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INFLUENCE OF TEMPERATURE, PRESSURE AND ELUENT COMPOSITION ON THE BEHAVIOUR OF BINARY ELUENTS UNDER SUB- AND SUPERCRITICAL CONDITIONS

PENTANE AND 1,4-DIOXANE

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SUMMARY

In supercritical fluid chromatography, the temperature- and pressure-dependent behaviour of the chromatographic parameters for binary eluent mixtures, containing pentane as the primary component and 1,4-dioxane as a secondary component, is similar to that of pure eluents. The isobars show maxima above the critical temperature for capacity factors and for resolution. With increasing content of the secondary component in the mixture, the critical temperatures rise and the maxima move to higher temperatures. The maxima are more pronounced at lower column pressures. Maxima appear also when plotting *versus* eluent composition at constant temperature and pressure. All maxima occur at both sub- and supercritical pressure. Temperature–pressure–composition triple points can be found where the capacity factors or the resolution reach maximum values. This can be used for optimizing separations.

INTRODUCTION

Recently, supercritical fluid chromatography (SFC) has come to be regarded as a complementary separation method to gas chromatography (GC) and high-performance liquid chromatography (HPLC). This is due to some special features of SFC, *e.g.*, higher separation efficiency when compared to HPLC and considerable solvent strength of the mobile phase as compared to GC.

Therefore, analysis under mild thermal conditions is possible. Nevertheless, the dissolution ability of many supercritical eluents, particularly those of low critical temperatures, is limited. By increasing the pressure, and thereby the density of the mobile phase, the solvent strength is improved, although the nature of the eluent–solute interactions is not altered.

In order further to alleviate the problem of low dissolution ability, cosolvents of higher solvent strength have been added to supercritical mobile phases. As in HPLC, gradients of eluent composition ("gradient elution") have also been shown to be applicable in SFC. On the other hand, the so-called "modifiers" exert their influence on the chromatographic process mainly by interaction with the stationary phase and not by altering greatly the solvent properties of the mobile phase. The addition of such modifiers, frequently at amounts below 3%, has been shown to shorten retention times greatly, particularly on adsorbent stationary phases[1–4]. For all of these binary systems, the optimization of SFC separations with respect to temperature, pressure and linear velocity requires more fundamental studies.

Pure one-component eluents in combination with bare silica stationary phases have more frequently been the subject of studies on chromatographic properties and their dependence on temperature and pressure[5,6]. In these studies, the variations of the capacity ratio, selectivity, plate number and resolution with pressure and temperature were found to be basically similar for all mobile phases employed.

The studies reported here were intended to clarify whether binary mobile phases behave similarly to one-component eluents, and to reveal the influence of the addition of a cosolvent with a higher solvent strength on the chromatographic properties of the mobile phase. Pentane was employed as the primary eluent component and the cyclic ether, 1,4-dioxane, as the secondary component. The substrate comprised four aromatic hydrocarbons naphthalene, anthracene, pyrene and chrysene, and the columns were packed with "normal-bare" silica. The binary mixture pentane–1,4-dioxane has frequently been used for separations with gradients of eluent composition[7–13], *e.g.* for the separation of oligomers from styrene[7,9,12],1- and 2-vinylnaphthalene[10,11,13] and 4-vinylbiphenyl[10,13]. Dioxane is known to be an aprotic solvent with high solvent strength.

EXPERIMENTAL

The SFC apparatus used was constructed from commercially available components, as described earlier[5]. The packing material for the stainless-steel column (25 cm × 4.6 mm I.D.) was LiChrosorb Si 100, 10 μm (Merck, Darmstadt, F.R.G.). UV detection of the four aromatic hydrocarbons, naphthalene, anthracene, pyrene and chrysene, was carried out at 254 nm. The flow-rate was regulated to 1 ml/min and measured at the outlet of the apparatus.

Pentane and heptane, the latter being used to determine the dead time, were purified by drying over sodium, distillation, filtration and degassing. 1,4-Dioxane was dried over calcium chloride and sodium and then distilled twice to a sufficient purity.

Capacity factors, k', were calculated in the usual manner from dead times, t_o, and retention times, t_r, according to eqn. 1:

$$k'_i = (t_{r,i} - t_o)/t_o \tag{1}$$

The resolution, R_{ij}, between two peaks i and j was calculated by use of eqn. 2

$$R_{ij} = \frac{f_{ij}}{g_{ij}} + \frac{d_{ij}}{w'_i + w'_j} \cdot \sqrt{\ln 4} \tag{2}$$

where f_{ij} is the depth of the valley between the two peaks i and j, g_{ij} is the average height of the two peaks, w' is the peak width at half height and d_{ij} is the distance between the baseline intercepts of the inner tangents to the two peaks[12]. The mean resolution, R_m, is the arithmetic mean of the resolutions R_{ij} over the n adjacent peak pairs:

$$R_m = \sum_{1}^{n} R_{ij}/n \tag{3}$$

It has been used instead of the individual resolutions of the three peak pairs for simplicity and because of its greater independence from erratic or special behaviour of the individual R_{ij} values. The present experiments with pentane–dioxane comprised 30 combinations of pressure and composition under isobaric–isocratic conditions. A series of isothermal experiments between ambient temperature and 300°C was carried out for each combination of pressure and composition. For completeness, pure pentane and pure dioxane were included in these series. The experimental conditions are listed in Table I.

Before use, the silica column packing was first modified by treatment with dioxane as described previously[14]. Dioxane-containing supercritical eluents have been found to effect a permanent chemical modification of silica and, thereby, to alter the behaviour of the column[14]. The changes in retention and peak symmetry were, however, only small with pentane as the mobile phase. They were comparable to the small effects observed for ethane[15], rather than to the pronounced changes obtained with carbon dioxide[14,15]. Still, more reproducible results were obtained for the pentane–dioxane mobile phases on pretreated columns.

RESULTS AND DISCUSSION

For evaluating experimental data obtained with mixed mobile phases in the critical region, knowledge is needed about the critical pressures and temperatures of the pure components as well as of the mixtures. For pure pentane and pure 1,4-

TABLE I

CONDITIONS FOR THE ISOBARIC–ISOCRATIC–ISOTHERMAL CHROMATOGRAPHY

Column temperature: 20–300°C.

Dioxane content % (v/v)	*Column outlet pressure (bar)*
0	20, 36 70
5	20, 36, 40, 70
10	20, 36, 44, 70
20	20, 36, 50
30	20, 36, 56
40	20, 36, 60
50	20, 36, 62
70	20, 36, 70
90	20, 36
100	20, 36

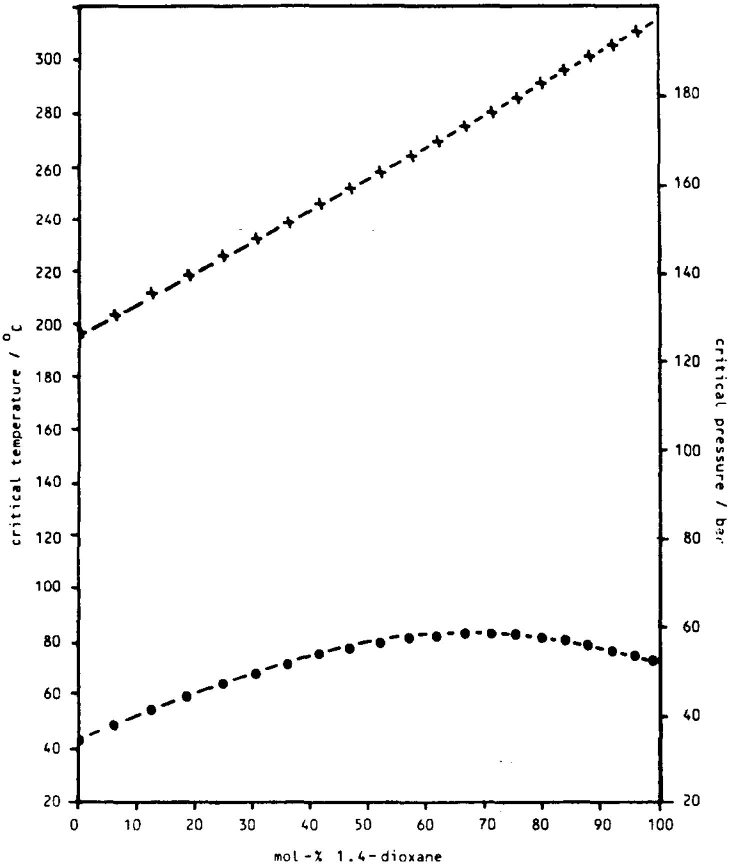

Fig. 1. Critical temperatures (+) and pressures (●) for *n*-pentane–dioxane mixtures plotted *versus* mol-% of dioxane.

dioxane, experimental data are available in the literature; however, no experimental data are known for their mixtures. A variety of methods exists for estimating the critical data for mixtures[16], and the method of Chueh and Prausnitz has been used in this work. Fig. 1 shows the critical temperatures and pressures for pentane–dioxane mixtures plotted *versus* the molar percentage of dioxane in the mixture. The critical temperature is seen to depend almost linearly on the molar percentage, whereas the critical pressure shows a maximum at about 70% (mol/mol) dioxane. The critical data for pentane, p_c = 33.7 bar and T_c = 196.5°C and for 1,4-dioxane, 52.1 bar and 313.9°C, were taken from the literature[16].

