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SUPERCRITICAL FLUID CHROMATOGRAPHY USING MIXTURES OF CARBON DIOXIDE OR ETHANE WITH 1,4-DIOXANE AS ELUENTS

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SUMMARY

In supercritical fluid chromatography (SFC), the permanent chemical modification of a silica stationary phase which occurs when eluent mixtures containing 1,4-dioxane are used leads to decreased retention with ethane and carbon dioxide as the mobile phases. Contrary to ethane, with carbon dioxide improved resolution is obtained on the dioxane-modified silica stationary phase. The chromatographic parameters capacity factor, selectivity, and mean resolution for a test mixture of aromatic compounds showed maxima as a function of temperature. The intensity of these maxima decreased with increasing pressure and increasing amount of 1,4-dioxane in mixtures with carbon dioxide or ethane. Furthermore, their positions were shifted to higher temperatures with increasing pressure and with increasing dioxane content, the latter effect being due to the much higher critical temperature of dioxane compared to carbon dioxide and ethane. The data presented can be used for optimizing SFC separations with mixed mobile phases.

INTRODUCTION

Similarly to liquid chromatography, the solvent strength of the mobile phase in supercritical fluid chromatography (SFC) can be increased by using mixed eluents in which at least one of the components of the mixture possesses high solvent strength for the substrate. In general, the critical temperature, T_c, of an eluent mixture is located between the critical temperatures of the components. Thus, if one of the components possesses a particularly high T_c, the T_c of the mixture usually increases greatly with increasing content of this component.

Mobile phases with high solvent strength and high solubility parameter generally also have high critical temperatures. In order to work with mixed eluents at supercritical, but relatively low, temperatures, it is therefore necessary to choose as the primary component of the mixture a compound of low T_c. Accordingly, carbon dioxide- or ethane-based mixed mobile phases are of particular interest. Carbon dioxide is inexpensive, has low toxicity, a lack of inflammability and a considerable dissolution ability for a variety of compounds.

To optimize separations with eluent mixtures, detailed knowledge is needed about the relationships between the mobile phase composition and chromatographic parameters and the dependence of these parameters on temperature and pressure. For characterizing a chromatographic separation, plate numbers, n, plate heights, h, and the resolution, R, should be determined in addition to retention times, t_R, capacity factors, k', and selectivities, α. Whereas for the determination of t_R, k' and α, only retention, *i.e.*, thermodynamic data are needed, for n, h and R additional, kinetic data on the width and shape of the peaks are required.

For one-component eluents, the dependence of the chromatographic parameters on temperature and pressure has been extensively studied, employing packed columns (see ref. 1 and references cited therein). Studies on binary mobile phases with pentane as the primary component have also been reported[2–5]. For the separation of vinyl oligomers with aromatic side groups, 1,4-dioxane has been found to be a suitable secondary component for alkane- or carbon dioxide-based mixed mobile phases[6–8]. Distinguishable from binary mobile phases, where the second component influences the chromatographic process mainly by altering the mobile phase properties, are mobile phases where the second component affects the chromatographic parameters mainly by interaction with the stationary phase. This type of second component is usually needed in much smaller amounts than the first type. The influence of such low "modifier" amounts on retention and peak shape has frequently been described[9–17]. With few exceptions where capillary columns have been employed[16–18], these binary mobile phases have been used with packed columns where the peak symmetry was often considerably improved upon addition of a modifier. For example, adding 1% methanol to carbon dioxide was found to improve the peak shape for the elution of 13H-dibenzo[*a,i*]carbazole on a C_{18} reversed-phase column[13].

In this communication, the results obtained with mixtures of carbon dioxide as well as of ethane with 1,4-dioxane are reported. The eluent composition, temperature and pressure were varied systematically, employing dioxane contents of up to 30%. As the test substrate, a mixture of the aromatics, naphthalene, anthracene, pyrene and chrysene, was used, the stationary phase being silica chemically modified by means of dioxane before use. The test mixture simulates a homologous series, and the results obtained may therefore be applicable also to the optimization of oligomer separations.

EXPERIMENTAL

A high-performance liquid chromatography (HPLC) instrument (1084B, Hewlett-Packard), modified for SFC as described[6], was used throughout this study. The separation column (stainless steel, 25 cm × 4.6 mm I.D.) was packed with LiChrosorb Si 100, 10 μm (Merck, Darmstadt, F.R.G.), using a slurry method[7]. In addition to measurement and control of the volume flow-rate (feed rate) of the liquid eluents at the pumps, the flow-rate was also determined at the instrument outlet. For that purpose, the eluent stream was depressurized and led into a simple device shown in Fig. 1. The secondary component of the eluent mixtures, 1,4-dioxane, was condensed by cooling and collected in a burette (1), enabling the determination of its volume flow per unit time. For measuring the flow of the gaseous primary component, the remaining gas stream was led into the top of a gas burette (2) filled with acidified

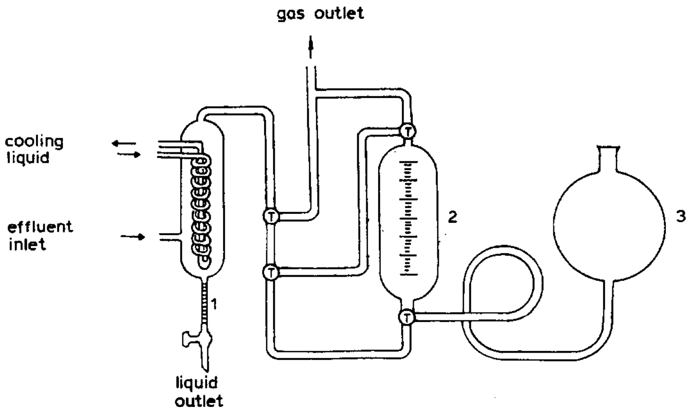


Fig. 1. Device for flow-rate measurements at the exit of an SFC apparatus (for explanations see Experimental section).

water and saturated with carbon dioxide or ethane prior to the measurement. The gas flow was measured by displacing water from the burette (2) into the niveau vessel (3).

Carbon dioxide (99.995%) and ethane (99.5%) were obtained from Messer Griesheim (Hürth, F.R.G.). Either eluent was supplied to the pumps as a liquid from a pressure cylinder, passing through a cascade of sintered metal filters of decreasing pore diameter from 40 to 5 μm. 1,4-Dioxane was distilled twice from sodium before use.

As in previous studies[1–5], an analyte mixture of the polycyclic aromatic hydrocarbons naphthalene, anthracene, pyrene and chrysene was employed.

