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Influence of linear velocity and multigradient programming in supercritical fluid chromatography

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ABSTRACT

The chromatographic plate height, resolution and time requirements in supercritical fluid chromatography depend on the physical properties of the mobile phase (pressure, mobile phase composition, temperature and linear velocity), on the chemical structure of the analyte, which determines vapour pressure and solubility, and on various properties of the stationary phase. If a given homologous series of compounds, or a test mixture simulating a homologous series, is used with a specific chromatographic column, the chromatographic properties for the compounds in these test mixtures will vary with pressure, mobile phase composition, temperature, velocity and the relative molecular mass of the individual compounds. If in addition to a suitable column a temperature is chosen which, for simplicity, is kept constant, and which is known to lead to good or even optimum resolution, the pressure, composition and relative molecular mass dependence of the Van Deemter plate height minimum, as determined experimentally at this temperature, can be used for predicting an optimum linear velocity programme. This velocity programme can be used either as a stand-alone programme or can be adapted to and superimposed on pressure or composition programmes, or even on combined pressure–composition programmes. The linear velocity dependence of plate height and resolution for a mobile phase composed of a mixture of carbon dioxide and methanol on a column packed with unbonded silica gel is presented. This dependence was measured at different pressures and compositions, employing four condensed aromatics as a test analyte. Each chromatogram of the analyte was therefore measured at constant temperature, velocity, pressure and composition, varying the last three physical properties between chromatograms. The data are presented as Van Deemter plots and as three-dimensional plots showing the dependence of resolution and capacity factor on velocity and either pressure or composition. Based on these data, the change in the linear velocity suitable for pressure programmes, mobile phase composition programmes and for increase in the relative molecular mass of the analyte is discussed. The conclusion is that a pressure (density) programme needs a superimposed negative linear velocity programme for the purpose of decreasing the plate height and increasing the resolution, whereas such a programme is not necessary to a comparable extent for composition programming. If compounds with a wide range of relative molecular masses are separated, the superimposing of a negative linear velocity programme on to a composition programme is also advantageous. For programming the physical properties of a mobile phase, *i.e.*, pressure, density, composition, temperature and velocity, a number of closely related equations are proposed and some corresponding programme curves are shown. Hardware needs for programming are also briefly discussed.

INTRODUCTION

Supercritical fluid chromatography (SFC) has been established as a routine method in some areas of analytical chemistry. For the further development of SFC, a general knowledge of the possibilities for the optimization of efficiency and resolution by gradient programming is important. The first generation of commercially available capillary SFC instruments were designed to achieve pressure (density) programmes by controlling and programming the pressure at the column inlet, thereby usually increasing the flow-rate through a capillary column whenever a "fixed" restrictor was employed at the column outlet. As is well recognized, the problem with this pressure programming technique is an increase in the linear velocity in the column, which may strongly decrease plate numbers, *i.e.*, efficiency.

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A second problem with the first generation of capillary SFC instrumentation was that the change in the nature of the mobile phase via programming of a composition gradient was not possible. A means of solving these problems was shown independently by different groups using pressurized gases or liquids as programmable counter pressure media at the outlet of open-tubular and other microcolumns [1–3]. These difficulties were not inherent in the use and commercialization of SFC instruments employing larger diameter packed columns because regulating valves at the column outlet could be used from the beginning of the development of SFC apparatus. Recently, self-controlling regulating valves as part of feedback loops have been introduced for larger diameter packed columns [4–7]. The regulation devices allow pressure and composition gradients independently of each other and of the feed rate of the pumps, which means that these devices allow also independent linear velocity programming for a continuous optimization of efficiency. Instruments using such valves have already become commercially available and more are expected to follow.

An SFC instrument that is capable of all these gradient programming techniques, including temperature programming, offers both the capability and the problem of finding the optimum single or combination gradient method. For instance, the optimum velocity programme to be superimposed on a composition programme, a pressure programme or a temperature programme is not entirely obvious and few relevant data are available in the literature. It can be expected, however, that the combination of a velocity programme with a temperature programme, or *vice versa*, should be applied, considering that a negative temperature programme reduces diffusion and diffusion coefficients, which is to be counteracted by a negative velocity programme, whereas a positive temperature programme increases diffusion and the velocity probably needs increasing. It might be expected also that in combination with other positive gradients, *i.e.*, pressure and composition, a usually negative velocity programme is useful if optimized efficiency and resolution are required throughout a separation.

This paper reports first the influence of linear velocity on plate height at different pressures and compositions, choosing a constant temperature of 150 C. The chromatographic system consisted of a column packed with unbonded silica which had been modified with methanol (see Experimental). The mobile phases were composed of mixtures of carbon dioxide (CO_2) and methanol and the test analyte consisted of a mixture of four condensed aromatic hydrocarbons. All individual chromatograms were obtained keeping the linear velocity, pressure, composition and temperature constant. Analogous work on the same system at constant pressure, composition and temperature at the more simply attainable constant flow-rates instead of constant linear velocities will be presented elsewhere [8]. The present results are expressed as the usual Van Deemter plots [9] of plate height *versus* linear velocity at constant pressure, composition and temperature. Because these Van Deemter plots were obtained at different pressures and compositions, it can be seen how a change in pressure or in composition affects the plots. This is equivalent to obtaining a first answer about how a pressure or a composition programme is to be combined with a velocity programme. Some information can even be obtained about suitable ternary combinations of programmes for velocity, pressure and composition. Previous studies of Van Deemter plots with different unbonded, bonded or coated silica phases in packed columns have shown significant differences in the HETP for different stationary phases and for the same stationary phase applied with different analytes [10–13]. Both results are to be expected on account of the different interdiffusion coefficients and capacity factors of the analytes. The mobile phases in these previous studies were pure CO_2 and CO_2–methanol mixtures.

This paper also reports on the influence of linear velocity on the capacity factors and resolution at different pressures and compositions at a constant temperature of 150 C. The results are presented in three-dimensional graphs, in which capacity factor and resolution are plotted against velocity and either pressure or composition. Again, information is obtained from this type of plot about suitable combinations of velocity, pressure and composition for binary gradient programmes.

Because gradient programmes having a good separation effect may follow relatively simple mathematical equations, a self-consistent set of simple equations appears desirable for programming the different parameters of the mobile phase. Therefore, a basic equation was developed that is applicable to

velocity, flow, pressure, density, composition or temperature programmes, singly or in combination as simultaneous or consecutive programmes. Emphasis was placed on employing a minimum of freely choosable constants in the equations, yet retaining maximum flexibility. Some hardware requirements for using the programmes are discussed with a view to combining GC with SFC by pressure or density programmes in the same chromatogram.

EXPERIMENTAL

The chromatographic equipment used was a modified Hewlett-Packard (Waldbronn, Germany) HP 1084B with a Model LC-75 UV detector (Perkin-Elmer, Düsseldorf, Germany) modified to be equipped with a high-pressure UV detector cell for pressures up to 350 bar as described previously [7,14]. The pressure-regulating system was composed of an electronically controlled Hitec valve (Bronkhorst Hitec, Ruurlo, Netherlands) [7], followed in series downstream by a Tescom (Elk River, MN, USA) back-pressure regulator analogous to published arrangements [4]. The complete pressure programming system consisted of these valves, an IBM AT PC and an ADDA converter as described previously [7].

The carbon dioxide eluent (99.995% purity) (Linde, Cologne, Germany) was prepressurized with helium in the storage cylinder and delivered as a liquid to the pump, the cylinder being turned upside down. The methanol (Merck, Darmstadt, Germany) was delivered from liquid storage bottles which were part of the original equipment of the HP 1084B chromatograph. A laboratory-made analytical column (250 mm × 4.6 mm I.D.), packed with Li-Chrospher Si 100 (5 μm particle diameter) (Merck) by a slurry packing procedure [15], was used. The column was conditioned with methanol at 300 C and 300 bar before use according to an earlier procedure employing 1,4-dioxane [16] instead of methanol. It is found that this conditioning leads to reaction of methanol with the silica, as is shown by the C content of the silica after conditioning. As the analyte a polyaromatic hydrocarbon mixture composed of naphthalene, anthracene, pyrene and chrysene was used at concentrations between $1 \cdot 10^{-2}$ and $1 \cdot 10^{-3}$ mol l of each component in heptane. The sample was injected by means of a Rheodyne Model 7125 valve (ICT Handelsgesellschaft, Frankfurt, Germany) equipped with a 20-μl sample loop. The temperature of the column was kept constant at 150°C by use of a Hereaus (Hanau, Germany) forced circulation air oven.