The dependence of the chromatographic parameters on temperature is found to be analogous to that of one-component eluents. This is exemplified in Figs. 2–9 for the capacity factors, k', of the four aromatic compounds employed as a test mixture, using pure pentane and mixtures of pentane and dioxane as the mobile phase. From isobaric plots at 36 bar of the capacity factors *versus* temperature, as shown in Figs. 2–7, k' is seen to decrease with increasing temperature as long as the

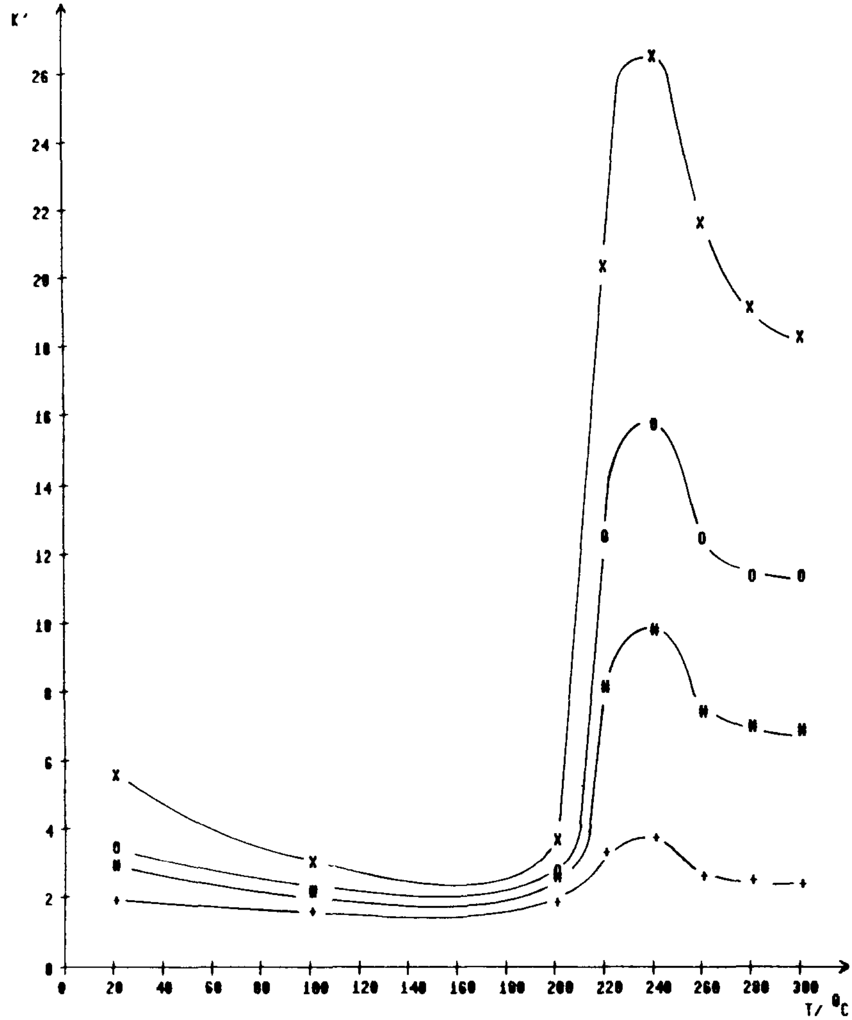

Fig. 2. Variation of the capacity factors, k', of aromatic compounds with the temperature, T. Mobile phase: 100% pentane. Column outlet pressure: 36 bar. Symbols: +, naphthalene; #, anthracene; 0, pyrene; X, chrysene.

mobile phase is in the liquid state. Having passed a minimum at a certain temperature while still below T_c, the capacity factor then increases with temperature above T_c, reaches a maximum and then decreases again. As with one-component eluents[5], this behaviour is due to: enhanced solubility of the substrate in a liquid mobile phase at rising temperatures, which explains the minimum; lowered solubility at temperatures above the boiling temperature (at pressures below the critical pressure, p_c) or above the critical temperature (at pressures above p_c), caused by a decrease in density, which explains the subsequent rise in k'; decreasing adsorption and increasing vapour pressure with increasing temperature, which causes the decrease in k' after the maximum.

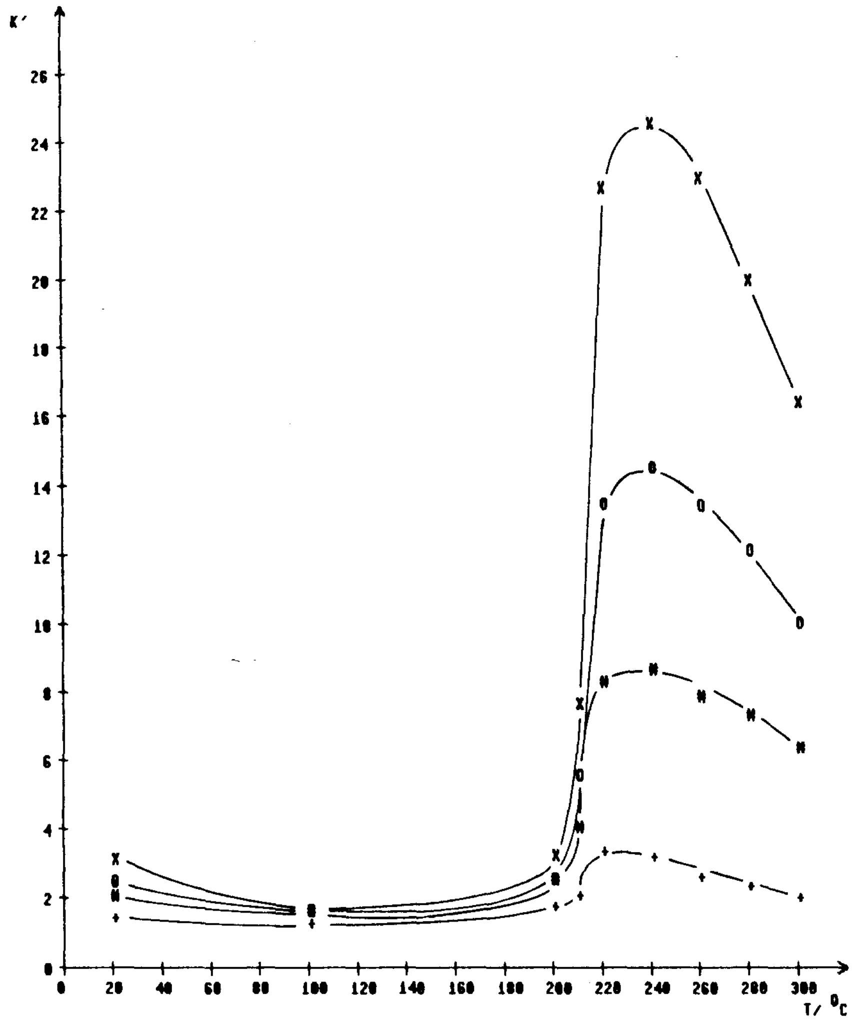

Fig. 3. Variation of k' with T. Mobile phase: pentane–dioxane (95:5, v/v). Other conditions as in Fig. 2.

The counteracting effects in the sub- and supercritical gaseous state are responsible for the formation of the k' maxima. These maxima are much more pronounced in height and area for the higher-molecular-weight members of the substrate.

As a comparison of Fig. 2–7 shows, the maxima in k' shift to higher temperatures with increasing dioxane content in the mobile phase. This is because the transition from the liquid to the gaseous state at sub- and supercritical pressures is shifted to higher temperatures when a second component of higher boiling and critical temperatures is added in increasing amounts. Correspondingly, the critical temperature of the mixture increases (Fig. 1). For dioxane contents of $\geqslant 50\%$ (v/v), the k' maxima are apparently located beyond 300°C, if they still exist, thus exceeding the experimental range of this study.