Capacity factors, k', selectivities, α, plate numbers, n, and resolutions, R, were calculated as described previously[19,20]:

$$k'_i = \frac{t_{\mathrm{R,i}} - t_0}{t_0} \tag{1}$$

$$\alpha_{ij} = k'_j/k'_i \tag{2}$$

$$n_i = \left(\frac{t_{R,i}}{w'_i}\right)^2 \cdot 8 \ln 2 \tag{3}$$

$$R_{ij} = \frac{f_{ij}}{g_{ij}} + \frac{d_{ij}}{w'_i + w'_j} \cdot \sqrt{\ln 4} \tag{4}$$

$$R_{\mathrm{m}} = \left(\sum_1^n R_{ij}\right)/n \tag{5}$$

TABLE I

EXPERIMENTAL REGIONS OF ELUENT COMPOSITION, COLUMN OUTLET PRESSURE AND TEMPERATURE

Eluent	*Composition (%)*	*Column end pressure (bar)*	*Column temperature (°C)*
Carbon dioxide–1,4-dioxane	0–30 (w/w)	150, 200, 250	20–240
Ethane–1,4-dioxane	0–30 (v/v)	100, 150, 200, 250	20–240

Here, t_0 is the elution time of a non-sorbed solute (heptane), $t_{R,i}$ is the retention time of solute i, f_{ij} is the depth of the valley between two peaks i and j, as measured from their average peak height, g_{ij} is the average peak height of the two peaks, d_{ij} is the distance between the baseline intercepts of the two nearest tangents to adjacent peaks at the point of inflection, w_i' is the width of peak i at half height and n is the total number of adjacent peak pairs (here: $n = 3$).

Experimental ranges of eluent composition, pressure and temperature are compiled in Table I. For ethane–dioxane mixtures, the two components were delivered separately by two pumps, then mixed in the mixing chamber of the 1084B instrument. Dioxane and carbon dioxide, however, were premixed and then delivered to the column by only one pump of the chromatograph. Premixing was done by weighing the two components into a pressure-resistant cylinder, introducing the dioxane first. Thus, for carbon dioxide–dioxane the percentages are given (w/w), while for ethane–dioxane the percentages are (v/v). The volume-based ratios are converted into weight-based or molar ratios in Table II. The conversion is based on the densities of the pure liquid components at 20°C under their own vapour pressures. At different pump pressures, variations of the liquid densities with pressure have to be taken into account.

TABLE II

RELATIONSHIPS BETWEEN % (v/v), % (w/w) AND % (mol/mol) FOR THE MIXED MOBILE PHASES

Data given as % dioxane. Conversion based on the density of the pure liquid components at 20°C under their own vapour pressures.

Mobile phase	*% Dioxane*									
Carbon dioxide–1,4-dioxane										
% (v/v)	3.8	7.7	11.7	15.8	20.0	24.3	28.8	33.3	38.0	42.9
% (w/w)	5.0	10.0	15.0	20.0	25.0	30.0	35.0	40.0	45.0	50.0
% (mol/mol)	2.6	5.3	8.1	11.1	14.3	17.7	21.2	25.0	29.0	33.0
Ethane–1,4-dioxane										
% (v/v)	5.0	10.0	15.0	20.0	25.0	30.0	35.0	40.0	45.0	50.0
% (w/w)	13.5	24.8	34.4	42.6	49.8	56.0	61.5	66.5	70.9	74.8
% (mol/mol)	7.2	14.2	20.8	27.2	33.1	38.8	44.5	49.7	54.8	59.9

RESULTS AND DISCUSSION

As shown recently, the use of binary supercritical eluents containing 1,4-dioxane as the secondary component leads to a permanent chemical modification of a silica stationary phase[21] and its properties are greatly changed. For instance, the retention of solutes is usually reduced. If carbon dioxide is employed as the primary component of the mobile phase, the peak shapes are also definitely improved, increasing thereby the resolution. This is demonstrated by plots of the capacity factor of chrysene, k' (C) (Fig. 2), and of the average resolution, R_m (Fig. 3), for the aromatic mixture *versus* temperature, using a column packed with a modified and an unmodified silica gel, and employing pure carbon dioxide or ethane without dioxane as the mobile phases. For both eluents, Fig. 2 shows the decreased retention on the modified compared to the unmodified stationary phase over the whole temperature range. Contrary to this, the effect of the modification on resolution is different for the two eluents (Fig. 3): whereas in carbon dioxide the resolution is considerably higher on the modified than on the unmodified stationary phase, the opposite is found for ethane. The former improvement is due to the elimination of the considerable peak tailing found on unmodified silica with carbon dioxide as the mobile phase[21]. Thus, the resolution increases due to the improvement in the peak symmetry.

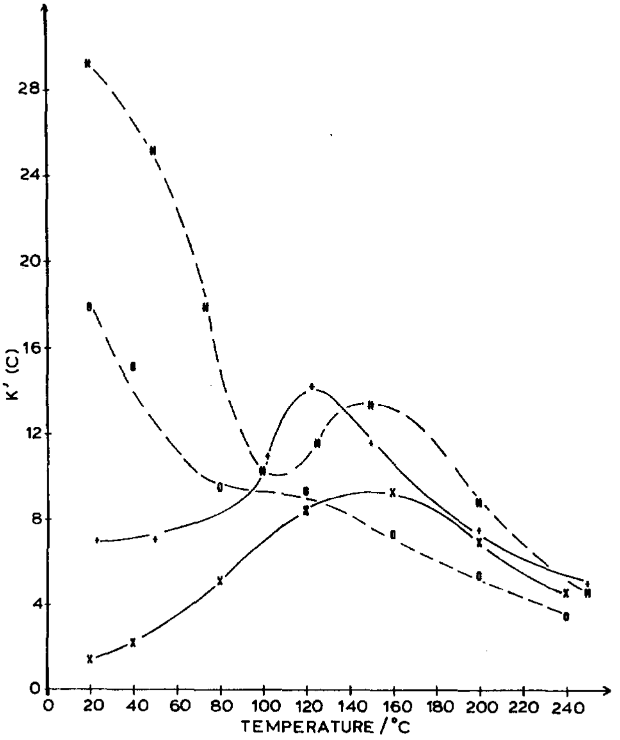


Fig. 2. Dependence of the capacity factor of chrysene, k' (C), on the column temperature, T. Column end pressure, p_e: 250 bar. Eluents and stationary phases: (#) ethane on unmodified silica; (O) ethane on modified silica; (+) carbon dioxide on unmodified silica; (×) carbon dioxide on modified silica.

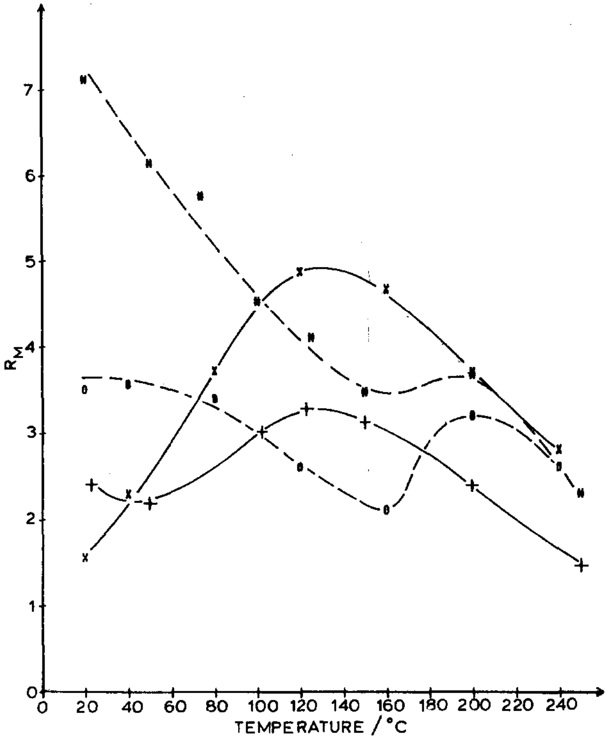
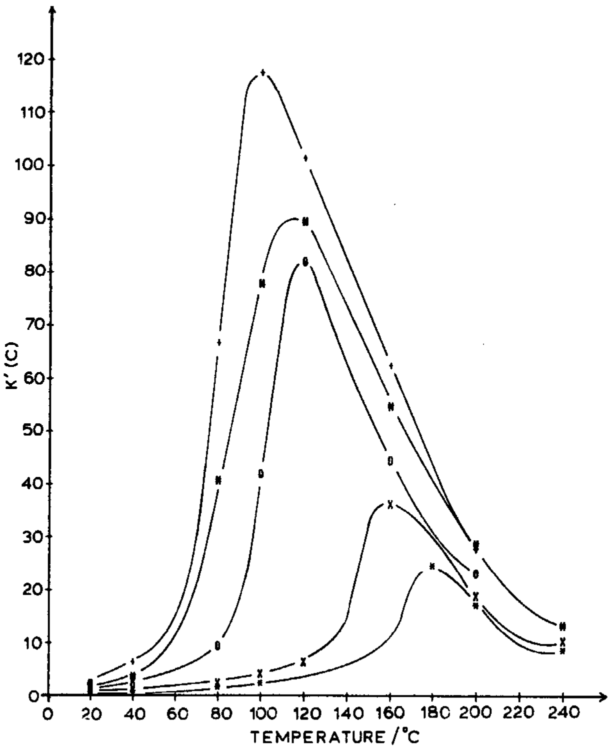


