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CHROMATOGRAPHIC BEHAVIOUR OF VARIOUS ELUENTS AND ELUENT MIXTURES IN THE LIQUID AND IN THE SUPERCRITICAL STATE

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SUMMARY

The influence of temperature, pressure, and density, as well as type and composition of the mobile phase on capacity factors and on resolution was studied. In general, a shorter analysis time and lower values for the resolution were observed when increasing either pressure, density or modifier content or when working at temperatures just below the critical point. However, there are combinations of distinct values for temperature, pressure, and composition, where capacity factors and resolutions reach maximum values. Knowledge of these combinations of values opens a variety of possibilities to optimize separations.

INTRODUCTION

Recently, interest in supercritical fluid chromatography (SFC) has grown significantly, and a need has arisen for methods to optimize separations. For that purpose, knowledge is required of the influence of chromatographic parameters on retention and separation efficiency.

Studies have been reported on the influence of the type of mobile^{1–12} and stationary phase^{1–3,13–18}, temperature^{1,2,6,7,10,16,19–27}, pressure^{2,3,7,20,23–29}, and density of the mobile phase^{7,25,28,30–32}. Due to this variety of parameters, the possibilities for optimizing SFC separations are considerable. Moreover, the analyst has to pay attention to the mutual interdependences between the different parameters. As an example, by varying the eluent composition at a given temperature and pressure, not only the critical temperature and the critical pressure of the mobile phase are affected, but also its chemical nature and the free volume between the eluent molecules³³.

In this work, the influence of temperature, pressure, density, and type and composition of the mobile phase on simple test separations is demonstrated, using packed columns. Their effects on the capacity factors (k') and the resolution (R) will be discussed with respect to the possibilities for optimizing SFC separations.

EXPERIMENTAL

The apparatus, columns and chemicals used were described previously³⁴.

TABLE I
CRITICAL DATA OF ELUENTS³⁶

<i>Eluent</i>	<i>T_c</i> (°C)	<i>p_c</i> (bar)
Propane	96.7	42.5
<i>n</i> -Butane	152.0	38.0
<i>n</i> -Pentane	196.5	33.7
1,4-Dioxane	313.9	52.1

The capacity factors were calculated according to

$$k' = \frac{t_R - t_0}{t_0}$$

using *n*-heptane for measuring the dead times t_0 .

Chromatographic resolution was calculated by a method also described previously²⁷, which is applicable to pairs of peaks which are either completely or partly resolved. The density at a given temperature and pressure was obtained from tables published by Löffler³⁵.

Pressures were measured both at the column inlet (p_i) and at the column outlet (p_e). In the graphs, column outlet pressures have been used as the parameter. Differences between pressures at column inlet and column outlet (column pressure drops) were of the order of 10 bar, similar to those reported previously²⁴.

Critical data of the eluents used in this study are summarized in Tables I and II.

RESULTS AND DISCUSSION

Chromatographic separations can be characterized by capacity factors, selectivity, and resolution. Whereas capacity factors and selectivity describe solute retention, resolution contains additional information about peak shapes. Therefore, discussion of both types of characteristics is useful. The systematic studies were carried out by using propane, butane, pentane, and mixtures of pentane and 1,4-dioxane as eluents, unmodified silica as stationary phase, and naphthalene, anthracene, pyrene, and chrysene (PAHs) as solutes.

Capacity factors of pyrene, $k'(p)$, show a strong dependence on column tem-

TABLE II
CRITICAL TEMPERATURES FOR MIXTURES OF PENTANE AND 1,4-DIOXANE

Calculated according to the method described by Chueh and Prausnitz³⁷.

<i>T_c</i> (°C)	<i>Dioxane</i> (% , <i>v/v</i>)								
	0	10	20	30	40	50	70	90	100
<i>T_c</i> (°C)	196.5	211.2	225.2	238.4	250.9	262.8	284.8	304.7	313.9

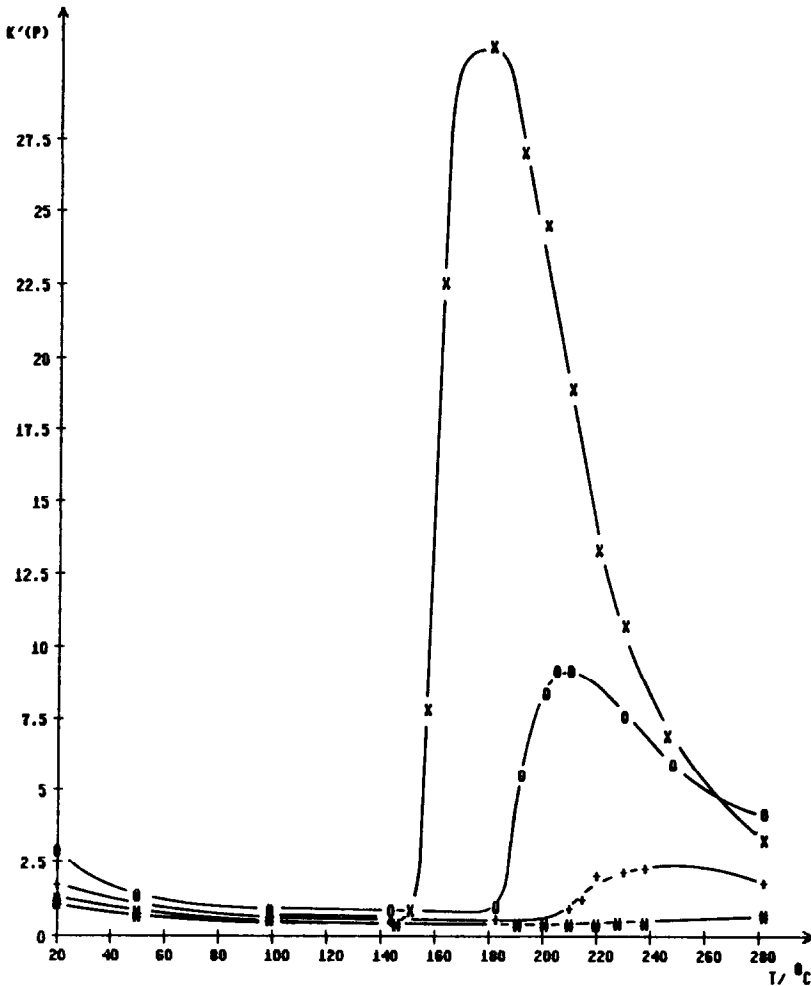


Fig. 1. Variation of the capacity factor with column temperature at four different column outlet pressures: 6 bar (\times), 20 bar (o), 36 bar (+), 70 bar ($\#$). Eluent: pentane. Solute: pyrene.

perature when working under isobaric conditions, especially at low pressures (Fig. 1). On increasing the temperature from ambient, where the mobile phase is a liquid, k' values decrease and reach a minimum. At temperatures above the boiling point of the eluent and pressures below critical (*i.e.*, at $p_e = 6$ and 20 bar) or above the critical temperature at pressures above critical the k' values increase. This increase is the more pronounced the lower the pressure and the higher the molecular weight of the solutes^{21,23,24}. All curves in Fig. 1 pass through a maximum, except the one at $p_e = 70$ bar. Thereafter the k' values decrease to values comparable to those at low temperatures.