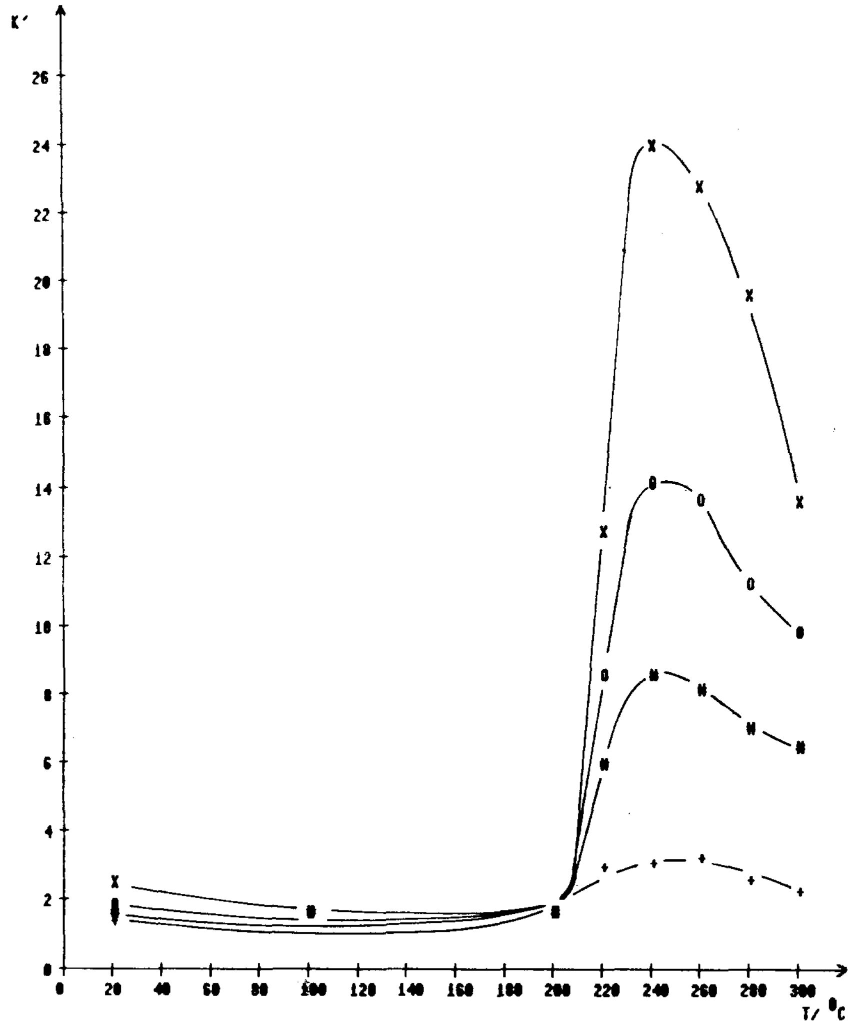

Fig. 4. Variation of k' with T. Mobile phase: pentane–dioxane (90:10, v/v). Other conditions as in Fig. 2.

Owing to the increase in the critical pressure with the amount of dioxane in the mobile phase in the range of 0–70% (mol/mol) dioxane, a pressure of 36 bar, as employed for the experimental series shown in Figs. 2–7, was above p_c only for pure pentane, but below p_c for molar dioxane contents of $\geqslant$ 5% (*cf.*, Fig. 1). It would be of interest to compare different eluent compositions at equal relative distance from their respective critical pressures, *i.e.*, to make a comparison at equal reduced pressures, p_r. This comparison is shown in Fig. 8, using the variations in k' for chrysene, k'(C), with temperature in the composition range of 0–40% dioxane (v/v), at a reduced pressure of 1.09. In this plot, the maxima in k' are shifted to higher temperatures and they decrease even more in height and area with increasing dioxane content than in Figs. 2–7.

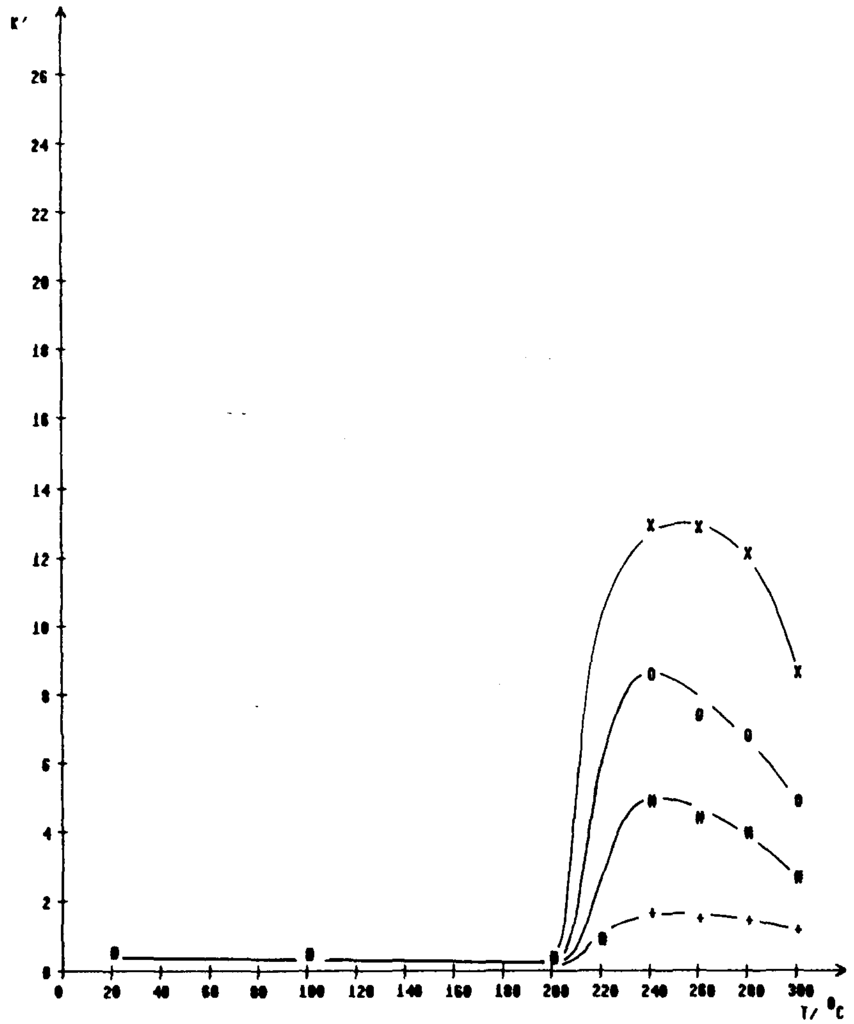

Fig. 5. Variation of k' with T. Mobile phase: pentane–dioxane (80:20, v/v). Other conditions as in Fig. 2.

The effect of pressure on retention is exemplified in Fig. 9, showing the variation of k'(C) with temperature at pressures of 20, 36, 44 and 70 bar for a mobile phase comprised of 90% pentane and 10% dioxane (v/v). With increasing pressure, the maximum becomes less pronounced and its position is shifted to higher temperatures, a behaviour which corresponds to that of one-component eluents. This effect of pressure has therefore already been discussed for one-component mobile phases. At higher pressures, the decrease in density occurring on passing the boiling or the critical temperature is less pronounced and shifted to higher temperatures, which is reflected by the behaviour of the k' values. When this decrease is small, *e.g.*, with pure pentane at 70 bar, no maxima in k' are observed with a pentane mobile phase

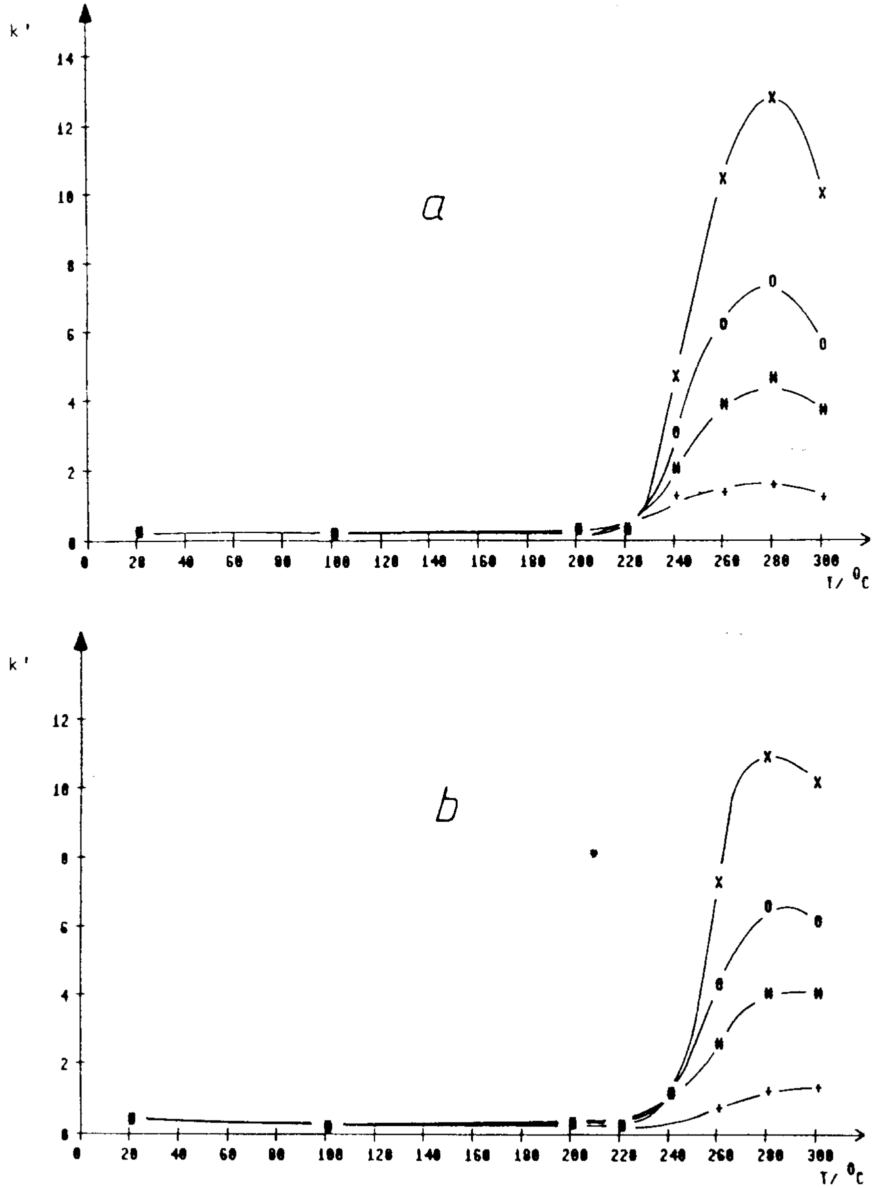

Fig. 6. Variation of *k'* with *T*. Mobile phases; (a) pentane–dioxane (70:30, v/v); (b) pentane–dioxane (60:40, v/v). Other conditions as in Fig. 2.

up to 300°C[1,5]. As Fig. 9 shows, this latter observation applies also to pentane–dioxane mixtures.

