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CHROMATOGRAPHY WITH SUB- AND SUPERCRITICAL ELUENTS

THE INFLUENCE OF TEMPERATURE, PRESSURE AND FLOW-RATE ON THE BEHAVIOUR OF DIMETHYL AND DIETHYL ETHER

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SUMMARY

In supercritical fluid chromatography, using dimethyl ether and diethyl ether as eluents, distinct maxima occur in capacity ratios and resolutions at temperatures somewhat above critical when separating polynuclear aromatic hydrocarbons, oligostyrenes or di-alkyl phthalates. They are caused by a decrease in eluent density and the opposing effect of an increase in the substrate vapour pressure. Dimethyl ether allows higher resolutions than diethyl ether. Higher pressures and flow-rates lead to faster analyses but poorer resolution.

INTRODUCTION

Gas chromatography is a standard method to separate substances of low molecular weight. However, substrates with higher molecular weights cannot be transported by gases of low density as their vapour pressure is not high enough. Increasing the pressure increases the dissolving power of the mobile phase to such an extent that heavier compounds may be dissolved. The saturation concentration of a poorly volatile substrate in a mobile phase increases by several orders of magnitude when the pressure is raised to the critical pressure and higher[1]. Thus, styrene oligomers and polymers can be separated by supercritical fluid chromatography (SFC)[2-6]. Substances of biological importance like peptides and ubiquinones have also been separated in this way[7-9].

The retention behaviour of substances in SFC depends on a variety of parameters, of which temperature and pressure — and thereby density —, flow-rate and eluent composition can easily be varied. When using *n*-alkanes as mobile phases, some authors have reported a distinct maximum in a plot of capacity ratios[10-14], k', and resolution[12] *versus* temperature at constant pressure. Klesper[15] concluded from separations of oligostyrenes that "for best resolution the operating temperature should well exceed the critical temperature". Upon raising the pressure at constant temperature, decreases in k' values[14,16] and log k' values[10,11,17-19] have been observed.

In this work, we report on the dependence of retention and resolution on temperature, pressure and flow-rate, using dimethyl ether and diethyl ether as eluents and polynuclear hydrocarbons, oligostyrenes or di-alkyl phthalates as substrates.

EXPERIMENTAL

The apparatus used for the investigation in the sub- and supercritical gaseous state was a modified high-performance liquid chromatographic (HPLC) instrument (Model 1084 B, Hewlett-Packard). The principles of its construction were described previously[20]. It was equipped with additional manometers and valves to measure and regulate the column inlet and outlet pressures as well as the pressure in the UV detector. The detector cell was reconstructed from stainless steel using a Hewlett-Packard conversion kit to obtain a higher resistance[18] pressure. The packings were made of Kel-F (3M Comp.). The stainless-steel column (25 cm × 4.6 mm I.D.) was packed with LiChrosorb Si 60, 10 μm (Merck, Darmstadt) using a slurry method.

Dimethyl ether (Merck; 99%, 0.3% methanol) was used without further purification. Diethyl ether was dried over calcium chloride, refluxed with sodium under nitrogen for 1 h, then distilled and treated with iron(II) sulphate heptahydrate in order to remove traces of peroxides. Naphthalene, anthracene, pyrene and chrysene were recrystallized several times. The concentrations of their solutions in n-pentane were in the range of 0.01–0.001 mol/l, chosen to yield approximately equal peak areas. Approximately 20 μl were injected for each chromatogram. Diethyl, di-n-butyl, di-2-ethylhexyl phthalate (Merck) and the oligostyrene sample PS 600 (Knauer, West Berlin) were not purified further.

The volume flow-rate, F (ml/min), refers to the pumping rate at ambient temperature and the specified column end pressure, p_e. The linear velocity, u (cm/sec), is valid for column temperature and p_e and is related to the dead time, t_0

$$u = L/t_0 \qquad (1)$$

where L = column length.

RESULTS AND DISCUSSION

For our studies on the separation behaviour under different conditions we used a mixture of four polynuclear aromatic hydrocarbons (PAHs) because of their easy detectability by an UV detector (254 nm). In order to obtain reasonable analysis times, even under operating pressures and temperatures where the solvent properties of the eluents are rather poor, the lower-molecular-weight representatives naphthalene, anthracene, pyrene and chrysene were chosen as substrates on account of their inherent vapour pressures.

