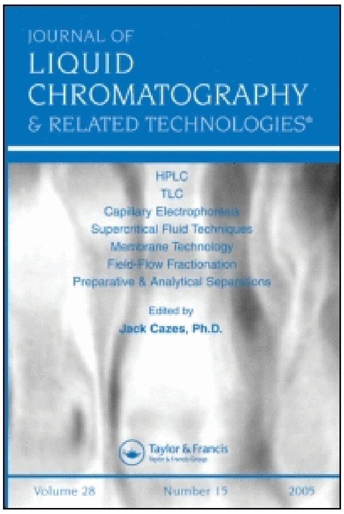

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Küppers, Stephan , Schmitz, Franz Peter and Klesper, Ernst(1991) 'Comparison of Different Compositions of Mixed Pentane/1,4-Dioxane Mobile Phases in SFC', Journal of Liquid Chromatography & Related Technologies, 14: 16, 3015 — 3043

To link to this Article: DOI: 10.1080/01483919108049374

URL: http://dx.doi.org/10.1080/01483919108049374

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(16&17), 3015-3043 (1991)

COMPARISON OF DIFFERENT COMPOSITIONS OF MIXED PENTANE/1,4-DIOXANE MOBILE PHASES IN SFC*

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ABSTRACT

The effective plate number has been chosen for a comparison between different mixed mobile phases composed of pentane and 1,4-dioxane. A column packed with unmodified silica gel and pyrene as the analyte was used. The dependence of the effective plate number on pressure and composition for a constant temperature, or a constant reduced temperature, both at constant mass flow, are shown in three dimensional network plots. The same dependence is shown at constant temperature and constant linear velocity.

For a detailed discussion, the dependence of the effective plate number on pressure, composition, capacity ratio, and other chromatographic parameters is given in two dimensional graphs. The main result for the present chromatographic system is that a

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[*] dedicated to Prof. Dr. W. Burchard, Freiburg, BRD, on the occasion of his 60th birthday

[**] to whom correspondence should be addressed

reduction of retention time by increase of the content of dioxane leads to higher plate numbers than the increase of pressure does. Highest plate numbers were found for a wide range of compositions at medium pressures.

INTRODUCTION

Chromatographic parameters such as capacity ratio, k′, selectivity, α, resolution, R, and effective plate numbers, N, are routinely used to describe a chromatographic system. Capacity ratio and selectivity depend on the thermodynamics of the system in equilibrium, whereas resolution and effective plate number depend in addition on the kinetic behavior which is manifested in the shape of the peaks, i.e. in peak widths and symmetry. Both types of parameters are needed for a reasonable description of a chromatographic system. In supercritical fluid chromatography (SFC), capacity ratios k′, selectivities α, and mean resolution R_m have previously been employed [1], [2], [3], as have been effective plate numbers [4]. Particularly the effective plate number is dependent on all physical parameters, pressure, temperature, binary mobile phase composition, and linear velocity. Together with the capacity ratio or the retention time, the knowledge of the effective plate number may be useful as a minimum information for the optimization of separations in SFC.

The aim of optimization in chromatography is often to combine short analysis time with high resolution. If, for instance, a specific maximum analysis time can be tolerated during an industrial production step, the chromatographic conditions of the analysis must be chosen such to obtain within this time a resolution sufficient for the analyte on hand. The resolution, R, is related to the effective plate number, N, and the selectivity, α, between peaks i and j by the standard equation:

$$R_{ij} = \frac{1}{4} \frac{\alpha_{ij} - 1}{\alpha_{ij}} \sqrt{N} \qquad (1)$$

Accordingly, a high N and a high α_{ij} lead to high R_{ij}. The effective plate number, N, is often called also the effective column efficiency. This might convey the impression that the efficiency is a constant for a specific column. However, both the effective plate number and the plate number are strongly dependent on the physical parameters of the mobile phase, as already pointed out. The extent of the dependence of the chromatographic on the physical parameters is particularly large for a system with a moderate interaction between analyte and stationary phase on one hand, and between analyte and mobile phase on the other. In this case, changing the physical parameters will lead to considerable changes for the chromatographic parameters.

EXPERIMENTAL

The chromatograms were obtained on a SFC instrument based on a commercially available high-performance liquid chromatograph, as described previously [5]. The pretreatment of the eluents pentane and 1,4-dioxane and the chromatographic conditions have also been described in detail [6]. The chromatograms were run either at a constant volume feed rate, as measured at the pumps in the liquid state, or at a constant linear velocity. For obtaining the chromatograms at constant linear velocity, the volume flow rate was changed until a constant dead time for heptane as the (non-retained) analyte was reached. The stainless steel columns (25 cm x 4.6 mm i.d.) were packed with LiChrosorb Si 100, 10 μm (Merck, Darmstadt, FRG). The ready packed columns were conditioned before use with 1,4-dioxane at 250°C and 60 bar as already described [7]. The mobile phase was pure pentane or binary mixtures of pentane and 1,4-dioxane. These binary mobile phases have a high solubility power as has been demonstrated before [8]. As the test analyte, pyrene in a concentration of 7.5 x 10^{-2} g/l was chosen as a typical polycyclic aromatic hydrocarbon of medium molecular weight. The injected volume was 20 μl. Detection was by UV, the detector being set to 254 nm. The back pressure device was either a Hitec P 532 valve (Bronkhorst High Tech B.V., AK Ruurlo, The Netherlands), as described in detail earlier [9], or a Tescom backpressure regulator (Tescom Corp. Minneapolis, Minnesota, USA), both being installed behind the UV-detector.

The capacity ratio k' was obtained from the retention time, t_r, and dead time, whereas the effective plate number N was calculated from k', t_r, and the peak width at half height, $w_{0.5}$:

$$N = n\left(\frac{k'}{(1+k')}\right)^2 = 5.54\left(\frac{t_r}{w_{0.5}}\right)^2\left(\frac{k'}{(1+k')}\right)^2 \qquad (2)$$

with n being the plate number. The method using the width of the peak at half height, $w_{0.5}$, was chosen, because this gives good results if the peaks are not too asymmetric [10]. For interpolation of the data grid and plotting of the three-dimensional network plots, in which a fourth variable is represented by shading the network, a computer program (Unimap, Uniras European Software Contractors A/S, Denmark 1985) was used, as described in detail before [11].

RESULTS AND DISCUSSION

Figures 1 to 3 show three-dimensional network plots of the capacity ratio of pyrene, k′(P), and the effective plate numbers for this analyte, N, in dependence of the physical parameters pressure, p/bar, and mobile phase composition in terms of vol-% 1,4-dioxane, %B. Fig. 1 gives a view of the behavior for a constant column temperature of 260°C and a constant volume feed rate of 1 ml/min. Fig. 2 shows the same type of network plot, but for a constant reduced temperature, T_r = 1.1 instead of T = 260°C, which means 215°C for pure pentane; 234°C for 90 vol-% pentane/10 vol-% dioxane; 248°C for 80 vol-% pentane/20 vol-% dioxane, and 263°C for 70 vol-% pentane/30 vol-% dioxane. Fig. 3 presents a three-dimensional plot for a temperature of 260°C again, as in Fig. 1, but with a constant linear velocity of 0.26 cm/sec. instead of a constant volume flow rate of 1