While the type of plot in Figs. 2–7 is of relevance for temperature programming and that in Fig. 9 for pressure programming, Fig. 10 is of interest for composition gradient elution. The capacity factors for chrysene are plotted as a function of eluent

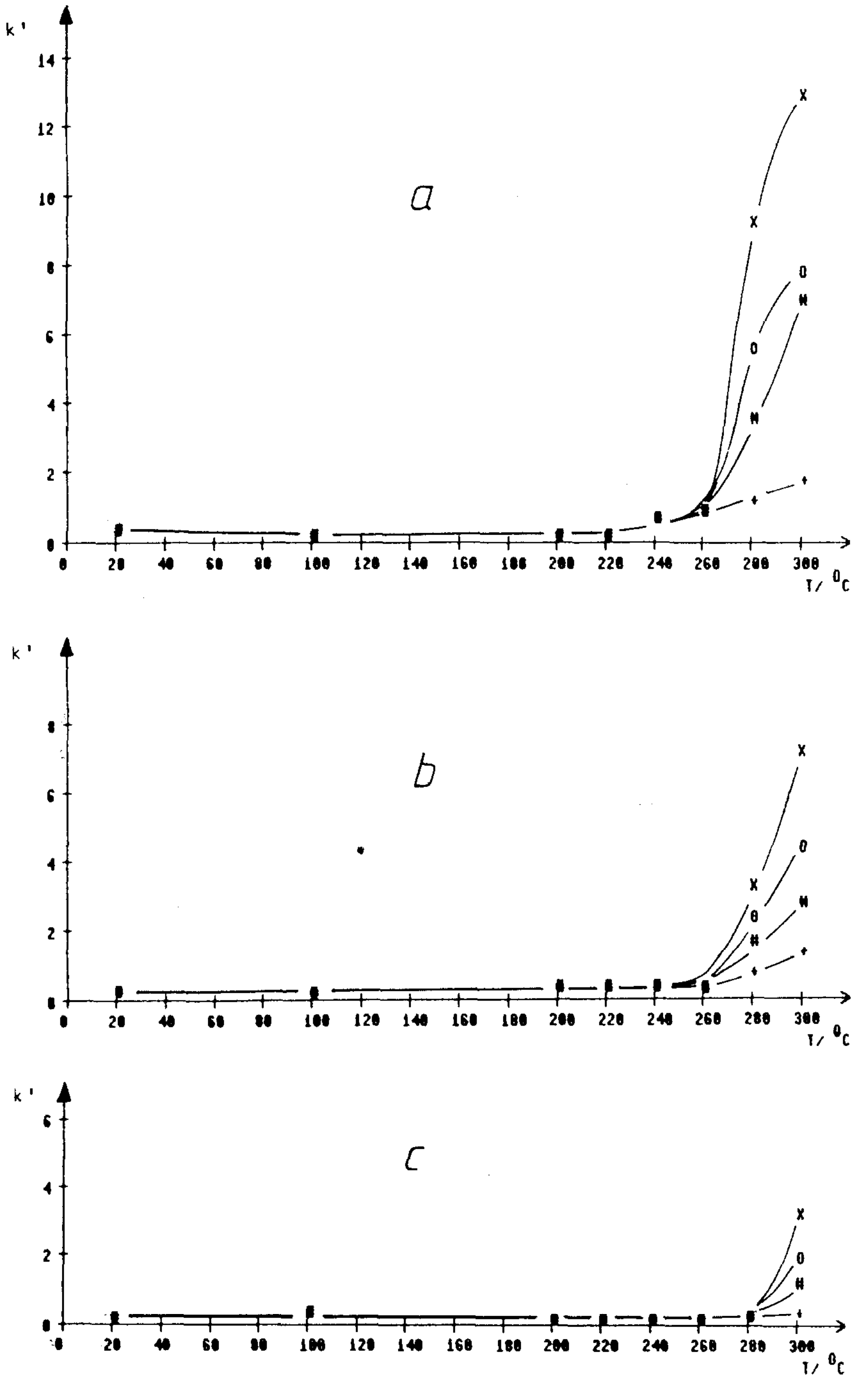

Fig. 7. Variation of k' with T. Mobile phases; (a) pentane–dioxane (50:50); (b) pentane–dioxane (30:70); (c) pentane–dioxane (10:90, v/v). Other conditions as in Fig. 2.

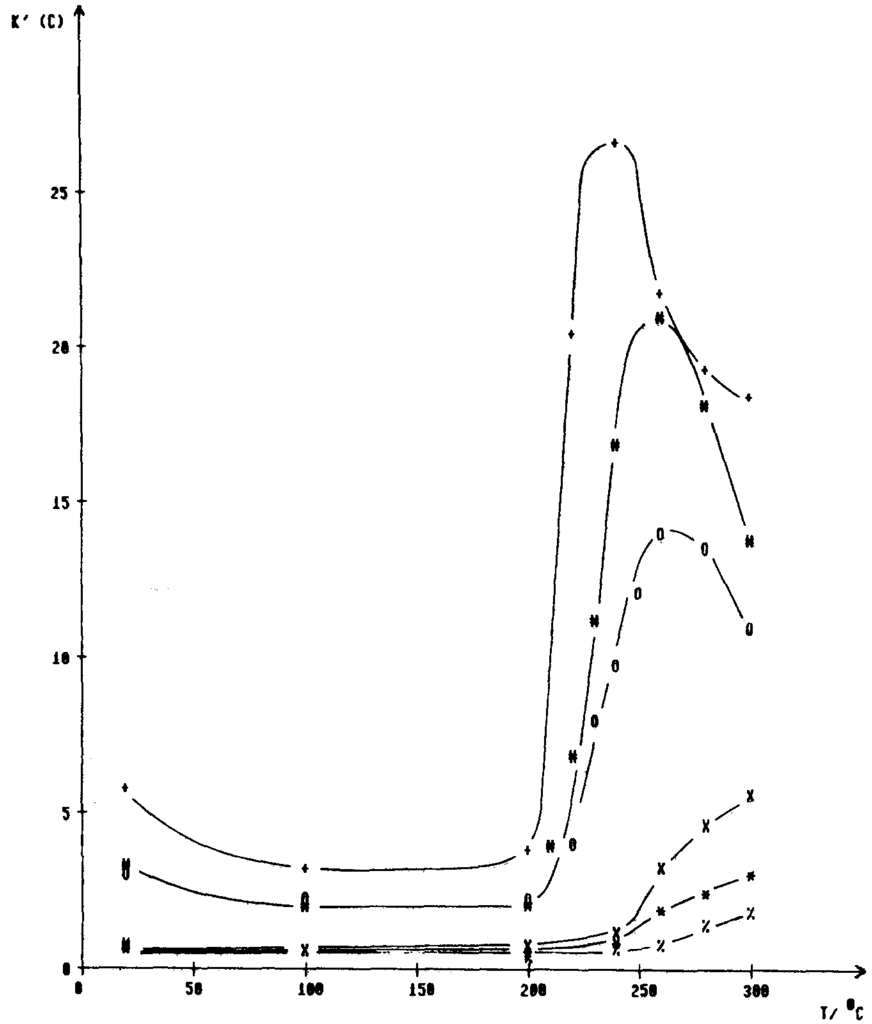

Fig. 8. Variation of the capacity factor of chrysene, k' (C), with the temperature, T, at a reduced pressure, p_r, of 1.09. Contents (%, v/v) of dioxane in the pentane mobile phase: 0% (+), 5% (#), 10% (0), 20% (X), 30% (*), 40% (%). Column outlet pressures (bar): 36 (+), 40 (#), 44 (0), 50 (X), 56 (*), 60 (%).

composition at different constant temperatures and a constant pressure of 36 bar. A rather complex behaviour is observed. At lower temperatures in the liquid state, k' (C) decreases steadily with increasing amount of dioxane. In contrast, at higher temperatures (260 and 300°C) in the supercritical state, k' increases intermediately, leading to the formation of maxima. The maxima are more pronounced and shifted to higher dioxane contents with increasing temperature. Thus, the use of a gradient of increasing amounts of dioxane in a pentane mobile phase, at a constant pressure of 36 bar and at a constant temperature in the range of 250–300°C, would not always lead to a diminishing k' and an acceleration of elution.