The elution time of the solvent heptane was taken as the dead time, t_0, whereby the pulse of heptane led to a negative peak in the UV detector. The linear velocity, u, determined from t_0 and $u = L/t_0$ (L = column length), was varied by changing the mobile phase pump flow-rate, and ranged from 0.07 to a maximum of 1.05 cm/s at 150 bar and 150 C. Both of these values arise because of hardware limitations of the instrument used. Thereby, the maximum linear velocity attainable was lower at higher than at lower pressures. The optimum linear velocity, u_{opt}, was obtained from the Van Deemter plots by both splines and best estimates. Similar results were obtained in both instances.

The plate height (HETP) was calculated from the column length, L, the retention time of the eluted peak, t_r, and its peak width at half-height, $w_{0.5}$, by means of the equation

$$\mathrm{HETP} = \frac{L}{5.54}\left(\frac{w_{0.5}}{t_r}\right)^2 \qquad (1)$$

The capacity factor, k', and the resolution between peaks i and j, R_{ij}^*, were calculated according to $k' = (t_r - t_0)\ t_0$ and [17]

$$R_{ij}^* = \frac{f_{ij}}{g_{ij}} + \frac{d_{ij}}{w_i' + w_j'} \sqrt{\ln 4} \qquad (2)$$

with $d_{ij} > 0$, 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 k_{ij} is the distance between the baseline intercepts of the tangents to the peaks. For determining d_{ij}, on each peak a tangent is drawn on that side of the peak which is adjacent to the other peak. The average resolution R_m^* is the arithmetric mean of the R_{ij}^*.

$$R_m^* = \sum_1^n R_{ij}^*\ n \qquad (3)$$

where n is the number of peak pairs in the analyte mixture, *i.e.*, $n = 3$ in the present case. For the plotting of the three-dimensional graphs, in which k' is plotted on one of the axes and R_m^* is represented by

shading the three-dimensional surface, a computer programme (Unimap; Uniras European Software Contractors, Lyngby, Denmark, and Düsseldorf, Germany) was used as described in detail previously [18].

RESULTS AND DISCUSSION

The HETP in SFC, as opposed to gas chromatography (GC), depends on physical properties of the mobile phase other than temperature and linear velocity. The HETP in SFC is in addition a function of pressure or density and mobile phase composition, besides also being dependent, as in GC, on the physical properties of the sample components, *i.e.*, vapour pressure and chemical structure, and on the stationary phase.

If in SFC a given stationary phase and a given homologous series of components, or a test mixture simulating a homologous series, is used, the HETP values for the compounds in these mixtures then vary only with pressure, mobile phase composition, temperature and velocity. At a given pressure, composition and temperature the experimentally determined Van Deemter curves show a plate height minimum at an optimum linear velocity, u_{opt}, for each compound in the analyte mixture. Provided that the dependence of the Van Deemter plate height minimum and of the corresponding u_{opt} on pressure, composition and temperature is known for a given analyte mixture, a suitable velocity programme can be derived for the separation either as a stand-alone programme or for superimposition of the velocity programme on a pressure, composition or temperature programme.

First, the linear velocity dependence of the HETP for a binary mobile phase composed of different relative amounts of CO_2 and methanol will be presented. All experiments were performed with a test mixture of naphthalene, anthracene, pyrene and chrysene on an unbonded, normal-phase LiChrospher 100 (5 μm) silica gel stationary phase. The experiments were carried out at a range of pressures and compositions but always at a constant temperature of 150°C. The reasons for choosing a constant temperature of 150°C were to reduce the number of chromatograms needed for the study and because it was found that the optimum resolution was obtained at *ca.* 150°C with, however, significant changes in the optimum temperature with pressure and composition.

In the Van Deemter plots in Figs. 1–5 increasing amounts of methanol (2.5, 5, 10, 20 and 30%, v/v) were employed for the CO_2–methanol binary mobile phase. In Fig. 1a–c the mobile phase is CO_2 with 2.5% of methanol. An increase in pressure from 150 (Fig. 1a) via 200 (Fig. 1b) and 250 bar (Fig. 1c) to 300 bar (Fig. 1d) leads generally to a decreased, and in part unchanged or even increased, plate height at u_{opt}. This decrease in HETP conflicts with the usual notion that increasing pressure tends always to increase the plate height. Chrysene shows increasing HETP with increase in pressure. The behaviour of chrysene may be explained by a larger decrease in the interdiffusion coefficient with pressure than occurs for the smaller molecules.

Turning to Figs. 2–5, with 5, 10, 20 and 30% of methanol in the mobile phase, the results with respect to the pressure dependence of HETP at u_{opt} are similar to those in Fig. 1. For Figs. 2–5 the HETP again decreases or remains about the same with increase in pressure as far as naphthalene, anthracene and pyrene are concerned, but tends to increase for chrysene. It should be noted, however, that the scatter of the data for the HETP in Figs. 1–5 is considerable. Nevertheless, the behaviour of the three lower relative molecular mass compounds is clear and that of chrysene stands out.

The second effect of pressure, which is also of significance, is the shift of the HETP minimum in the Van Deemter curves to lower values of the optimum velocity, u_{opt}, when the pressure increases. At the same time, there is a tendency for narrowing the range of the low HETP around the location of u_{opt}, steepening the slopes of the low- and the high-velocity branches of the Van Deemter curves. Nevertheless, there is still a reasonably wide range of velocity that can be used even for the highest pressure (300 bar). The curves indicate also that, generally, a lower linear velocity for the minimum is found when the relative molecular mass or the size of the sample molecule increases. Considering that naphthalene is eluted first and chrysene last during the separation and taking the Van Deemter plots for 10% methanol as an example (Fig. 3), the velocity might be reduced during the separation by pressure programming from about $u = 0.4$ cm/s for the elution of naphthalene to 0.15 cm/s for the elution of chrysene to