Fig. 3. Dependence of the mean resolution, R_m, between naphthalene, anthracene, pyrene and chrysene on the temperature, T, at p_e = 250 bar. Symbols as in Fig. 2.

In contrast, ethane yields symmetric peaks on the unmodified stationary phase, and the modification of the stationary phase has little effect on the peak widths. Therefore, decreasing capacity factors lead to decreasing resolution.

In order to obtain reproducible results throughout this study, the silica packed columns were conditioned with dioxane at elevated temperature (200°C) as described previously[21]. Such conditioned columns showed long-term stability, both with dioxane-containing and non-dioxane-containing mobile phases.

In Figs. 4–7 plots are shown for the variation of the chrysene capacity factor with temperature for mixtures of carbon dioxide or ethane with 1,4-dioxane at different column outlet pressures of 150 bar (Figs. 4 and 6) and 200 bar (Figs. 5 and 7). The curves show characteristic shapes, similar to those obtained earlier with pure eluents[1] and binary eluent mixtures[2–5]. In particular, with carbon dioxide, the plots (Figs. 4 and 5) exhibit capacity factor maxima which shift to lower temperatures and become more pronounced with decreasing dioxane content and decreasing pressure. These tendencies were confirmed by series of additional measurements at 100 and 250 bar. The shift of the maximum with decreasing dioxane content is due to the concurrent decrease in the critical temperature, T_c, of the binary mobile phase. For calculating the critical data for mixtures, the procedure of Chueh and Prausnitz[22] has been used. The calculated data for the critical temperatures and pressures of mixtures of carbon dioxide or ethane with 1,4-dioxane are shown in Fig. 8. The



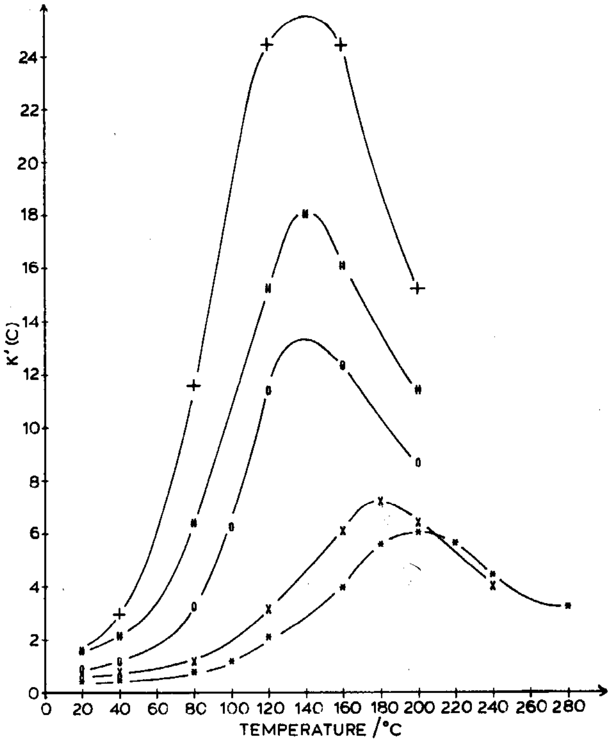
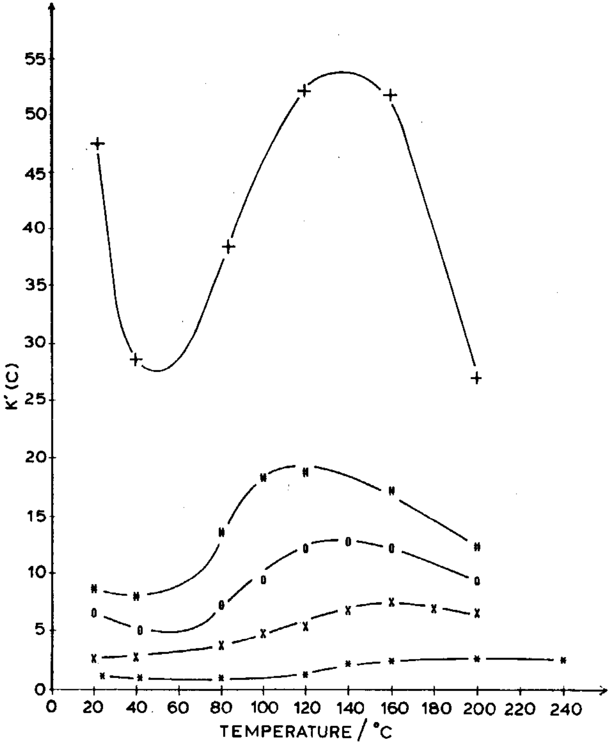


Fig. 4. Dependence of the capacity factor of chrysene, k' (C), on the column temperature, T, at p_e = 150 bar. Eluents (carbon dioxide–1,4-dioxane): (+) 100:0; (#) 95:5; (O) 90:10; (×) 80:20; (*) 70:30 (w/w).

Fig. 5. Dependence of k' (C) on the column temperature, T, at p_e = 200 bar. Eluents as in Fig. 4.