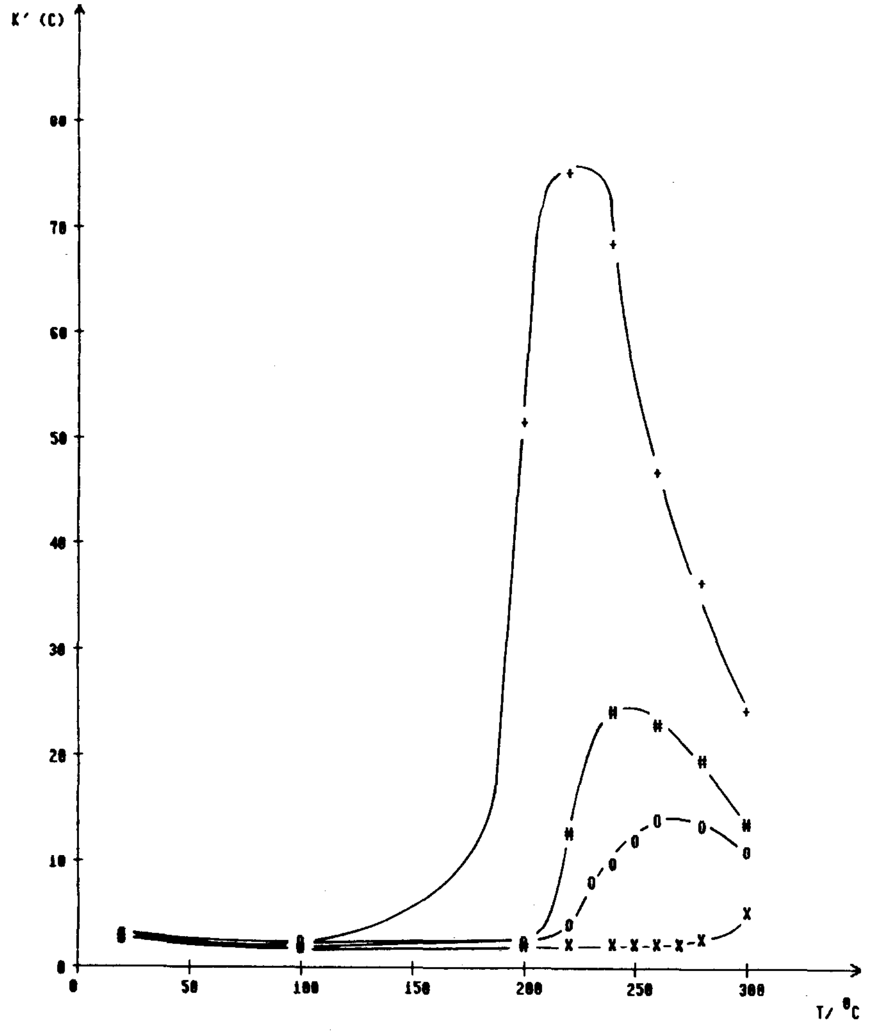

Fig. 9. Variations of k' (C) with T at different pressures. Mobile phase: pentane–dioxane (90:10, v/v). Column outlet pressures (bar): 20 (+), 36 (#), 44 (0), 70 (X).

When the plotting of k' *vs.* eluent composition is not done at a constant pressure but at a constant reduced pressure, the maxima are reduced or disappear almost completely. This is demonstrated in Fig. 11, for a constant reduced pressure of 1.09 in the temperature range of 240–300°C. Plotting at a constant reduced pressure, $p_r > 1$ implies that at all compositions the mobile phase is in the supercritical state with respect to pressure.

The appearance of the maxima in k' in Fig. 10 is a consequence of the temperature shift of the position of the maxima with increasing dioxane content at constant pressure, as can be seen by comparing Figs. 2–7. Thus, at a given temperature,

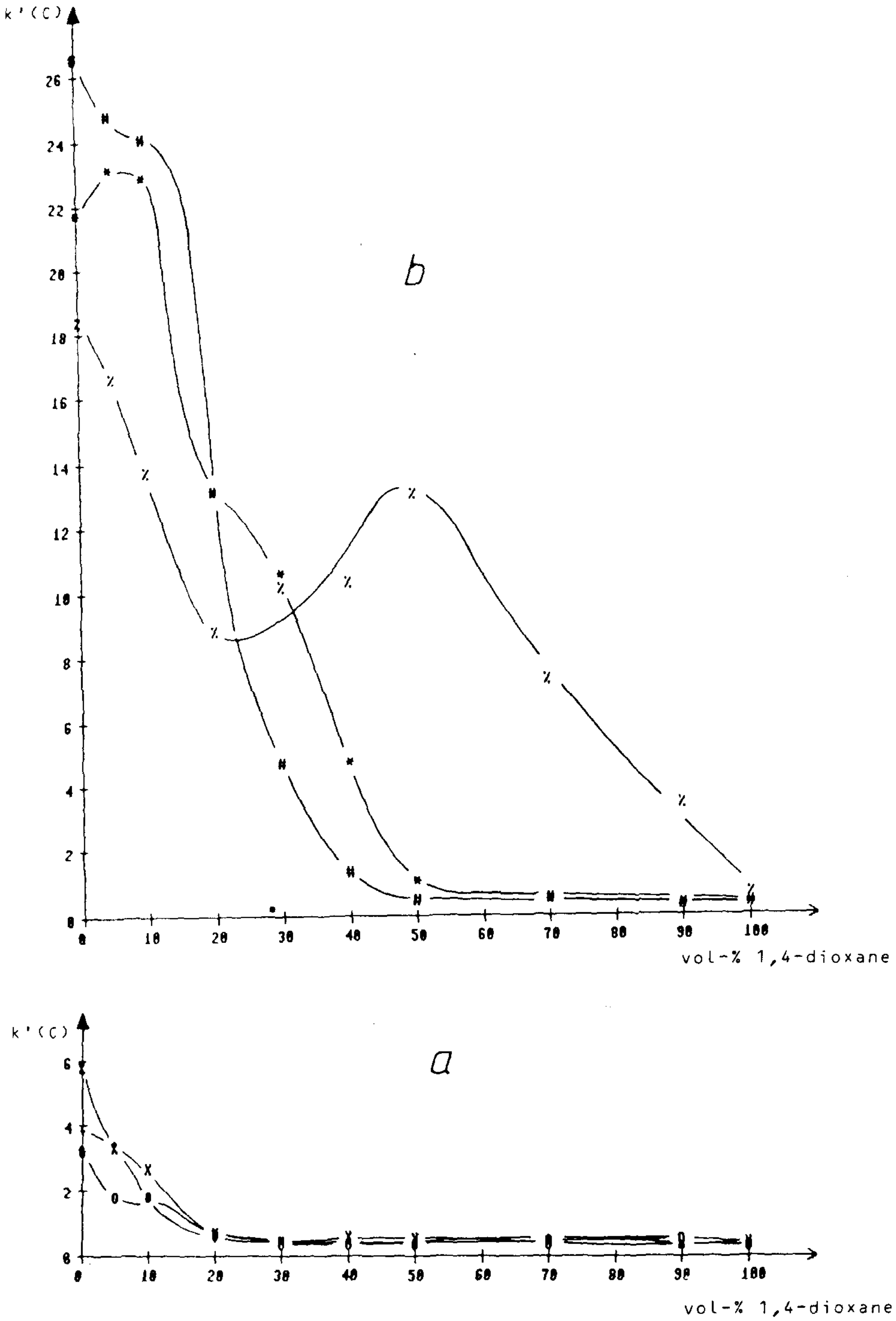

Fig. 10. Variation of *k'* (C) with the content of dioxane in the mobile phase at a constant column outlet pressure of 36 bar. Temperatures (°C): (a) ambient (X), 100 (0), 200 (+); (b) 240 (#), 260 (*), 300 (%).

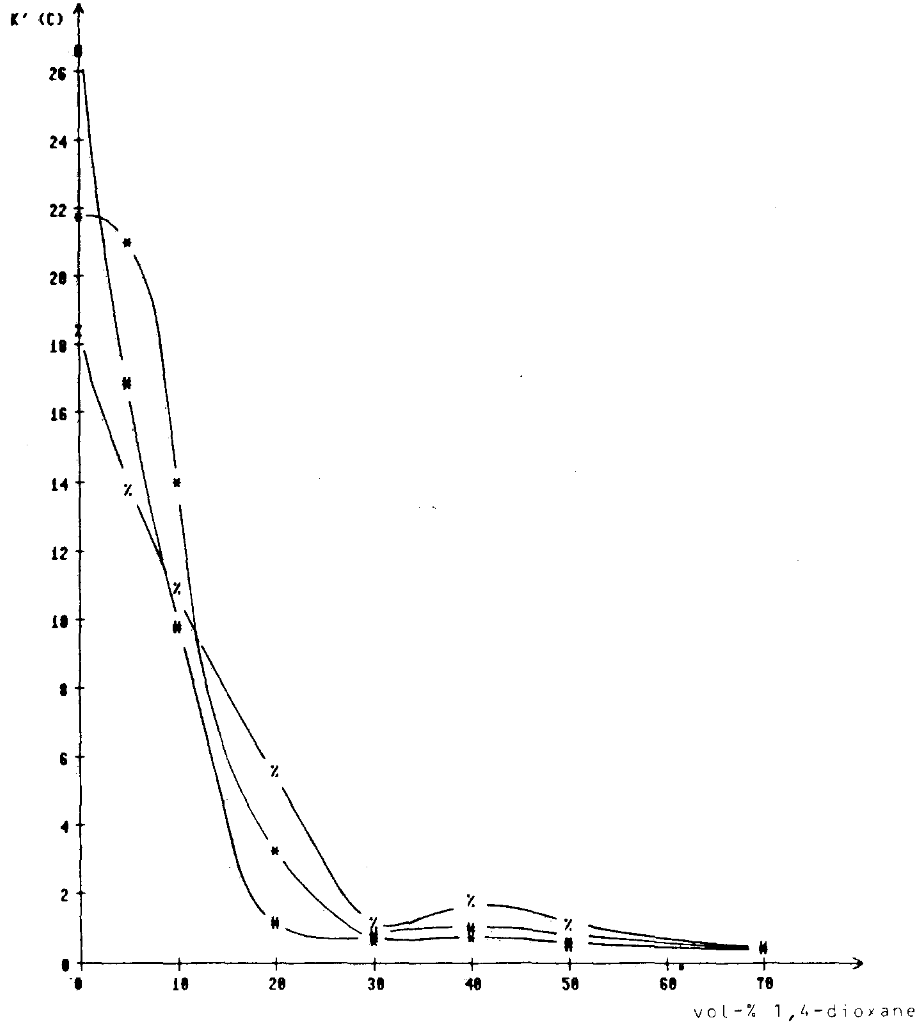

Fig. 11. Variation of k' (C) with the content of dioxane in the mobile phase at a constant reduced pressure, p_r = 1.09. Temperatures (°C): 240 (#), 260 (*), 300 (%).

relatively high k' values are found for dioxane contents which show maxima at this temperature, and these k' values may be higher than those at lower or higher dioxane contents.

