

Isocratic networks in supercritical fluid chromatography

III^a. Dependence of capacity factor, selectivity and resolution on temperature, pressure, density and free volume of pentane as shown in multi-dimensional plots

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SUMMARY

A plotting program for drawing three-dimensional graphs in supercritical fluid chromatography was employed which allows a fourth variable to be represented by colours. The graphs contain two physical parameters of the mobile phase pentane, e.g., temperature and pressure or temperature and density, and, on the same plot, one or two chromatographic parameters, e.g., capacity factor, selectivity or resolution of polycyclic aromatic hydrocarbons as the analyte. The plotting program is useful for showing interrelations between physical and chromatographic parameters on the one hand, and between chromatographic parameters themselves on the other.

INTRODUCTION

In preceding papers²⁻⁴, we have reported on the dependence of chromatographic parameters, such as retention, selectivity, plate number and resolution, on the physical parameters temperature and pressure for pure, one-component mobile phases on bare, unmodified silica. All these studies were performed with an analyte test mixture composed of the polycyclic aromatic hydrocarbons naphthalene, anthracene, pyrene and chrysene. In some instances, the dependence of chromatographic parameters on the density and free volume of the mobile phase was also studied^{6,9,12,13}. In general, the behaviours of different mobile phases such as alkanes, ethers, carbon dioxide, nitrous oxide and trifluoromethane were found to be very similar.

For pentane as the mobile phase, the network of available data was dense enough to allow three-dimensional plotting^{5,7}. These three-dimensional plots yield an overall impression of the dependence of capacity factors⁵, selectivity⁷, plate number⁷ and resolution⁷ on both pressure and temperature over the pressure and temperature

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ranges studied. Hence, they can guide the chromatographer towards the optimization of a separation, as long as the pressure-temperature dependence of the chromatographic behaviour of the sample and of the column under investigation resembles that of the test mixture and the test column.

In this paper we report the use of a computer program that is capable not only of three-dimensional regression of data and of forming a regular grid from irregularly placed experimental data, in addition to smoothing of the grid, but also permits colour shading of the three-dimensional contour¹⁴. The last feature can be used for facilitating the approximate reading of data from the plots, or for adding additional information by superimposing data of a second z-variable in the form of coloured bands on the three-dimensional contour.

EXPERIMENTAL

The supercritical fluid chromatographic (SFC) apparatus, including the UV detector, the columns and the chemicals, have been described previously⁵. The mobile phase was pentane, the stationary phase unbonded LiChrosorb Si 60 (10 μm) and the analyte mixture consisted of naphthalene, anthracene, pyrene and chrysene (PAH-1). Heptane, which gave a small but definite response in the UV trace, was used as an inert compound for measuring dead times. The liquid volume flow-rate of the pumps at ambient temperature was maintained at 1 ml/min, and confirmed by measurement at the outlet of the apparatus.

The capacity factor of chrysene, $k'(C)$, and the selectivity between pyrene and chrysene, $\alpha(PC)$, were calculated as usual by means of the equations

$$k'(C) = [t_r(C) - t_0]/t_0 \quad (1)$$

$$\alpha(PC) = [t_r(C) - t_0]/[t_r(P) - t_0] \quad (2)$$

where $t_r(C)$ and $t_r(P)$ are the retention times of chrysene and pyrene, respectively, and t_0 is the dead time. As a measure of separation quality, the average resolution $R_m^{*1,5}$ for the three peak pairs of PAH-1 was chosen. The resolution values for the individual pairs were calculated as described previously⁵.

The free volume, f_r , is defined relative to the volume required for the close packing of the mobile-phase molecules in the crystalline state, i.e., as a reduced parameter according to the equation

$$f_r = (V_{p,T} - V_0)/(V_0) = (V_{p,T}/V_0) - 1 = \rho_0/\rho_{p,T} - 1 \quad (3)$$

where V_0 is the specific volume of the crystalline state at the melting temperature, $V_{p,T}$ the specific volume of the gaseous mobile phase at the pressure p and the temperature T of the chromatographic experiment and ρ_0 and $\rho_{p,T}$ are the corresponding densities.