The calculation of capacity ratios, k', according to

$$k' = (t_R - t_0)/t_0 \qquad (2)$$

requires the exact determination of the dead time, t_0. For this, the retention behaviour of cyclohexane, benzene, methylene chloride, n-heptane, n-hexane and n-pentane was

examined under conditions known to yield good resolution of the PAHs; even small retentions should be apparent.

Cyclohexane and benzene showed significant retention, while methylene chloride and the straight chain alkanes were not retarded; however, methylene chloride caused a baseline shift. We chose n-pentane for measuring the dead time because the smaller the alkane molecule the more inert it is expected to be. n-Pentane is also a good solvent for the lower PAHs, so that it can serve both as an inert substance for determination of t_0 as well as a solvent for substrate injection.

The temperature-dependent changes in the separation behaviour were studied by performing series of analyses, keeping constant the flow-rate (1 ml/min) and the pressure, p_e, at the end of the column. For diethyl ether the column end pressures were 6 bar (corresponding to a reduced pressure, p_r, of 0.17), 38 bar ($p_r = 1.06$) and 70 bar ($p_r = 1.97$), for dimethyl ether, 56 bar ($p_r = 1.06$) and 70 bar ($p_r = 1.25$). When the pressure was kept above critical, the temperature was varied from the critical temperature (diethyl ether, 192.6°C; dimethyl ether, 126.9°C) up to about 280°C. For diethyl ether at $p_e = 6$ bar the series started at a temperature of 140°C, which is somewhat above the boiling temperature. The obtained $k'(T)$ isobars are shown in Fig. 1 for diethyl ether and Fig. 2 for dimethyl ether.

All curves have a similar shape for the four components of the mixture. The elution sequence is in accord with the increasing molecular weights and boiling points of the components, i.e., naphthalene elutes first, followed by anthracene, pyrene and chrysene. At temperatures near the transition to the gas state, i.e., the boiling temperature or the critical temperature, the k' values are small. The k' values increase with increasing temperature and all curves, save the one for diethyl ether at $p_e = 70$ bar, have a distinct maximum. The maximum was found to be most pronounced at the lowest pressure of 6 bar. In this curve there is a gap between 160 and 200°C, because the diethyl ether was in the liquid state at the beginning of the column but gaseous at its end due to the pressure drop along the column. The position of the maximum shifted to higher temperatures for the higher-molecular-weight PAHs. After passing the maximum, the k' values of all components decrease again at still higher temperatures.

The observed behaviour can be explained by the counteraction of two effects. At the phase transition liquid–subcritical gas or liquid–supercritical gas the density of the eluent decreases, this decrease becoming more pronounced at lower working pressures. The density decrease corresponds to lower dissolution power leading to increased k' values. At higher temperatures, however, substrate transport along the chromatographic column is increasingly determined by the vapour pressures of the substrate components. Due to the increase in vapour pressure with temperature, raising the temperature then results in more rapid elution and smaller k' values.