An explanation for the maxima in k' in Fig. 10, may be a preferential adsorption of dioxane as molecular layers on the silica surface. At a given temperature and pressure, the layers build up with increasing dioxane content of the mobile phase. At still higher dioxane contents, k' decreases again because the layer may be progressively removed on account of the higher dissolution power of the mobile phase. Moreover, the partition coefficient diminishes, because the composition of the layers

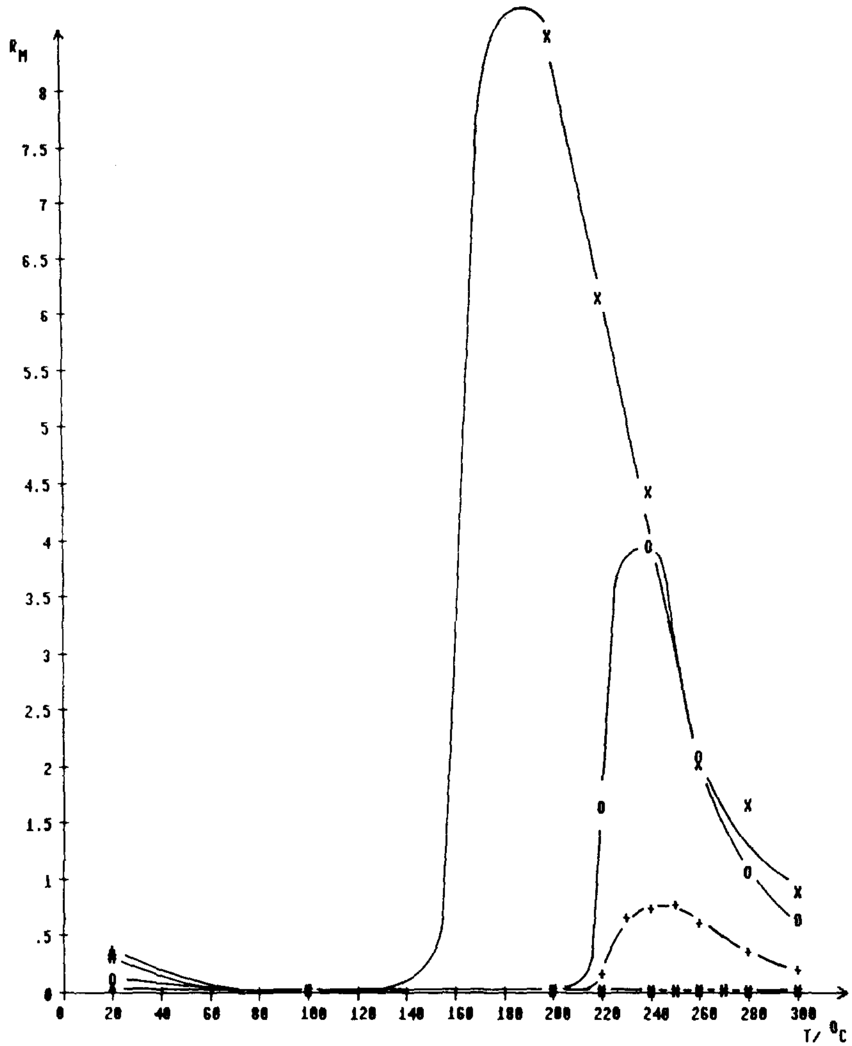

Fig. 12. Variation of the average resolution, R_m, with T. Mobile phase: pentane–dioxane (90:10, v/v). Column outlet pressures (bar): 20 (X), 36 (0), 44 (+), 70 (#).

and of the mobile phase become progressively more similar. When the pressure is increased simultaneously to the dioxane content, as in Fig. 11, the removal of the layers and the decrease in partition coefficient may be more pronounced, suppressing the formation of the maxima.

A comparison of Figs. 10 and 11 suggests that a gradient elution in SFC may be performed at increasing pressure rather than at constant pressure, if reduced retention is desired. Thereby, the pressure increase would probably compensate for the increase in the critical pressure due to the admixing of the higher boiling component. In fact, SFC separations with gradient elution using the eluent pair pentane–dioxane

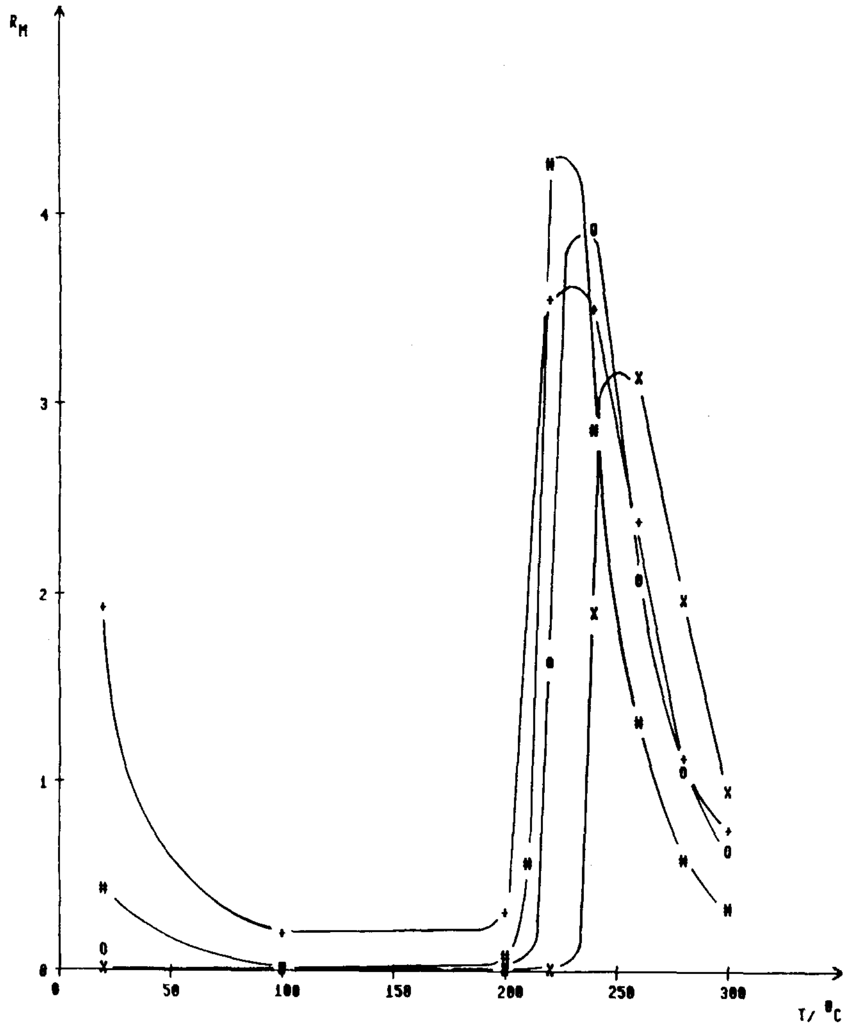

Fig. 13. Variation of R_m with T at a constant column outlet pressure of 36 bar. Contents (%, v/v) of dioxane in the pentane mobile phase: 0 (+), 5 (#), 10 (0), 20 (X).

have been proven to be applicable; during these separations "inherent" pressure gradients occurred which were due to a column outlet valve with a fixed setting, in addition to the higher viscosity of dioxane compared to pentane$^{7-13}$.