The occurrence of maxima in k' vs. T plots is caused by the combined action of two opposing effects. First, when the temperature increases from a low value the

eluent density and, thereby, the solute concentration in the mobile phase decreases. Second, at higher temperatures the vapour pressure of the solute increases and, moreover, the adsorption power of the stationary phase decreases. The second effect overcompensates the first if the temperature exceeds a certain level, which is considerably above the temperature of either the boiling point or the critical point. At high pressures, the density decrease is too small for the first effect to become efficient and, therefore, the capacity factors do not show a maximum.

The strong increase in the k' values with increasing temperature is accompanied by an increase in the resolution between two adjacent peaks and in the mean resolution, R_m , of the four PAHs (Fig. 2). In previous studies we found that the maxima in R_m were mainly caused by maxima in the number of theoretical plates²⁴. When the pressure is not too high, R_m values also pass through maxima, the positions

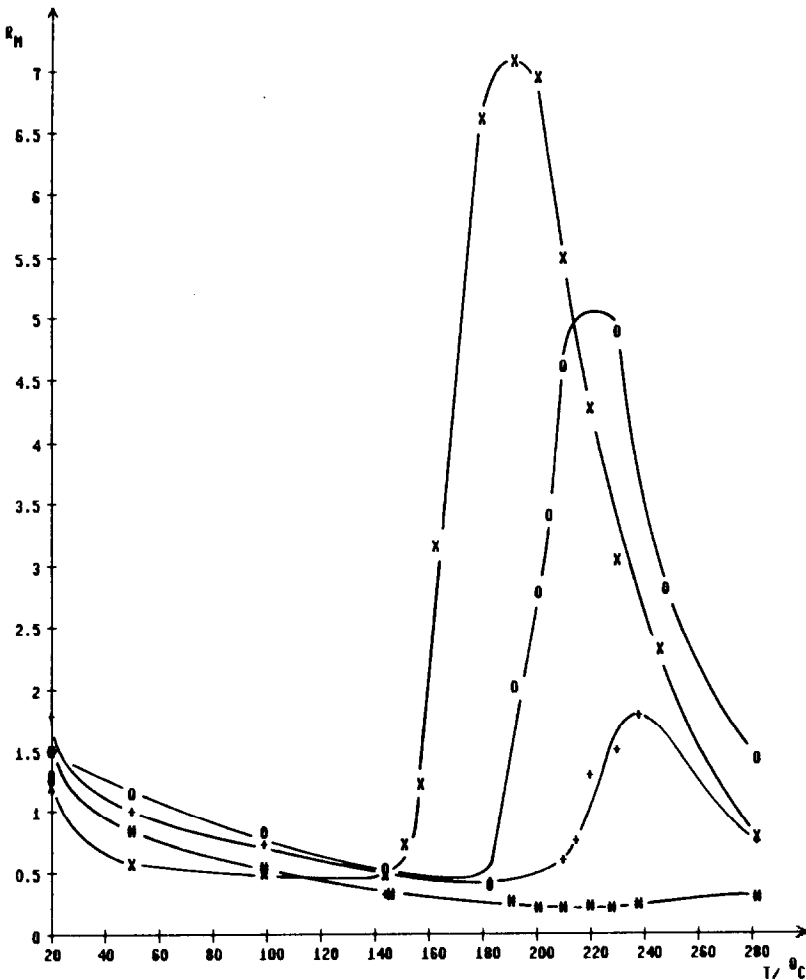


Fig. 2. Variation of the mean resolution with the temperature at four different column outlet pressures (symbols as in Fig. 1). Eluent: pentane. Solutes: naphthalene, anthracene, pyrene, chrysene.