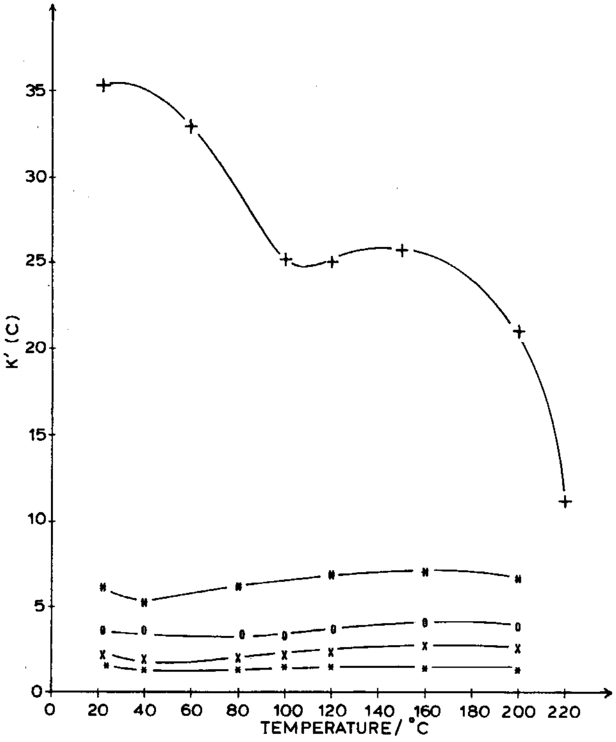


Fig. 6. Dependence of k' (C) on the column temperature, T, at p_e = 150 bar. Eluents (ethane–1,4-dioxane): (+) 100:0; (#) 95:5; (O) 90:10; (×) 80:20; (*) 70:30 (v/v).

Fig. 7. Dependence of k' (C) on the column temperature, T, at p_e = 200 bar. Eluents as in Fig. 6.

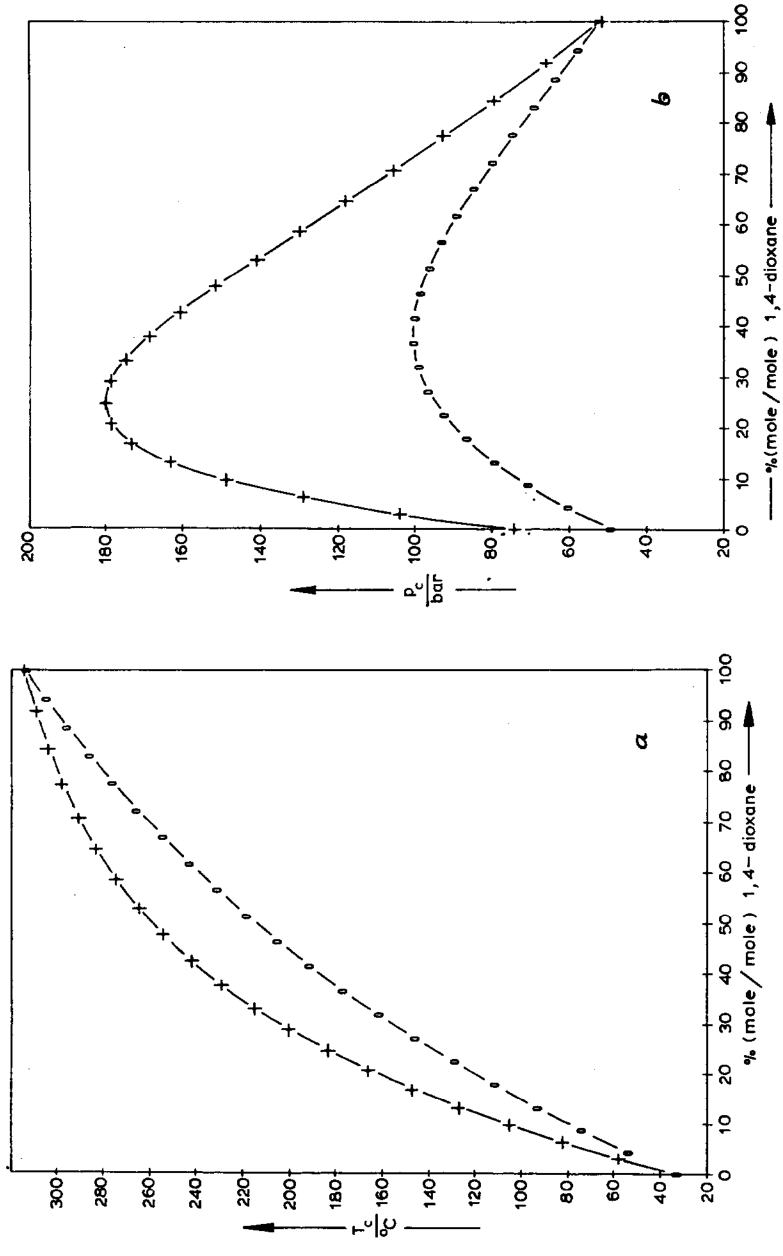


Fig. 8. Dependence of (a) the critical temperature, T_c, and (b) the critical pressure, p_c, on eluent composition. Eluents: (+) carbon dioxide–1,4-dioxane; (O) ethane–1,4-dioxane.

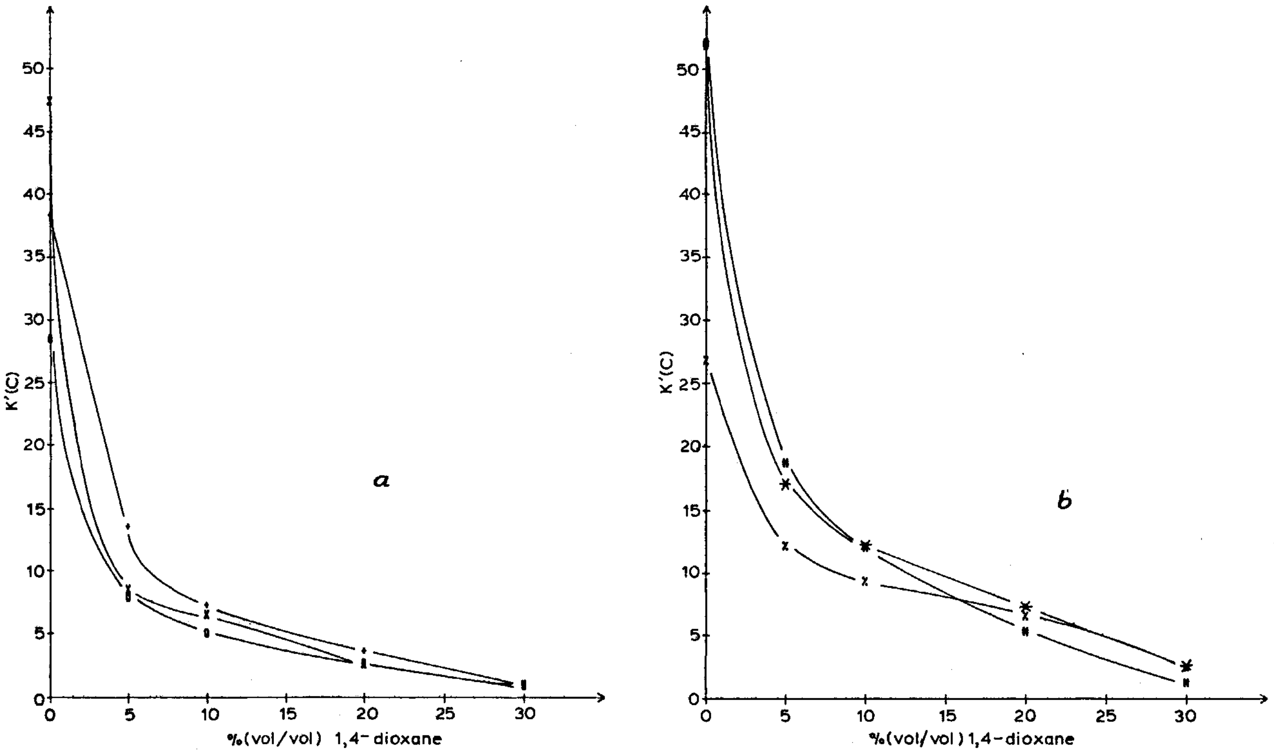


Fig. 9. Dependence of k' (C) on the eluent composition. Eluent: ethane–1,4-dioxane. p_e: 150 bar. Column temperature: (a) (×) 20; (O) 40; (+) 80; (b) (#) 120; (*) 160; (%) 200°C.