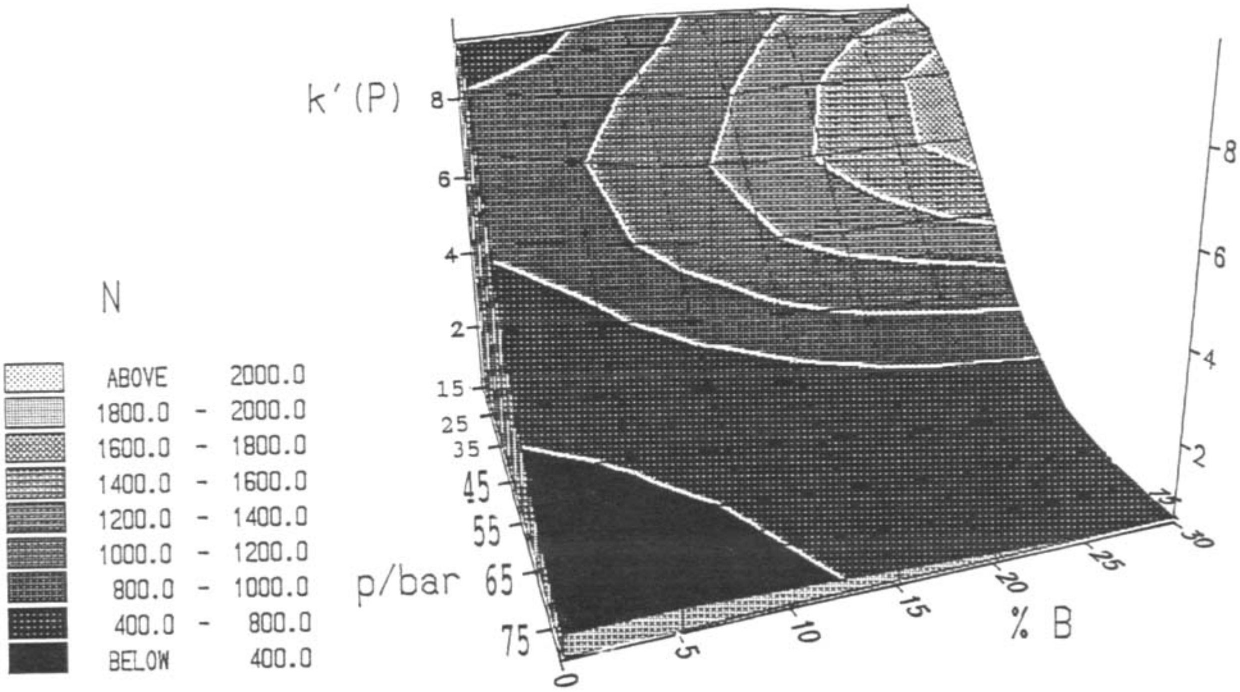


Figure 1: Capacity ratio of pyrene, k′(P), and effective plate number of pyrene, N, as a function of pentane/1,4-dioxane mobile phase composition, %B (= vol. % 1,4-dioxane), and pressure, p/bar, at T = 260°C, and at a constant liquid volume feed rate of 1 ml min^{-1}. at the pumps. Other conditions see experimental part.

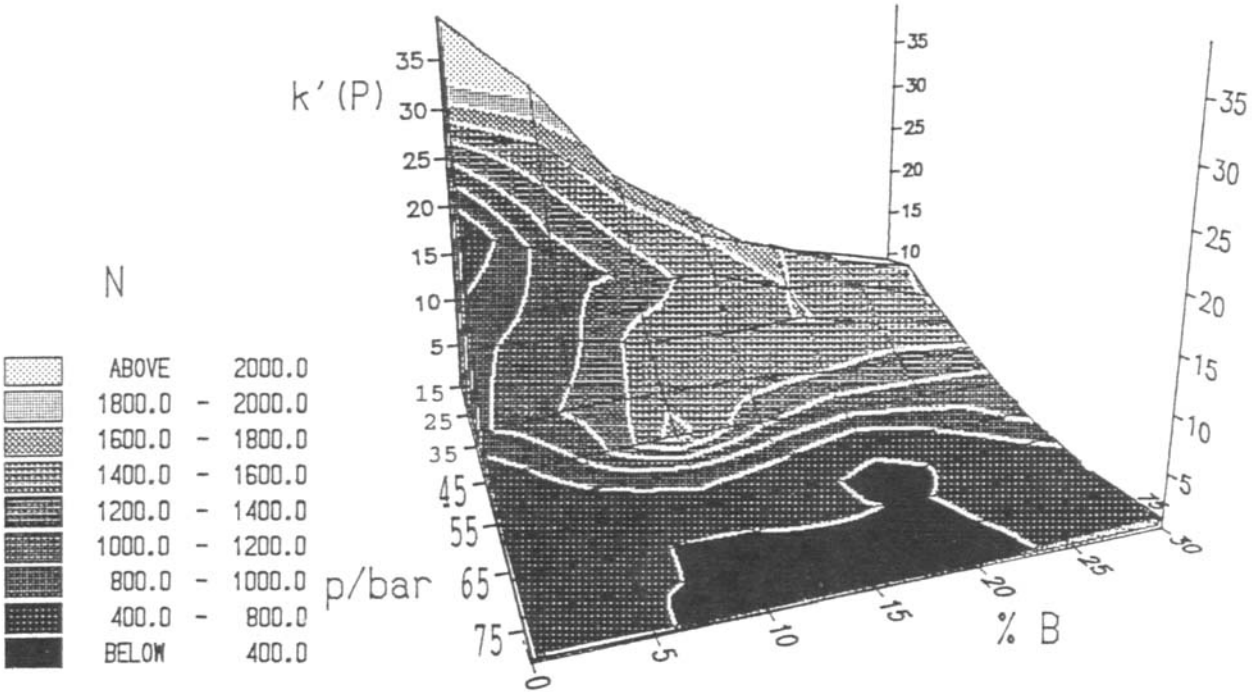


Figure 2: Pyrene capacity ratio, k'(P), and effective plate number, N, as a function of mobile phase composition, %B, and pressure, p/bar, at a reduced temperature of $T_r = 1.1$ and a liquid volume feed rate of 1 ml min^{-1}. Otherwise as for Fig. 1.

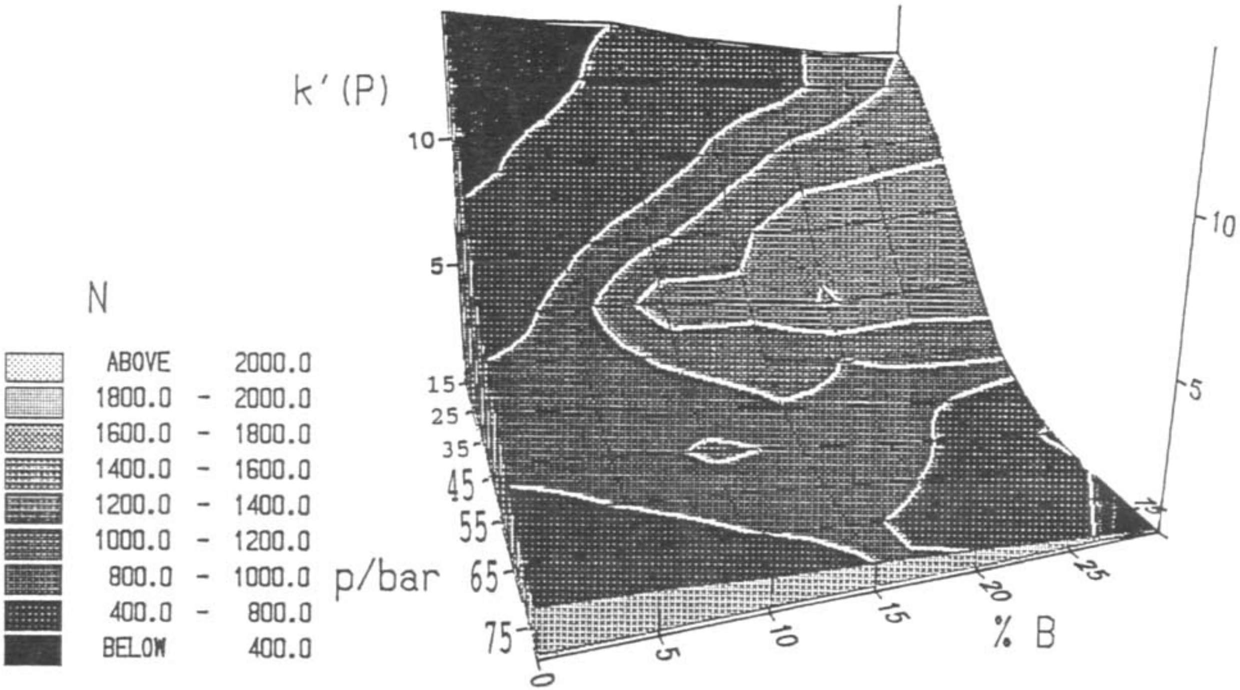


Figure 3: Pyrene capacity ratio, k′(P), and effective plate number, N, as a function of mobile phase composition, %B, and pressure, p/bar, at T = 260°C, and at a constant linear velocity of 0.26 cm sec^{-1}. Otherwise as for Fig. 1.

ml/min. as in Figs. 1 and 2. The plots in Figs. 1 to 3 demonstrate by way of an overview the dependence of the effective plate number on the parameters pressure, mobile phase composition, and to a more limited extent, on temperature and linear velocity.