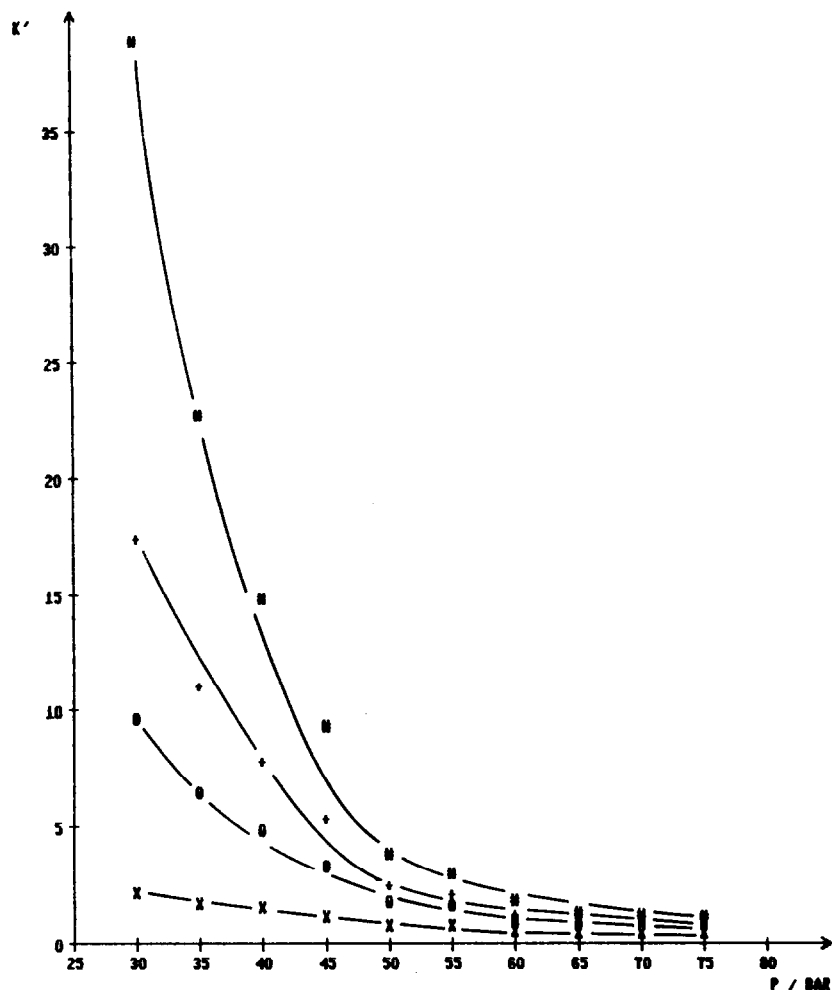


Fig. 3. Variation of the capacity factor with the column outlet pressure. Eluent: pentane. Solutes: naphthalene (\times), anthracene (o), pyrene (+), chrysene (#). Column temperature: 240°C.

and intensities of which depend on the pressure. The temperature at which maxima occur for R_m and k' are about the same. Like the capacity factors, the resolution decreases with increasing pressure and at a column exit pressure (p_e) of 70 bar, no maximum was observed for mobile phases containing pentane within the temperature range of this study. Like the maximum k' , the maximum R_m is shifted to higher temperatures with increasing pressure.

In SFC, pressure plays a role comparable to that of temperature in GC. By changing or programming the pressure, SFC separations can be varied considerably. As Fig. 3 shows for pyrene as the solute, capacity factors decrease upon increasing the pressure under isothermal conditions (240°C). Thus, analysis times are shortened, *i.e.* separations are accelerated. The influence of the pressure on the mean resolution,

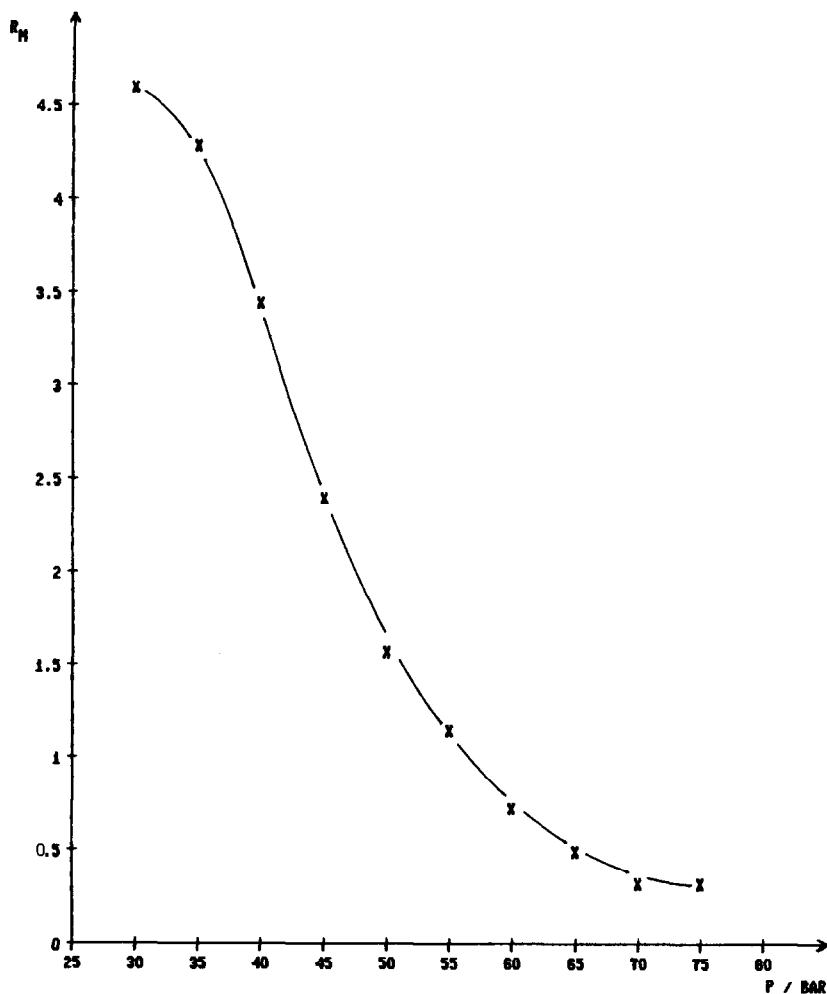


Fig. 4. Variation of the average resolution with the column outlet pressure. Eluent: pentane. Solutes: PAHs. Column temperature: 240°C.

R_m , is shown in Fig. 4 for the same conditions as in Fig. 3. Resolution is seen to decrease considerably with increasing pressure. The effects as illustrated in Figs. 1–4 for temperature and pressure are similar for other mobile phases and for other solutes of low molecular weight. High-molecular-weight solutes usually require rather high pressures (densities) to be eluted.

The three parameters pressure, temperature, and density are related by an equation of state, *i.e.* every combination of temperature and pressure defines the density. For the most common eluents, especially for alkanes and carbon dioxide, density data are available in the literature. The density is more directly related to solubility, viscosity and to the diffusion coefficient than is the pressure for a given mobile phase. Therefore, k' may be plotted *vs.* density (ρ) rather than *vs.* pressure.