critical temperatures of the mixtures slope steeply with increasing dioxane content at low amounts of dioxane, but decrease in slope at higher amounts. Although the critical temperatures of carbon dioxide and ethane are almost identical, the critical temperatures of carbon dioxide–dioxane mixtures can exceed those of ethane–dioxane mixtures by about 50 K, at the same molar percentage. For both binary mobile phases, the critical pressures show distinct maxima, located at dioxane molar percentages of 25 and 37% for mixtures of dioxane with carbon dioxide or ethane, respectively. At its maximum, the critical pressure of the mixture may exceed the critical pressure of the pure components by a factor of 2 or more. Thus, in a set of experiments at varying eluent composition, but constant column outlet pressure, as in Figs. 4–7, the reduced pressure, p_r, decreases significantly with increasing dioxane content.

In principle, the variations of k' with temperature and dioxane content for the ethane–dioxane mobile phase resemble those observed with carbon dioxide–dioxane. There are, however, some differences. As a liquid, pure ethane shows a very low transport ability for the aromatic analytes, which is reflected in high k' values for the liquid state, which exceed even those at the k' maximum under supercritical conditions (Figs. 6 and 7). Moreover, the k' maxima are distinctly less pronounced than with carbon dioxide–dioxane and disappear earlier when the pressure and dioxane content are increased. Since the calculated critical pressures for ethane–dioxane mixtures are lower than those for carbon dioxide–dioxane, the same absolute pressure corresponds to a higher reduced pressure, which in part may be connected to the differences in behaviour between ethane–dioxane and carbon dioxide–dioxane binary mobile phases. For instance, the earlier disappearance of the k' maxima at the same absolute pressure for ethane–dioxane *versus* carbon dioxide–dioxane may be connected with the higher reduced pressure for the former mixed eluent.

With carbon dioxide–dioxane as well as with ethane–dioxane, combinations of composition, pressure and temperature were observed where reproducible chromatograms could not be obtained. Such conditions were found for carbon dioxide–dioxane at 20% (w/w) dioxane and 150 bar at temperatures between 40 and 240°C, and at 30% (w/w) dioxane and 150 bar between 100 and 180°C. For ethane–dioxane at dioxane contents exceeding 20% (v/v) and 150 bar the temperature range was from 200 to 260°C. It is assumed that these results are caused by demixing of the supercritical eluent mixture in the column rather than by demixing of the mixture in the liquid state. Demixing in the liquid state at ambient temperature would have been manifested by an erratic detector response, irrespective of the separation temperature in the column, because detection (and pumping) is carried out at ambient temperature.

Under isothermal–isobaric conditions, the capacity factors generally decrease upon increasing the amount of 1,4-dioxane in ethane (Fig. 9) or carbon dioxide (Fig. 10). At different temperatures the curves differ from each other, this differentiation becoming more pronounced at lower pressures (not shown). The plots show some differences between ethane–dioxane and carbon dioxide–dioxane. Whereas with ethane–dioxane the decrease in k' is considerable and shows a similar shape at different temperatures, the carbon dioxide–dioxane plots have a more diverse behaviour. In particular, at 80 and 120°C a strong decrease in k' is observed when the mobile phase attains a critical temperature which is as high as the temperature at

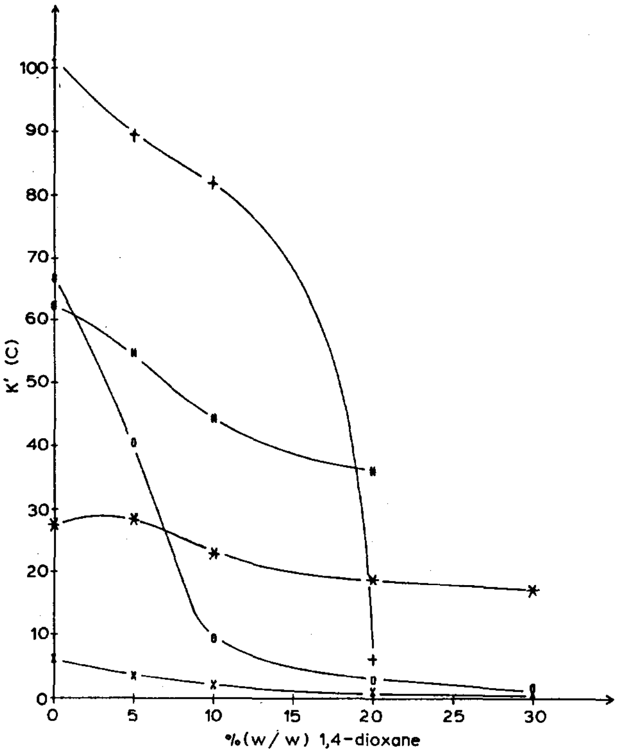


Fig. 10. Dependence of k' (C) on the eluent composition. Eluent: carbon dioxide–1,4-dioxane. p_e: 150 bar. Column temperature: (×) 40; (O) 80; (+) 120; (#) 160; (*) 200°C.

which the isothermal–isobaric experiments were carried out (Fig. 10). At 200°C, the dependence of k' on the dioxane content is only moderate, and k' even increases to a small extent when going from pure carbon dioxide to a mixture of 5% dioxane in carbon dioxide (Fig. 10). In this special case, an eluent composition gradient applied under isothermal–isobaric conditions would not lead to lower retention. Similar observations were made in a study by Randall[11] on the dependence of k' on the amount of 2-methoxyethanol in carbon dioxide. It was demonstrated that the k' values of anthraquinone, xanthone and 1,2-dihydroxyanthraquinone do not continuously decrease with increasing modifier amount but, intermediately, remain at a plateau or even show a small maximum.

Like k', the selectivities, α, exhibit maxima when plotted against T. This is demonstrated in Figs. 11 (carbon dioxide–dioxane) and 12 (ethane–dioxane) for the selectivities of the peak pairs naphthalene–anthracene, α (NA), and anthracene–pyrene, α (AP). The position and the intensity of these maxima show changes with respect to the composition and pressure, like those of k'. There are, moreover, differences between the selectivities of these two peak pairs. The selectivity between the homologues naphthalene and anthracene is generally higher and is found to be more dependent on temperature. Also, α (NA) assumes higher values with carbon dioxide-based eluents compared to ethane–dioxane mixtures, a difference which is less pronounced for α (AP). These observations, exemplified in Figs. 11 and 12 for 95:5

mixtures of carbon dioxide or ethane with dioxane at 150 bar, have been found to be valid over the whole experimental range of Table I, irrespective of the mobile phase composition and the pressure, and they correspond to results with binary mobile phases comprised of pentane and 1,4-dioxane[5] as well as with pentane–diglyme[4].