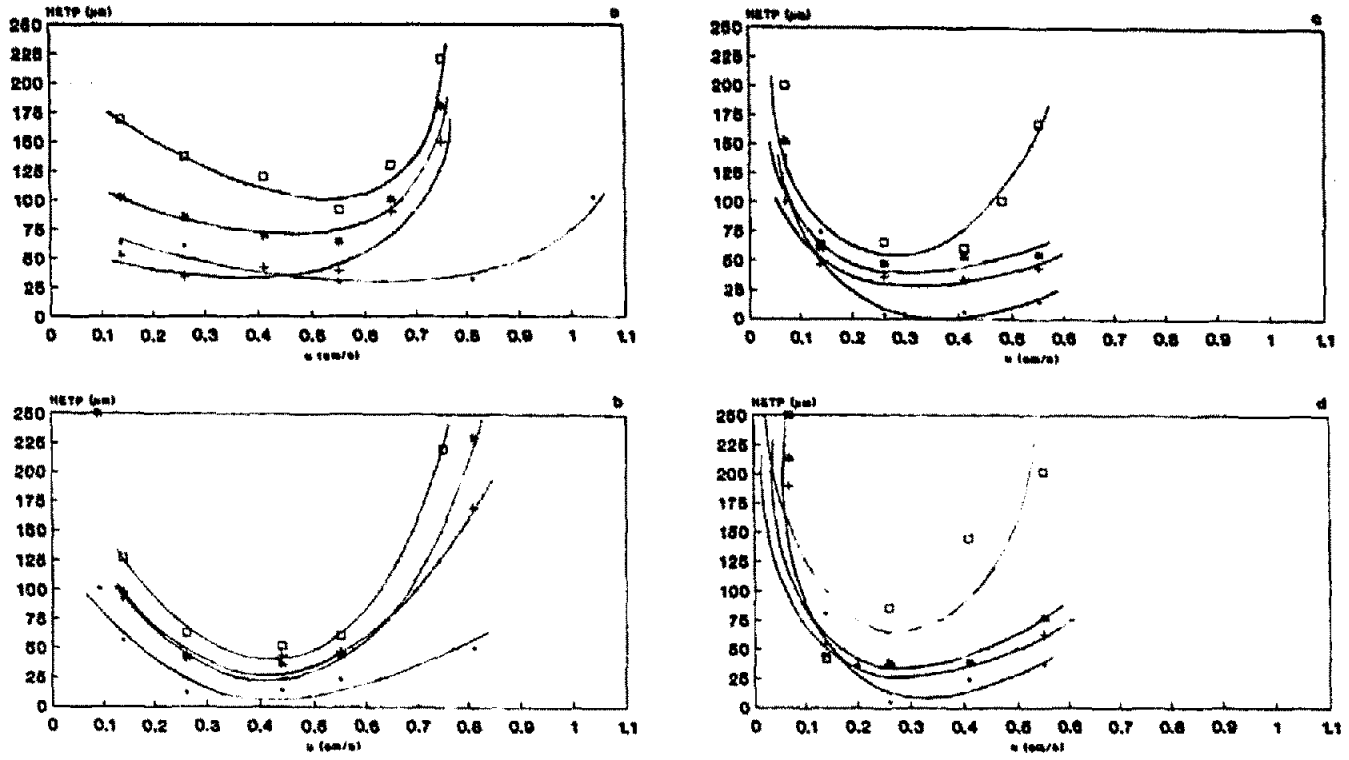

Fig. 1. Van Deemter plots for a mobile phase of carbon dioxide–2.5% (v/v) methanol for (a) 150, (b) 200, (c) 250 and (d) 300 bar and 150 C. Column, 250 mm × 4.6 mm I.D., packed with unbonded silica gel (5 μm). Test mixture: ■ = naphthalene; + = anthracene; * = pyrene; □ = chrysene. Additional conditions as given in the text.

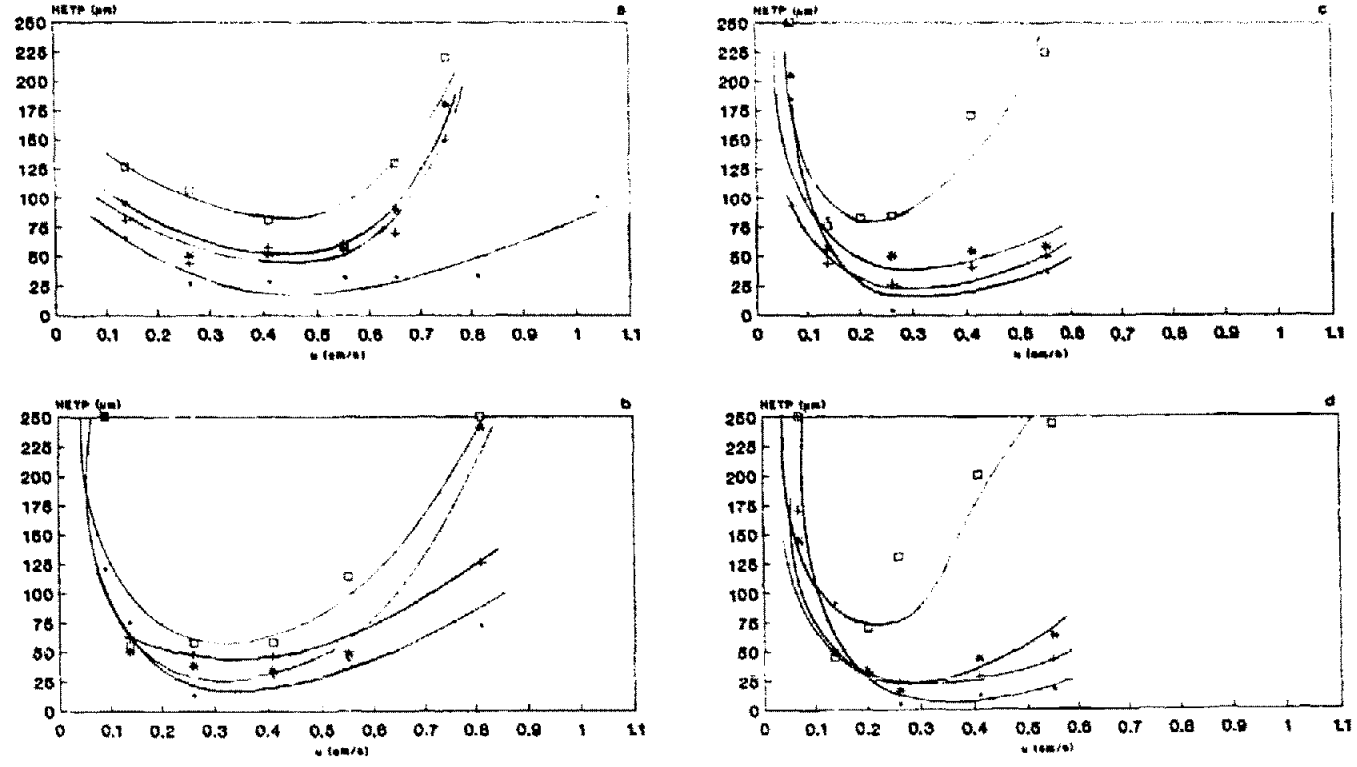

Fig. 2. Van Deemter plots for a mobile phase of carbon dioxide–5% (v/v) methanol for (a) 150, (b) 200, (c) 250 and (d) 300 bar. Other conditions and symbols as in Fig. 1.

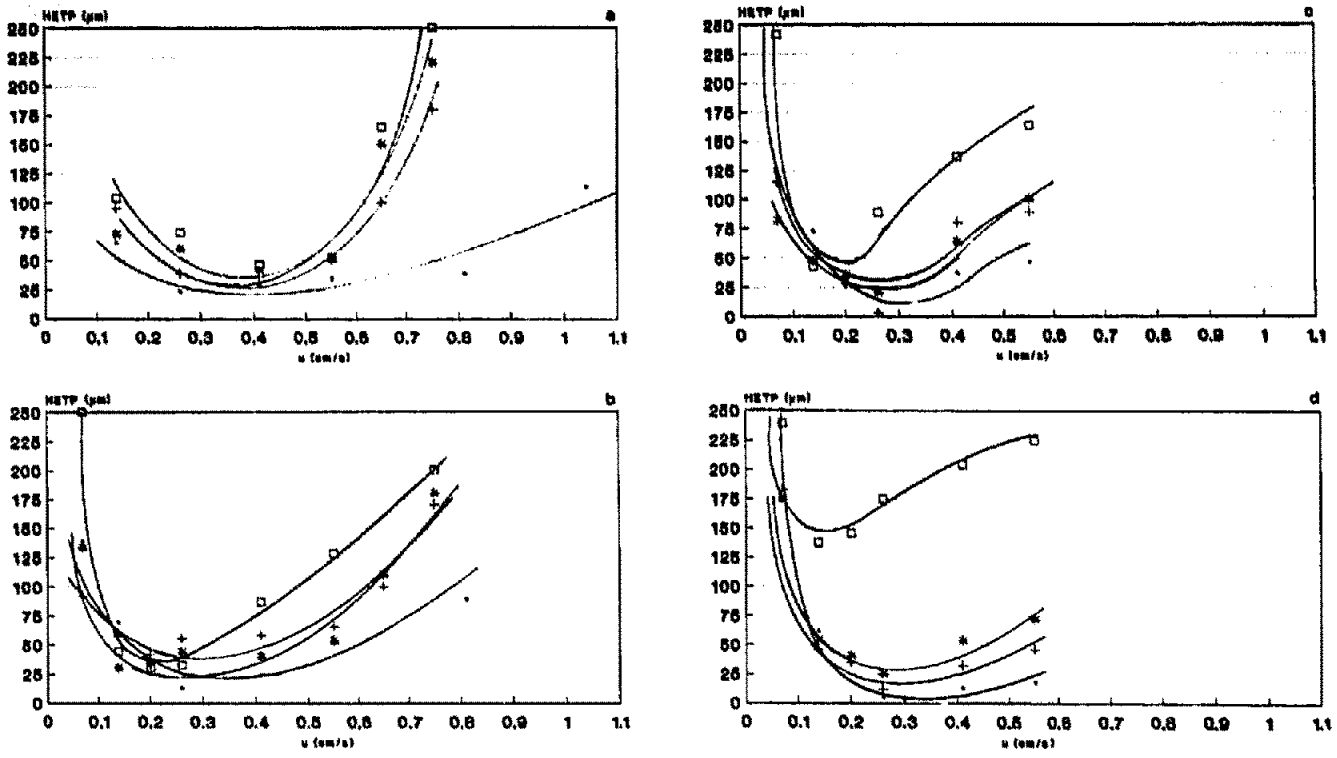

Fig. 3. Van Deemter plots for a mobile phase of carbon dioxide–10% (v/v) methanol for (a) 150, (b) 200, (c) 250 and (d) 300 bar. Other conditions and symbols as in Fig. 1.

obtain low HETPs for all compounds in the mixture. Even if the pressure is kept constant, a decrease in the linear velocity during the separation appears to be advisable.