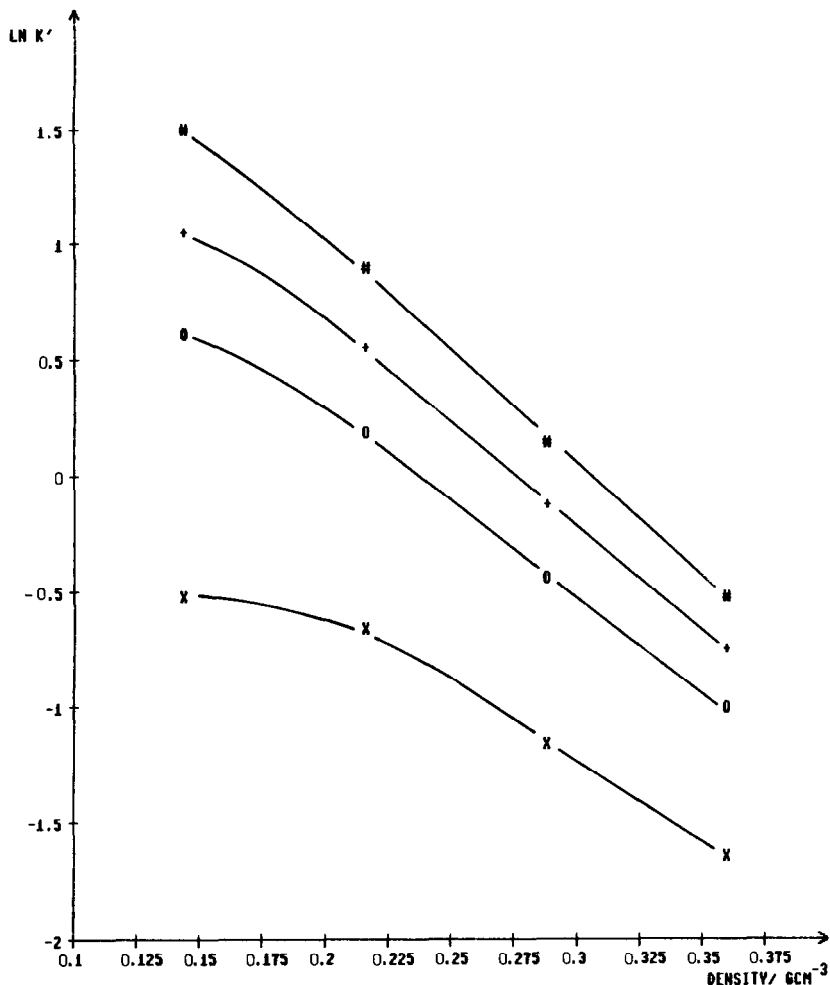


Fig. 5. Variation of the capacity factor with the density. Eluent: pentane. Solutes: PAHs (symbols as in Fig. 3). Column temperature: 240°C.

As shown in Fig. 5, the plots of the logarithm of capacity factors *versus* density at a constant temperature of 240°C, using pentane, show more or less straight lines. The negative slope reflects a decrease in retention with increasing density and a concurrent decrease in the distribution ratio. The variation of the capacity factor with the density is a function of the temperature and of the solute.

The influence of the density on the mean resolution is similar to that of the pressure, as is shown in Fig. 6 using the same conditions as in Fig. 5. Thus, upon increasing the column pressure and thereby the mobile phase density, separations are speeded up at the expense of resolution. Since the liquid volume flow-rate was always 1 ml/min, a twofold increase in density entails a twofold decrease in linear velocity in the column. As Fig. 6 shows, this decrease does not suffice to prevent a strong decrease in R_m .

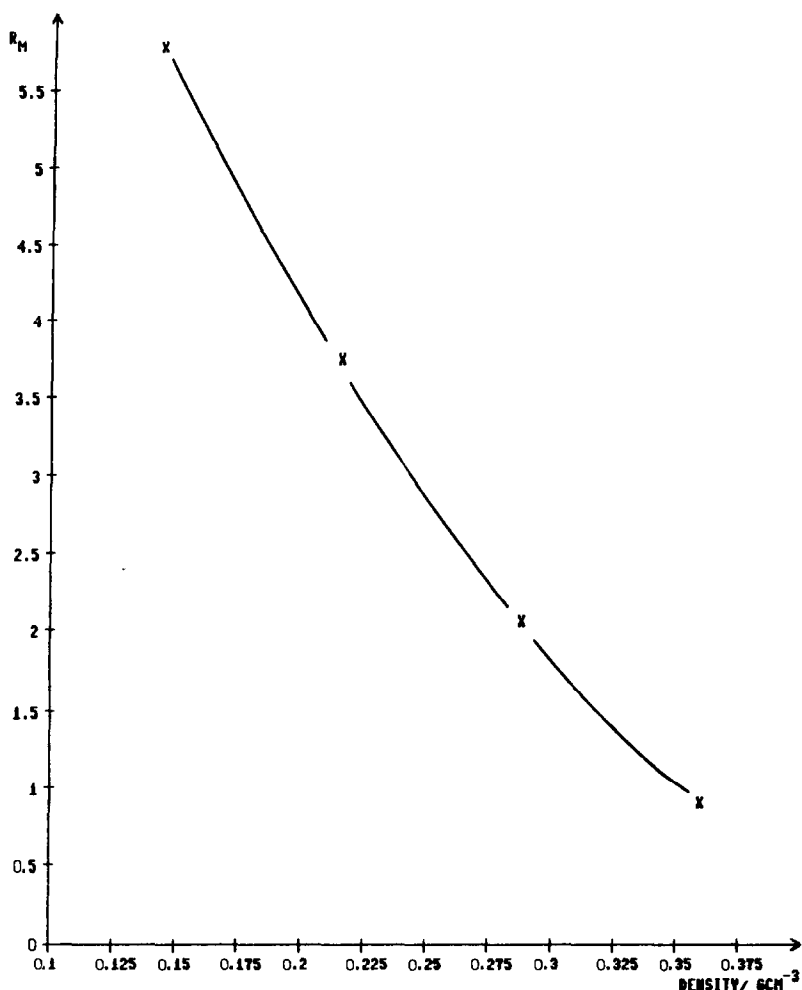


Fig. 6. Variation of the average resolution with the density. Eluent: pentane. Solutes: PAHs. Column temperature: 240°C.

As is the case in HPLC, the type of mobile phase has a decisive influence on the separation behaviour in SFC. By choosing different eluents, the solvating power for a given solute can be varied considerably. Besides carbon dioxide, which is frequently used because of its low critical temperature of 31.3°C, alkanes are common eluents in SFC. Homologues of pentane, *e.g.* butane and propane yield results similar to pentane for the effects of temperature and pressure. The $k'(T)$ -isobars at similar reduced pressures (Fig. 7) also show maxima. Indeed maxima were obtained with all other eluents examined. However, differences exist with respect to the positions and the magnitudes of these maxima. Due to the decreasing critical temperatures, they are shifted to lower temperatures in the order pentane, butane, and propane. Moreover, the smaller the solvent molecule, the higher the maximum.