The regression and plotting program (Unimap; Uniras European Software Contractors, Düsseldorf, F.R.G. and Lyngby, Denmark) used in this work offers a number of possibilities. Different interpolation methods can be selected to transform a set of chromatographic data which is not regularly distributed with respect to the physical parameters, e.g., temperature and pressure, to a set which is regularly

distributed, that is, which forms a regular network or grid. Here a bilinear interpolation was used to form the regular network. In all cases shown, a grid was chosen which, first, adheres as closely as possible to the experimental data points available, and second exhibited nodes (crossing points) not more numerous than the number of data points. Additionally, a low smoothing level was selected which was based on quadratic interpolation. The smoothing consisted of improving the estimate of the bilinear interpolation by computing gradients at the points and using quadratic interpolation, and finally by refining the values by distance weighting methods. For a given set of original data, different interpolation methods and different smoothing levels were compared with each other and with the original data. It was ascertained that the graph represented the original untreated data within a reasonable range of experimental error. The three-dimensional plots, for which any viewpoint, i.e., perspective, can be selected, show the z-parameter as a three-dimensional contour in space, on which coloured contour bands can be drawn to allow an easier reading of the z-parameter. Alternatively, it is possible to present two different z-parameters simultaneously. In this instance, the three-dimensional contour represents one z-parameter, leaving the information conveyed by the coloured bands for the second z-parameter.

For a pretreatment of the irregularly distributed original data points", to adapt them beforehand to the regular interpolation grid, it was of advantage first to plot these experimental data, e.g., k' versus p and k' versus T . From these curves, the data were read which directly fitted the physical parameters in the selected regular grid. For the density-based plots, 'the experimental p - T pairs were converted to densities using data tables¹⁵.

RESULTS AND DISCUSSION

Chromatographic parameters such as retention, selectivity and resolution have been shown to depend strongly on both temperature and pressure. This is found again in three-dimensional plots for the simultaneous dependence on temperature and pressure of the capacity ratio of chrysene (not shown), of the selectivity between pyrene and chrysene (Fig. 1) and of the average resolution (not shown), all of them calculated from the chromatograms for the PAH-1 test mixture. For each of these three chromatographic variables, a distinct maximum is seen above the critical temperature of the mobile phase [T_c (pentane) = 196.5°C], whereas a minimum is formed below T_c . Although the height of the maximum of k' (C) is greatly reduced if the pressure is above p_c [p_c (pentane) = 33.7 bar], the corresponding maximum for the selectivity between pyrene and chrysene, α (PC), is still considerable. At temperatures below T_c , α (PC) rises strongly again, even at pressures above p_c . This is in contrast to the selectivity between naphthalene and anthracene or that between anthracene and pyrene', but it corresponds to results obtained with enantiomer separations^{16,17}, where below T_c the selectivity was found to increase with decreasing temperature of the liquid mobile phase. Nevertheless, Fig. 1 shows that at low pressures the highest values for α (PC) occurs in the region above T_c .

Fig. 1 demonstrates that the three-dimensional graphs are useful not only for gaining a qualitative overview of the behaviour of a chromatographic variable over a given pressure-temperature range, but also for obtaining easier readings from the

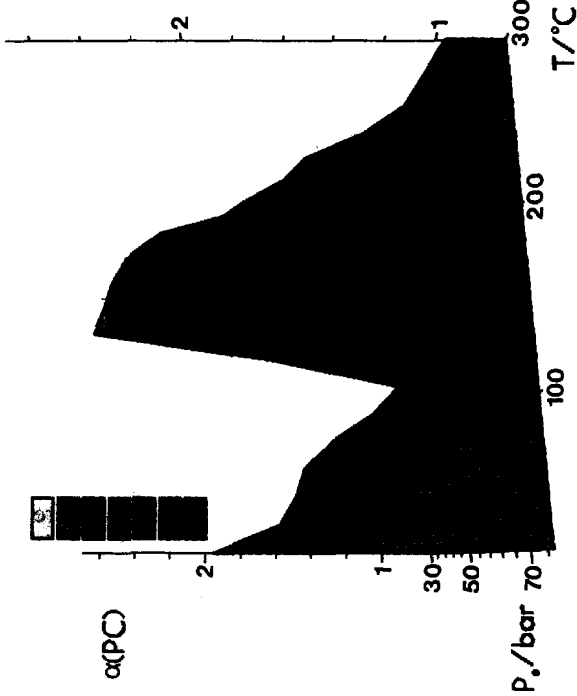
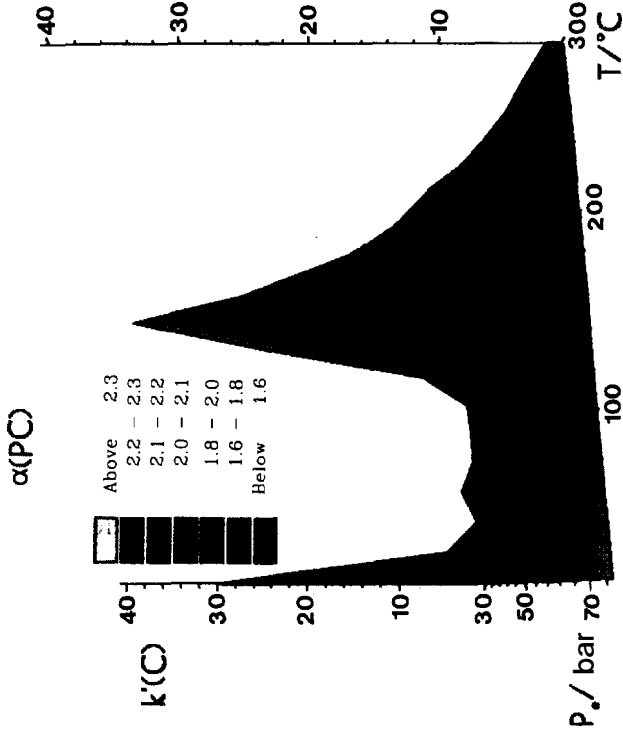