Turning now explicitly to the influence of the composition of the mobile phase on the Van Deemter curves, one might compare different compositions at the same pressure, that is, 2.5, 5.0, 10.0, 20.0 or 30.0% (v/v) methanol at, for instance, 200 bar (Figs. 1b, 2b, 3b, 4b and 5a). If one compares the different compositions at 150 bar, a trend for the HETP minimum to move to lower linear velocities is observed when the proportion of methanol increases. This trend is smaller, however, than that observed when at a constant composition the pressure is increased from 150 to 300 bar. Moreover, comparing the movement of the HETP minima to lower u_{opt} when the methanol content is increased, it is seen that at lower pressures the shift of u_{opt} is more pronounced than at higher pressures. The first observation, *i.e.*, the smaller influence of composition on u_{opt}, can possibly be explained by smaller decrease in the free volume and the diffusion coefficient of the mobile phase when the more powerful solvent, here methanol, is added at concentrations up to 30% at constant pressure, as compared with a larger decrease in the free volume when the pressure is raised from 150 to 300 bar at constant composition. To give an example of the decrease in linear velocity when employing a composition programme at constant pressure: if a composition programme is run from 2.5 to 30% methanol at 200 bar, the velocity might be decreased from about 0.4 to 0.2 cm/s. Thereby, the starting velocity (0.4 cm/s) is derived from the Van Deemter curve of the first-eluting compound, naphthalene, and the final velocity (0.2 cm/s) is obtained from the last-eluting compound, chrysene.

The effect of the composition, *i.e.*, of an increasing concentration of methanol, on the magnitude of the HETP at u_{opt} is not clear from the present data. On the whole, there is no increase in HETP with an increasing content of methanol. In detail, at 150 bar an increase in methanol content from 2.5 to 30%

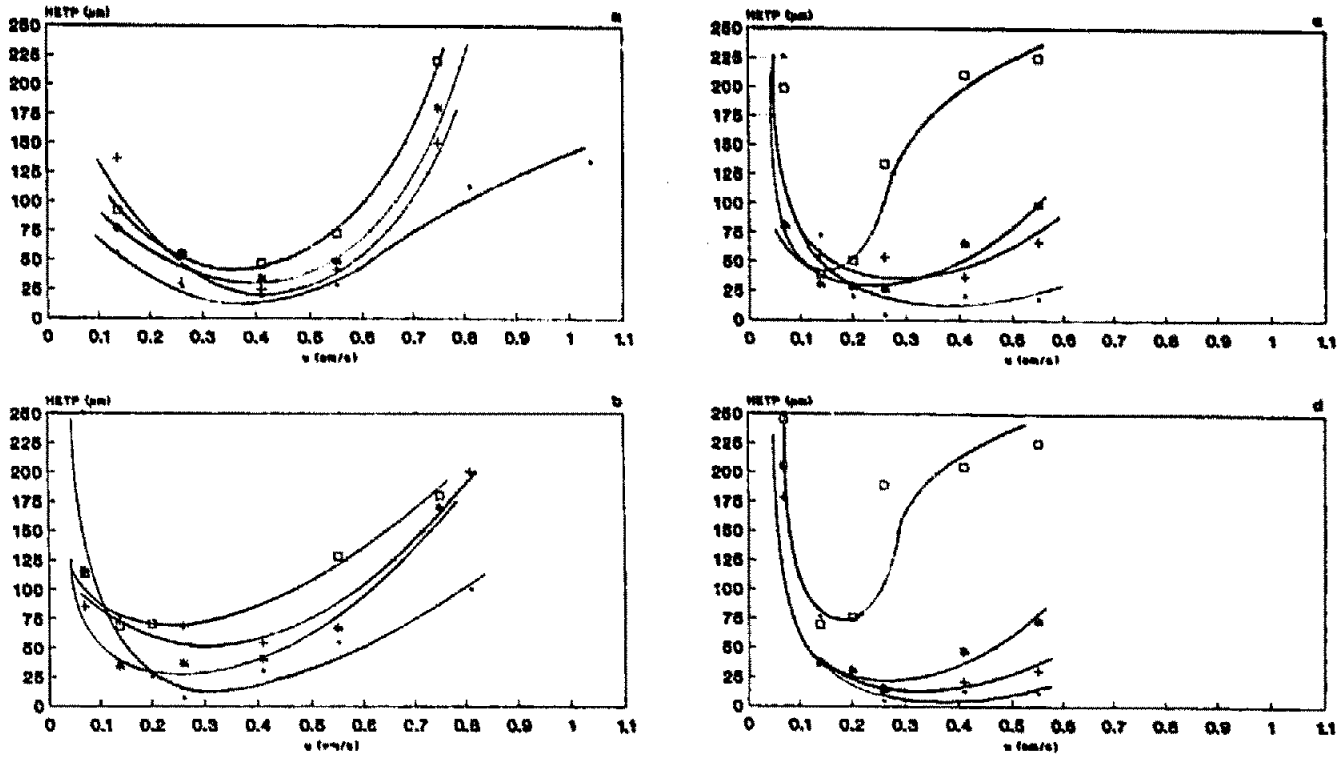

Fig. 4. Van Deemter plots for a mobile phase of carbon dioxide–20% (v/v) methanol for (a) 150, (b) 200, (c) 250 and (d) 300 bar. Other conditions and symbols as in Fig. 1.