The curves obtained by plotting the average resolution, R_m, *versus* temperature are generally similar in shape to those of the k'-plots (Figs. 12–14). In the gaseous state above the boiling and critical temperatures T_b and T_c, particularly at low column outlet pressures and at low dioxane contents, R_m shows a very strong dependence on temperature. After having passed T_b or T_c, the resolution increases with temperature, attains a maximum, then decreases again. With increasing pressure, the intensity of the maxima decreases and finally the maxima disappear at a pressure of

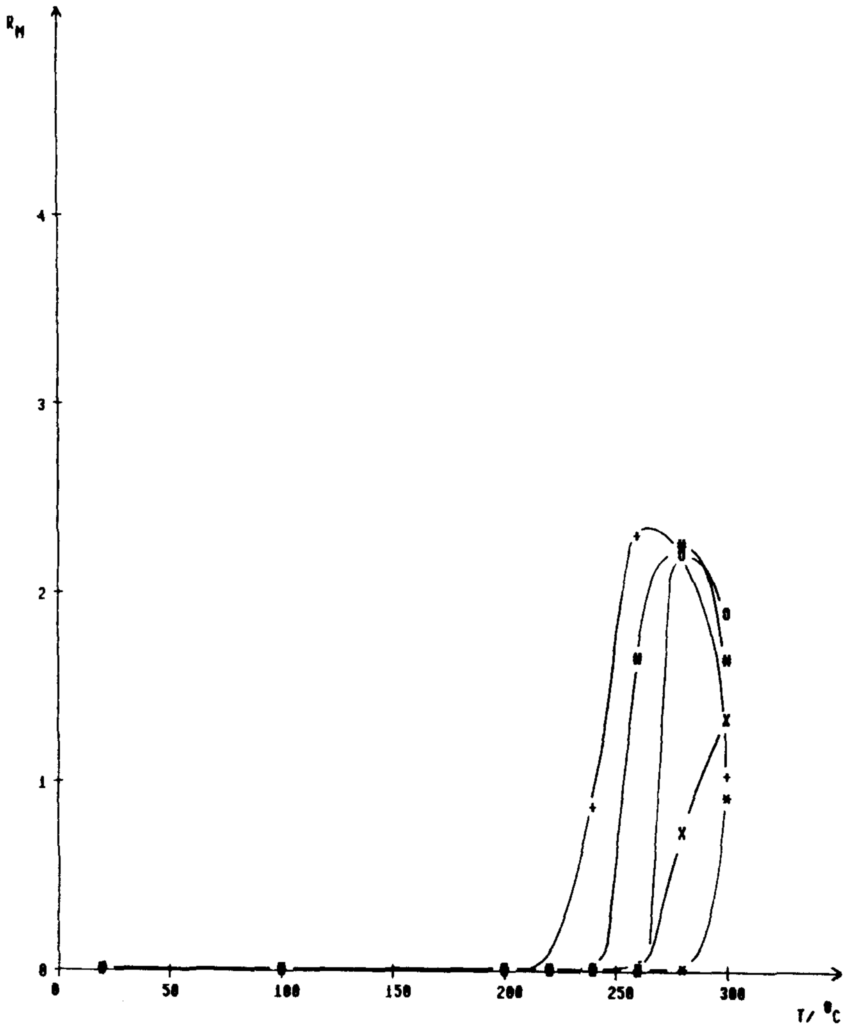

Fig. 14. Variation of R_m with T at a constant column outlet pressure of 36 bar. Contents (%, v/v) of dioxane in the pentane mobile phase: 30 (+), 40 (#), 50 (0), 70 (X), 90 (*).

70 bar (Fig. 12). The highest resolution values are observed with the mixtures containing 5 and 10% dioxane (Fig. 13). Furthermore, higher pressures and higher dioxane concentrations in the mobile phase shift the resolution maximum to higher temperatures (Figs. 12–14). Thus, the positions of the maxima may even be located above 300°C (Fig. 14). In the liquid state below T_b and T_c, the resolution decreases with increasing temperature until a minimum is reached shortly below the boiling or the critical temperature. Contrary to pure pentane, showing high R_m at ambient temperature (Fig. 13), the aromatic compounds are only poorly separated with liquid pentane–dioxane mixtures (Figs. 13 and 14). Similarly, an increase in pressure also

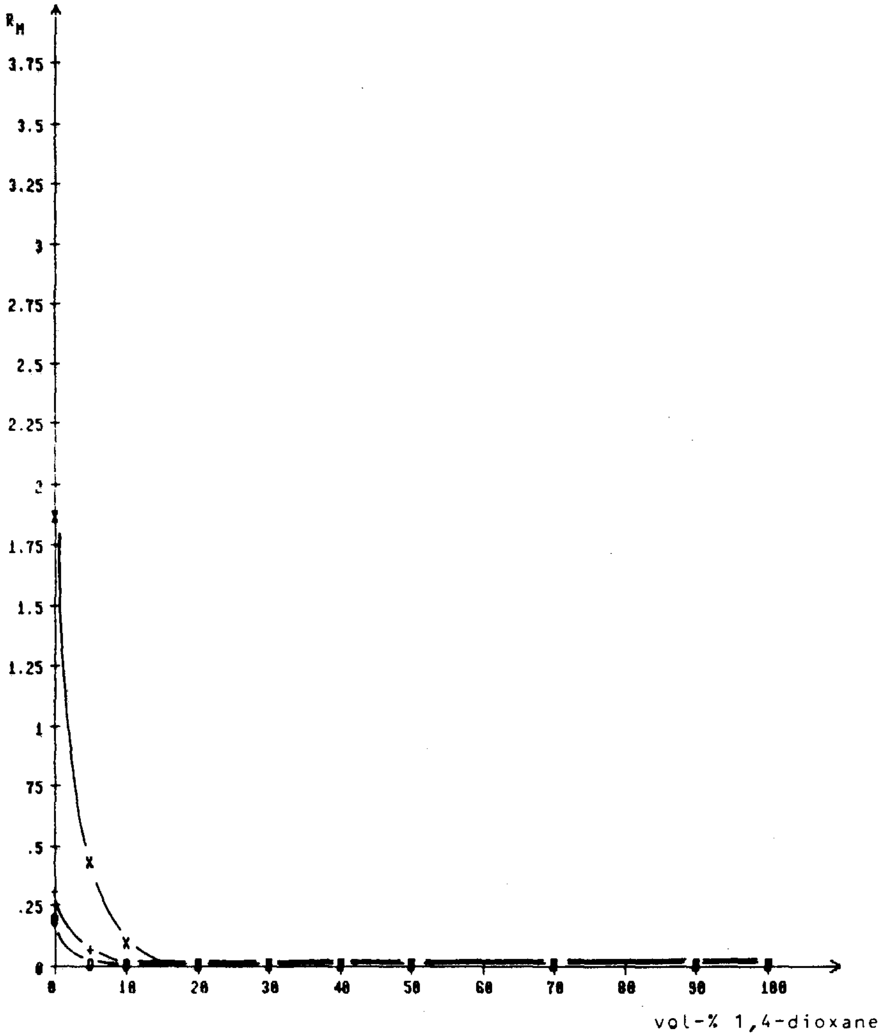

Fig. 15. Variation of R_m with the content of dioxane in the mobile phase at a constant column outlet pressure of 36 bar. Temperatures (°C): 20 (X), 100 (0), 200 (+).

reduces the resolution (Fig. 12). This again shows that varying the column outlet pressure in HPLC has a significant effect and could be utilized to solve practical problems.[5].

The dependence of R_m on the mobile phase composition at constant pressure or constant reduced pressure and constant temperature is similar to that for k', as shown in Figs. 15–17. Plots of the average resolution, R_m, *versus* mobile phase composition at constant column outlet pressure, with temperature as a parameter, are curves of similar shape to those of the k'-plots. The resolution is seen to decrease continuously with dioxane content when the mobile phase is in the liquid state at lower temperatures from 20 to 200°C (Fig. 15). At the higher temperatures of 240,

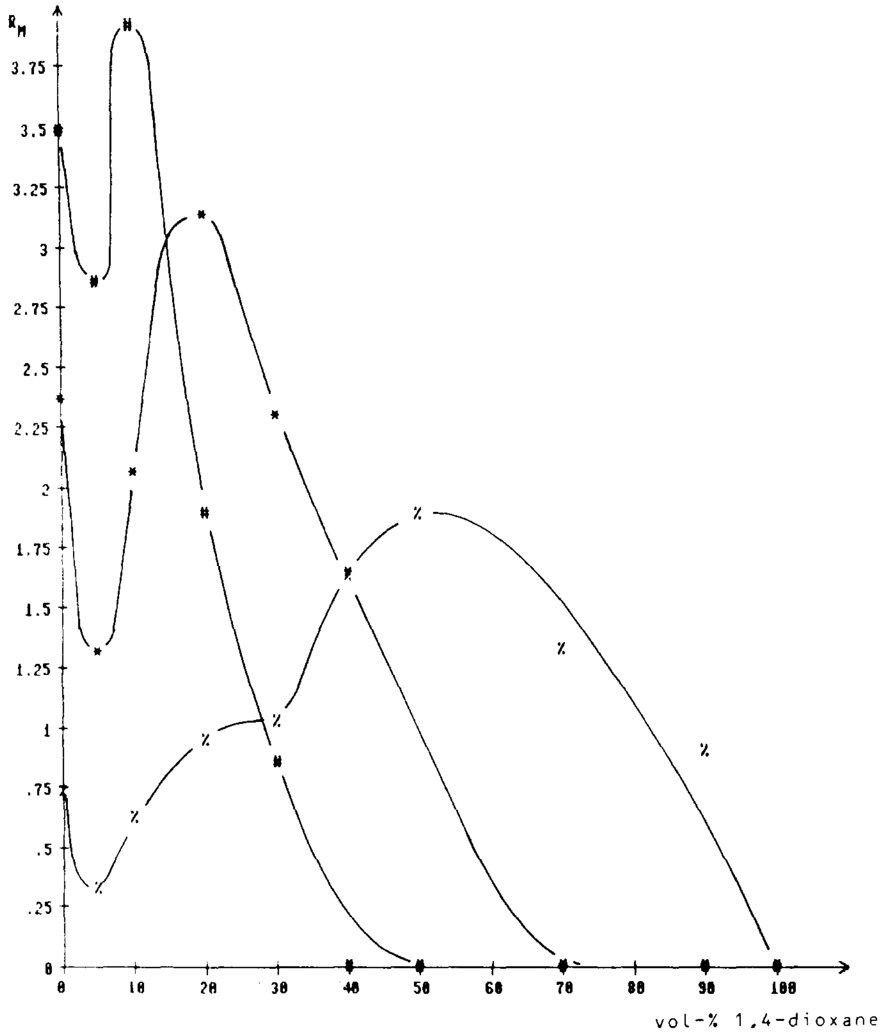

Fig. 16. Variation of R_m with the content of dioxane in the mobile phase at a constant column outlet pressure of 36 bar. Temperatures (°C): 240 (#), 260 (*), 300 (%).