Even a cursory inspection of Figs. 1 to 3 reveals that for a given k'(P) the N may differ widely. Thus, although the analysis time may be acceptably short, the efficiency may not be sufficient to lead to a satisfactory resolution. Specifically for Fig. 1, a high N at $k'(P)<10$ for pyrene, or for a type of analyte behaving similarly to pyrene, may be obtained in the lower range of p combined with the higher range of %B. Staying at T_r = const. = 1.1 throughout the plot, as in Fig. 2, is equivalent to effecting a temperature rise when increasing %B and, therefore, to a simultaneous composition-temperature program. At T_r = 1.1 the temperature at the highest investigated 1,4-dioxane content of 30% is close to 260°C (i.e. 263°C). This leads to a temperature which is generally lower than 260°C for Fig. 2 and, therefore, the behavior of k'(P) and N is significantly different from Fig. 1. High N may again be obtained in the lower range of p, but the region of higher N spreads now over a wider range of %B. A disadvantage seen in Fig. 2 is that the k'(P) rise to values which are unacceptably high for practical purposes, if the pressure is chosen too low. Fig. 3 is again obtained at a constant temperature of 260°C, but now at a constant linear velocity. A constant linear velocity in the supercritical state in the column is not at all equivalent to a constant volume flow rate in the liquid state at the pump. The higher the temperature and the lower the pressure or the 1,4-dioxane content, the higher is the linear velocity in the column if the volume flow rate is kept constant. If, on the other hand, the linear velocity is kept constant, the volume flow rate must decrease whenever the temperature increases and the pressure or the 1,4-dioxane content decreases. By Fig. 3 it is found that the behavior of N is, however, not very different from that of Fig. 1, although the spread in the magnitude of the values of N is smaller in Fig. 3 than in Fig. 1.

While the plots of the type of Figs. 1 to 3 are useful to foresee the chromatographic behavior during isocratic-isobaric-isothermal separations at constant volume flow or at constant linear velocity, they are also useful for gradient separations. This is immediately obvious for mobile phase composition or pressure gradients because composition and pressure are plotted as x- and y-axis of the three-dimensional graphs. It appears that it is of advantage during a composition or a pressure program to remain in the region of high N for the individual compounds of a given analyte. With the present triad, mobile phase, stationary phase, and analyte, this region is found at about 15 - 45 bar and at about 10 to 30%B in Figs. 1 to 3. Thereby, regions of too high k'(P) are left out since these are of lesser practical importance. An additional consideration is appropriate for Fig. 2 because the condition T_r = const. = 1.1 requires a specific temperature program to be superimposed on the composition program. Implicitly, of course, it is presupposed for all components of an analyte that basically similar dependencies of N on %B and p at a given temperature and linear velocity exist. With respect to programs of temperature or linear velocity, additional three-dimensional plots at different constant temperatures or different linear velocities are required which show more fully the changes occurring when these physical parameters of the mobile phase are being changed.

While the three-dimensional plots of Figs. 1 to 3 are suitable to provide an overview about the behavior of k'(P) and N in dependence of %B and p and, by comparison between plots, also to a small extent about the dependence on temperature and linear velocity, the three-dimensional plots are perspectivic and therefore less suited to read numerical values of k'(P) and N from them. For the latter purpose two-dimensional graphs are preferable, which graphs are given in Figs. 4 to 9. Figs. 4 and 5 correspond to Fig. 1 in as much as they are derived again from isocratic-isobaric-isothermal chromatographic runs at a volume flow rate of 1 ml/min. They represent data sets different from Fig. 1, however. In plotting, either p or %B may be chosen as the independent variable, the other being kept constant. In Fig. 4, the p is the independent variable, and according to four constant %B values, the figure consists of 4 individual graphs (a) to (d). Peaks with maxima of N are found whose higher parts of the peaks are located at about k'(P) = 4 to 9 at all 1,4-dioxane contents. The non-linear scales of the linear k'(P)-axis demonstrate what retention times, t_r, pressures, p, and average linear velocities, u, belong to specific k'(P) values. Thus one can see, for instance, that the peak maxima of N at different %B are located in the range of 33 to 43 bar. The pressure is, in fact, the experimental variable whose changes at constant volume feed rate result in the concomitant changes of k'(P), t_r, and u. Besides N, the effective

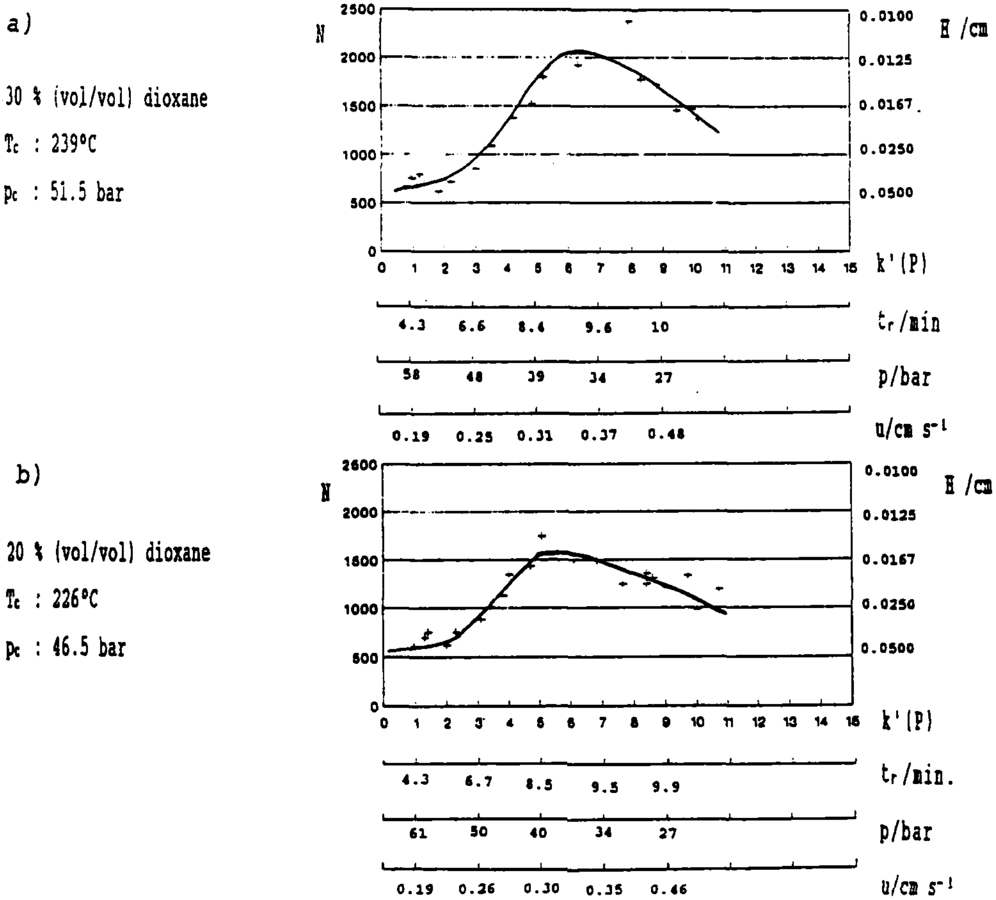


Figure 4: Pyrene effective plate number N in dependence of capacity ratio k'(P). Mixed mobile phase of pentane and 0%, 10%, 20%, 30% (vol/vol) 1,4-dioxane, temperature 260°C, liquid volume flow rate 1ml/min. Additional x-axes: retention times, t_r/min., column outlet pressure, p/bar, and average linear velocity, u/cm s^{-1}. Additional y-axes: effective plate height, H/cm. Otherwise as for Fig. 1.