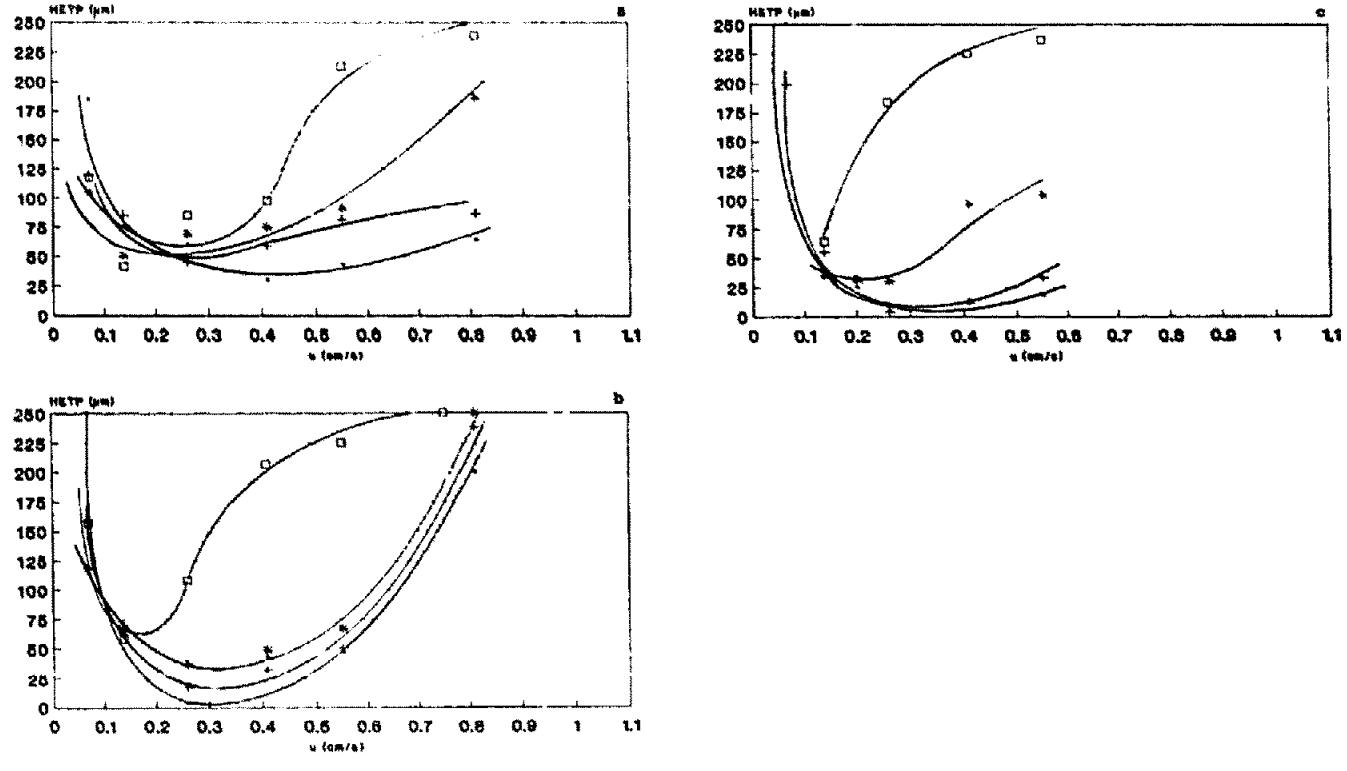

Fig. 5. Van Deemter plots for a mobile phase of carbon dioxide–30% (v/v) methanol for (a) 200, (b) 250 and (c) 300 bar. Other conditions and symbols as in Fig. 1. Data for 150 bar are not presented because at 150°C and 30% (v/v) methanol the area of phase separation was close according to experimental observations, calculation of the critical temperature, T_c, following Chueh and Prausnitz in Reid *et al.* [24] and to the literature data [25].

leads to a smaller HETP at u_{opt}. At 200, 250 and 300 bar no clear trend to either higher or smaller HETP can be discerned.

It is of interest, of course, to establish what influence a change in temperature has on the Van Deemter curves. It can be expected, for instance, that at higher temperatures higher velocities appear to be tolerable. Not many Van Deemter plots in which the temperature has been varied have been published so far. Therefore, the possibilities for comparison with respect to temperature are limited. Two published Van Deemter plots for different methanol contents in CO_2 (3000 psi and 80°C), employing Deltabond CN as the stationary phase and carbazole and naphthalene as analytes, show that increasing the content of methanol does not significantly increase the HETP at u_{opt} but raises the slope of the high-velocity branch [10]. Another Van Deemter plot with pure CO_2 at 50°C, employing octadecyl-bonded silica as the stationary phase and phenanthrene as the analyte, shows a decrease in HETP on increasing the pressure from 170 to 240 bar both for u_{opt} and the high-velocity branch [11]. The results of both studies appear to be compatible with the present results.

At the same pressure, composition and temperature, *i.e.*, for a given Van Deemter plot, the order of the HETP for the different analytes is as expected from their interdiffusion coefficients. Naphthalene has the lowest and chrysene the highest plate height, with anthracene and pyrene being intermediate, as seen in Figs. 1–5. In the literature an opposite order is found in several instances, *i.e.*, an analyte of a lower molar volume has a larger plate height than that with a higher molar volume [11,19–21]. One may possibly explain this opposite order by the type

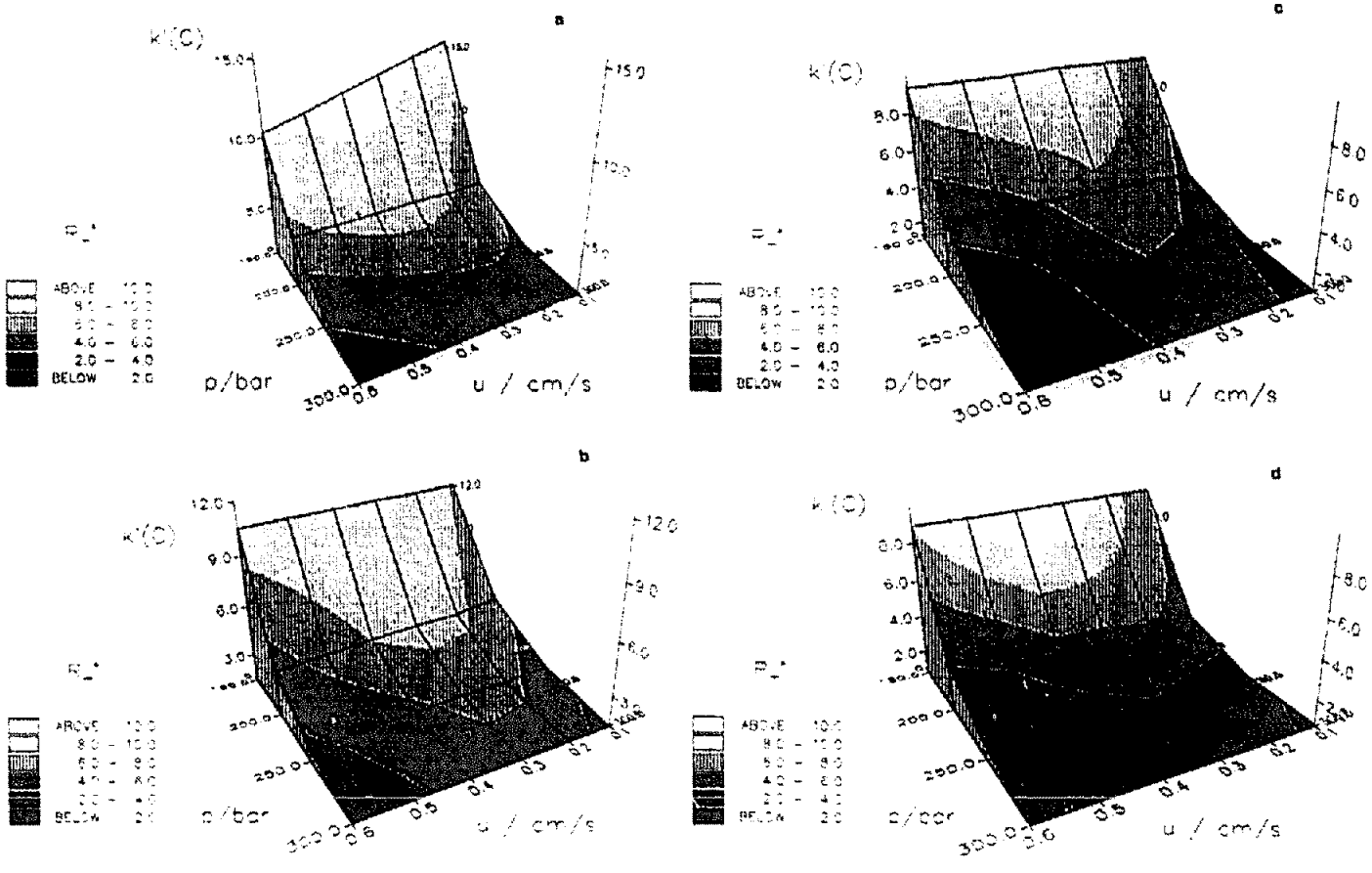

Fig. 6. Graphs of the capacity factor of chrysene, $k'(C)$, *versus* column end pressure, p, and average linear velocity, u. Arithmetic average of resolution between naphthalene, anthracene, pyrene and chrysene, R_m^*, represented as shading of surface. Mobile phase composition: (a) 2.5%; (b) 5%; (c) 10%; (d) 20% methanol in CO_2. Other conditions as in Fig. 1.

of pores, the pore size and the pore size distribution. With on average narrow pores, a narrow pore size distribution and restrictions on cross-section down the length of the pores, it is conceivable that the larger analytes are ad- or absorbed mainly near the entrance of the pores, whereas the smaller analytes may penetrate more deeply into the pores. This may lead to an apparently higher interdiffusion coefficient for the larger analytes and an apparently smaller interdiffusion coefficient for the smaller analytes. The stationary phase used in this work does apparently not fulfil these conditions as the normal order of HETP for analytes of different molecular sizes is observed.