The course of the density changes can be demonstrated by the temperature dependence of the dead volume, which is shown in Fig. 3 for diethyl ether as the eluent. The dead volume only depends on the amount of the eluent in the given free volume of the chromatographic system. This amount increases with increasing pressure and, thereby, increasing density. Fig. 3 also explains the disappearance of the maximum in k' values at high pressures. Especially for the transition liquid–subcritical gas, the density decreases drastically. High pressures, on the contrary, compress the supercritical gas to such an extent that when passing from the liquid to the
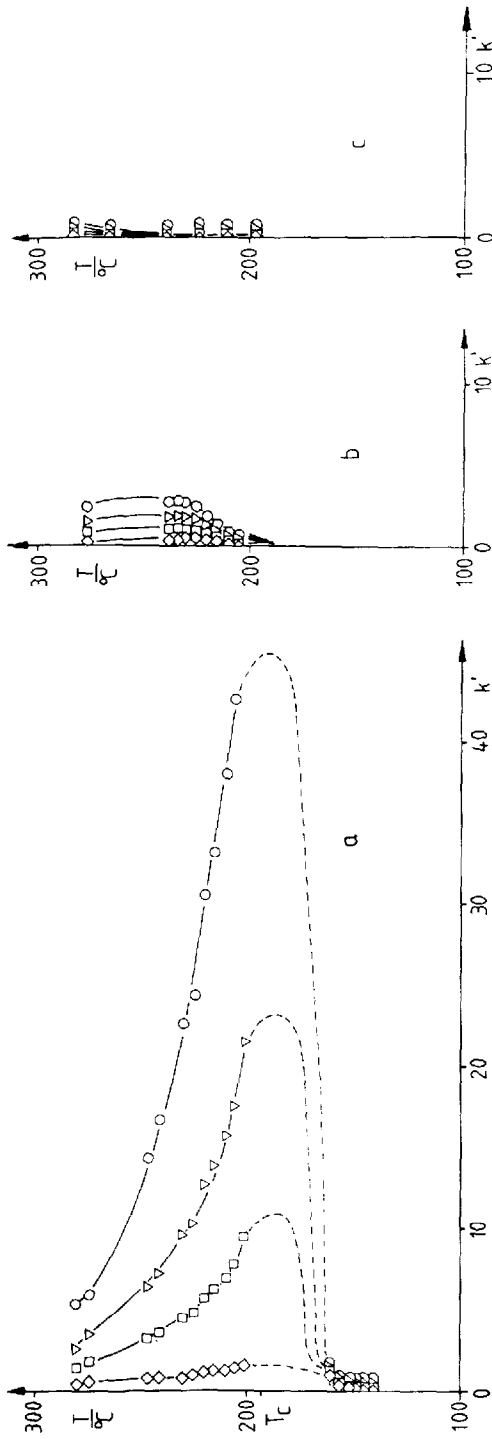
Fig. 1. Plots of temperature *versus* capacity ratios, k', with diethyl ether as the mobile phase at different p_c (isobars): a, 6 bar (reduced pressure, p_r, = 0.17); b, 38 bar (p_r = 1.06); c, 70 bar (p_r = 1.97). Substrate: mixture of naphthalene ($\diamond$), anthracene ($\square$), pyrene ($\triangledown$) and chrysene ($\bigcirc$) (PAHs). Volume flow-rate: 1 ml/min.
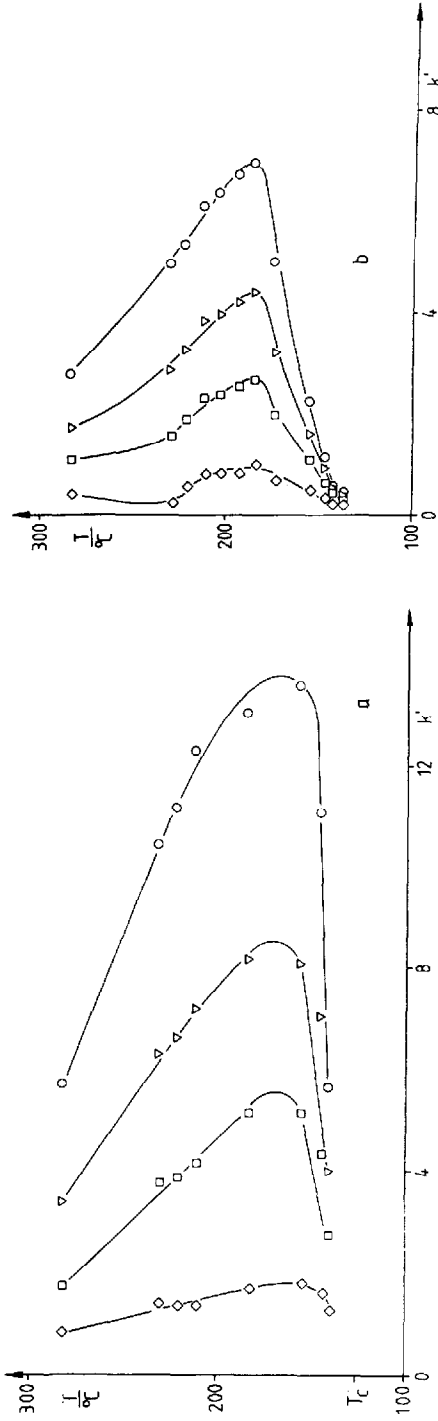
Fig. 2. Plots of temperature *versus* capacity ratios, k', with dimethyl ether as the mobile phase at different p_c: a, 56 bar (p_r = 1.06); b, 70 bar (p_r = 1.25). Substrate and flow-rate as in Fig. 1.
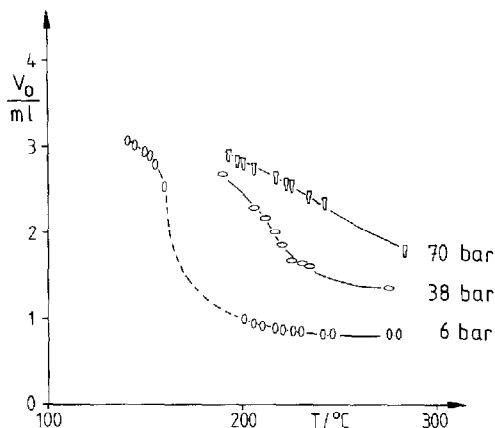
Fig. 3. Plot of dead volume *versus* temperature with diethyl ether as the mobile phase at different pressures, p_e. Substrate: *n*-pentane. Volume flow-rate: 1 ml/min.