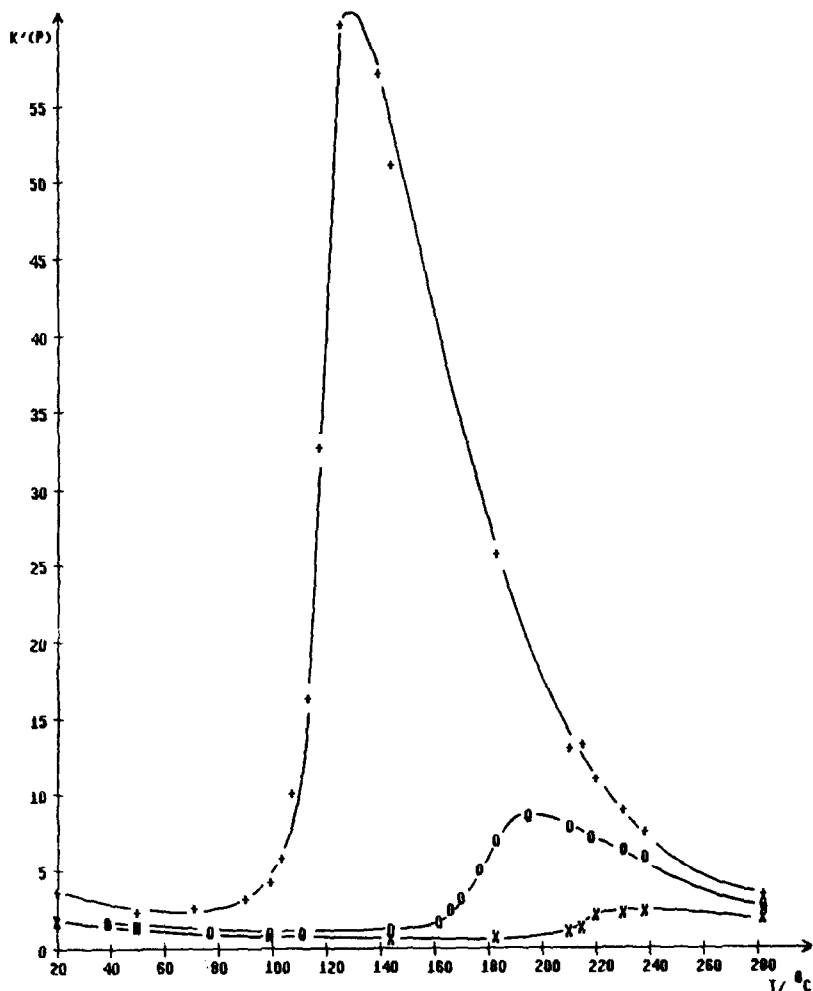


Fig. 7. Variation of the capacity factor with the column temperature, comparing three homologous alkanes at similar reduced pressures. Eluents and column outlet pressures: propane (+) at $p_c = 43$ bar, butane (0) at $p_c = 39$ bar, pentane (x) at $p_c = 36$ bar. Solute: pyrene.

The same statements can be made with regard to the chromatographic resolution (R_m) comparing different alkanes. Using propane, significantly higher resolutions at lower temperatures can be obtained than using pentane (Fig. 8). Consequently, the use of lower members of a homologous series permits working at lower temperatures and provides increased resolution at the same time. On the other hand, if a higher solvent strength of the eluent is needed, one may increase the pressure (density) of the same eluent, or else one may change to a higher homologue. This applies also if one desires a shorter analysis time.

Not only by changing the mobile phase, but also by adding a second component to a primary eluent, can the properties of the mobile phase be altered consider-

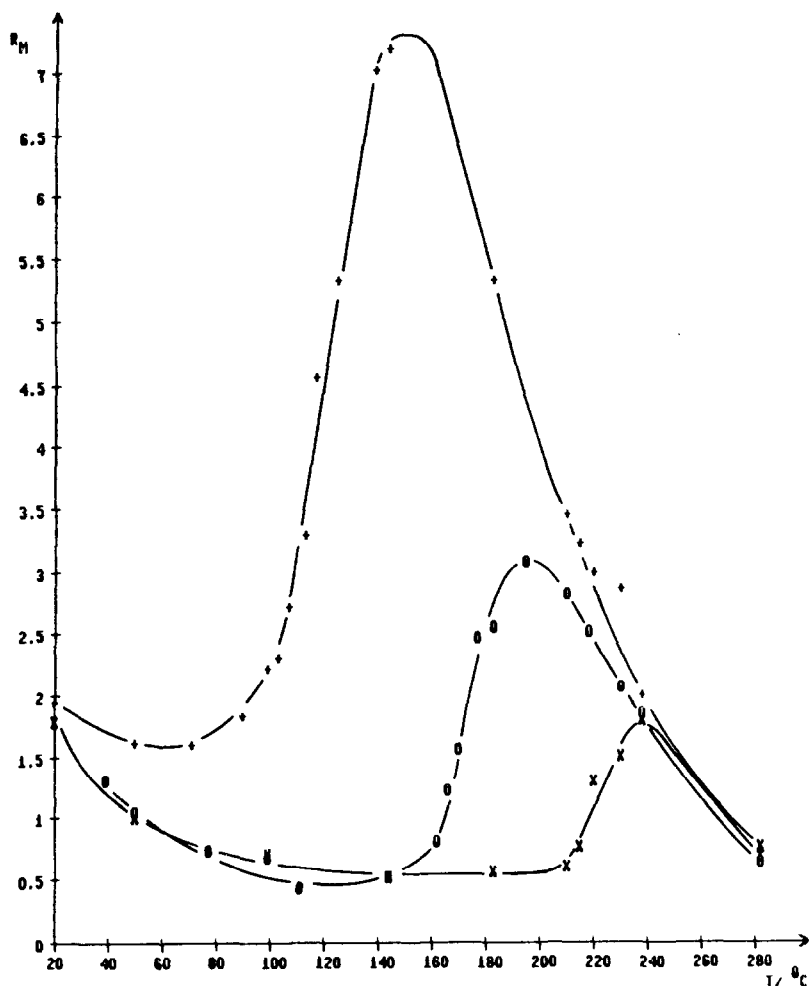


Fig. 8. Variation of the average resolution with the column temperature, comparing three homologous alkanes. Eluents: pentane, butane, propane (symbols and column outlet pressures as in Fig. 7). Solutes: PAHs.

ably. If the second component dioxane shifts the distribution of the solute towards the mobile phase, then the capacity factors decrease with increasing dioxane content in the eluent mixture at constant temperature (Fig. 9). Eventually at high dioxane concentration, coelution of the sample components may be observed. The capacity factors of larger molecules are reduced more than those of smaller molecules. For the chromatographic analysis of a homologous series, this implies that the elution of the higher members will be accelerated more strongly than that of the lower members.