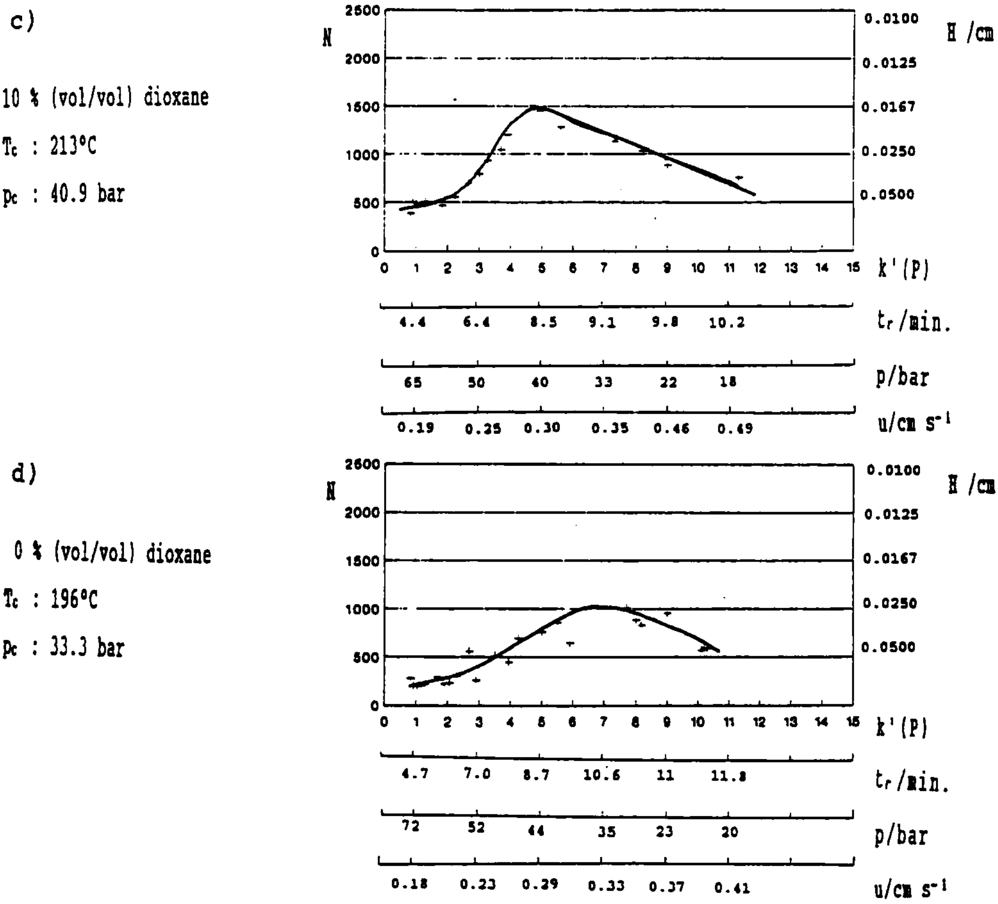


Figure 4 (continued)

plate height, H, is plotted on an additional, non-linear scale. Although H and u are plotted on the x- and y- axis, this is not a van Deemter like graph because p changes. In fact, the decreasing p is the cause of the increase in u.

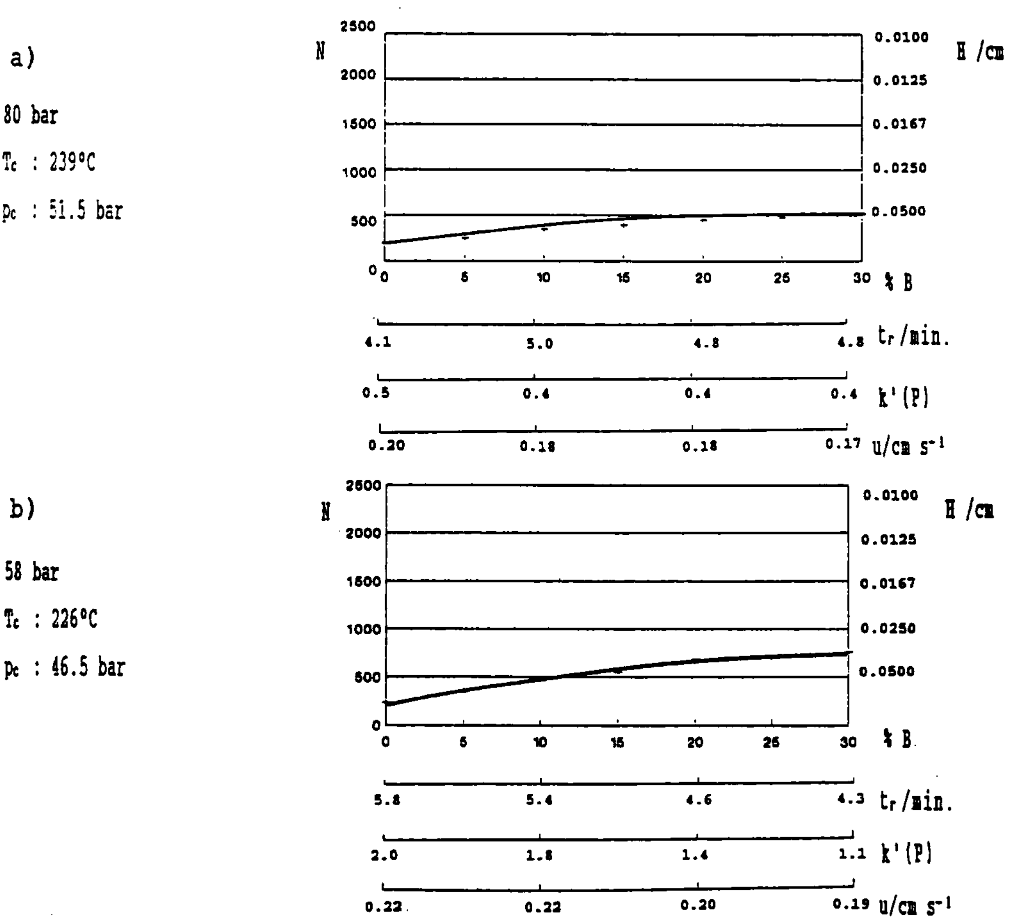


Figure 5: Pyrene effective plate numbers N in dependence of mobile phase modifier, %B, at pressures of 15, 37, 58, and 80 bar, temperature T = 260°C, liquid volume flow rate 1 ml min^{-1}. Additional x-axes: retention times, t_r/min, capacity ratio, k'(P), and average linear velocity, u/cm s^{-1} Additional y-axes: effective plate height, H/cm. Otherwise as for Fig. 1.