supercritical gas there is no such significant decrease in density.

The comparatively larger decrease in the elution volumes of the higher- compared to the lower-molecular-weight PAHs in those parts of the curves which are determined by the vapour pressure is explained by the Clapeyron equation, which describes the slope of the vapour pressure curve

$$\mathrm{d}p/\mathrm{d}T = \Delta H_{vap}/T\Delta V \tag{3}$$

where ΔH_{vap} = enthalpy of vaporization and ΔV = volume change on vaporization. The slope of the vapour pressure curve increases with increasing ΔH_{vap}. This leads to a faster decrease in the k' values for the higher PAHs.

Fig. 4 shows some chromatograms obtained with dimethyl ether as mobile phase. At slightly supercritical temperatures the peaks are incompletely separated. Increasing the temperature results in complete separation of the substrate, the peaks becoming sharper and more symmetrical. Further increase in temperature leads to shorter analysis times. Finally, at 280°C the resolution becomes poorer again. These qualitative observations can be expressed in terms of the resolution, R_i, or the average resolution, $\bar{R}$, calculated according to

$$R_i = \frac{t_{n+1} - t_n}{w'_{n+1} + w'_n} \sqrt{\ln 4} \tag{3a}$$

$$\bar{R} = \frac{\sum\limits_{i}^{j} R_i}{j} \tag{3b}$$

where t_n = retention time of the nth peak, w' = peak width at half height, R_i = resolution for ith pair of peaks, $j = 3$ and $i = 1$–3.

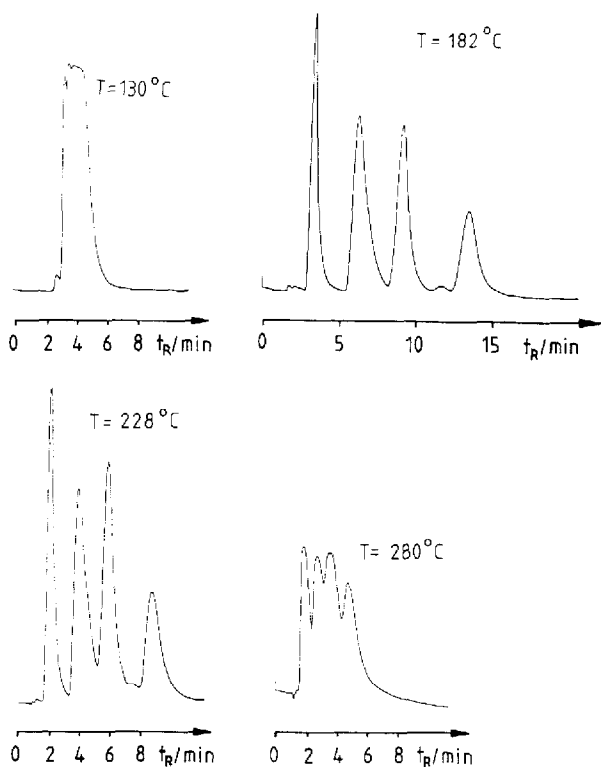Figs. 5 and 6 show the temperature dependence of the average resolution, $\bar{R}$,