Fig. 1. Three-dimensional network plot for the selectivity between pyrene and chrysene, $\alpha(\text{PC})$, versus column exit pressure, P_e , and temperature, T . The $\alpha(\text{PC})$ are shown both as one of the three axes (z -axis) and as colour bands (key to colours as given), the latter facilitating the reading of numerical values from the plots.
 Fig. 2. Three-dimensional network plot for the capacity factor of chrysene, $c'(C)$, versus P_e and T . The corresponding selectivity between pyrene and chrysene, $\alpha(\text{PC})$, is shown as colour bands.

graphs by means of the colour bands. Nevertheless, the main benefit of such three-dimensional graphs is not to allow readings of data, but rather to show instantly general, qualitative features about the interrelations between physical and chromatographic parameters. It should be emphasized, however, that the chromatographic data will depend not only on the physical conditions of the mobile phase such as temperature and pressure, but also on the analyte and on the stationary phase material, although the general behaviour may often resemble fairly closely that shown here.

The possibility of plotting two different z-variables simultaneously is demonstrated by Fig. 2. One z-variable is displayed as the three-dimensional contour, while the other is superimposed on that contour by coloured bands. Thus, interrelations between two chromatographic variables can be made directly visible. Fig. 2 shows such an interrelation between $k'(C)$ and $\alpha(PC)$. Similarities and differences of the behaviour of these two variables as functions of p and T become obvious from this diagram. There are maximum values for both $k'(C)$ and $\alpha(PC)$ above T_c . These maxima become lower and are shifted to higher temperatures with increasing pressure. A broad $\alpha(PC)$ maximum compared with the sharp maximum for $k'(C)$ and differences in behaviour at high pressures and low temperatures can also be seen.

The dependence of retention on density and temperature is shown in Fig. 3, on reduced density and reduced temperature in Fig. 4 and on free volume and reduced temperature in Fig. 5, all in the temperature region above T_c . The retention k' decreases monotonously with increasing temperature (at constant density), with increasing density (at constant temperature) and with increasing reduced density. The free volume shows a basically similar behaviour, although, as expected, in the opposite direction of the density or the reduced density. The use of the free volume instead of the density has the advantage, however, that different mobile phases can be compared directly. In comparison with the reduced density, the free volume gives a more realistic measure of the space between molecules. Together with the structure, configuration and conformation of the mobile-phase molecules, this factor determines the chromatographic properties of the mobile phase at a given temperature. High free volumes give rise to decreasing solvent strength of the mobile phase. Higher temperatures lead to the opposite effect. Thus, $k'(C)$ rises with increasing free volume (at constant temperature) and with decreasing temperature (at constant free volume). The qualitative information in these three plots is similar, because the physical parameters are related to each other.

The dependences of selectivity and resolution on density and temperature are illustrated in Figs. 6 and 7, respectively. The selectivity between pyrene and chrysene shows similar features to the retention of chrysene (Fig. 3). This is reasonable, because these compounds closely resemble each other in chemical structure and molecular weight. Hence the influence of varying the density of the mobile phase and the temperature on the k' values of the two compounds is expected to be similar. The resolution, on the other hand, shows a maximum with respect to temperature. Whereas retention and selectivity are influenced by thermodynamics alone, resolution is also influenced by kinetic effects. These kinetic effects, which determine band broadening, differ in their dependence on pressure and temperature from the thermodynamics effects. Temperature-dependent maxima of resolution at constant density have also been observed previously using propane⁷ and carbon dioxide¹³ as mobile phases.