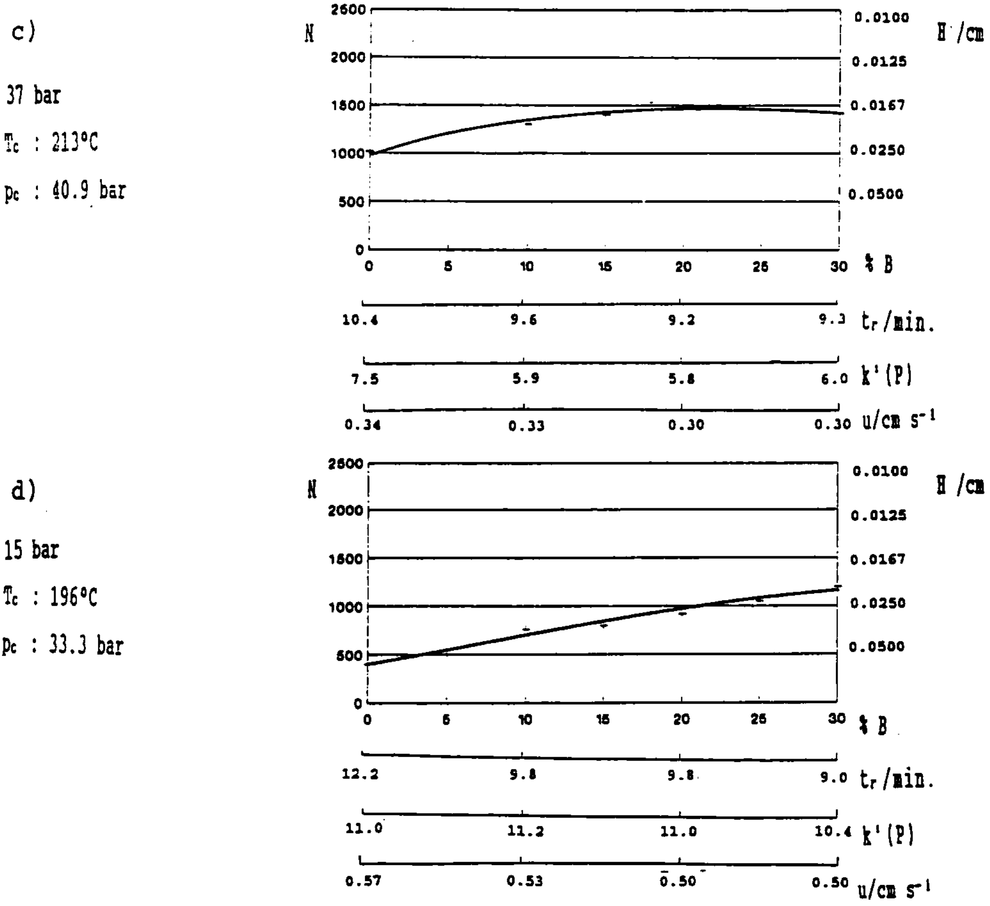


Figure 5 (continued)

In Fig. 5 the %B is the independent variable and p is kept constant at 4 different values resulting again in 4 individual graphs(a) to (d). According to Fig. 5, working at medium pressure leads to higher N, while at the lowest pressure at 15 bar the N appears to drop again. Also, up to a certain level a higher

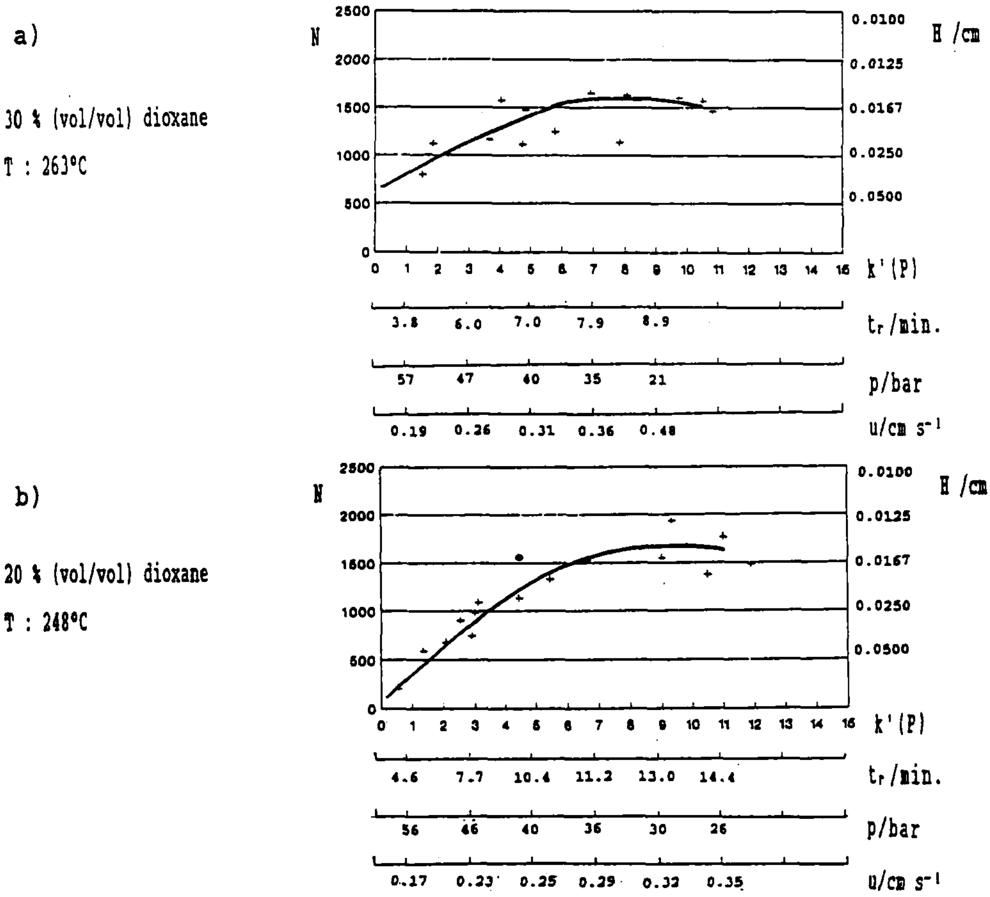
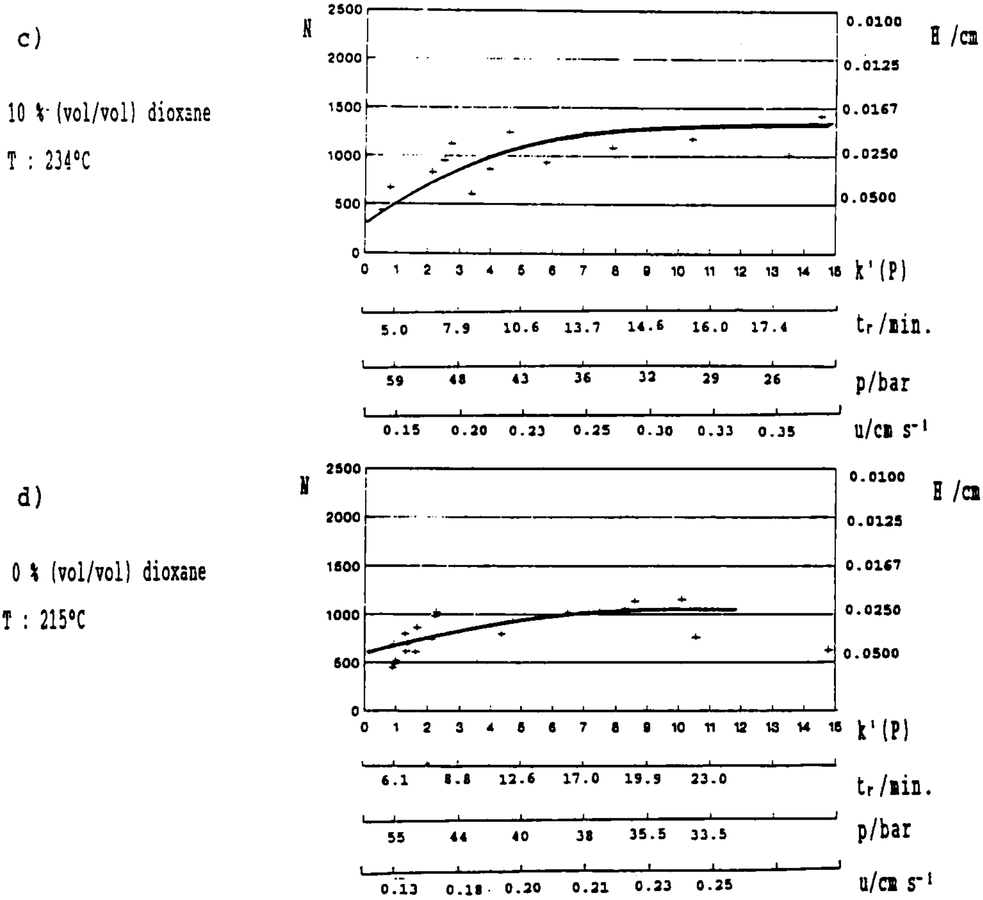