Fig. 4. Chromatograms of PAHs with dimethyl ether as the mobile phase at different temperatures: 130°C (slightly above T_c = 126.9°C); 182°C (maximum in $\bar{R}$; cf., Fig. 6); 228°C ($\bar{R}$ = 1.5); 280°C (highest temperature). p_e = 70 bar. Volume flow-rate: 1 ml/min.
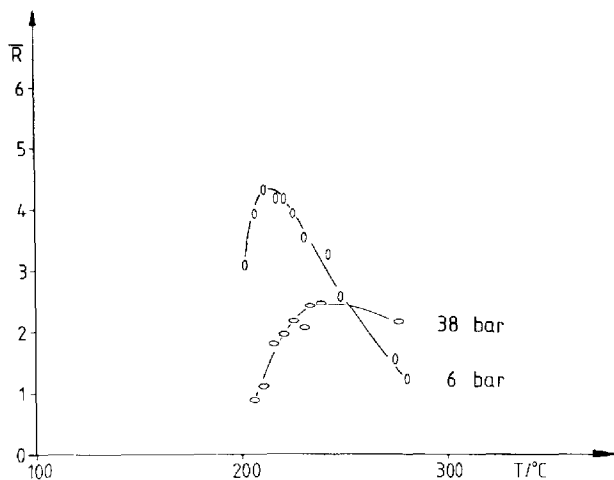

Fig. 5. Average resolution, $\bar{R}$, of PAHs in diethyl ether at different temperatures and pressures, p_e. Volume flow-rate: 1 ml/min.
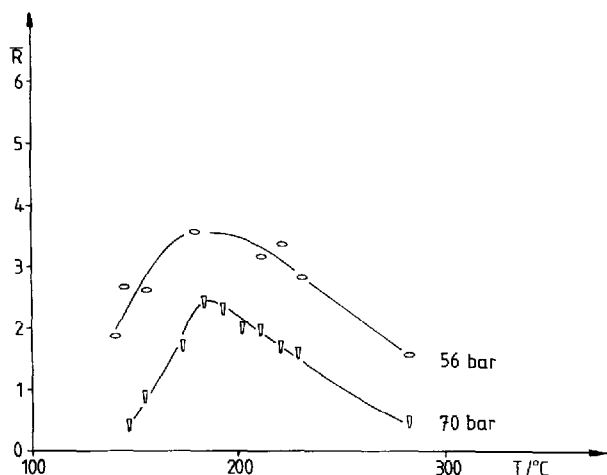
Fig. 6. Average resolution, $\bar{R}$, of PAHs in dimethyl ether at different temperatures and pressures, p_e. Volume flow-rate: 1 ml/min.

at constant pressures. The course of these plots is similar to that of the k' curves. At a certain temperature the resolution becomes a maximum. Lower pressures apparently render better resolutions. The maximum resolution does not correspond exactly to the maximum in the k' values, but is shifted to somewhat higher temperatures due to increasing peak broadening at high k' values having a detrimental effect on resolution. With diethyl ether as mobile phase and a column end pressure of 70 bar, the peaks are poorly separated, so that peak widths and, consequently, the resolution, $\bar{R}$, could be determined only at high temperatures. The comparison of dimethyl and diethyl ether shows more pronounced maxima in the k' and $\bar{R}$ values using dimethyl ether, both at the same reduced and the same absolute column end pressure. Also, the maxima with dimethyl ether were found at lower temperatures, corresponding to its lower critical temperature.

    The assumption that an optimum separation temperature exists for a given mobile phase at a fixed working pressure was checked for two more classes of compounds: a standard mixture of oligostyrenes ($\bar{M}_w = 600$ g/mol) as an example of an homologous series and a mixture of di-alkyl phthalates (alkyl = ethyl, n-butyl and 2-ethylhexyl) representative of molecules containing polar groups. For these separations diethyl ether was chosen as the mobile phase. The results obtained with the aromatic test mixture (PAHs) yielded an optimum temperature of about 240°C with a column end pressure of about 38 bar. Both the oligostyrenes and di-alkyl phthalates were chromatographed at this pressure and temperature and at lower and higher temperatures.

    Fig. 7 shows the separations of the oligostyrene sample. At 238°C it was completely separated into the components of increasing degree of oligomerization. The analyses were arbitrarily stopped at a degree of oligomerization of $n = 6$–7. At 280°C the resolution was better than at 210°C, but distinctly poorer than at the optimum of 240°C. Analogous results were obtained with the mixture of phthalates. This confirms the existence of a temperature at which the resolution is maximal.