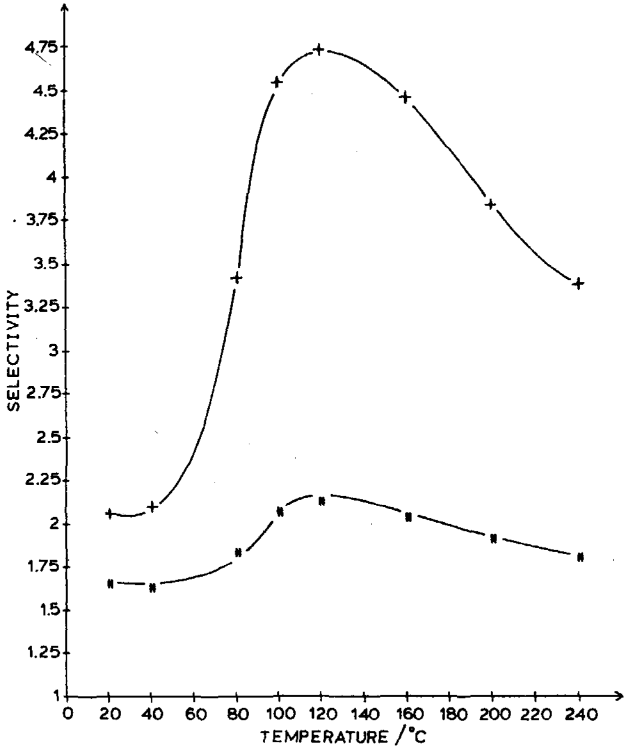
As is seen from Table III, maxima are also exhibited by the plate numbers, *n*, *versus* temperature. Examples are given for chrysene in carbon dioxide–dioxane mobile phases at three different pressures. Furthermore, *n* tends to increase with increasing dioxane content, especially at higher temperatures. Maxima for *n versus* temperature are also observed for ethane–dioxane mobile phases. However, *n* decreases with increasing dioxane content.

By comparing the plots of the mean resolution, R_m, *versus* temperature in Figs. 13 and 14 to the corresponding plots of k' (Figs. 4 and 6), it is observed that the curves show similar shapes. Maxima occur, the positions of which are shifted to higher temperatures with increasing dioxane content in the mixtures. An exception

TABLE III

EXPERIMENTAL PLATE NUMBERS FOR CHRYSENE AT DIFFERENT PRESSURES, TEMPERATURES AND CONTENTS OF DIOXANE IN CARBON DIOXIDE

T (°C)	*Dioxane (%, w/w)*				
	0	*5*	*10*	*20*	*30*
150 bar					
20	830	730	1680	580	1690
40	1000	1140	1790	1110	1570
80	1700	1340	1520	1650	2020
100	1020	2690	1440	2330	–
120	1650	1470	1380	2880	–
160	1700	1290	1250	6180	–
200	760	1070	350	2560	1640
240	–	400	–	1130	1780
200 bar					
20	970	600	1680	550	1070
40	1550	850	1860	900	1400
80	2450	1210	1550	1430	1940
100	–	–	1140	–	2400
120	1220	1270	1490	1760	2130
160	1240	1250	1350	1570	1570
200	1200	960	1100	1540	1580
240	–	–	–	1010	1210
250 bar					
20	940	620	1790	610	840
40	1250	720	1670	1090	250
80	1510	1040	1940	1940	1550
100	–	–	1620	1830	2030
120	1900	1050	1350	1640	2040
160	1040	930	1380	1640	1780
200	950	920	1200	1210	1620
240	–	730	730	1140	1200



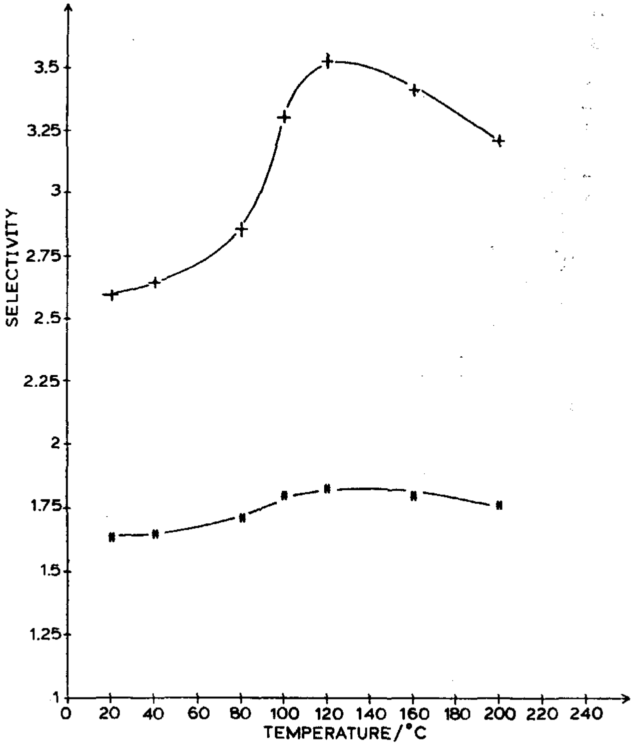
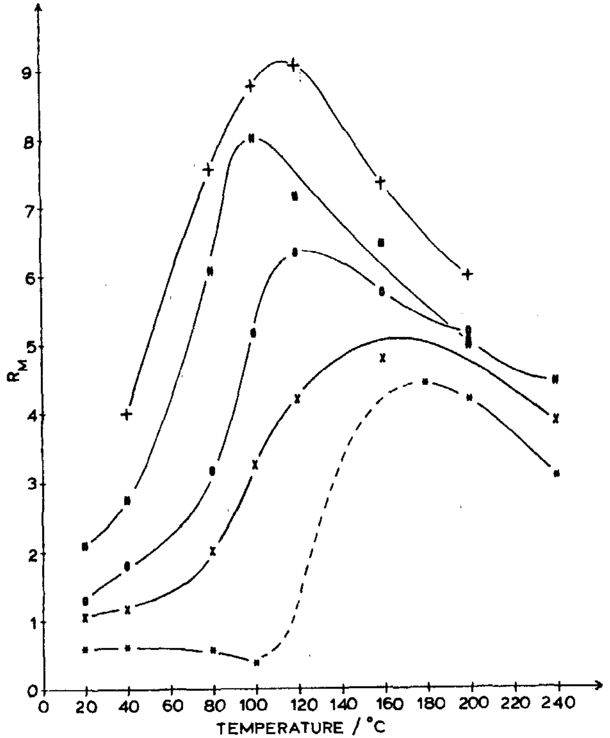


Fig. 11. Dependence of the selectivity between naphthalene and anthracene, α (NA) (+), and between anthracene and pyrene, α (AP) (#), on temperature, T. Eluent: carbon dioxide–1,4-dioxane (95:5, w/w). p_e: 150 bar.

Fig. 12. Dependence of the selectivity, α (NA) (+) and α (AP) (#), on temperature, T. Eluent: ethane–1,4-dioxane (95:5, v/v). p_e: 150 bar.