Figure 6: Pyrene effective plate number N in dependence of capacity ratio k′(P) at a reduced temperature of T_r = 1.1. Other conditions see Figure 4.


c)

10 % (vol/vol) dioxane

T : 234°C

d)

0 % (vol/vol) dioxane

T : 215°C

Figure 6 (continued)

1,4-dioxane content improves N. Because u extends only over a small range in this type of plot, nothing can be said about the influence of the average linear velocity, However, according to the small change in the linear velocity, the volume contraction of the gaseous mobile phase is only reasonably small when

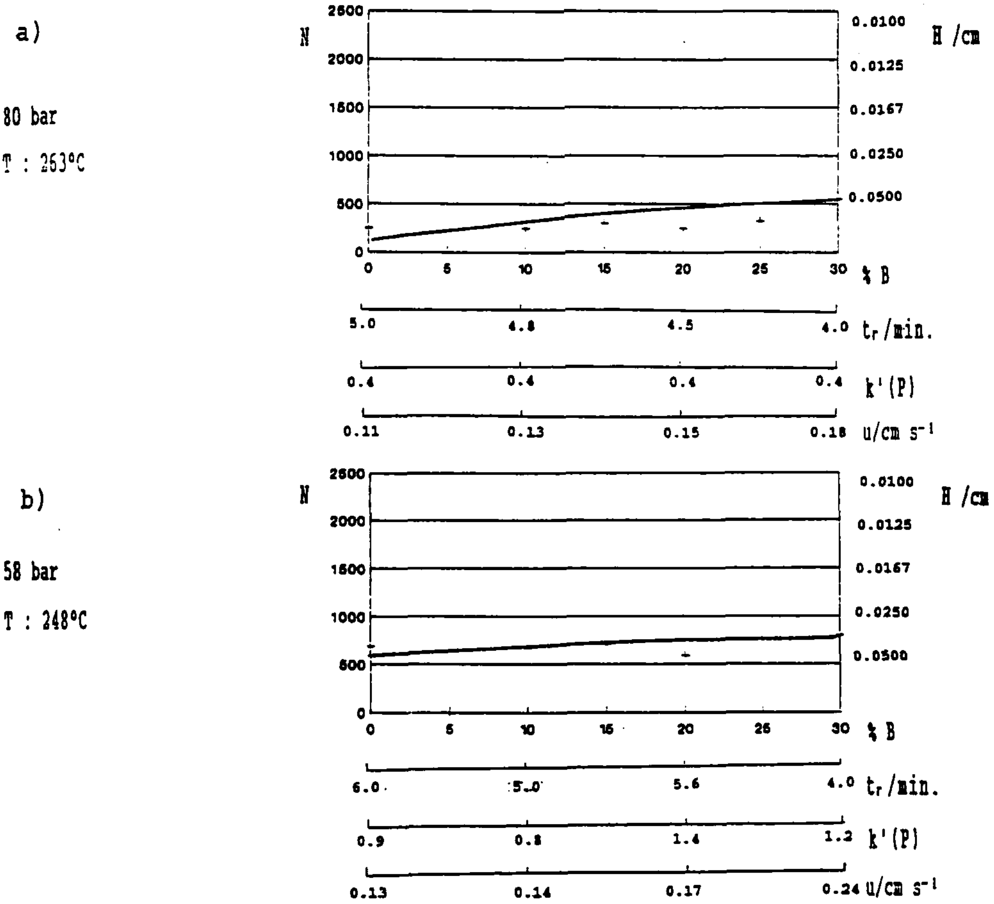


Figure 7: Pyrene effective plate number N in dependence of mobile phase modifier, % B, at a reduced temperature of T_r = 1.1. Other conditions see Figure 5.

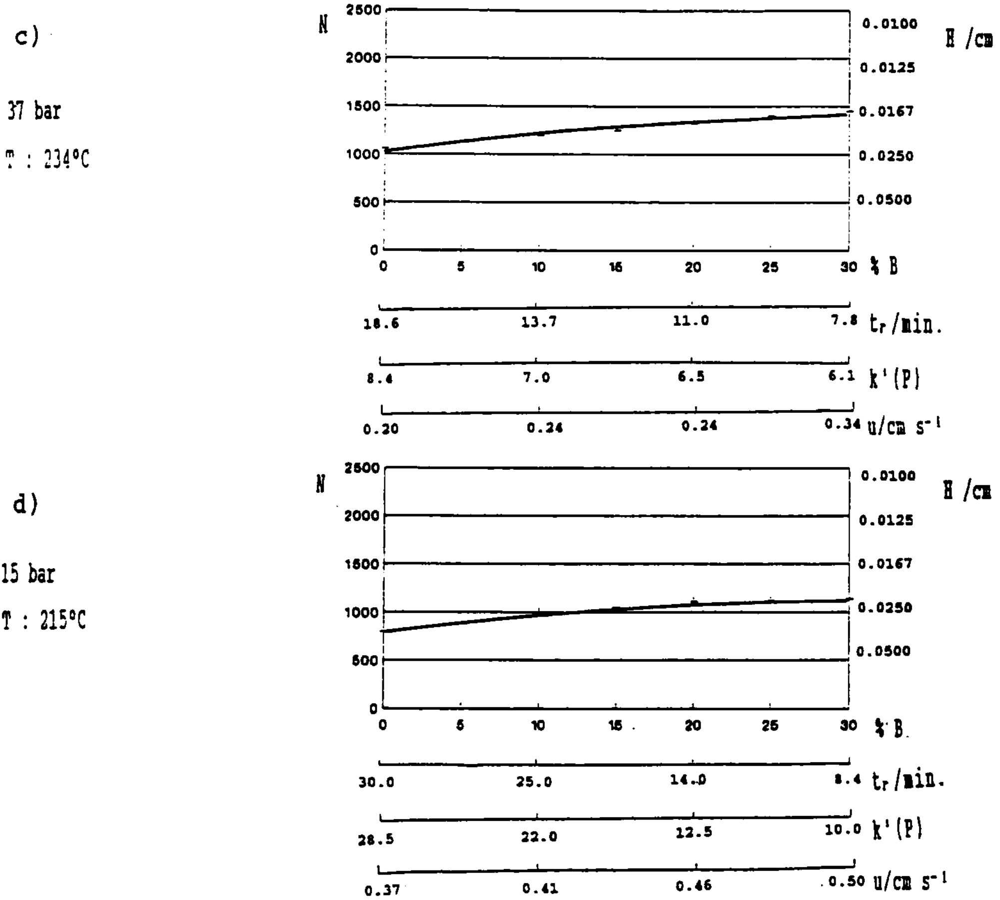


Figure 7 (continued)

substituting part of the pentane by an equal liquid volume of 1,4-dioxane.