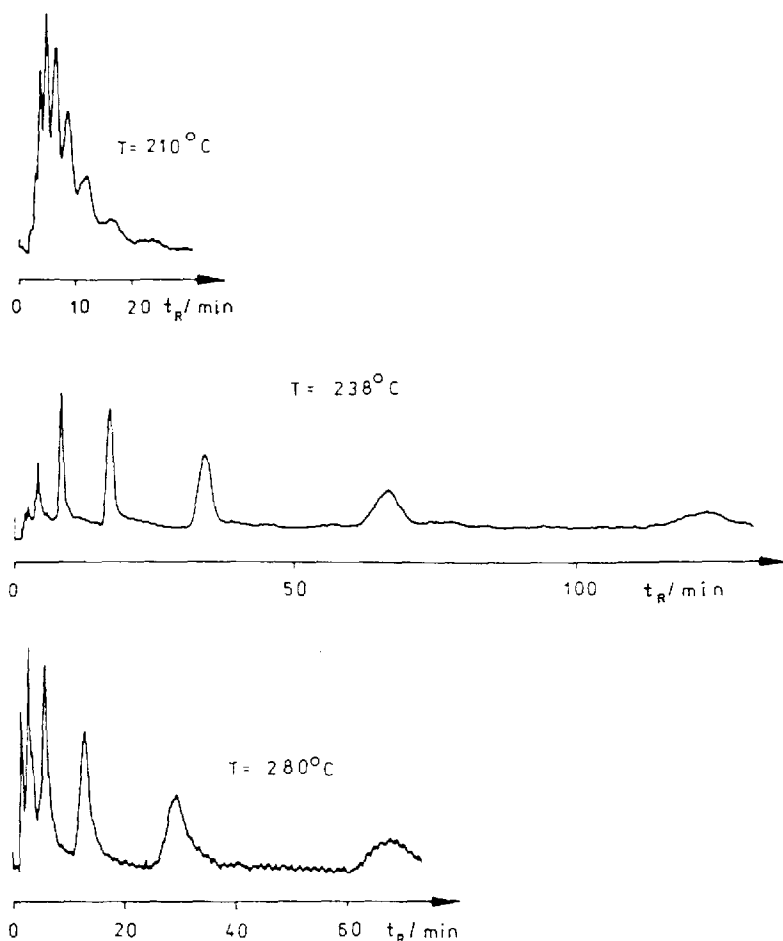   As the preceding results show, analysis times can be reduced by increasing the

Fig. 7. Chromatograms of oligostyrenes (PS 600) in diethyl ether as the mobile phase at different temperatures: 210°C (17.4°C above T_c); 238°C (maximum in $\bar{R}$; cf., Fig. 5); 280°C (highest temperature). p_e = 38 bar. Volume flow-rate: 1 ml/min.

pressure. Another possibility is to raise the flow-rate. We carried out our studies on the influence of the flow-rate on k' values and resolution with dimethyl ether as the eluent because of the higher resolutions obtained with this mobile phase. The column end pressure of 70 bar should give short analysis times *per se*. At this pressure a temperature of 172°C was found to yield the highest $\bar{R}$ values. As expected, the retention times decreased under these conditions with increasing flow-rates. At first the k' values decreased considerably (Fig. 8). At a flow-rate, F, of 3 ml/min, corresponding to a linear velocity, u, of about 0.57 cm/sec, the curves showed an inflection. This may be due to a transition from a laminar to a turbulent flow, having an influence on the mass transport in the separation column. Novotný et al.[14] reported a similar observation.

The average resolution, $\bar{R}$, could not be determined over the range of flow-rates because of the poor separation and immeasurable peak widths at higher flow-
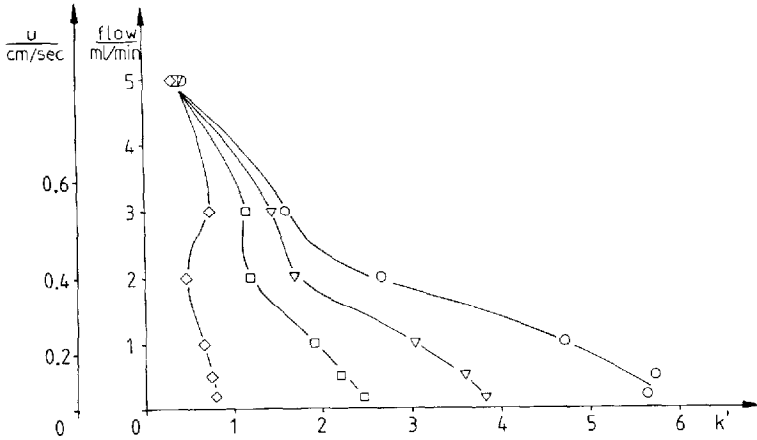
Fig. 8. Dependences of capacity ratios, k', on volume flow-rate, F, and linear velocity, u, for PAHs (see Fig. 1) in dimethyl ether at 172°C and $p_e = 70$ bar.