With packed columns, especially with modified (bonded) silica, it has frequently been observed that small modifier concentrations (below 1%) already show a considerable reduction in k' due to the interaction of the modifier with the stationary phase. The effect is larger than what may be expected from a change in mobile

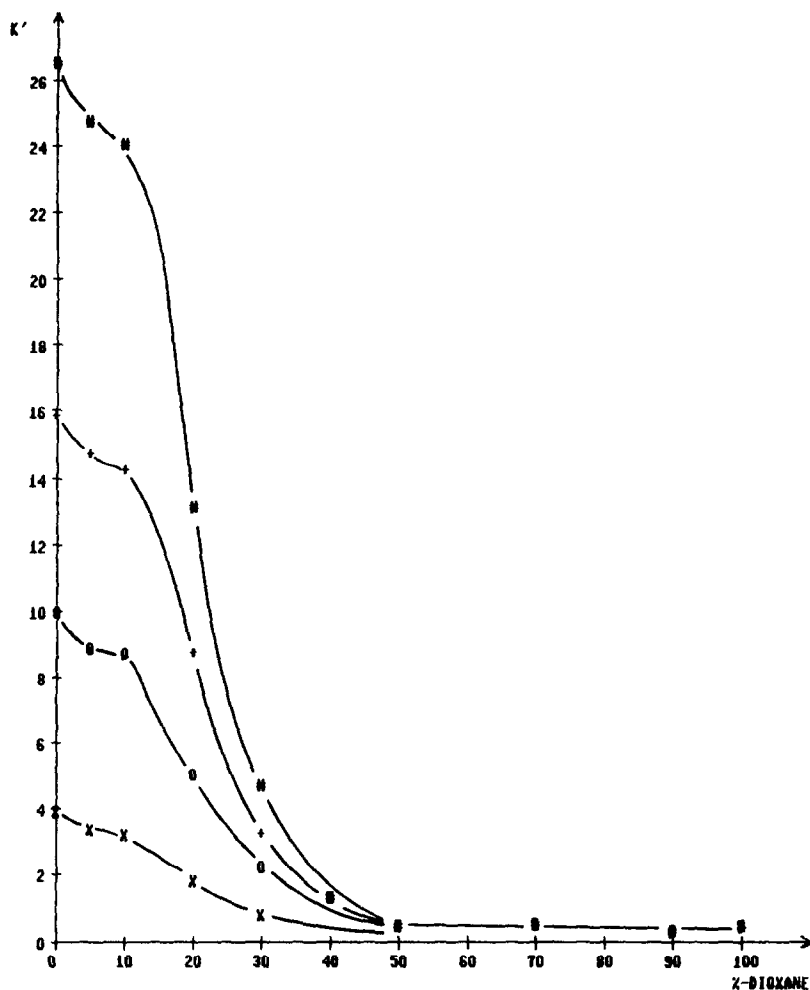


Fig. 9. Variation of the capacity factor with the mobile phase composition. Eluent: pentane-1,4-dioxane. Solutes: PAHs (symbols as in Fig. 3). Column temperature: 240°C. Column outlet pressure: 36 bar.

phase composition alone^{8,9}. This “modifier effect” has been ascribed to interactions with highly active sites of the stationary phase. This has been confirmed by the work of Wright and Smith¹², who failed to obtain similar effects on polysiloxane coated capillary columns. In our present work, these “modifier effects” have not been studied explicitly; the experiments concentrated on studying variations in solvent strength.

The shape of the curves of resolution vs. dioxane content (Fig. 10) is more complex than those for the capacity factors. Adding small amounts of dioxane not only led to shorter analysis times, but also to a decrease in peak widths. Therefore, the resolution reached a maximum at about 10% (v/v) of 1,4-dioxane for the example shown here. A further increase in the amount of dioxane then causes resolution to decrease considerably.

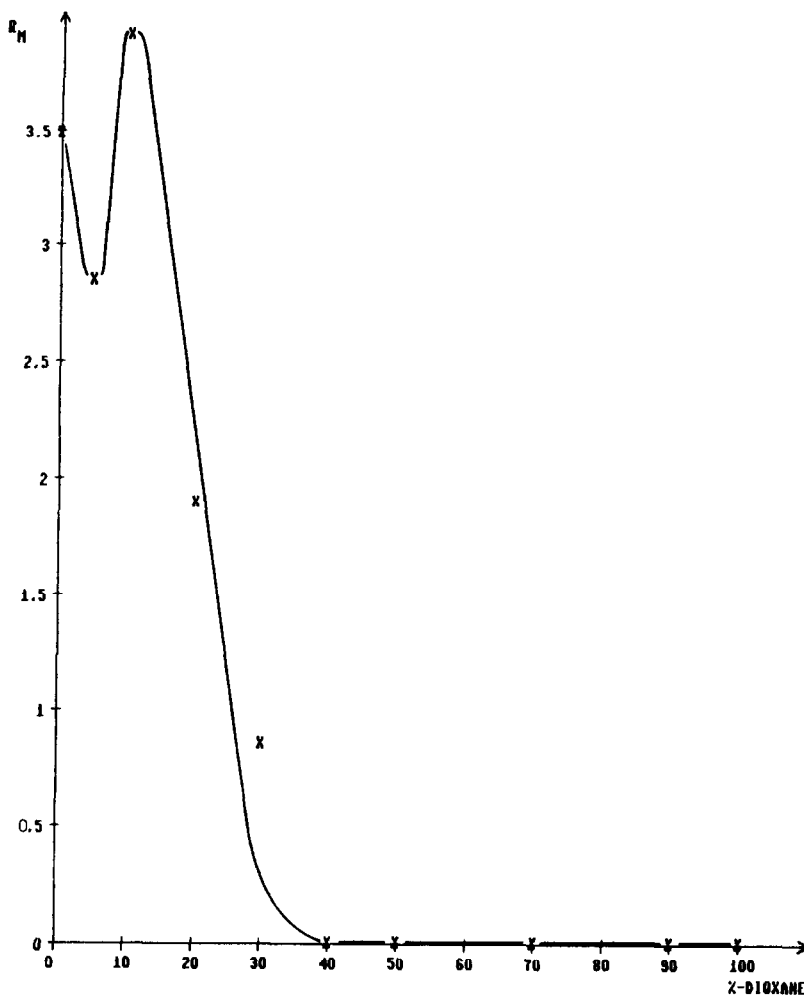


Fig. 10. Variation of the average resolution with the mobile phase composition. Eluent: pentane-1,4-dioxane. Solutes: PAHs. Column temperature: 240°C. Column outlet pressure: 36 bar.