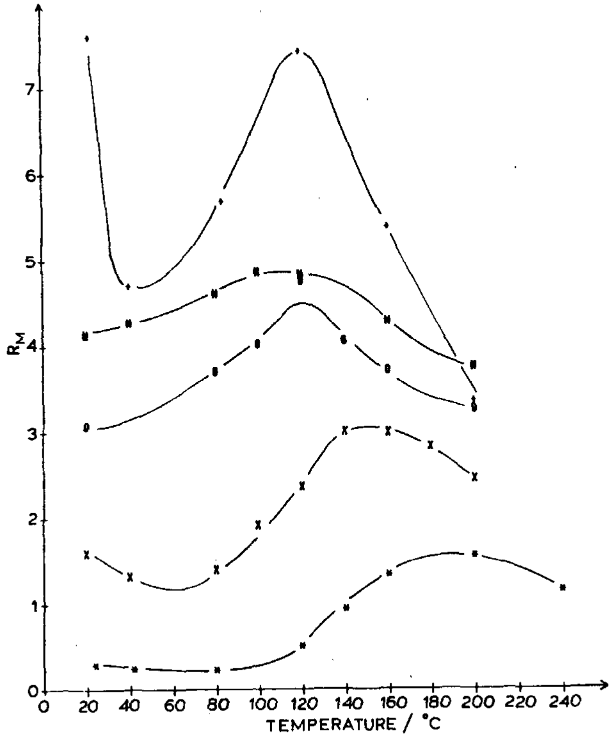


Fig. 13. Dependence of the mean resolution, R_m, on temperature, T, at different eluent compositions. Eluent: carbon dioxide–1,4-dioxane. For details see Fig. 4. – – – –, Uncertain parts.

Fig. 14. Dependence of R_m on temperature, T, at different eluent compositions. Eluent: ethane–1,4-dioxane. For details see Fig. 6.

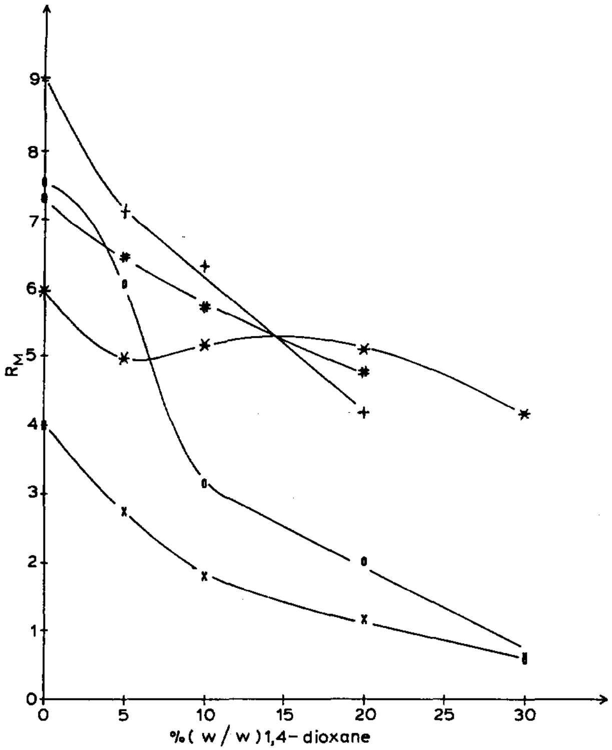


Fig. 15. Dependence of R_m on eluent composition at different temperatures. Eluent: carbon dioxide–1,4-dioxane. For details see Fig. 10.

is that the k' maxima for the pure eluents carbon dioxide and ethane are located at the same (ethane) or at a higher temperature (carbon dioxide) than those for the mixtures containing 5% dioxane. The intensity of the maxima decreases with increasing dioxane content. The dependence of R_m on pressure follows the usual pattern[1–5], an increase in pressure leading to smaller maxima shifted to higher temperatures.

In Fig. 15, R_m is plotted *versus* dioxane content for the carbon dioxide–dioxane mobile phase and in Fig. 16 for the ethane–dioxane phase, both at 150 bar. On the whole, there is a monotonous decrease in R_m with increasing dioxane content. However, with carbon dioxide–dioxane and at certain temperatures, the decrease in R_m is less strong than the decrease in k'. Particularly at 120°C, k' decreases by a factor of about 10 and R_m only by a factor of 2 when going from pure carbon dioxide to a mixture of 20% dioxane in carbon dioxide. Thus, using a mobile phase of 80% carbon dioxide and 20% 1,4-dioxane at 120°C, fast elution and high resolution are obtained. Comparing the R_m plots for ethane–dioxane (Fig. 16) with the corresponding k' plots (Fig. 9), it is seen that a monotonous decrease in R_m with dioxane content, of the type which has been observed for k', occurs only at the lower temperatures of 40–80°C (Fig. 16a). At higher temperatures small plateaus or maxima appear, similar to those with pentane-based binary eluents[3–5].

To facilitate the optimization of a separation, the resolution data may be plotted as shown in Fig. 17 for carbon dioxide–dioxane. In this plot, the temperature of

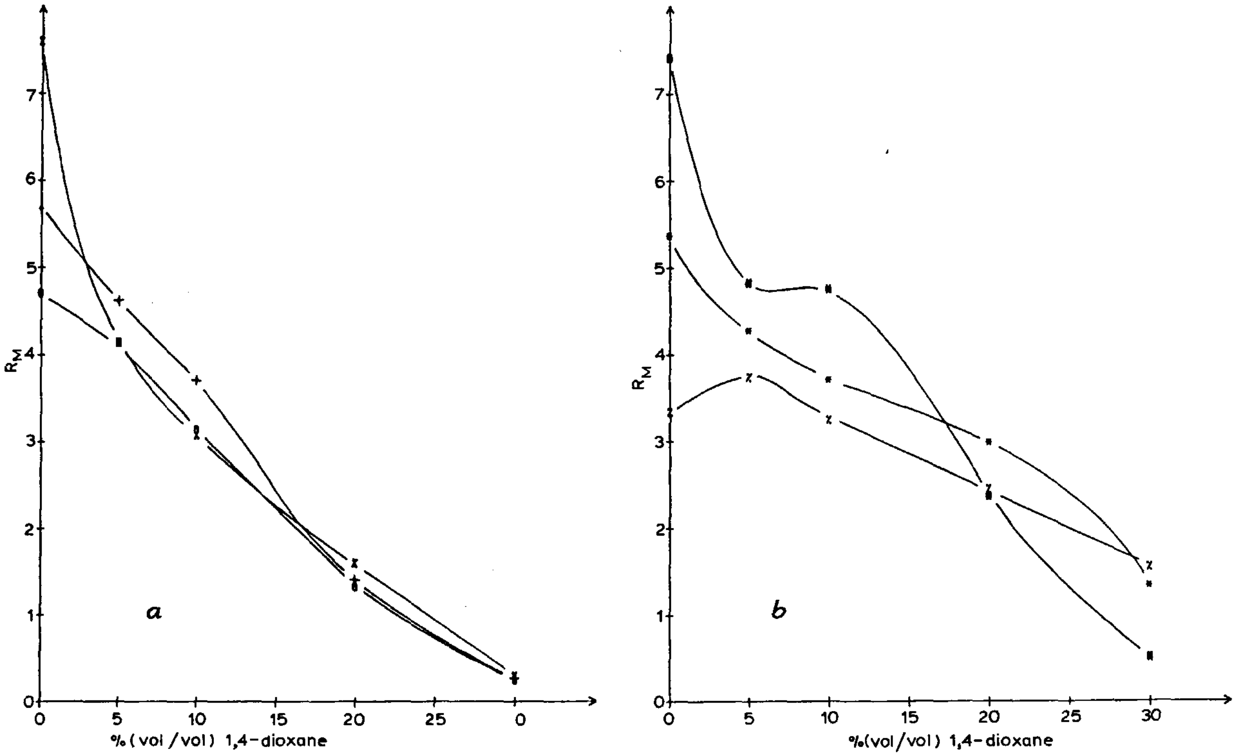


Fig. 16. Dependence of R_m on eluent composition at different temperatures. Eluent: ethane–1,4-dioxane. For details see Fig. 9.