In Figs. 6 and 7 data are shown which have been obtained at $T_r = 1.1$ instead of T = 260°C, other conditions being the same as for Figs. 4 and 5,

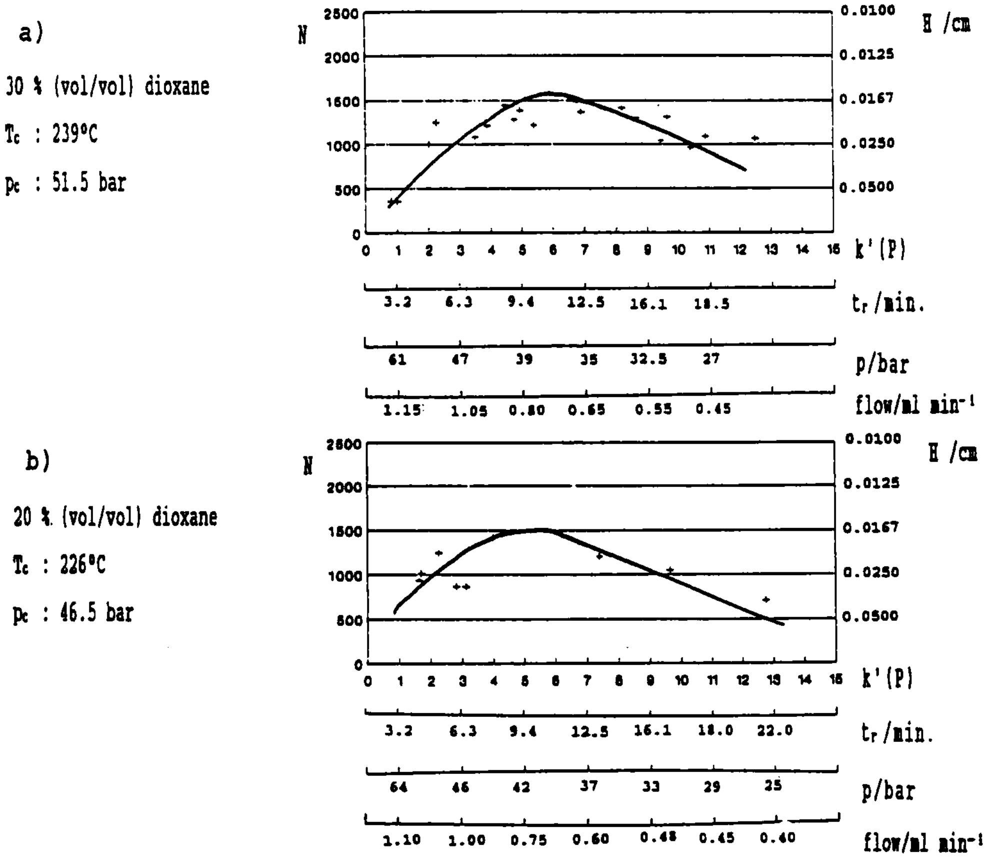


Figure 8: Pyrene effective plate number N in dependence of capacity ratio k'(P), at a temperature of T = 260°C and a linear velocity of u = 0.26 cm sec^{-1}. Additional x-axes: t_r/min, p/bar, and liquid volume flow rate, flow/ml min^{-1}.
Other conditions see Figure 4.

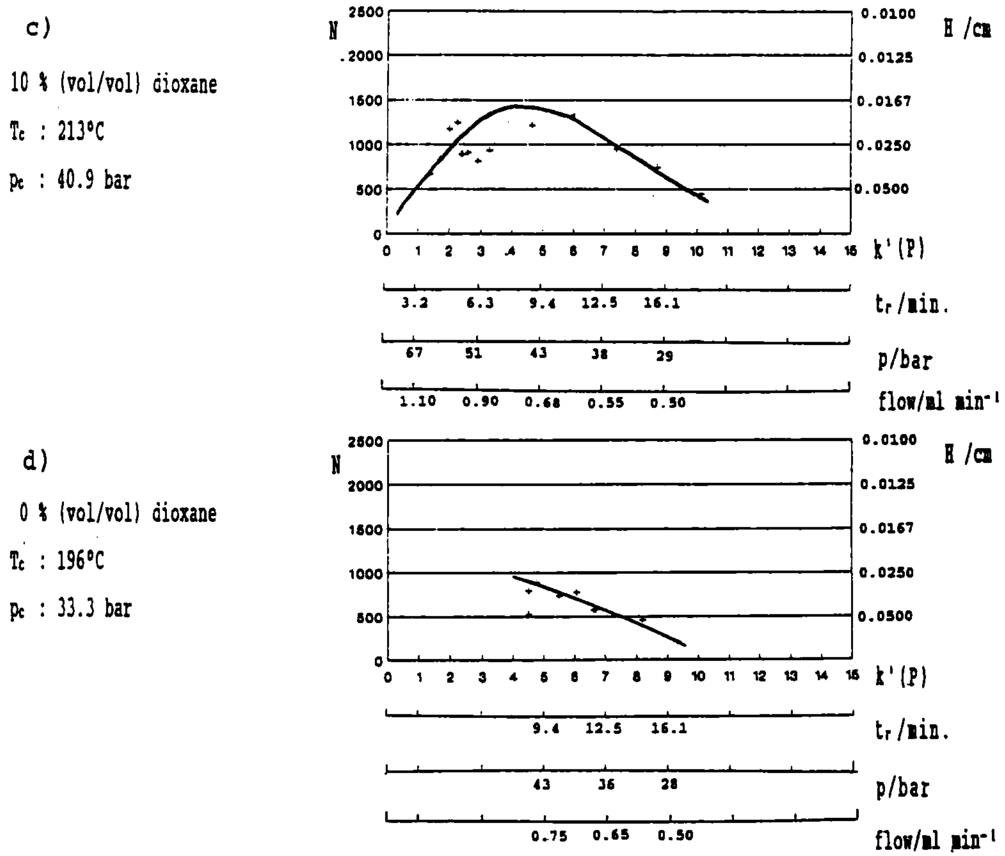


Figure 8 (continued)

respectively. In Fig. 6, p is again the factual independent variable, whereas in Fig. 7 it is %B. Both figures correspond to the three-dimensional plot of Fig. 2. Fig. 6 again shows a rise in N, going up with decreasing p and increasing k'(P) either to a maximum or to a plateau. The maxima or the start of the