Although the HETP is the preferred measure for judging the quality of a column, the retention time and the resolution are the final criteria for judging the time requirement and quality of a separation for a given analyte mixture on a specific column. The capacity factor is to a first approximation independent of linear velocity and column length, not only in GC and HPLC but also, to a lesser extent, even in SFC, and is given by the ratio of the retention times in the stationary and mobile phases. Therefore, one may consider the capacity factor as a dimensionless retention time measure. In Fig. 6, three-dimensional plots are shown with the capacity factor of chrysene, k'(C), plotted on the z-axis and the pressure at the column end, p, and the linear velocity, u, on the x- and y-axes, respectively. The average resolution, R_m^*, is shown as shading on the three-dimensional surface of the graph. The average resolution, R_m^*, shows a definite maximum at low pressures and at intermediate linear velocities. If the pressure is increased, the maximum tends to move toward lower velocities. This is connected with the decrease in the interdiffusion coefficient at higher density. The capacity factor k'(C) tends to decrease at higher velocities, which may be due to the higher pressure drop over the column arising at higher velocities. An increase in methanol content from 2.5% (Fig. 6a) to 20% (Fig. 6d) leads to considerable decreases in both k'(C) and R_m^*. The same is seen in Fig. 7 with similar three-dimensional graphs in which, however, the p axis is replaced by a %B axis and p = constant in Fig. 7a (200 bar), Fig. 7b (250 bar) and Fig. 7c (300 bar). The R_m^* values show, as in Fig. 6, a maximum which in this instance is located at low %B and again at a medium linear velocity. Also, the

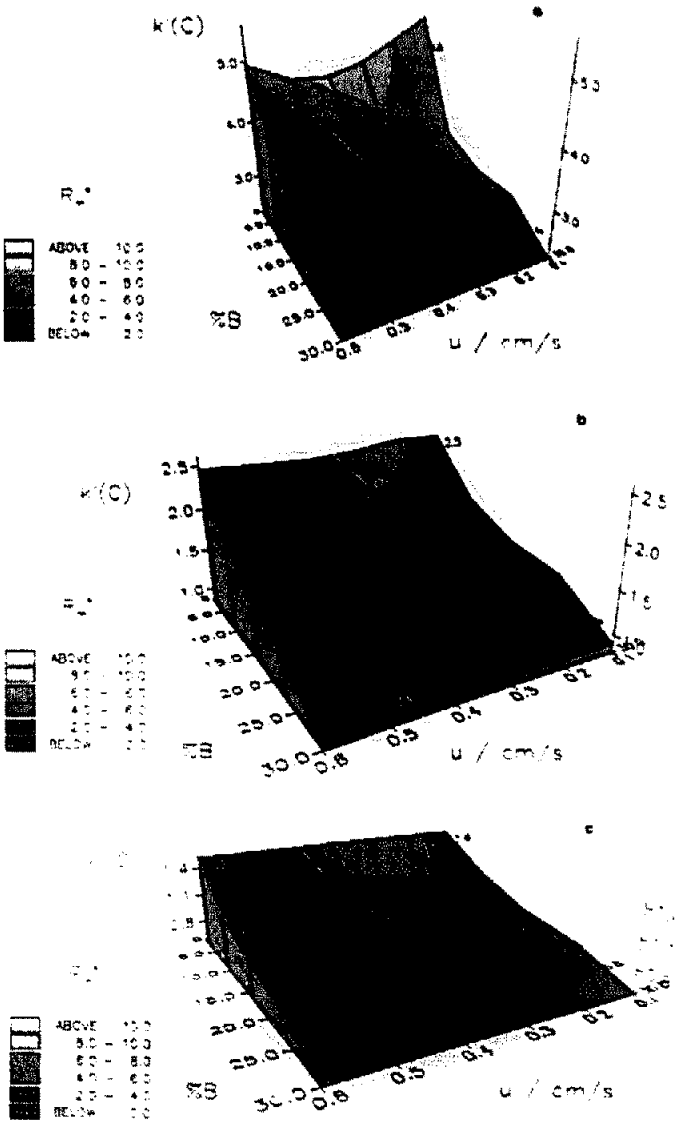

Fig. 7. Graphs of k'(C) versus concentration of methanol in CO_2 (%B) and linear velocity (u). R_m^* is shown as shading of surface. Column end pressure: (a) 200; (b) 250; (c) 300 bar. Other conditions as in Fig. 1.

k'(C) values tend to decrease at higher velocities. Inspection of both Figs. 6 and 7 points to a larger change in k'(C) and R_m^* with p than with %B over the ranges investigated. In general, however, the question of p or %B having a larger influence on k' and R_m^* will depend not only on the size of the range, but also on the nature of the two components making up the mobile phase. For instance, it is seen in other cases that the %B of a component with a higher

dissolution power for a specific analyte than methanol will decrease k'(C) more than the pressure p over the chromatographically useful range of %B and p.

Fig. 6 shows that for positive pressure gradient programming at a constant %B and at 150°C, a negative velocity programme is advisable in order to remain at optimum resolution throughout the pressure programme. This appears to be less the case with a positive composition programme as is indicated by Fig. 7, because the optima of k'(C) and R_m^* remain essentially at the same velocity during a %B programme.

Three-dimensional diagrams of the type in Figs. 6 and 7, wherein chromatographic parameters such as capacity factor k', selectivity α, plate number N, plate height H and resolution R are plotted *versus* two physical properties, selected from pressure/density, composition, temperature and linear velocity, contain much useful information for SFC. The information exceeds that available from Van Deemter two-dimensional diagrams, not exhibiting different p, %B or T as parameters. Nevertheless, important information is not contained in the three-dimensional plots, even if by a sufficient number of plots the influence of all four of the physical properties on the chromatographic parameters is shown. The missing information is the time, or time increment, during which a specific set of physical properties of the mobile phase holds during programming. For instance, for pressure programming no information is contained in the three-dimensional plots about the time which is allotted by a programme to a specific pressure level, or pressure increment at a given pressure level. This information can only be supplied by a pressure–time function, that is, by a pressure programme curve. On the other hand, a favourable pressure–time function may be selected from the three-dimensional plots and then both resolution and time requirement for the chromatogram may be estimated. It is thereby understood that the reading of quantitative values cannot be made from three-dimensional plots but that the two-dimensional plots which are the precursors of the three-dimensional diagrams must be utilized. Obviously, it is of major importance to choose a suitable programme curve.

The question of whether a pure mobile phase consisting of component A or a mixed mobile phase consisting of a constant ratio of components A and B is to be preferred for pressure programming with the present or any other system depends to an approximation on the dissolution ability and the diffusion coefficient of component B relative to component A at the same pressure and temperature. If, for instance, by adding B to A there is a significantly higher dissolution ability to be gained with not too great a decrease in diffusion coefficient, then pressure programming with a mixture of A and B should be of advantage. The question of whether a constant mixture of A and B or a composition programme in A and B is to be preferred is related to the difference in the diffusion coefficients between A and B, *i.e.*, as a rule on how much smaller the diffusion coefficient of B is. If there is a large difference, it is of advantage to elute as many compounds in the analyte mixture as possible with zero or low contents of B in the mobile phase. The remaining compounds in the analyte mixture may require steadily increasing contents of B for elution. In such a case a composition programme may yield better resolution in a shorter time than a pressure programme with a constant mixture of A and B.

For the chromatographic system used here, Figs. 6 and 7 can be utilized for a comparison of pressure and composition gradient programmes. Pressure programmes going from 200 to 300 bar with a mobile phase of constant composition of A (CO_2) and B (methanol), *i.e.*, 2.5, 5, 10 or 20% B (Fig. 6) are available for comparison with composition programmes going from 2.5 to 30% B at constant 200, 250 or 300 bar (Fig. 7). For a suitable comparison, it may be considered that both the pressure and the composition programmes should start at the same pressure and composition, *e.g.*, at 200 bar–2.5% B, or at 200 bar–5% B; or at 250 bar–2.5% B or at 250 bar–5% B; etc. Starting a pressure programme at 300 bar is not possible for this comparison because no experiments above 300 bar were conducted. The temperature is comparable, as it is 150 C for all data. As a result of the indicated identical conditions, the compared pressure and composition programmes start at the same k' for the analytes. As the end point for the pressure and composition programmes to be compared one should probably use the same retention time, t_r. Because in the three-dimensional diagrams not t_r but k' is plotted, we are led to use k'. Because both the

starting and the ending k' cannot be read with sufficient accuracy from the three-dimensional plots, the two-dimensional plots of k'(C) *versus* u and of R_m^* *versus* u, which provided the precursors of the three-dimensional diagrams, were considered. At optimum velocities, the composition programmes in Fig. 7 lead on average to about same resolutions as the pressure programmes in Fig. 6, but exhibited higher capacity factors. This is probably connected with the relatively small increase in dissolution power for the analytes used here when methanol is added to CO_2 in the supercritical mobile phase.