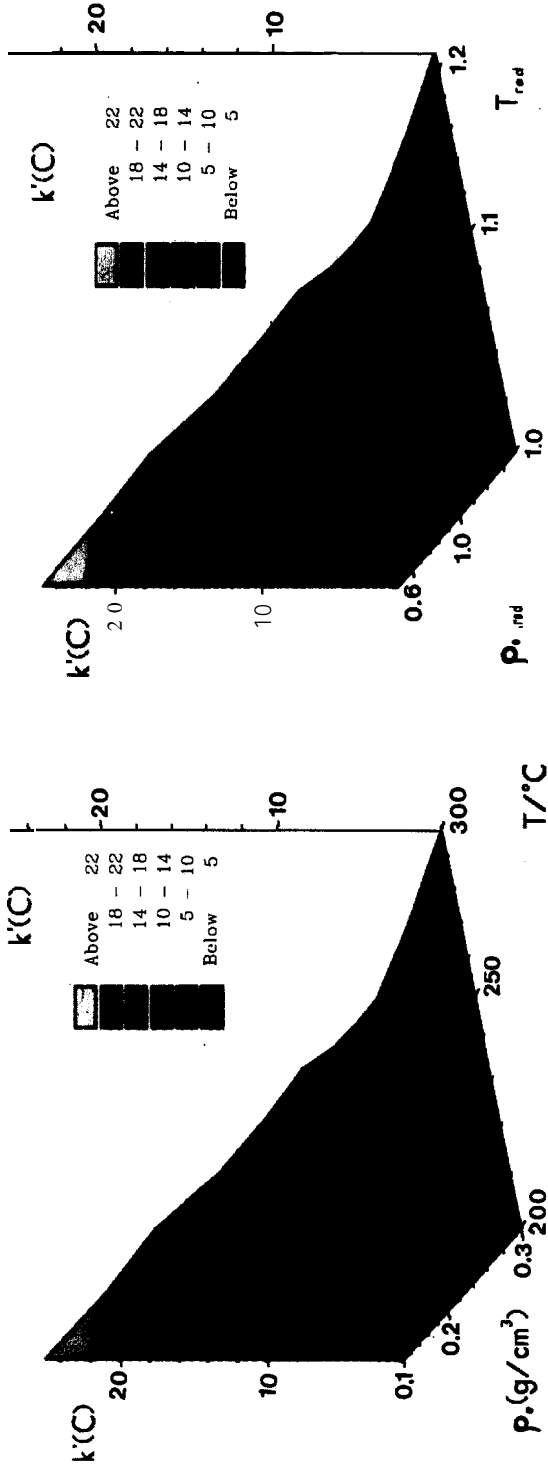


Fig. 3. Three-dimensional network plot for $k'(C)$ versus the density at the column exit, ρ_s , and temperature, T . The $k'(C)$ are shown both as one of the axes and as colour bands.

Fig. 4. Plot of $k'(C)$ versus the reduced density at the column exit, $\rho_{s,red}$, and the reduced temperature, T_{red} . The $k'(C)$ are seen both as one of the axes and as colour bands.