260 and 300°C, maxima are observed (Fig. 16) which shift to higher dioxane contents with increasing temperature. Maxima are also observed, although less pronounced and at lower dioxane contents, when R_m is plotted *versus* eluent composition at a constant reduced pressure, $p_r = 1.09$ (Fig. 17).

As shown in the preceding figures, maxima are observed in the isobaric–isocratic plots of k' or R_m *versus* temperature, if the pressure does not exceed a certain value. The temperatures, T_R^m, at which these maxima in the average resolution occur are plotted *versus* eluent composition at different pressures in Fig. 18. The T_R^m are seen to increase with increasing dioxane content and with increasing pressure. Such

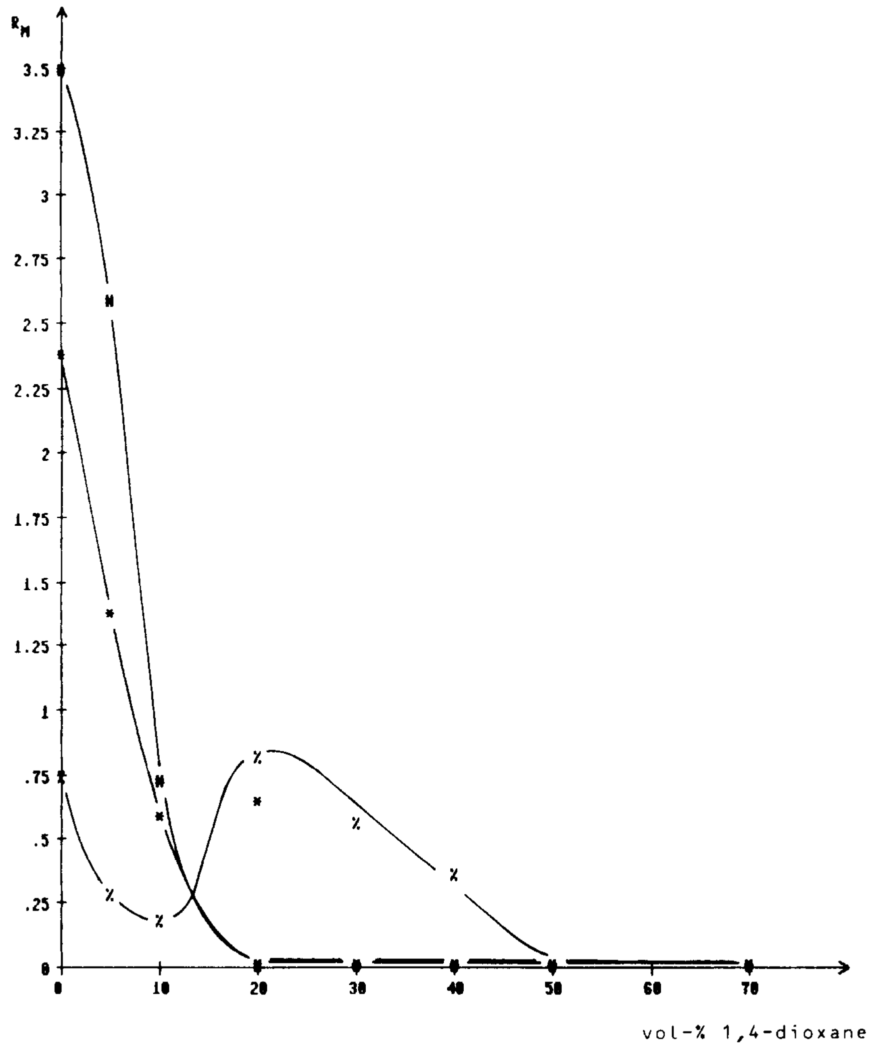

Fig. 17. Variation of R_m with the content of dioxane in the mobile phase at a constant reduced pressure of 1.09. Temperatures as in Fig. 16.

plots yield the temperature at which the resolution reaches its maximum value, for given values of the eluent composition and pressure. They may therefore be used for maximizing the resolution during a separation, if the separation is performed by means of a gradient of eluent composition. For that purpose, a temperature gradient is superimposed on a given eluent gradient, the temperature gradient being obtained from the temperature–composition–pressure triple points which can be read directly from Fig. 18. For pressures intermediate between those shown in Fig. 18, an interpolation may be carried out. Using carbon dioxide–dioxane mobile phases, it has been shown that this procedure can actually yield improved resolution throughout gradient separations[17].

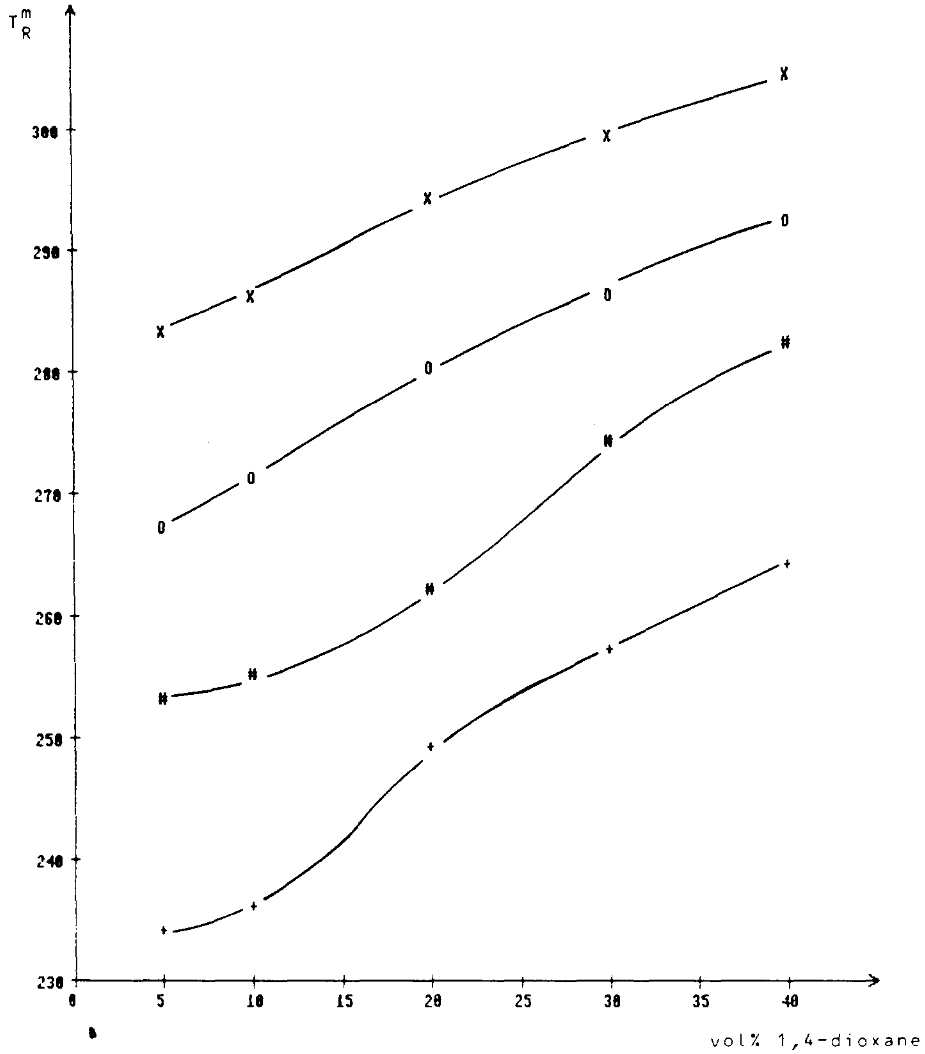

Fig. 18. Variation of the temperature of the resolution maximum, T_R^m, with the content of dioxane in the mobile phase. Pressures (bar): 30 (+), 40 (#), 50 (0), 60 (X).

In conclusion it may be emphasized that, when working with mixed mobile phases in SFC, a knowledge of the critical data for the mixtures to be employed is necessary. In particular, fundamental data on the interdependences between the physical parameters temperature, pressure and eluent composition on the one hand and retention as well as resolution on the other are needed. These data are of considerable value for optimizing separations, including those involving gradient elution. Thus, it was shown that the addition of a component of higher solvent strength, a co-fluid, to the mobile phase at constant temperature and pressure does not necessarily lead to enhanced elution at all co-fluid contents.

Besides the capacity factors, the selectivities, α, and the plate numbers, n, contribute to the maxima found for the chromatographic resolution and they should therefore also be considered. This will be addressed in a separate communication.

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