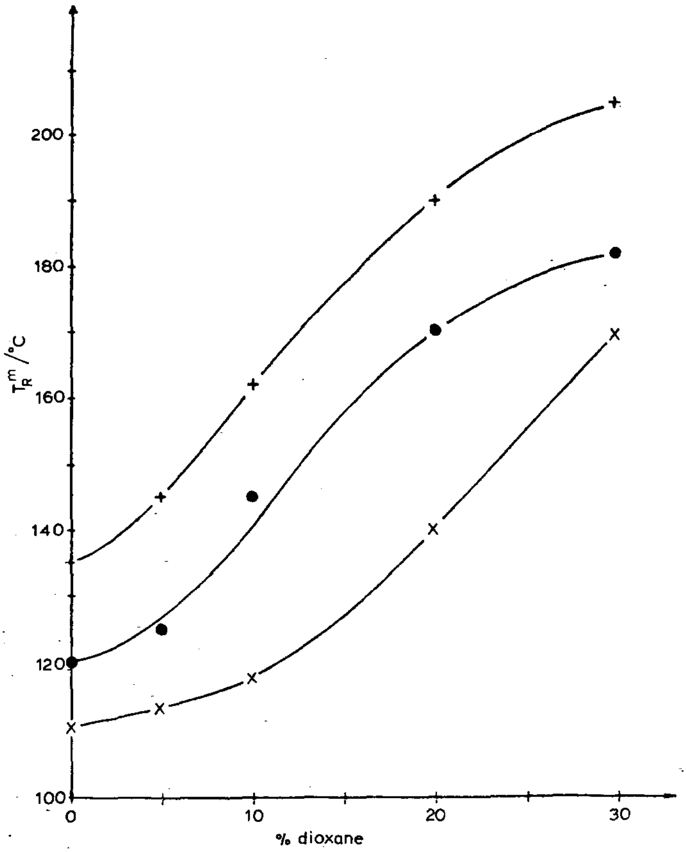


Fig. 17. Dependence of the temperature of the mean resolution maximum, T_R^m, on eluent composition and column outlet pressure: (×) 150; (●) 200; (+) 250 bar. Eluent: carbon dioxide–1,4-dioxane.

maximum resolution (*cf.*, Fig. 13) is plotted *versus* the dioxane content of the mobile phase for three different column outlet pressures of 150, 200 and 250 bar. This temperature, T_R^m, increases with increasing dioxane content and also with increasing pressure. From such plots one may obtain for a given set of two experimental parameters the third parameter which allows the maximum resolution of the mixture of aromatic compounds on a silica column. Thus for a given pressure and a given mobile phase composition, the temperature of highest resolution can be read from Fig. 17. This was exemplified previously[23,24] by superimposing a temperature gradient on a binary gradient of eluent composition and pressure in order to maximize the resolution of vinylarene oligomers throughout a gradient elution separation.

In conclusion, the eluent pair carbon dioxide–dioxane appears to be superior to the pair ethane–dioxane for resolving aromatic compounds on a packed column of silica gel. However, at a given eluent composition, the elution with the former eluent pair is slower than with the latter pair. As a consequence, the column pressure must be higher for carbon dioxide–dioxane in order to effect equal retention. In view of the higher critical pressure of carbon dioxide and carbon dioxide–dioxane mixtures, as compared to ethane and ethane–dioxane mixtures, lower reduced pressures are employed for the former than for the latter, if the absolute pressure is the same. At such low reduced pressures, considerable effects of temperature and pressure on the capacity factor and resolution are observed. For example, resolution maxima are found with carbon dioxide–dioxane at pressures and dioxane contents where the curves for ethane–dioxane do not show maxima and are flattened. This provides a larger variety of possibilities for optimizing a separation when carbon dioxide is used as the primary mobile phase.

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REFERENCES

1 D. Leyendecker, D. Leyendecker, F. P. Schmitz and E. Klesper, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 566.
2 D. Leyendecker, D. Leyendecker, F. P. Schmitz and E. Klesper, *J. Chromatogr.*, 371 (1986) 93.
3 D. Leyendecker, D. Leyendecker, F. P. Schmitz and E. Klesper, *J. Chromatogr.*, 392 (1987) 101.
4 D. Leyendecker, F. P. Schmitz and E. Klesper, *Chromatographia*, 23 (1987) 171.
5 D. Leyendecker, F. P. Schmitz, D. Leyendecker and E. Klesper, *J. Chromatogr.*, 393 (1987) 155.
6 F. P. Schmitz, H. Hilgers and E. Klesper, *J. Chromatogr.*, 267 (1983) 267.
7 F. P. Schmitz, H. Hilgers, B. Lorenschat and E. Klesper, *J. Chromatogr.*, 346 (1985) 69.
8 F. P. Schmitz, H. Hilgers and B. Gemmel, *J. Chromatogr.*, 371 (1986) 135.
9 R. Board, D. McManigill, H. Weaver and D. Gere, *CHEMSA*, June (1983) 12.
10 D. R. Gere, *Application Note 800-2, Publication No. 43-5953-1691*, Hewlett-Packard Co., Avondale, PA, 1983.
11 L. G. Randall, *Technical Paper No. 102, Publication No. 43-5953-1722*, Hewlett-Packard Co., Avondale, PA, 1983.
12 L. G. Randall, *ACS Symp. Ser.*, 250 (1984) 135.
13 A. L. Blilie and T. Greibrokk, *Anal. Chem.*, 57 (1985) 2239.
14 J. M. Levy and W. M. Ritchey, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 503.
15 J. M. Levy and W. M. Ritchey, *J. Chromatogr. Sci.*, 24 (1986) 242.
16 B. W. Wright and R. D. Smith, *J. Chromatogr.*, 355 (1986) 367.
17 C. R. Yonker and R. D. Smith, *J. Chromatogr.*, 361 (1986) 25.
18 W. P. Jackson, R. C. Kong and M. L. Lee, in M. Cooke and A. J. Dennis (Editors), *Polynuclear Aromatic Hydrocarbons*, Battelle Press, Columbus-Richland, 1985, p. 609.
19 F. P. Schmitz, *J. Chromatogr.*, 356 (1986) 261.
20 D. Leyendecker, D. Leyendecker, F. P. Schmitz and E. Klesper, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 141.
21 F. P. Schmitz, D. Leyendecker and D. Leyendecker, *J. Chromatogr.*, 389 (1987) 245.
22 P. L. Chueh and J. M. Prausnitz, *AIChE J.*, (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.
23 D. Leyendecker, D. Leyendecker, F. P. Schmitz and E. Klesper, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 525.
24 F. P. Schmitz, D. Leyendecker, D. Leyendecker and B. Gemmel, *J. Chromatogr.*, 395 (1987) 111.