With mixed eluents, maxima are observed in the temperature dependence of capacity factors and resolution, just as described above for pure eluents. Thus, with mixed eluents, for a given composition and pressure, capacity factors and resolution show maxima at certain temperatures. These temperatures are shown in Fig. 11 for the capacity factors of pyrene and in Fig. 12 for the resolution of the peak pair anthracene-pyrene. The mobile phase was a mixture of pentane and 1,4-dioxane. With increasing dioxane concentration and with increasing pressure, the maxima in the capacity factors and the resolution are shifted to higher temperatures. Above a certain dioxane concentration, no maximum in k' and R_m could be observed within the examined temperature range. From the graphs of Fig. 12, conditions of maximum resolution can be deduced as follows. For a given set of two parameters (*e.g.*, eluent

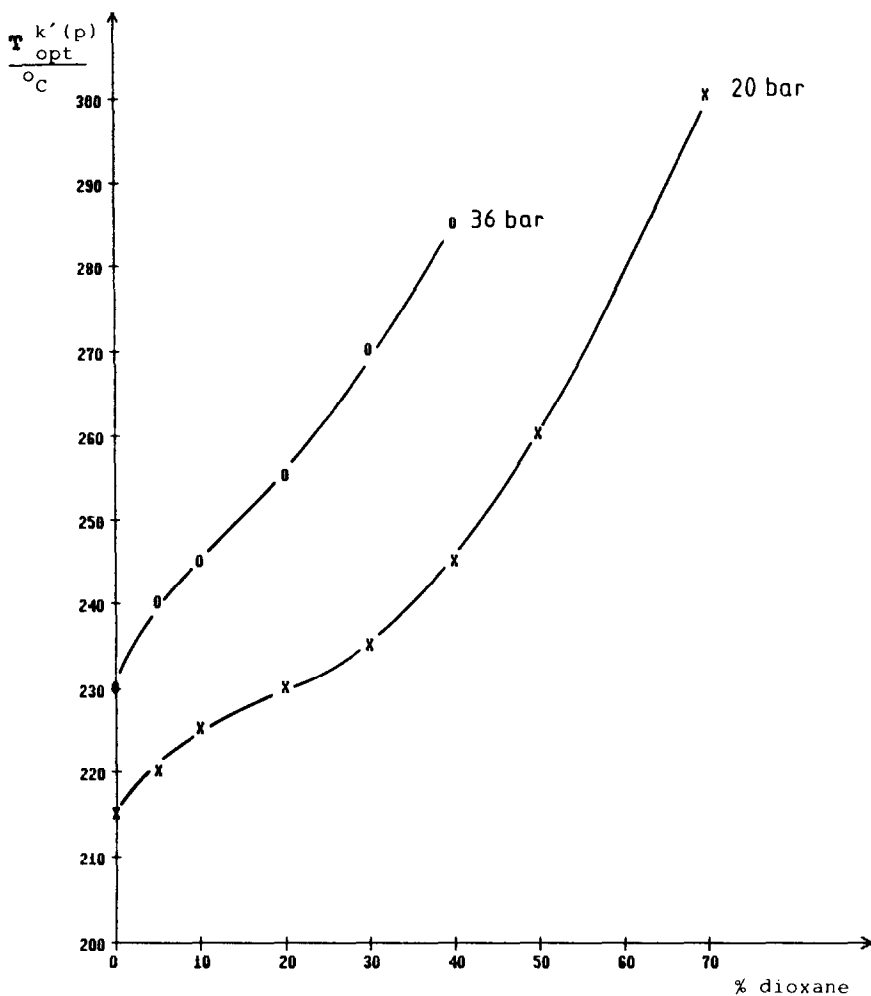


Fig. 11. Temperature of the maximum in the plots of capacity factor vs. eluent composition. Eluent: pentane-1,4-dioxane. Solute: pyrene.

composition and pressure), the value for the third parameter (temperature) can be read from the diagram. Although the diagram gives no information about the absolute resolution values, it represents the location of the maxima. The figure can be applied for optimizing multiple gradient separations.

CONCLUSION

SFC offers a wide range of possibilities for optimizing separations by improving resolution as well as by reducing analysis time. The resolution increases with decreasing pressure (Figs. 2 and 4) or density (Fig. 6) and is highest at the temperature of the resolution maximum (Figs. 8 and 12). The reduction of capacity factors can

