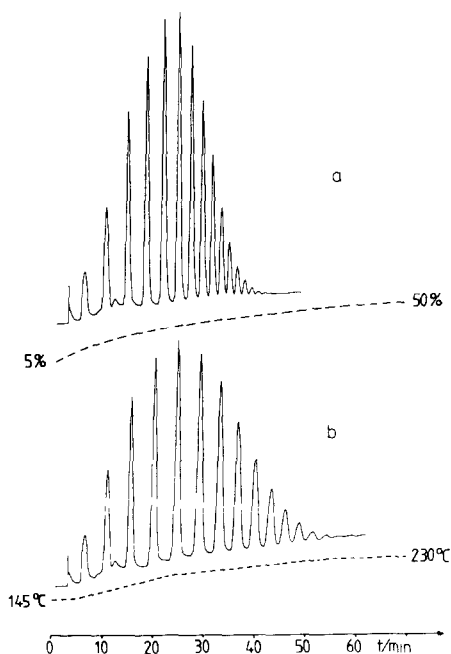
Fig. 10. Separations of styrene oligomers (PS 800), (a) by means of isothermal composition programming at 145°C, and (b) by combined composition and temperature programming. Mobile phase: carbon dioxide (A)–dioxane (B). Pressure: 250 bar at the start, and 295 bar at the end of the separation. The gradients are shown, the composition programme being valid for both (a) and (b). Other conditions as in Fig. 6.

interest. However, there are methods for calculating critical properties of mixtures. Fig. 8 shows the critical curves for two binary mixtures of interest in the present work: pentane–dioxane and carbon dioxide–dioxane. Calculations were carried out according to the methods described by Chueh and Prausnitz[42]. These curves show the typical shapes: more or less bent curves for the critical temperatures, and a pressure maximum which is more pronounced the higher is the difference between the critical pressures of the pure components. For SFC with isothermal composition gradients, the critical temperature of the mixture at the end of the separation usually determines the minimum working temperature.

Just like pure eluents, binary eluent mixtures show temperature-dependent maxima for the capacity ratio and resolution, the T_k^m and T_R^m being shifted to higher values not only with increasing pressure but also with increasing content of the higher-boiling component in the eluent mixture[43]. In Fig. 9, T_R^m is shown as a function of the dioxane content in mixtures with pentane or carbon dioxide at different pressures. These plots suggest that superposing a temperature programme on a composition programme should lead to an enhancement of resolution, similar to that seen in Fig. 5 for combined pressure and temperature programming. This is shown for a separation of styrene oligomers in Fig. 10, the mobile phase being carbon dioxide–dioxane. Keeping the composition gradient and the inherent pressure gradient the same, a fair enhancement of resolution is obtained with a multiple composition–pressure–temperature gradient[43].
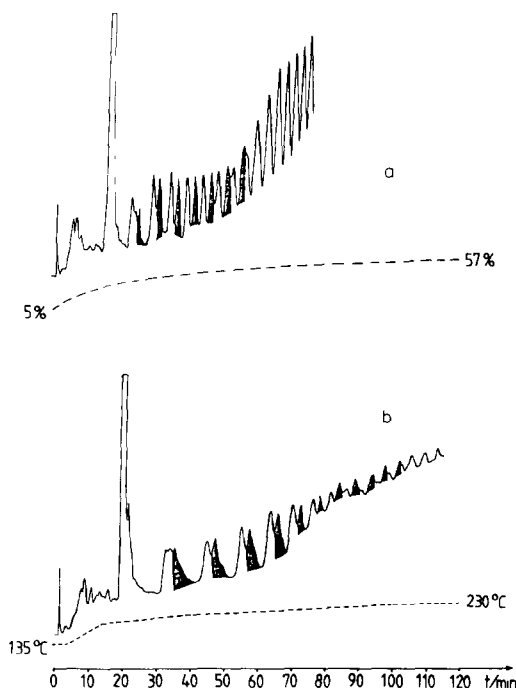
Fig. 11. Separations of 2-vinylnaphthalene oligomers, (a) by means of isothermal composition program-ming at 145°C and (b) by combined composition and temperature programming. Pressure: increasing from 220 to 240 bar for (a) and from 220 to 250 bar for (b). Gradients as shown; other conditions as in Fig. 10.

It has been observed by means of SFC that the anionic-initiated oligomeri-zation of 2-vinylnaphthalene can lead to several parallel homologous series[11,12]. This can also be observed from the chromatogram shown in Fig. 11a, where for seven subsequent degrees of oligomerization a peak splitting is seen. When the same com-position gradient is coupled with a temperature gradient, parallel series are resolved for eleven degrees of oligomerization (Fig. 11b). Although there have been as yet few applications of the multiple gradient techniques, the results presented show that mul-tiple gradients can be used for improving the efficiency of a separation.

CONCLUSIONS

Along the guidelines described, strategies can be envisaged for optimizing the conditions for the SFC separation of oligomers. These guidelines and some additional points for the optimization by means of temperature, pressure or composition gra-dients are now summarized.

Gradients of decreasing temperature (negative temperature gradients) can suc-cessfully be applied only if the dependence of k' on temperature is known. For the elution of compounds of higher molecular weight, a negative temperature gradient can be extended down below the critical temperature of the mobile phase into the liquid region. Nevertheless, the molecular weight range which can be covered by negative temperature programming appears to be limited.

For pressure-programmed separations, the appropriate initial pressure depends on the composition of the sample; a pressure is usually chosen which leads to good separation of the early-eluted components. When an isothermal, pressure-programmed separation is desired, the resolution may be enhanced by working at a temperature in the region of the k' maximum. Combining the pressure gradient with a temperature gradient may lead to a further substantial enhancement of the resolution.

With composition-programmed separations, the initial amount of eluent B is chosen to be as low as possible in order to maintain a wide latitude for the gradient. The selection of the initial pressure depends on the composition of the sample and the eluent. When eluent A shows a significant solvent strength for the lower members of the oligomer sample, an initial pressure slightly above the critical pressure of the initial eluent composition is appropriate for maintaining good efficiency for the separation of the early-eluted components. The appropriate temperature depends on the complexity of the sample. If only one homologous series is present, a rather low temperature can be chosen. For more complex samples it may become necessary to work near the temperature of the resolution maximum for the initial pressure and eluent composition, in order to achieve good resolution, at least for early-eluted compounds. In order to maintain optimum chromatographic efficiency throughout the experiment, it may be necessary to combine the composition programme with a temperature programme. For the chromatography of oligomers that are difficult to elute, it may be useful to combine the composition programme with a pressure programme to speed up the separation.

ACKNOWLEDGEMENTS

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