rates. Therefore, another parameter, the resolution function, Q, was used[21,22]

$$Q = \frac{\sum_{i}^{j} f_i/g_i \, (\Delta V_e)_i}{\sum_{i}^{j} (\Delta V_e)_i} \tag{4}$$

where f_i = average peak height of the ith peak pair, g_i = average peak depth (between peaks of the ith peak pair), $(\Delta V_e)_i$ = difference in elution volumes of the ith peak pair, $j = 3$ and $i = 1$–3. At small flow-rates Q remained approximately constant (Fig. 9), but above a flow-rate of 1 ml/min the resolution decreased. Therefore, it
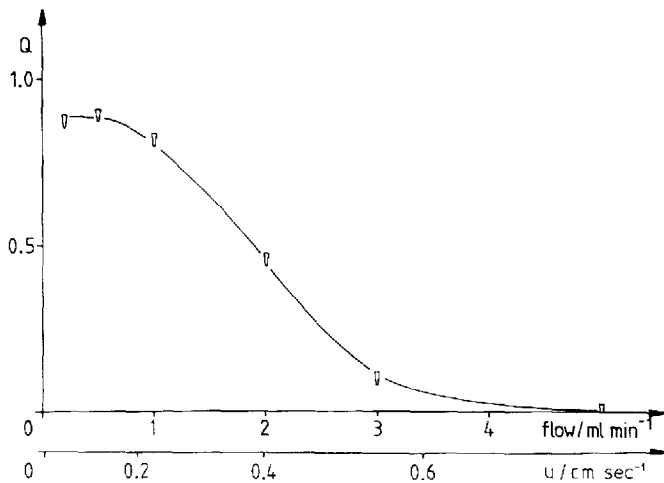

Fig. 9. Dependence of resolution, Q, on the volume flow-rate, F, and linear velocity, u, for PAHs in dimethyl ether at 172°C and $p_e = 70$ bar.
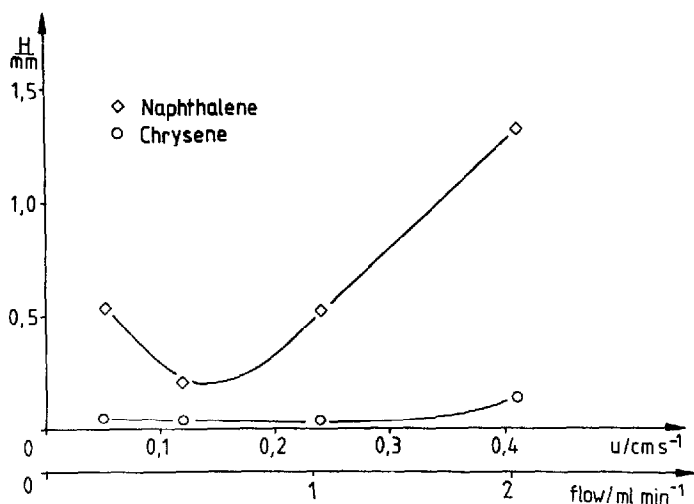
Fig. 10. Plots of effective plate height, H, *versus* linear velocity u for naphthalene ($\diamond$) and chrysene ($\bigcirc$) in dimethyl ether at 172°C and p_c = 70 bar.

appears that for our chromatographic system a flow-rate of 1 ml/min represents a good compromise between high resolutions and short analysis times.

The van Deemter plot of the effective plate height, *H, versus* the linear velocity, *u*, shows that for chrysene smaller values are attainable than for naphthalene (Fig. 10) and the increase in plate heights starts at higher linear velocities.

In conclusion it may be stated that with dimethyl and diethyl ether as mobile phases in the sub- or supercritical gaseous state there exist optimum temperatures above the boiling and the critical temperature, respectively, where maximum capacity ratios and resolutions are obtained. Higher pressures shift the maxima to higher temperatures and the larger dissolving power also results in smaller k' and $\bar{R}$ values. The influence of temperature and pressure on the separation behaviour results from two effects: the increase in the vapour pressure with increasing temperature and the dependence of the eluent's density on both temperature and pressure. When, a certain range is exceeded, increasing flow-rate results in a considerable decrease in resolution.

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