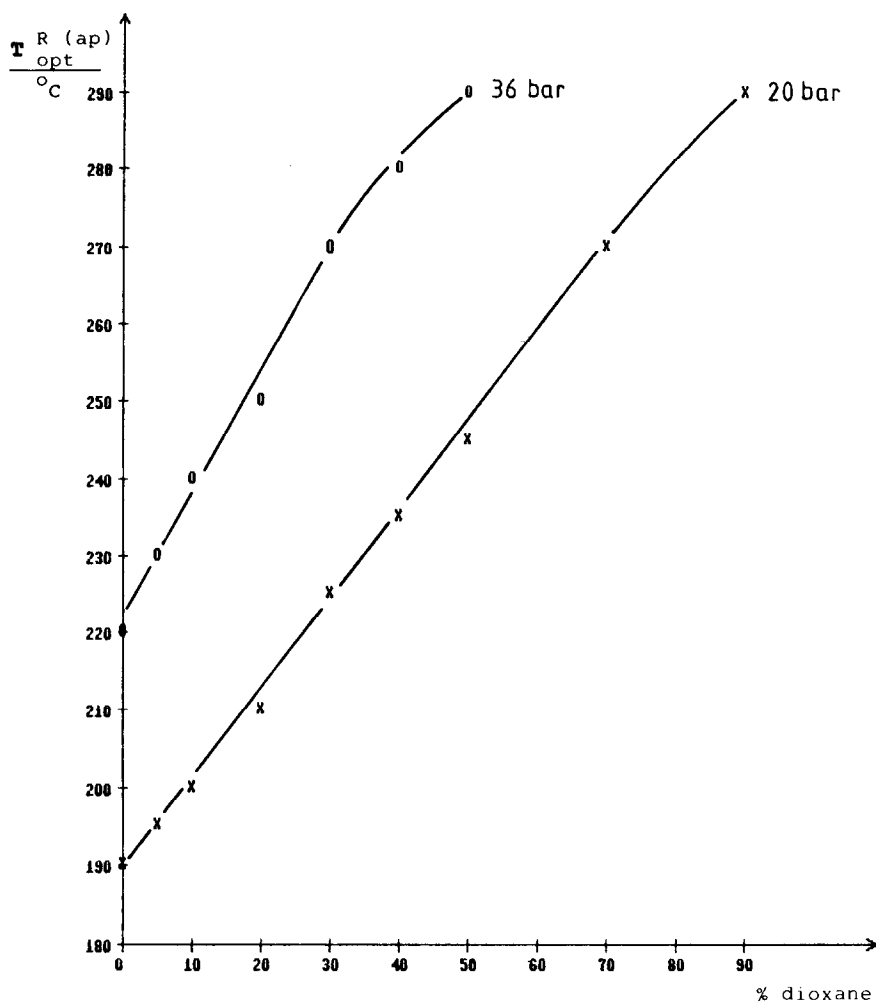


Fig. 12. Temperature of the maximum in the plots of vs. eluent composition. Eluent: pentane-1,4-dioxane. Solutes: anthracene, pyrene.

be achieved by increasing pressure and, thereby, density (Figs. 1, 3 and 5), or by choosing a mobile phase with enhanced solution power, *e.g.*, applying higher members of a homologous series (Fig. 7) or binary eluents (Fig. 9).

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REFERENCES

- 1 M. Novotný, W. Bertsch and A. Zlatkis, *J. Chromatogr.*, 61 (1971) 17.
- 2 J. A. Nieman and L. B. Rogers, *Sep. Sci.*, 10 (1975) 517.
- 3 J. E. Conaway, J. A. Graham and L. B. Rogers, *J. Chromatogr. Sci.*, 16 (1978) 102.
- 4 R. Board, D. McManigill, H. Weaver and D. Gere, *Publication No. 43-5953-1647*, Hewlett-Packard Co., Avondale, PA, 1982, p. 17; CHEMSA, June 1983, p. 12, 21.
- 5 L. G. Randall, *Technical Paper No. 102, Publication No. 43-5953-1722*, Hewlett-Packard Co., Avondale, PA, 1983.
- 6 Y. Hirata, *J. Chromatogr.*, 315 (1984) 31.
- 7 P. Mourier, M. Caude and R. Rosset, *Analisis*, 13 (1985) 299.
- 8 J. M. Levy and W. M. Ritchey, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 503.
- 9 A. L. Blilie and T. Greibrokk, *Anal. Chem.*, 57 (1985) 2239.
- 10 P. A. Mourier, E. Eliot, M. H. Caude, R. H. Rosset and A. G. Tambute, *Anal. Chem.*, 57 (1985) 2819.
- 11 B. W. Wright, H. T. Kalinoski and R. D. Smith, *Anal. Chem.*, 57 (1985) 2823.
- 12 B. W. Wright and R. D. Smith, *J. Chromatogr.*, 355 (1986) 367.
- 13 J. A. Graham and L. B. Rogers, *J. Chromatogr. Sci.*, 18 (1980) 75.
- 14 D. R. Gere, R. Board and D. McManigill, *Publication No. 43-5953-1647*, Hewlett-Packard Co., Avondale, PA, 1982, p. 7.
- 15 J. C. Kuei, B. J. Tarbet, W. P. Jackson, J. S. Bradshaw, K. E. Markides and M. L. Lee, *Chromatographia*, 20 (1985) 25.
- 16 F. Bickmann and B. Wenclawiak, *Fresenius' Z. Anal. Chem.*, 320 (1985) 261.
- 17 M. W. Ogden, and H. M. McNair, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 816.
- 18 W. R. West and M. L. Lee, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 161.
- 19 S. T. Sie and G. W. A. Rijnders, *Anal. Chim. Acta*, 38 (1967) 31.
- 20 B. P. Semonian and L. B. Rogers, *J. Chromatogr. Sci.*, 16 (1978) 49.
- 21 F. P. Schmitz, D. Leyendecker and E. Klesper, *Ber. Bunsenges. Phys. Chem.*, 88 (1984) 912.
- 22 F. P. Schmitz, H. Hilgers, D. Leyendecker, B. Lorenschat, U. Setzer and E. Klesper, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 590.
- 23 D. Leyendecker, F. P. Schmitz and E. Klesper, *J. Chromatogr.*, 315 (1984) 19.
- 24 D. Leyendecker, F. P. Schmitz, D. Leyendecker and E. Klesper, *J. Chromatogr.*, 321 (1985) 273.
- 25 T. L. Chester and D. P. Innis, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 561.
- 26 C. R. Yonker, B. W. Wright, R. C. Petersen and R. D. Smith, *J. Phys. Chem.*, 89 (1985) 5526.
- 27 F. P. Schmitz, *J. Chromatogr.*, 356 (1986) 261.
- 28 U. van Wasen and G. M. Schneider, *Chromatographia*, 8 (1975) 274.
- 29 C. R. Yonker, S. L. Frye, H. R. Udseth, B. W. Wright and R. D. Smith, *Prepr. Pap. Am. Chem. Soc., Div. Fuel Chem.*, 30 (3) (1985) 183.
- 30 A. Wilsch and G. M. Schneider, *Fresenius' Z. Anal. Chem.*, 316 (1983) 265.
- 31 C. R. Yonker and R. D. Smith, *J. Chromatogr.*, 351 (1986) 211.
- 32 B. Wenclawiak, *Fresenius' Z. Anal. Chem.*, 323 (1986) 492.
- 33 E. Klesper, D. Leyendecker and F. P. Schmitz, *J. Chromatogr.*, 366 (1986) 235.
- 34 F. P. Schmitz, H. Hilgers and E. Klesper, *J. Chromatogr.*, 267 (1983) 267.
- 35 H. J. Löffler, *Thermodynamische Eigenschaften binärer Gemische leichter gesättigter Kohlenwasserstoffe im kritischen Gebiet*, C. F. Müller, Karlsruhe, 1962.
- 36 A. P. Kudchadker, G. H. Alani, B. J. Zwolinski, *Chem. Rev.*, 68 (1969) 659.
- 37 P. L. Chueh, J. M. Prausnitz, *AIChE J.*, 13 (1967) 1099; cited in R. C. Reid, J. M. Prausnitz and T. K. Sherwood, *The Properties of Gases and Liquids*, McGraw-Hill, New York, 3rd ed., 1977, p. 140.