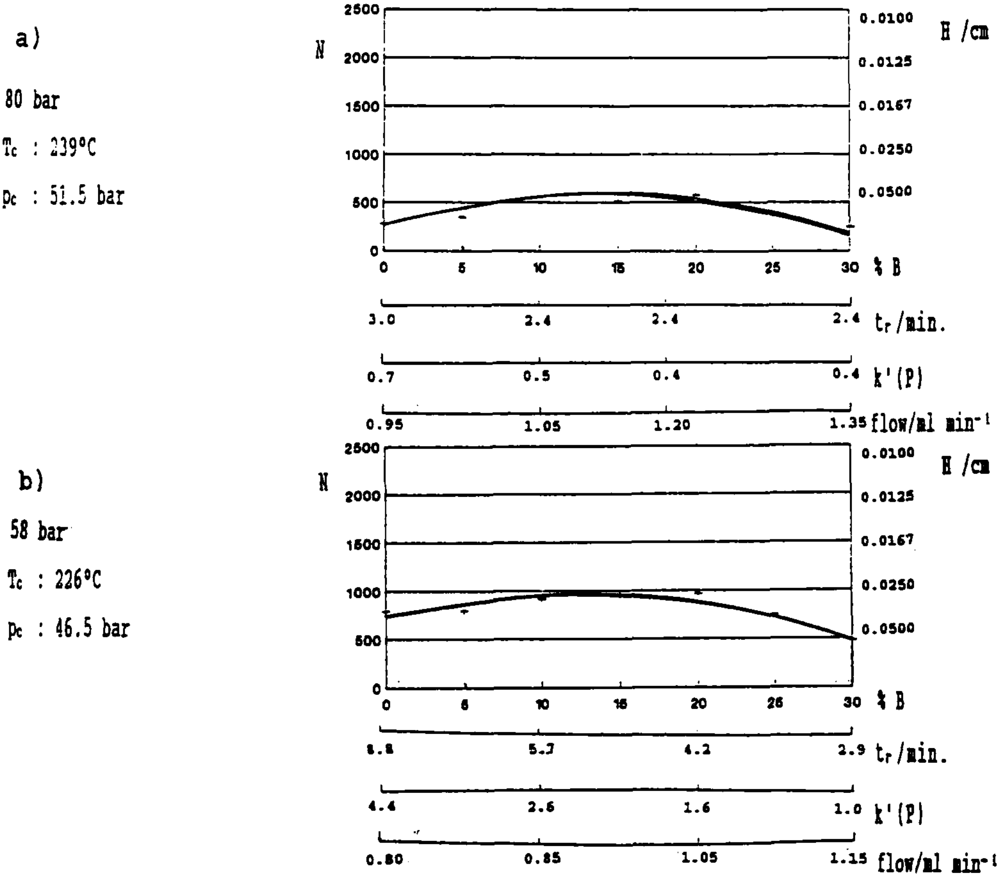


Figure 9: Pyrene effective plate number N in dependence of mobile phase modifier, %B, temperature T = 260°C, average linear velocity u = 0.26/cm sec^{-1}. Additional x-axes: t_r/min, k'(P), and liquid volume feed rate, flow/ml min^{-1}.
Other conditions see Figure 5.

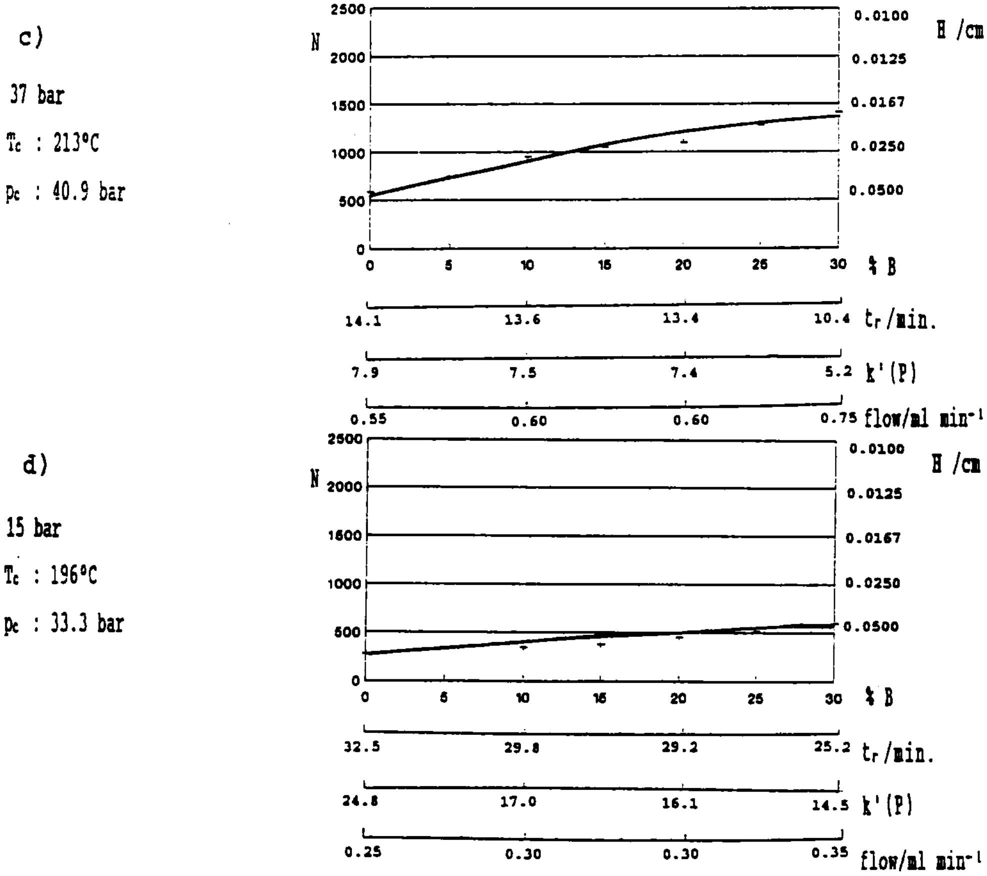


Figure 9 (continued)

plateaus are found at k′(P) = 7 - 9 and rise to higher levels of N with higher %B. Fig. 7 shows again, and similarly to Fig. 5, the tendency of obtaining higher N at higher %B. Because the variation of u with a change in %B in Fig. 7 is considerable, and because the substitution of pentane by an equal volume of 1,4-

dioxane alone leads only to smaller changes in u which are, moreover, in the opposite direction (cf. Fig. 5), the observed increase of u with increasing %B should be due to the rise in temperature. This rise takes place according to the condition of $T_r = 1.1$.

Instead of keeping the volume feed rate of the mixed mobile phase constant at 1 ml/min. as for Figs. 4 - 7, the linear velocity is kept constant at u = 0.26 cm/sec. for Figs. 8 and 9 at T = 260°C. The independent variables are p and %B, respectively, as before, and both figures correspond to the three-dimensional plot of Fig. 3. A linear velocity of u = 0.26 cm/sec. corresponds to the standard 1 ml/min volume flow rate at 260°C when $p \approx 44 - 48$ at 0 to 30 %B. A higher pressure leads to a smaller and a lower pressure to a higher linear velocity at 1 ml/min volume flow rate. The chosen value of 0.26 cm/sec. is on the high side of the pressure range which yields high N values (cf. Fig. 4). Fig. 8 shows that, starting on the high pressure side and then reducing the pressure, a maximum of N is found at medium pressures. In Fig. 4, a maximum in N is found also when reducing the pressure, but in contrast to Fig. 8, the u increases in Fig. 4, while it remains the same in Fig. 8. Because the N rises to comparable levels in

Fig. 4 and Fig. 8, the velocity increase in Fig. 4 does not appear to be of a disadvantage. In Fig. 9, the intermediate pressures of 37 and 58 bar exhibit the highest N, which confirms the result of Fig. 8. Turning to the kind of change in N, when the 1,4-dioxane content is increased, one finds maxima at higher pressures (80 and 58 bar) and plateaus at lower pressure (37 and 15 bar). Figs. 8 and 9 point to the possibility to estimate the interdiffusion coefficient, D_{12}, in dependence of pressure or composition, if a van Deemter equation is employed which contains only k', u, the particle diameter, d_p, and other known constants.

The main conclusions which may be drawn for the present chromatographic system from the data of Figs. 1 to 9, are that a higher content of 1,4-dioxane in the pentane/1,4-dioxane mobile phase appears to be generally beneficial for obtaining a high N at retention times of up to about 13 min. With respect to pressure, it appears that a medium pressure, i.e. between about 33 and 43 bar, over the whole range of dioxane contents, leads to maxima of N at retention times between 7 and 13 minutes. As to temperature, it is found that a series of chromatographic runs carried out at a constant temperature T_1 on one hand and at a constant reduced temperature $T_r = T_2/T_c$ on the other, whereby $T_1 = T_2$ (max) and where composition and pressure vary, may give superior N in the second case of a constant reduced temperature. It remains to be seen whether analogous observations, which are similar in a general way, may also be made for such physical conditions of composition, pressure, temperature, and linear velocity which are far from the ranges of physical conditions used here. This qualification applies even more to other types of mobile and stationary phases and to other analytes.

ACKNOWLEDGEMENT

Financial support by the Arbeitsgemeinschaft Industrieller Forschungsvereinigungen (AIF) is gratefully acknowledged.

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