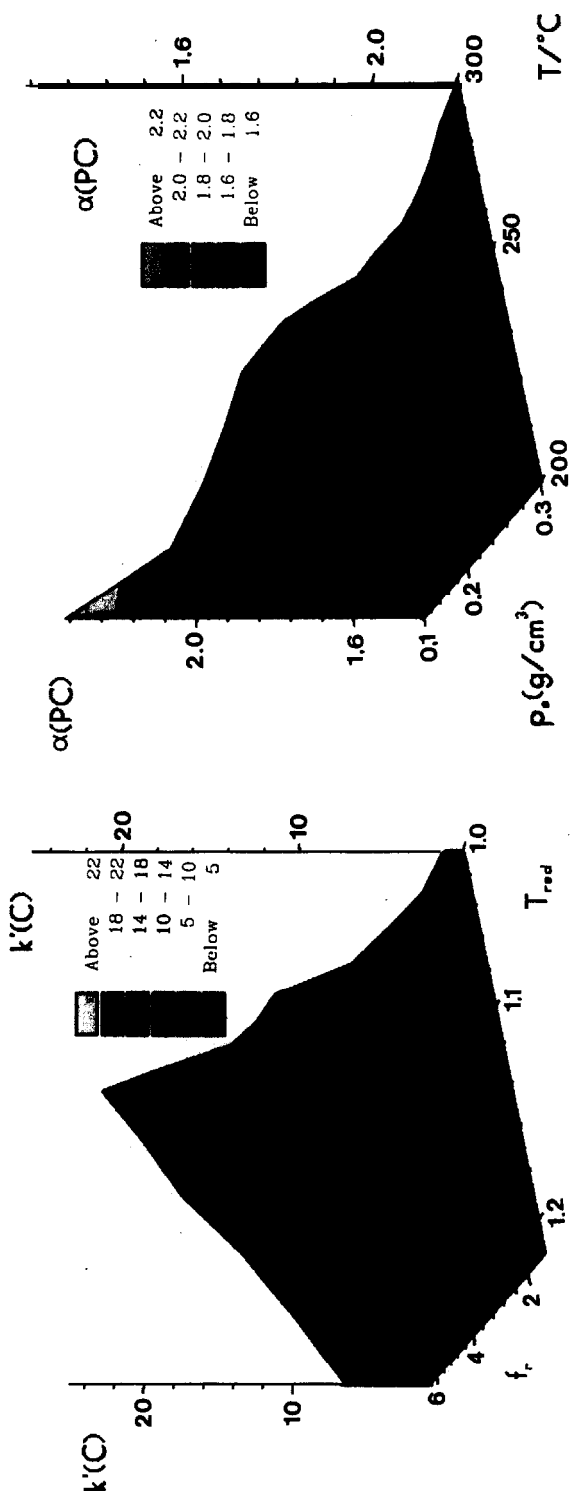


Fig. 5. Plot of $k'(C)$ versus the reduced free volume, f_r , and the reduced temperature, T_{red} . The $k'(C)$ are given both as one of the axes and as colour bands.
 Fig. 6. Plot of $\alpha(PC)$ versus p_e and T . The $\alpha(PC)$ are shown both as one of the axes and as colour bands.

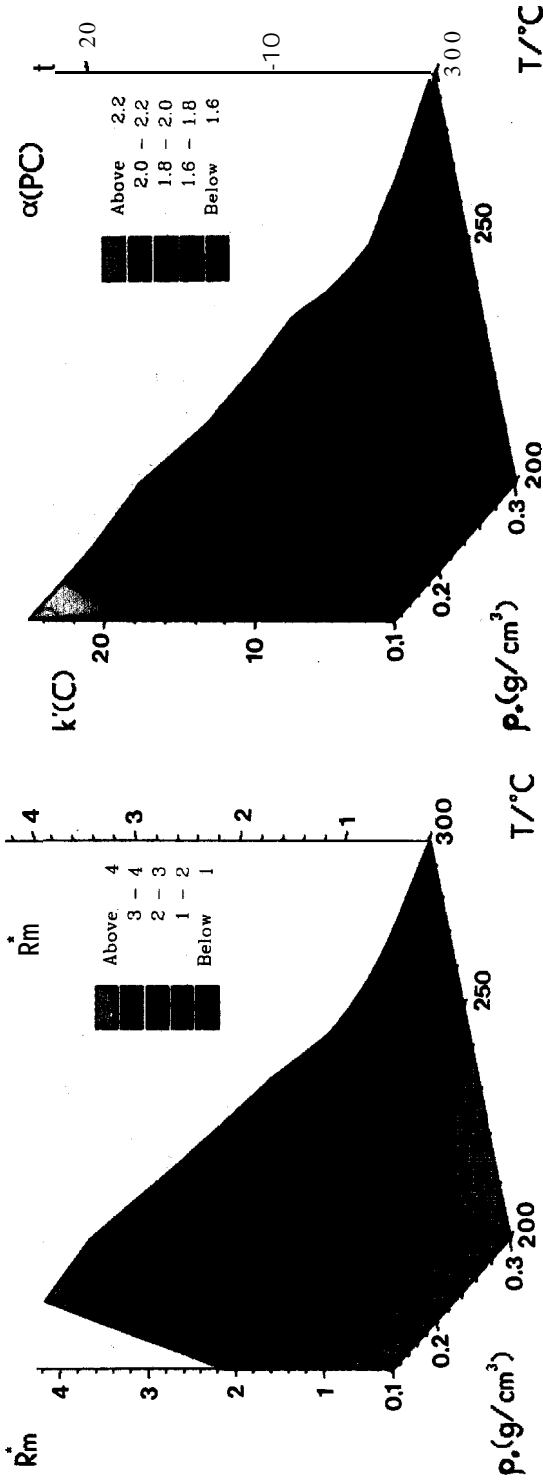


Fig. 7. Plot of R_m^* versus ρ_e and T . The R_m^* are seen both as one of the axes and as colour bands.