Equations useful for gradient programming have rarely been proposed. One equation has been employed for composition [22] and one for density [23] programmes. It would be of interest, however, for practical use, for the comparison of the results of different workers and for clarity, if simple equations could be found that can be employed for all types of gradient programming, *i.e.*, for the programming of pressure, density, composition of binary mobile phases, temperature, volume flow-rate or average linear velocity. Thus, one or a few equations should be adaptable by appropriate changes of definitions to the programming of all gradients. A composition programme that has frequently been used previously is [22]

$$Q'_B = \frac{P_B}{1 - P_B} = \frac{P_B}{P_A} \tag{4}$$

with

$$P_A + P_B = 1 \tag{5}$$

where Q'_B is an arbitrary, but proportional, measure of real time whose proportionality constant may be freely chosen for each chromatographic run. The real time may be expressed in hours, minutes, seconds or other units, as convenient. The P are fractions of volume flow, the volume flow usually taken as the feed rates of the pumps. The definitions of P are

$$P_B = \frac{F_B}{F_A + F_B} \tag{6a}$$

$$P_A = \frac{F_A}{F_A + F_B} \tag{6b}$$

where F_A and F_B are the volume flow-rates of components A and B, respectively, of the binary mobile phase. Whenever it holds that $F_A + F_B = F_t$ = constant, where t = total and F_t is the total volume flow-rate, during a chromatographic run, this programming equation fulfils the condition for an asymptotic increase in the content of component B in the mobile phase during the programme. Component B usually is chosen to possess the higher migrating (solubility) power for the analyte as compared with the base component A. An asymptotic increase is desirable because with increasing relative molecular mass or increasing polarity of the compounds in the analyte mixture, the differences in physical properties between compounds in the analyte mixture often become smaller and smaller as the molecular size and/or the polarity of the compounds increase. This is immediately obvious, for instance, for homologues and oligomers. However, not all SFC hardware allows the additional condition needed for running a composition programme of eqn. 4, *i.e.*, $F_A + F_B$ = constant. In this event another programme equation is needed. Moreover, eqn. 4 is not applicable to the other gradients because a pressure gradient, for instance, starts at a constant pressure level which does not change during a run like P_A does. Also, the desirability of an asymptotic, or at least progressively slower, increase in a programme applies not only to composition programmes but probably also to programmes of pressure, density, temperature, volume flow-rate or linear velocity.

For example, an equation for a volume flow-rate programme may tentatively be written in the same general form as eqns. 4 and 5 for the composition programme, *i.e.*,

$$Q'_F = \frac{P_F}{1 - P_F} = \frac{P_F}{P'_F} \tag{7}$$

with

$$P_F + P'_F = 1 \tag{8}$$

Here Q_F is again the arbitrary time scale and P_F and P'_F are fractions of flow-rate as defined by

$$P_F = \frac{F_a}{F_0 + F_a} \tag{9a}$$

$$P'_F = \frac{F_0}{F_0 + F_a} \tag{9b}$$

where F_0 is the volume flow-rate at which the programme starts and F_a is the additional flow-rate which increases during the programme. F_0 is a constant and $F_0 + F_a = F_t$; F_t is the total flow-rate, which changes as F_a changes. However, $F_0 + F_a = F_t$ leads to a programme curve that is not asymptotic but linear. In order to introduce a curvature again, the equation must be raised to powers different from 1, *i.e.*,

$$Q'_F = \left(\frac{P_F}{1 - P_F}\right)^a = \left(\frac{P_F}{P'_F}\right)^a \qquad (10)$$

With exponents $a > 1$ the curvature is downward (towards the Q axis) and with $a < 1$ it is upward, the curvature becoming more pronounced the more the exponent a differs from 1.

Instead of writing eqns. 4 and 10 in terms of the reduced quantities P, they may also be written in the corresponding absolute quantities. Starting from eqn. 7, substituting eqn. 9a and b, raising to the power of a and introducing an arbitrary constant factor k yields for eqn. 10

$$Q_F = k\left(\frac{F_a}{F_0}\right)^a = k\left(\frac{F_t - F_0}{F_0}\right)^a = k\left(\frac{F_t}{F_0} - 1\right)^a \qquad (11)$$

One might be of the opinion that the introduction of a constant factor such as k is superfluous because Q_F is connected with time by any arbitrary factor. However, if more than one programme is applied to the same chromatographic run it is convenient to have at hand an additional means for expanding or compressing each programme curve individually, and by this means being able to retain a single, overall time scale applicable to all programmes of the chromatographic run.

When wishing to programme the other physical parameters, *i.e.*, pressure, density, temperature or linear velocity, one has only to redefine the terms in eqn. 11. In turn, F_0 then becomes the starting pressure, p_0, or the starting density, ρ_0, or the starting temperature, T_0, or the starting average linear velocity, u_0, all of which are constants for a given programme. F_a becomes then, respectively, the additional pressure, p_a, the additional density, ρ_a, the additional temperature, T_a, or the additional linear velocity, u_a, all of which are the parameters that change during a given programme. Finally, F_t becomes the total pressure, p_t, the total density, ρ_t, the total temperature, T_t, or the total linear velocity, u_t. Otherwise, eqn. 11 remains the same, considering, however, that the factor k and the exponent a may be chosen differently for each programme and might therefore also be renamed as, for instance, l, m, n, ..., and b, c, d, ..., respectively.

The flexibility of the hardware is of concern not only for composition programming but also for pressure programming. When one is able to start pressure programmes at a few bars, say at $p_0 = 3$ or 10 bar, and then increases the pressure above the critical pressure p_c ($p_0 + p_a = p_t > p_c$, with $T > T_c$), one is in the position to combine GC and SFC without needing to change the mobile phase. One could start, for instance, with CO_2, ethane, or trifluoromethane at 3 bar and proceed by means of a pressure programme to leave the GC and enter the SFC region. However, liquified gases such as CO_2 possess a considerable vapour pressure at ambient temperature which must be exceeded in the pumping device if one desires to pump the mobile phase in its liquid state. With the pumps currently used, the mobile phase is metered as a liquid and not as a gas as by a compressor or from a pressure cylinder. This vapour pressure prevents a chromatogram being started at a pressure lower than the vapour pressure of the liquid mobile phase at the temperature of the pumping device. Therefore, a back-pressure controller immediately downstream of the pumping device, which keeps the pressure in the pump well above the vapour pressure and constant, is necessary. Keeping the pressure constant has the added advantage that no changes in the compressibility of the pumped liquid occur when the pressure is changing in the column, *e.g.*, during a pressure programme. This lack of compressibility change is even more convenient when two or more liquids need to be pumped separately, as is the case for composition programmes. In Fig. 8 a schematic diagram of an SFC apparatus is shown which possesses a pressure-controlling device downstream of two pumps. The apparatus is capable of independent programming of all physical variable which affects the mobile phase and the column.