Fig. 8. Three-dimensional network plot for $k'(C)$ versus ρ_e and T . The corresponding $a(PC)$ are given as colour bands.

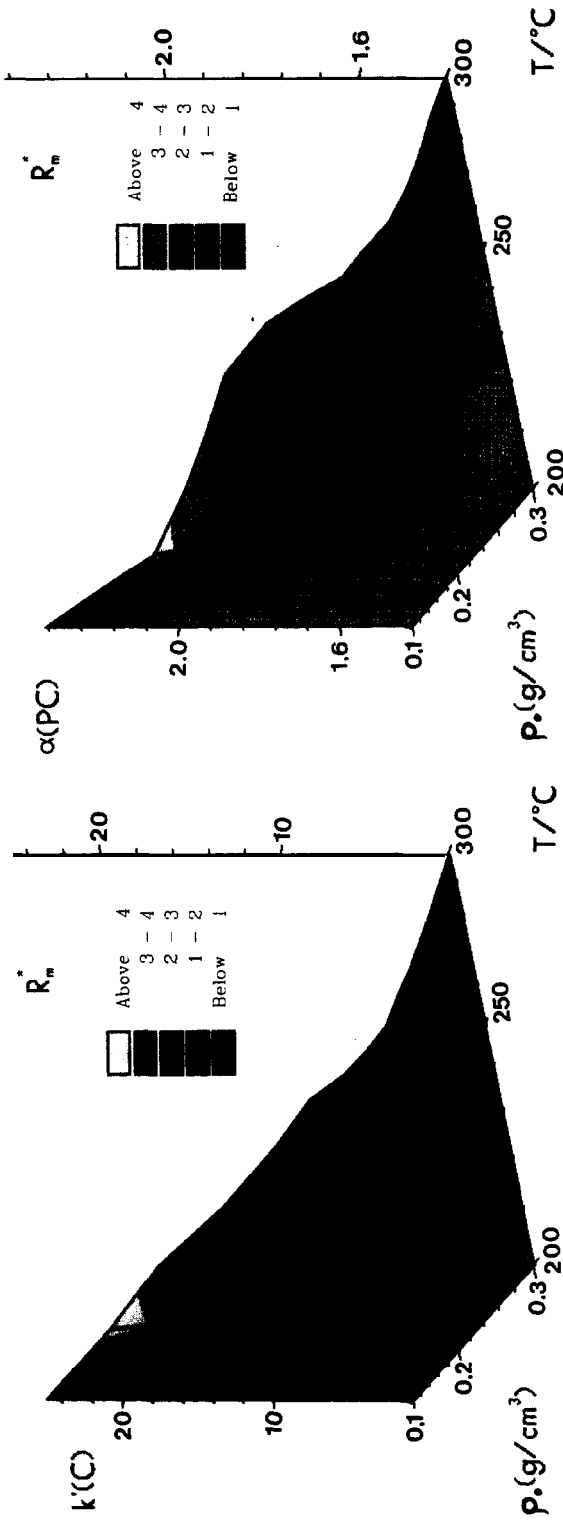


Fig. 9. Plot of $k'(C)$ versus ρ_s and T . The corresponding R_m^* are shown as colour bands.

Fig. 10. Plot of $\alpha(PC)$ versus ρ_s and T . The corresponding R_m^* are seen as colour bands.

'The similarities between the density-temperature dependence of retention and selectivity are clearly evident from Fig. 8, which shows $k'(C)$ as the three-dimensional contour and $\alpha(PC)$ as coloured bands. Also directly visible are the strong differences in behaviour between retention and resolution (Fig. 9) and between selectivity and resolution (Fig. 10), showing R_m^* as the coloured bands. The coloured bands are shaped as irregular half-circles and, therefore, do not behave like contour lines with respect to height, as approximated, for instance, in Fig. 8.

CONCLUSION

The applications of the plot program presented here demonstrate that this and similar programs can be of value for generating informative three-dimensional plots. Specifically, the possibility of presenting two chromatographic variables in one three-dimensional diagram can be used for demonstrating interrelations between different chromatographic variables.

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