Taking the gradient programme of the average linear velocity, u, as a further example and to demonstrate the effect which exponent c and factor m have on the shape of the programme curve of Q_u *versus* u_t, we can write

$$Q_u = m\left(\frac{u_a}{u_0}\right)^c = m\left(\frac{u_t - u_0}{u_0}\right)^c = m\left(\frac{u_t}{u_0} - 1\right)^c \qquad (12)$$

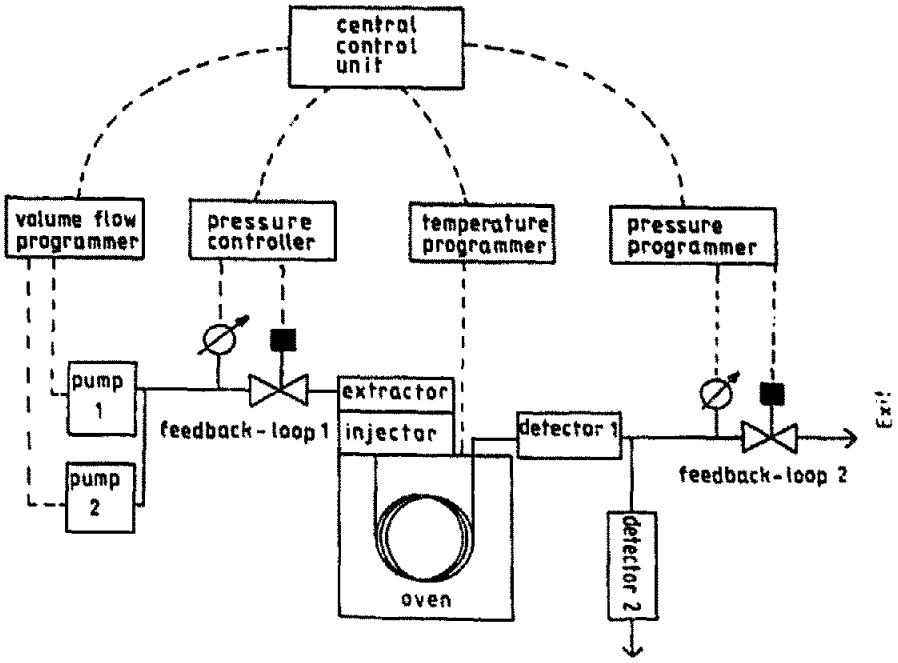

Fig. 8. Schematic diagram of SFC apparatus capable of combining GC and SFC during the same chromatographic run, without changing the pumping (delivery) arrangement and with or without changing the mobile phase during a given run, as made possible by a pressure-controlling feedback loop downstream of the pumps. The apparatus is capable of independently controlling and programming pressure, density, composition, temperature and volume flow-rate (linear velocity).

In Fig. 9a, the effect of the exponent c on the shape of the programme curve is shown, assuming the values $c = 3.0$, 2.0, 1.5, 1.0 and 0.5 with $m = 1$ and $u_0 = 0.1$ cm/s. As is obvious from eqn. 12, an exponent $c = 1.0$ must produce a linear programme, *i.e.*, a straight line, exponents $c > 1.0$ lead to curvatures towards the Q_u axis and $c < 1.0$ produce curvatures towards the u_t axis. There is a common intersection of all curves at $Q_u = 1$ and $u_t = 0.2$ cm/s. Such a common intersection of all curves occurs whenever $(u_t - u_0)/u_0 = 1$. A common intersection can be utilized as a common end-point for different programmes. Together with a common starting point, this will simplify comparison of different programmes because they may then differ only by their curvature. Also, any section of any curve may

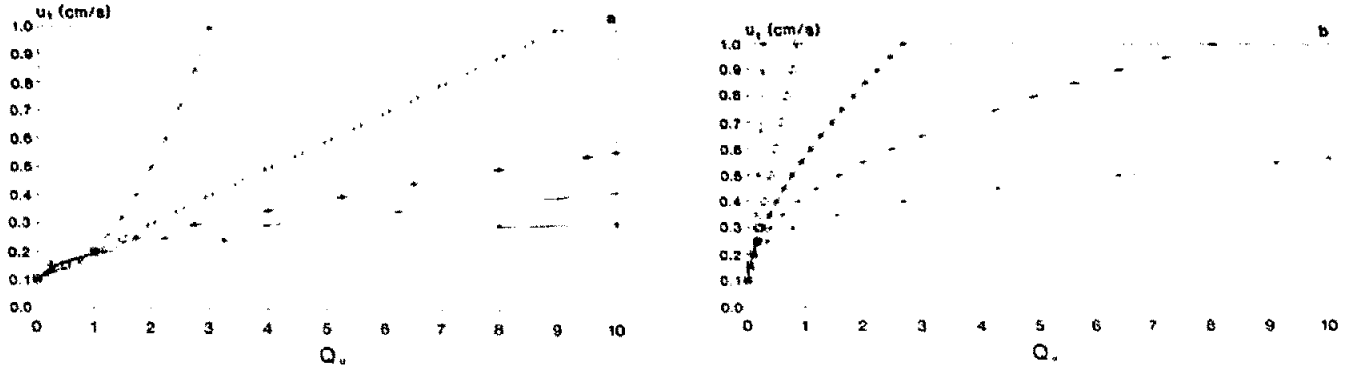

Fig. 9. Curves for gradient programming as examples of programming the average linear velocity u_t *versus* Q_u units, the latter units being proportional to time. Curves in Fig. 9a and b calculated by eqn. 12. (a) Factor $m = 1$, velocity $u_0 = 0.1$; (b) $m = 0.1$, $u_0 = 0.1$. The exponent c in eqn. 12 was varied as follows: ■ = 3.0; + = 2.0; ✱ = 1.5; □ = 1.0; × = 0.5.

be used as a full programme, for instance the section between $Q_u = 0$ and 1, or between $Q_u = 1$ and 10. Moreover, the factor m may be utilized to compress the curves by choosing factors $m < 1$ or to expand the curves with $m > 1$. The compression is seen in Fig. 9b, where $m = 0.1$ instead of $m = 1$ in Fig. 9a, leading to a compression by factor 10 in comparison with Fig. 9a.

Variability in choosing different shapes of the programme curve arises not only from the exponent and factor and by selecting a desirable section of the curve, within or beyond their common point of intersection, but also by using curves in their reverse direction. In the latter instance a positive programme turns into a negative programme and *vice versa*, whereby the shape of the programme in the reverse direction is different from the shape of that in the forward direction, because the greatest change of slope is not at the beginning but at the end of the programme. This type of programme curve may be of interest whenever the solubility differences between the compounds of an analyte mixture become larger the later the compounds elute. This behaviour of an analyte mixture is opposite to that found in homologous series.

CONCLUSIONS

The results of this study can be divided into three parts, *i.e.*, those concerning plate height, resolution and equations for gradient programming. With respect to plate height, it can be concluded that for a positive pressure programme at constant mobile phase composition and constant temperature, a negative linear velocity programme should be superimposed, *i.e.*, run simultaneously, in order to stay within the area of the optimum plate height in the Van Deemter diagrams. The higher the relative molecular mass and molecular size, the stronger this effect might become and, therefore, when separating higher relative molecular mass compounds by pressure or density programming in SFC, improvement is possible by superimposing an appropriate linear velocity programme. The second basic gradient besides pressure which was explicitly considered in this study is that of composition programming. A positive composition gradient at constant pressure does not need to the same extent as a pressure gradient an additional negative linear velocity programme to improve plate height. Nevertheless, and even without a composition or a pressure programme, the influence of the relative molecular mass of the sample itself on the plate height makes it desirable to have the capability of applying a negative linear velocity programme, especially if an analyte mixture with a wide relative molecular mass range of the individual compounds is to be separated.

Second, with respect to resolution and capacity factor, three-dimensional graphs show that for positive pressure programmes negative linear velocity programmes improve the resolution. For positive composition programmes a negative velocity programme is not needed, or only to a lesser extent. If the temperature stability of the analytes allows, it is speculated that a negative linear velocity programme may be wholly or in part substituted by a positive temperature programme to keep the resolution at a high level. This applies not only to a positive composition programme but also to a positive pressure programme, or to an analyte mixture of wide relative molecular mass range without a pressure or composition programme. The reason is that the negative velocity programme is to compensate for a decrease in interdiffusion coefficient with increasing pressure, composition and relative molecular mass range. This decrease in the interdiffusion coefficient may be counteracted also by increasing the temperature.

Third, the results obtained by pressure and composition programmes, or by velocity and temperature programmes, depend to a considerable extent on the shape and the total time of the programme, even for an otherwise similar programme. "Similar" means in this case identical start and end-point for, *e.g.*, the pressure programmes to be compared among each other. A set of related equations (essentially only one equation) has been presented that allows programme curves of different shapes for the programming of pressure, density, composition, temperature, flow-rate and average linear velocity. Because the equations are of simple form and easy to use, they might be of help in practical method development.

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